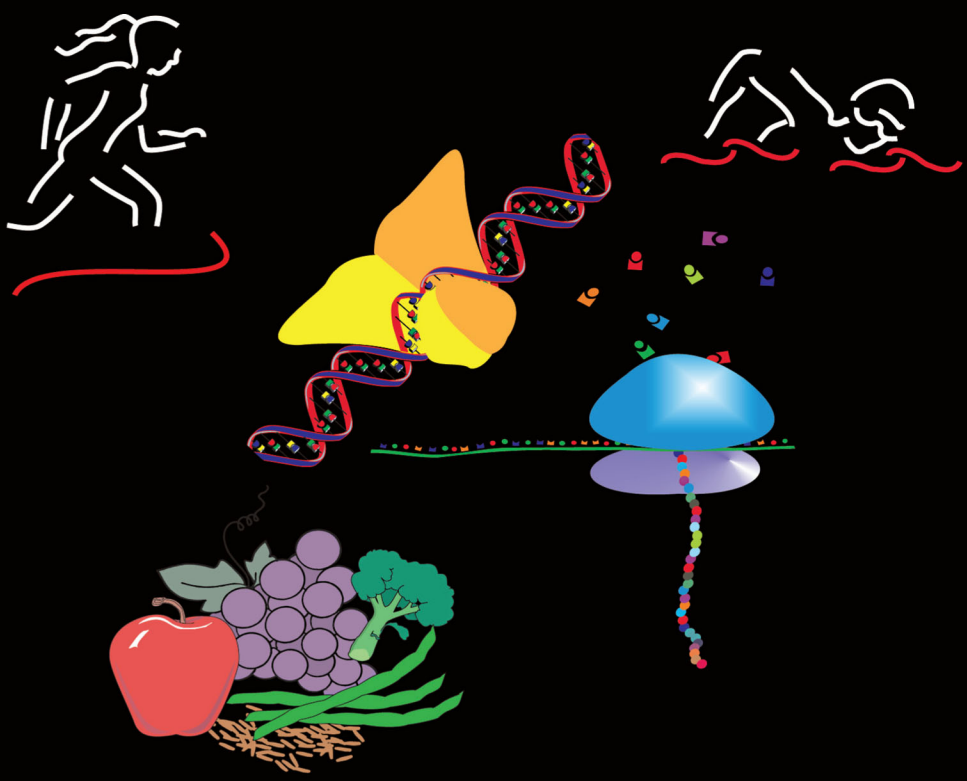
Diet, Exercise, and Chronic Disease

The Biological Basis of Prevention



Edited by
C. Murray Ardies

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Contents

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Preface

The idea of producing a book on the prevention of chronic diseases through exercise and diet was intriguing for me when I was first approached with the idea. I had been teaching nutrition, exercise, and health science courses with a focus on cellular aspects of prevention for many years and had to develop all of my own materials because very few books were available. And of those that were, they had a decided clinical approach with very little discussion about the actual biochemistry or molecular mechanisms involved in prevention. When I broached the concept with several potential coauthors, the prevailing opinion was that books that covered biochemical and molecular aspects of disease etiology also were in very short supply. Thus this book was born: an attempt to collate the latest cellular- and molecular-based research on the etiology of chronic diseases with how these mechanisms of cause are modified by various aspects of diet and exercise. Essentially, we have tried to produce a text that translates molecular-based data on etiology and prevention into a clinical prescription for the prevention of chronic disease.

The focus on diabetes, atherosclerosis, osteoporosis, cancer, and degenerative neurological disease is because they are the major causes of morbidity and mortality by chronic disease and also because there is sufficient molecular evidence for a strong dietary and activity (or rather, an insufficiency of both) component to their etiology. The inclusion of a separate chapter on "Inflammation" became necessary when it was clear that inflammatory signaling is a fundamental component of each of these diseases and that reducing inflammation is key to reducing risk for all of these diseases. At the time we started, obesity had not yet been declared a disease, so it did not get its own chapter. It is discussed as a major contributor to other diseases rather than a disease in and of itself. The chapter on "Hunger and Satiety Signaling" provides a very interesting and probably the most realistic take on the regulation of eating behavior available. As it becomes more obvious from reading this and the other chapters, many things may not really be what "everyone" seems to believe.

Ultimately, we hope that this book can help readers to develop a broader understanding of how chronic disease "works"; to better integrate molecular, biochemical, and cellular mechanisms of cause and prevention into the study of chronic disease; and to get excited about developing new research into the etiology of prevention and into clinical methodologies that translate that research into successful prevention strategies. With recent advances in techniques to determine various aspects of proteomics, metabolomics, epigenetics, and functional genomics, we can anticipate great things in the near future for prevention research.

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Acknowledgments

In the end, I am very grateful to all for the tremendous amount of work necessary to complete this text. From Wendy Ward and David Dodington, Sonia Najjar and Raymond Bourey, and Christian Roberts and "my" graduate student Jonathan Cannizzo who all signed on to this project at the very beginning to Stephen Woods and Denovan Begg and Zahoor Shah who helped rescue it (and then made it even better) when other authors had to leave the project for other commitments; I thank you all for your insight, dedication, and hard work in this very difficult undertaking in the face of never-ending grant deadlines, site visits, innumerable meetings with administrators and graduate students, and the constant laboratory work that never seems to end before midnight.

I also thank Tony Treston, director of manufacturing at Unither Virology (past research scientist at National Cancer Institute (NCI) and VP product development at EntreMed), for his many insightful editorial comments that helped make this text an easier "read"; Milie Fang, Biology Major at University of Illinois at Chicago, for her expert help in converting figure drawings into Adobe Illustrator; and Randy Brehm, senior editor, Chemical and Life Sciences Group at Taylor & Francis Group/CRC Press, for putting up with our ever lengthening schedule and ensuring that the project made it to completion.

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Editor

C. Murray Ardies earned a bachelor of physical education from the University of Manitoba in Winnipeg, Canada, in 1975 following which he worked for the Government of Manitoba as a counselor and then as codirector of Operation ReNu, a public health education program that emphasized proper diet and activity as preventive medicine. In 1978, he earned a master's degree with a major in health education and a major in physical education along with a minor in nutrition from Northern Michigan University, Marquette. He then earned a multidisciplinary doctoral degree at The University of Texas in 1985 with majors in pharmacology, nutrition, and exercise physiology. His dissertation concerned the interactive effects of exercise and chronic ethanol consumption on mitochondrial metabolism in liver, where for the first time exercise was demonstrated to prevent alcohol-induced toxic reactions in nonmuscle tissues.

Dr. Ardies subsequently worked at the Icahn School of Medicine at Mount Sinai in New York City researching biochemical mechanisms of ethanol toxicity, where he developed new methodologies for CYP and CYP reductase purification and the determination of oxygen radical production; then in the Department of Endocrinology at Stanford University Medical School to purify steroid-binding proteins and clone their genes; and next in the Department of Anesthesia with Dr. James Trudell, where he helped characterize molecular aspects of alcohol toxicity involving direct autoimmune attack on liver cells.

In 1989, he joined Northeastern Illinois University, where he worked on defining mechanisms through which repeated endurance exercise reduces risk for chemical toxicities and cancer. As part of this work, he was among the first to demonstrate that beneficial alterations in cellular function were tied to a generalized stress response mediated by the activation of the AP-1 response element in nuclear DNA.

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1 Introduction

C. Murray Ardies

CONTENTS

1.1 INTRODUCTION

The focus of this chapter is on prevention of chronic diseases through diet and exercise. Chronic diseases comprise a major source of mortality from disease for the world and are by far the greatest source of mortality among the high-income countries.[1,2] In the United States, chronic diseases account for about 70% of all deaths and 75% of health-care costs[3] and their prevention is extremely important in terms of alleviating personal suffering and reducing economic impact, for individuals and for the nation. In addition, and possibly even more importantly, various aspects of a poor diet and physical inactivity account for the majority of risk factors among the top 15 sources of risk for both all-cause mortality and disease burden. These risks factors include overweight and obesity, high cholesterol, zinc deficiency, iron deficiency, alcohol use, child underweight, physical inactivity, vitamin A deficiency, and low fruit and vegetable intake.[1]

Because issues related to diet and physical activity are so important for disease risk, it is certainly possible that developing preventive methods that focus solely on these parameters also could have a huge impact on morbidity and mortality from disease and, of course, on the economic issues as well. This is not to say that pharmacological- and nutraceutical-based management of disease risks has no place in prevention. They can be very important in reducing genetic- or environmental-based risks as well as in correcting serious dietary deficiencies, regardless of the cause. They are, however, outside the scope of this chapter.

1.1.1 Definition of Disease

In order to create an emphasis on the biological (cellular and molecular) basis of prevention, the concept of disease used in this chapter is a slight variation of that commonly used elsewhere in a variety of books on prevention,[4–8] medical textbooks,[9–11] and medicine-related Websites.[12–16] For our purposes, disease is a complex process that is initiated by detrimental chemical, biochemical, or cellular processes (endogenous or exogenous), either alone or in some combination thereof,

that if allowed to continue for long enough or are severe enough will result in some form of cellular damage, cellular dysfunction, or cell death. An array of symptoms that are dependent on the specific type of cellular dysfunction and the organ/tissue in which the dysfunction (or cell death) exists will occur only if the cellular dysfunction is prolonged and extensive enough (or if a sufficient number of cells die).

The major difference between the aforementioned definition and other definitions is that in many of the references listed both clinical symptoms and (cellular or organ) dysfunction coexist. Clinically observable symptoms are obviously a component of disease and are especially useful for the purposes of diagnosis. However, if symptoms are a necessity for disease to exist, then there can be no disease if there are no symptoms. For most experts in the biological and medical sciences this distinction may not be important, but for those who are less well versed in human biology it may make a large difference in terms of how they view prevention of disease. For the purposes of prevention in this chapter, the processes of disease that lead to symptomatic disease are the focus.

For a layperson who might be familiar only with those concepts promoted by the experts who write for Web-based media (or, as is far more likely, who has watched innumerable television advertisements that feature middle- to late-middle-aged people newly concerned about preventing coronary atherosclerosis, stroke, osteoporosis, or prostate cancer), it may appear that many diseases just do not *happen* until around 40 years of age or later and that preventing diseases does not start until then either. This incorrect interpretation creates a dilemma for those trying to elucidate and correct the root causes of preventable diseases. On the other hand, many people simply may not be interested in prevention until it is much too late just because they do not feel sick in spite of the fact that they know that various disease processes may have been going on for many years.

In order to create a timely focus on prevention, symptomatic disease needs to be separated from the processes of disease. To use a well-understood example, in atherosclerosis the build-up of plaque in the walls of coronary arteries occurs over a period of several decades before clinical symptoms actually appear. If the concept of disease requires symptoms, then this particular disease does not really start for the *average* person until about the fourth decade of life.[17] The reality, however, is that the processes that produce symptoms of coronary artery disease are ongoing continuously for decades prior to the appearance of the symptoms with asymptomatic lesions already evident in early childhood.[18–21]

Therefore, prevention also should be considered to be a continuous process, one that starts long before symptoms appear. From the aforementioned atherosclerosis example, because the processes of disease start before the appearance of fatty lesions in early childhood prevention must start before early childhood as well. From this standpoint, prevention is a *lifelong* issue and not a middle-age issue. Further, because poor diet and a lack of exercise are integral to high risk for chronic diseases, prevention is clearly a *lifestyle* issue.

1.1.2 Disease Etiology and Prevention

This returns us to the definition of disease. By broadening the concept of disease to include the processes that produce cellular dysfunction, which can then progress to produce symptoms of disease, a focus on cellular and molecular mechanisms of

disease etiology (or cause) is forced on us. Attenuating these mechanisms, which ultimately lead to enough dysfunction to cause symptoms, then becomes the focus of prevention. To clarify, the term *disease* refers to symptomatic disease, whereas the term *disease processes* refers to various mechanisms of disease etiology that occur prior to the appearance of symptoms (Figure 1.1).

A molecular approach to studying the etiology of diseases has revealed that there are cellular mechanisms that are common to many different diseases, which leads us to our approach to prevention. Rather than looking for specific treatments to prevent specific diseases, we look at integrating common mechanisms of disease etiology into a (single) cell-function model that can then be applied to a variety of different cell types and different disease outcomes. This cell-function model of disease should then allow us to develop a (single) lifestyle model of prevention that is applicable to most, if not all, diseases.

To continue with the example of atherosclerosis, the physical perturbation of membrane and cellular proteins of the endothelial cells of coronary arteries caused by turbulent flow, along with chemical-mediated and oxygen radical–mediated trauma, leads to the production of proinflammatory signaling molecules: various eicosanoids and cytokines. These, in turn, lead to the attraction and activation of monocytes to the intima at the site of initial trauma. These monocytes develop into (lipid-filled) foam cells that also produce additional inflammatory molecules as well as more damage-causing radical species. The continuing (low-level) production of inflammatory cytokines leads to the gradual accumulation of more foam cells, smooth muscle cell proliferation, and synthesis of fibrotic tissue, a vicious cycle of inflammatory responses progressing into the development of atherosclerotic plaque.

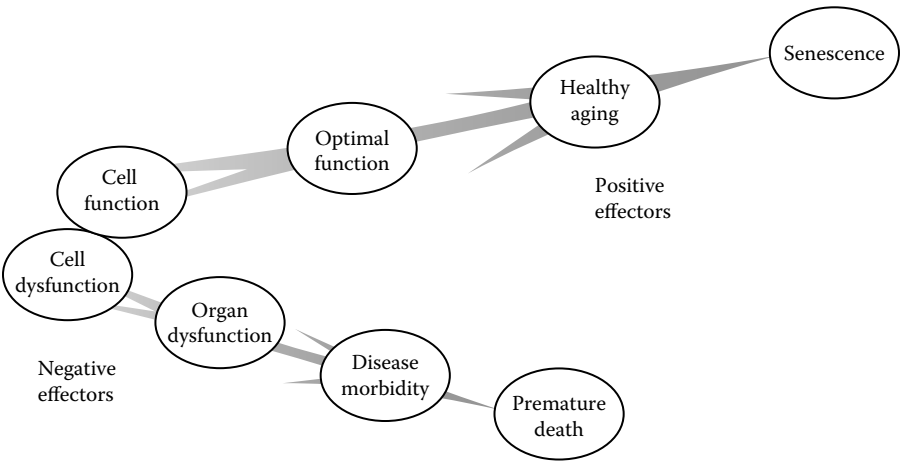

FIGURE 1.1 Effects of positive and negative effectors on morbidity and death: a variety of factors produce positive or negative effects on cell function with some being able to produce both, depending on the amounts produced and the duration of effects. Those factors with predominantly negative effects on cell function can enhance the rate of our inevitable progression toward death while increasing morbidity due to disease. Those factors with predominantly positive effects can optimize function and reduce risk for morbidity to promote healthy aging to senescence.

Eventually, the plaque grows sufficiently large to impede or block blood flow through the coronary artery or to break off to produce a thrombus, thus producing symptoms of the disease.[18–29]

Inflammation-related cellular signaling molecules (and their effects on cell function) and reactive molecules (a variety of reactive oxygen and other molecular species) produced by activated inflammatory cells are therefore integral to the development of atherosclerosis.[19,22,24–29] They are also involved in many cancers,[30–35] and in degenerative neurological disorders such as Parkinson's[36–39] and Alzheimer's.[40–42] In addition, they also contribute to osteoporosis[43–46] and type 1 and 2 diabetes,[47–50] with the inflammatory mechanisms in type 1 diabetes most likely being a result of autoimmune attack on B cells of the pancreas.[51–53] With such global effects on disease etiology mechanisms associated with inflammation should be common primary targets for prevention of chronic diseases.

The details of inflammation are discussed in Chapter 2, whereas the inflammatory mechanisms associated with diabetes, atherosclerosis, osteoporosis, cancer, and neurodegenerative disease are discussed in Chapters 3 through 7. As described in these chapters, cell functions that are affected by various inflammation-related signaling molecules play a contributory role in all of these diseases, as does the molecular damage caused by radical species produced by activated inflammatory cells. In addition to inflammatory factors, noninflammatory factors related to poor diet and inactivity also are important components of many disease processes.

Although the general concepts of poor diet and inactivity might seem straightforward, they actually are fairly complex. Regarding dietary issues, *poor diet* might refer to the consumption of insufficient amounts of specific nutrients leading to nutritional imbalances. It might also refer to the consumption of large amounts of refined carbohydrates, or to excessive alcohol consumption, too little fiber, too many calories, or any one of a myriad number of excesses or specific nutrient insufficiencies that may occur in spite of consuming macronutrients in adequate amounts. From our prevention standpoint, it also refers to consuming too few phytochemical-rich foods while maintaining both caloric balance and proper nutrition.

In general, detrimental alterations in cell function due to a combination of poor diet, excessive calorie intake, and lack of physical activity are the fundamental causes of obesity and metabolic syndrome.[54–56] Although not usually considered a disease per se, metabolic syndrome is a collection of coexisting risk factors associated with atherosclerosis, type 2 diabetes, and stroke that includes abdominal obesity, hypertension, insulin resistance, and dyslipidemia.[54,56] Metabolic syndrome and obesity, and by default poor diet, excess calories, and inactivity, are also causally associated with many cancers.[55,57–61] Metabolic syndrome, along with other dietary factors, is also causally associated with neurological disease[62–65] and osteoporosis.[66–68] Further, when studied in isolation of other risk factors a lack of physical activity seems to be associated with an increased risk for just about any chronic disease,[69–74] so much so that some of the leading experts in our field have suggested that inactivity should be considered a disease in itself.[74]

The overall consumption of calories and the amount of calories expended through various activities are fundamental issues in weight control. As detailed in Chapters 2

through 8, it is how these variables affect disease etiology through altering cellular metabolism and activities of signal transduction pathways that, in part, reveals how prevention through weight control works. In addition to the roles of proper diet and caloric balance in the prevention of diseases, specific components of foods called phytochemicals are also very important for prevention.

Phytochemicals are nonnutritive molecules from plants that produce biological effects that are beneficial to health.[75–77] The roles that phytochemicals play in reducing risk for disease are very complex. For some phytochemicals the molecular mechanisms of prevention may be related to their antioxidant or prooxidant properties, whereas for others the ability to modify enzyme activity or the cellular expression of various cytokines, enzymes, and transcription factors appears to be integral to their protective effects.[77–85] In terms of overall prevention of disease, it is most likely through the additive and synergistic effects of many *different* phytochemicals that their preventive effects are realized.[77,79,82,83,85] This implies, of course, that incorporating a variety of phytochemical-rich foods into an otherwise nutritionally adequate and calorie-balanced diet may enhance the preventive effects of the said diet, a concept that is directly in line with one of the major objectives of this chapter: the development of a (single) lifestyle model of optimal prevention that is applicable to most, if not all, chronic diseases.

When it comes to exercise and prevention, it is important to recognize that exercise is not just a *calorie burner.* Exercise can also produce changes to cell function that in some respects are similar to those observed with many phytochemicals. Activation of signal transduction pathways and transcription factors along with altered expression of various functional proteins have all been observed in skeletal muscle cells[86–90] and in nonmuscle cells such as adipose, liver, lung, and brain,[91–96] to reference just a few of the available studies. Just to clarify, for our purposes, exercise is much more than physical activity. *Physical activity* refers to any bodily movement produced by skeletal muscles that results in an energy expenditure that is substantially greater than that at rest[97] and refers to a single session of activity. The term *exercise* or *physical exercise* as defined by Courneya and Friedenreich[98] is any physical activity that is performed repeatedly over prolonged periods of time with the intent of improving fitness or health. Because improvement of health is central to the concept of prevention, it is the incorporation of exercise into our daily lifestyle that is relevant for prevention. How exercise affects cell function is the means through which exercise alters health.

A simplified general model of some of the molecular aspects of cellular function is illustrated in Figure 1.2. Changes in the activity of various signal transduction pathways due to responses to extracellular signaling molecules (hormones, cytokines, etc.), to damage from reactive oxygen species and other chemical oxidants, and to changes in the intracellular redox environment lead to alterations in the expression of various proteins that are important in maintaining cellular integrity and function. Deleterious cellular responses can lead to disruptions in cell function that may then lead to tissue dysfunction and ultimately to symptoms of disease. Cellular responses to different forms of exercise (or lack thereof) and to various compounds obtained from the diet and the environment can modify these responses to subsequently alter risks for cellular dysfunction and disease.

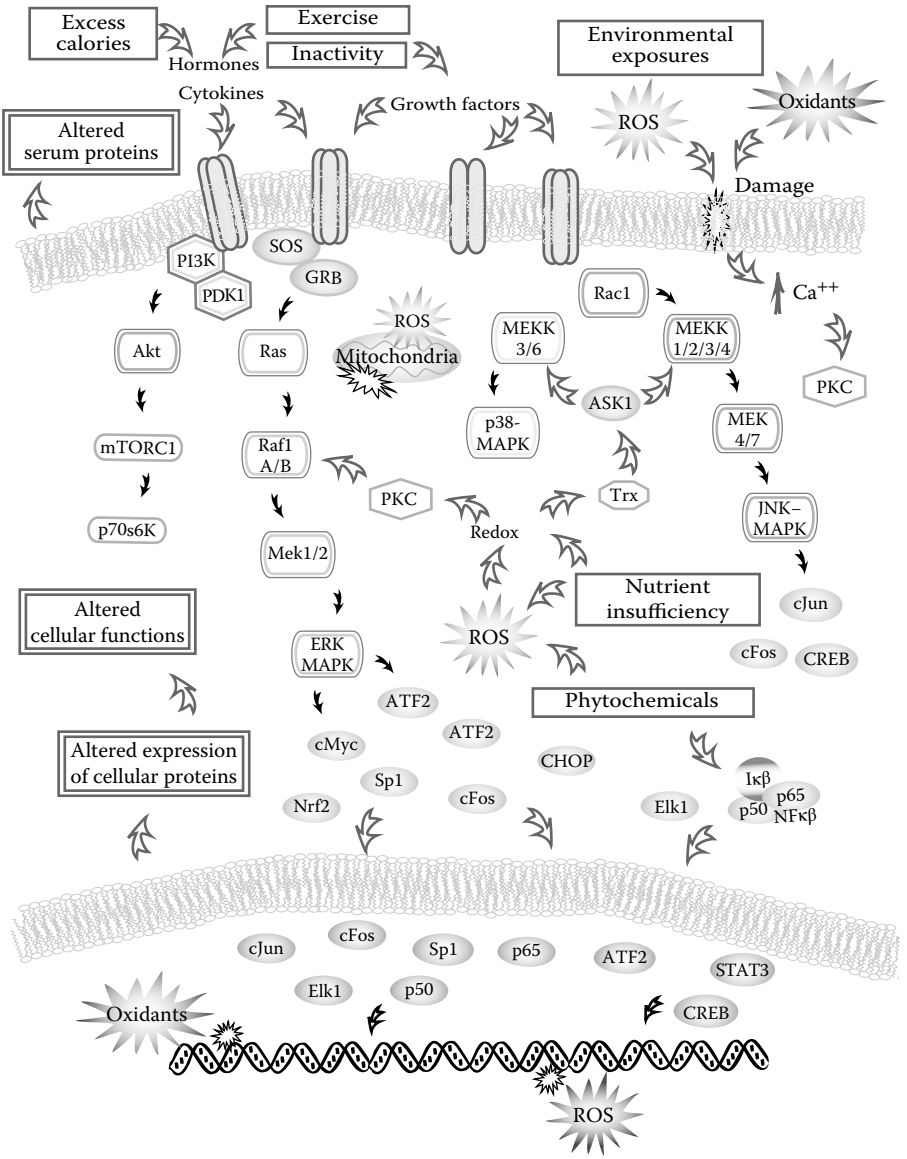

FIGURE 1.2 A model for the molecular aspects of cell function: alterations in activity of various signaling pathways that regulate the activation of transcription factors are fundamental to the mechanisms through which disease processes result in cellular dysfunction as well as how physical activity and dietary components alter risk for chronic disease. Filled small arrows indicate signal transduction pathways that lead to activation of various transcription factors. Open arrows indicate some of the mechanisms through which the activity of these pathways is modified by both intra- and extracellular factors.

Although such a model may have utility for aiding in understanding a complex topic, it can also be used to assist in testing hypotheses related to disease prevention by highlighting potential biomarkers of risk and effect. Biomarkers are simply any molecular marker obtained from tissues or biological fluids that can be used to indicate a biological event has occurred, and they can be used for a variety of purposes including indicating some sort of cellular damage has occurred or some risk exists for future events.[99] For example, changes over time in the level of urinary 8-hydroxy-deoxyguanosine (8-OH-dG), a hydroxyl radical–caused DNA adduct, can be used to indicate that genotoxic exposures of different degrees have occurred, that there have been changes in the amount of repair of oxidative damage to DNA, as well as a marker for potential future risk for cancer.[100] On a cellular or tissue basis, alterations in the activity of redox-control enzymes and antioxidant enzymes may indicate changes in risk for cellular and tissue damage and dysfunction due to exposures to cellular oxidants from either endogenous or exogenous sources. Acute changes in redox and antioxidant status may result in altered serum levels of certain proteins if the changes alter the expression of proteins that are exported from the cell. Stress-response signaling molecules such as certain cytokine antagonists, for example, appear in the blood following long-term metabolic stress due to exercise, and they initiate a variety of cellular responses that are anti-inflammatory in nature and may then influence many disease processes.[101,102]

The use of such biomarkers in prevention research would require detailed knowledge of how they specifically relate to both cell function and the etiology of disease. Fortunately, as discussed in detail in this book, the various roles that many potential biomarkers play in the etiology of chronic diseases is known. One advantage of utilizing biomarkers in research is that hypothesized paradigms for prevention can be tested in a relatively short time based on the following premise: if there are no changes in mechanisms, there are no changes in risks. For example, changes in activation patterns of transcription factors or in the activity of antioxidant enzymes can be observed within minutes to hours after the start of exercise.[86–88,90–92,103] Conversely, looking for an actual change in incidence of a particular disease in order to experimentally determine the clinical effectiveness of a specific form of exercise may take many years or even decades before any indication of an actual preventive effect might be observable. This does not mean that random assignment, placebo-controlled clinical trials should not be performed; they are essential and are the gold standard for definitive evidence of effectiveness. Judicious use of biomarkers in experiments can, however, provide valuable information that can lead to the development of more refined prevention paradigms that are based on observed alterations in mechanisms that are part of the etiology of disease rather than on associations observed in epidemiology surveys.

The actual amount and intensity of exercise required to reduce risk for various cancers, for example, is not well understood.[104] There have been conflicting recommendations for exercise, depending on which professional body is making the recommendations.[105–108] The recommendations range from a minimum of 60 minutes of continuous moderate-intensity activity each day for children and adolescents to a minimum total of 20 minutes of moderate-intensity activity spread throughout the day on most days, a very wide range. There is also a general recognition by

these same groups that additional benefits are gained by following the longer, more intense guidelines, raising the issue of why one set of recommendations would be made when a different one will result in greater health gains.

The aforementioned recommendations for physical activity are based on epidemiological assessments of reported patterns of physical activity and incidence of disease,[105,106,108] and they all suffer the limitations of such efforts: inaccurate memories of just how much activity was performed, lack of precise control over a large array of specific variables (including diet), and no actual measurement of cellular function variables directly affected by exercise and associated with cause of disease, to name a few. In fact, one of the major factors in producing some of the guidelines was the type of exercise that increases maximum oxygen consumption,[105] which is hardly a cellular mechanism associated with the etiology of any disease. It is even plausible to interpret many of the observed preventive benefits of exercise as happening simply because the detrimental effects of obesity/overweight were avoided. Only through understanding the molecular mechanisms of prevention obtained through weight management and those obtained exclusively from exercise is it even possible to determine whether it is the exercise, weight management, or a combination of both that promotes optimal prevention. For the sake of argument, if it is weight management that *does the job* of prevention, and there are no additional beneficial effects from exercise per se, then exercise would not even be necessary for prevention. Maintaining a healthy body weight through diet alone is certainly possible; it just may not be as easy without exercise.

Of course, the bulk of research evidence certainly does suggest that both exercise and diet can have a profoundly positive effect on health. Further, as described in Chapters 2 through 8, the molecular mechanisms of how those effects are realized are now being delineated. These molecular events not only help to explain how prevention works but also form the basis for the development of specific biomarkers of effect that provide more meaningful measurements of the preventive effects of dietary components and of various types, amounts, and intensities of exercise. As suggested by Rundle,[104] the concept of using molecular markers (or biomarkers) of risk is well established in the field of cancer epidemiology,[109–111] and a similar approach should be followed by investigators in the exercise–health field as well.

Only through a comprehensive analysis of the molecular etiology of chronic diseases can targets for effective prevention be determined. Further, effective prevention strategies can be devised only through an understanding of how various dietary components and forms of exercise affect these molecular mechanisms. In addition, it is only through molecular approaches to epidemiology and experimental clinical trials that effective prevention protocols can be validated and refined without waiting for decades for symptomatic disease to (not) appear.

Ultimately, we have to start somewhere in our quest for a definitive model for a prevention-based lifestyle. Where known, each chapter will delineate mechanisms through which specific dietary components and different forms of exercise may reduce risk for the specific chronic disease or syndrome. Because inflammatory mechanisms underlie many chronic diseases, a recommendation for an anti-inflammatory lifestyle may present a reasonable model from which to start. In the end, all of the recommendations will be consolidated into a comprehensive prevention diet complete with

recommendations for specific foods and numbers of servings, as well as for specific forms of exercise based on the preventive benefits of each. Because these recommendations are made on the basis of molecular-based evidence on how etiology of disease is affected by dietary components or specific forms of exercise, they will differ in both quality and quantity from the current recommendations that are predominantly based on epidemiological associations between diet, exercise, and disease risk. As such, the recommendations might be considered to comprise a set of research hypotheses that are designed to translate existing evidence of how dietary components and exercise stresses alter molecular mechanisms of cell function to alter the etiology of chronic disease into practical and effective strategies for the prevention of chronic disease.

ACKNOWLEDGMENT

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2 Inflammation

C. Murray Ardies

CONTENTS

2.1 INTRODUCTION

From the standpoint of health and chronic diseases, inflammation certainly appears to be the scourge of humankind. Inflammation is not, however, considered to be a disease. This is in spite of the fact that a variety of processes that are associated with inflammation are fundamental to the cause of many chronic diseases,[1–15] including those detailed in this book. Traditional immunology views inflammation as a component of the innate immune system and a local response to the entry of infectious agents into a tissue and to cellular damage resulting from the infection.[16–19] Inflammation comprises a well-defined set of cellular responses that are designed to eliminate the damaging pathogen and initiate repair of the damage caused by the pathogen. The responses are (usually) localized to the specific tissue involved in the infection/damage, and while it typically occurs as a result of some type of infection the same array of inflammatory processes can be initiated by cellular damage that is independent of infection, which is called sterile inflammation. We put this in context with the initiation component of our definition of disease: a disease is a complex process that is initiated by detrimental chemical, biochemical, or cellular (endogenous or exogenous) processes, either alone or in some combination thereof, that if prolonged for long enough or are severe enough, will result in some form of cellular damage, cellular dysfunction, or cell death. Inflammation is a response to those detrimental processes that initiate disease, and it is started at the same time as those initiating events. In this context, inflammatory responses comprise what might be termed a *precautionary* response, a set of responses designed to limit the cause of impending damage and to "ramp up" repair processes on first appearance of that cause.

As an immune function issue, the inflammatory response is necessary for initiating a series of events that leads to removal of the infectious agent and repair of the damage. Removal of the infectious agent means killing the infectious agent, and the killing processes are a component of innate as well as adapted immune responses. Although immune responses are necessary to kill off infectious agents to avoid cellular death, the killing function can also have negative effects on unaffected cells near the site of infection. In the same context, repair processes that are initiated by an inflammatory response, processes that are designed to promote the repair of cells damaged (or replacement of those killed) by the infection, can also have negative effects on other cells.[16–19] Thus, the inflammatory response is a double-edged sword: if an appropriate inflammatory response occurs, then the damaging agent is removed and the damage is repaired. On the other hand, if the inflammatory response is too aggressive, or insufficiently aggressive and continues too long, then additional problems are created, problems that include increased risks for a variety of chronic diseases.

Inflammatory responses to infectious pathogens and to damage from noninfectious sources contribute to the development of chronic diseases through a variety of complex interactions. The contribution of inflammatory responses to atherosclerosis illustrates this nicely and might be summarized briefly as follows: inflammation-associated responses to vascular stresses due to the physical characteristics of blood flow in the coronary arteries are mechanistically involved in the initiation of atherosclerosis, and a variety of molecules have been implicated in enhancing the responses, molecules including oxidized lipoproteins, oxidized cholesterol, homocysteine,

and damaging reactive oxygen species (ROSs) from various exogenous and endogenous sources.[20–26] In some cases, inflammation-related processes that are produced in conjunction with various infections (herpesvirus, *Chlamydia pneumonia, Porphyromonas gingivalis, Helicobacter pylori*, and hepatitis A) have also been implicated as contributing factors to atherosclerosis with both innate and adaptive responses to the infectious agents being involved in the production of atherosclerotic plaque.[20,21,27–34] As a result of a plethora of research studies in this particular area, the proximate cause of atherosclerosis is now known to be a complex interplay of local inflammation-associated responses (see Chapter 4 for details) and not disordered lipids in the blood or cholesterol (although they are among the many factors that can influence the disease process, they are not the cause of the disease process).

From the standpoint of prevention, inflammatory processes are responsive to a variety of dietary components as well as to exercise. Further, because inflammatory processes are a component of cause for a large number of chronic diseases, attenuating inflammatory processes should reduce risk for these diseases. The ω-3 fatty acids from fish oils, for example, predominantly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), comprise one well-known dietary component that has anti-inflammatory properties. These ω-3 fatty acids are commonly known to compete with arachidonic acid (AA) for incorporation into membrane phospholipids and, as a substrate for cyclooxygenase (COX)-1 and COX-2, lead to a reduced production of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) and an enhanced production of LTB_5.[35,36] These alterations in inflammation signaling reduce the adhesion of peripheral blood leukocytes to endothelial cells and diminish inflammatory responses. In a variety of epidemiology-based studies, consumption of these ω-3 fatty acids from marine sources appears to be strongly associated with lowered markers of inflammation in serum[37–42] as well as lowered risks for a variety of chronic diseases, including atherosclerosis,[38,43–49] Alzheimer's,[50–52] various cancers,[53–56] and osteoporosis.[57–59] Other dietary components that appear to have anti-inflammatory effects include olive oil; red wine; green and black teas; and a variety of whole grains, fruits, nuts, and vegetables.[38,60–67] In addition to these dietary components, physical exercise is also well known for both promoting anti-inflammatory effects[68–75] and reducing risk for a variety of inflammation-associated diseases, including various cancers, atherosclerosis, type 2 diabetes, obesity, Alzheimer's, and osteoporosis.[76–92]

In order to see how inflammatory responses can contribute to chronic diseases and how dietary components and exercise can contribute to prevention, a detailed understanding of the molecular aspects of inflammatory signaling is required. For the purposes of this discussion, the innate immune responses of inflammation are emphasized; aspects of adaptive immunity are discussed only briefly to provide context. For additional details relating to the aspects of immune function not covered here, many excellent texts and reviews are available.[16–19,93–102]

2.2 SYNTHESIS OF PROINFLAMMATORY MOLECULES

As previously mentioned, the overall purpose of inflammatory response appears to be to initiate a variety of cellular responses that lead to removal of the damaging agent and repair of any damage. As described in detail here, a variety of proinflammatory

signaling molecules are produced by an array of cells in order to initiate and regulate the different processes that comprise an inflammatory response. Once the damaging agent is removed and the tissue is repaired, the production of proinflammatory signaling molecules stops and the inflammatory response disappears.[16–18]

In general, an acute inflammatory response to infectious agents typically results in an array of responses that are localized to the area of infection, including the following:

1. Vasodilation (and increased permeability of capillaries) to increase the flow of neutrophils, monocytes, and plasma proteins (antibodies, complement, and various acute-phase proteins) into the tissue vasculature; the activation and attraction of any local tissue macrophages and dendritic cells in the immediate area to kill the infectious agents as well as the attraction (and activation) of circulating neutrophils and monocytes to infiltrate the tissue and kill the pathogens
2. Activation of platelets to enhance clotting in order to close damaged areas of blood vessels and the attraction of fibroblasts into the area to enhance the healing process and synthesize scar tissue to permanently close wounds
3. The release of various growth factors from a variety of cells to increase rates of synthesis of a variety of cellular components for repair and rates of cell division and cell growth in order to replace cells that were killed by the infectious agent
4. Initiation of various cellular functions that lead to an adaptive immune response resulting in far more efficient killing of the pathogen

2.2.1 Pathogen-Associated Molecular Patterns, Damage-Associated Molecular Patterns, and Cytokines

Each of the four responses that are localized to the site of infection is initiated by an array of signaling molecules produced by cells that have receptors for various components of infectious agents and by various cells that have receptors for one or another of the signaling molecules that are produced, molecules that include a variety of cytokines, predominantly tumor necrosis factor (TNF)-α, interferon (IFN)-β, interleukin (IL)-1β (IL-1β), and IL-6; prostaglandins (PGs), leukotrienes (LTs), and thromboxanes (TXs); histamines; chemokines, predominantly IL-8, monocyte chemoattractant molecule (MCP)-2, and macrophage inflammatory protein (MIP)-2; and adhesion molecules, predominantly P-selectin, E-selectin, intracellular adhesion molecules (ICAMs), and vascular cell adhesion molecules (VCAMs). In addition to the infectious agents themselves, cellular components from damaged or necrotic cells can also initiate the same array of inflammatory signaling through similar receptor-mediated processes by essentially the same cells involved in the pathogen-stimulated inflammatory response.[99,101,103] These damage-associated inflammatory responses are also important to understand how inflammation can play a role in a variety of chronic diseases.

The major cells that are capable of directly responding to components of infectious agents or cellular components released by damaged or dying cells include dendritic cells (found in skin, lymphoid tissue, mucosal epithelium, and organ parenchyma), natural killer (NK) cells (found mainly in blood, spleen, and liver), neutrophils, tissue macrophages, mast cells (in most tissues, with higher amounts in skin, airway, and intestinal

mucosa), as well as endothelial cells and fibroblasts.[93,99,103–105] More specifically, these cells respond when specific cellular receptors bind to various components from pathogens, components known as pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs: TLR1, TLR2, TLRs4-6) on the plasma membrane bind bacterial lipopolysaccharides (LPSs), bacterial peptidoglycans, bacterial flagellin, envelope proteins from viruses, and hemagglutinin protein from viruses, whereas TLR3, and TLRs 7–9, in the intracellular endosome membranes and endoplasmic reticulum, bind a variety of pathogen-associated nucleotides (such as ssRNA, dsRNA, and CpG DNA) as well as endogenous nucleotides. TLRs 2 and 4 also recognize mammalian HSPs and fibrinogen, TLR9 binds mammalian DNA, and TLR3 binds mammalian RNA. These latter compounds are commonly released from cells that have been severely damaged and/or are dying and are therefore called damage-associated molecular patterns (DAMPs). Figure 2.1 shows a summary of PAMP- and DAMP-mediated cellular responses.

Other pattern recognition receptors (PRRs) include a variety of cytoplasmic nucleotide oligomerization domain (NOD)-like receptors (NLRs)[103,106] and the cytoplasmic retinoic acid–inducible gene I (RIG-I)-like receptor family, which includes the RIG-I-like receptor, melanoma differentiation–associated gene 5 receptor, and the laboratory of genetics and physiology 2 receptor, collectively referred to as RLRs.[93,107–109] Similar to TLRs, NLRs and RLRs also bind various PAMPs. The major PAMPs for NLRs include peptidoglycan (PGN) (components of cell walls of all bacteria), bacterial flagellin, bacterial LPS, bacterial RNA, viral RNA, and viral DNA.[103,106,107] RLRs, on the other hand, appear to be restricted to recognizing viral nucleotides such as dsRNA and uncapped 5′-triphosphate ssRNA.[108,109] TLRs, NLRs, and RLRs comprise the three main classes of PRRs that are known, and they are discussed further, although there are others, including the membrane-bound C-type lectin receptors and absence in melanoma 2-like receptors.[103]

When various pathogen or damage-associated particles bind to PRRs, signal transduction pathways are activated, which then lead to the synthesis of proinflammatory cytokines. The major cytokines produced following the activation of TLRs are IL-6, IL-1β, TNF-α, IFN-β, IL-8, and IP-10, with the individual cytokines that are produced being dependent on the specific TLRs that are activated. Once activated, TLRs will dimerize and then form (various) complexes with adapter molecules. These TLR complexes then activate one or another of four major signaling pathways: nuclear factor κ-β (NFκβ), p38–mitogen-activated protein kinase (p38–MAPK) pathway, JNK–MAPK, and/or IFN regulatory factors. Heterodimers of TLRs, TLR2/1 and TLR2/6, and homodimers of TLRs 4, 5, and 7–9 form complexes with the adapter protein MyD88, whereas both heterodimers and homodimers of TLR4 can bind with the additional adapter protein Mal. TLR4 can also form complexes with the adapter proteins TRAM and TRIF to activate a MyD88-independent pathway, and TLR3 is unique in that it only seems to form a complex with the (MyD88-independent) adapter protein TRIF.[108,110,111]

Although every detail of the signal transduction pathways is not discussed, TLR–adapter protein complexes mediate the activation of the NFκβ and MAPK signaling pathways through the activation of TAK1 kinase, which occurs through both the MyD88-dependent and the MyD88-independent pathways.[103,108,112,113] TAK1 subsequently activates NFκβ (comprised of the heterodimer p65, p50 complexed to the inhibitory unit IκB in the cytosol) by phosphorylating Iκκγ, which subsequently

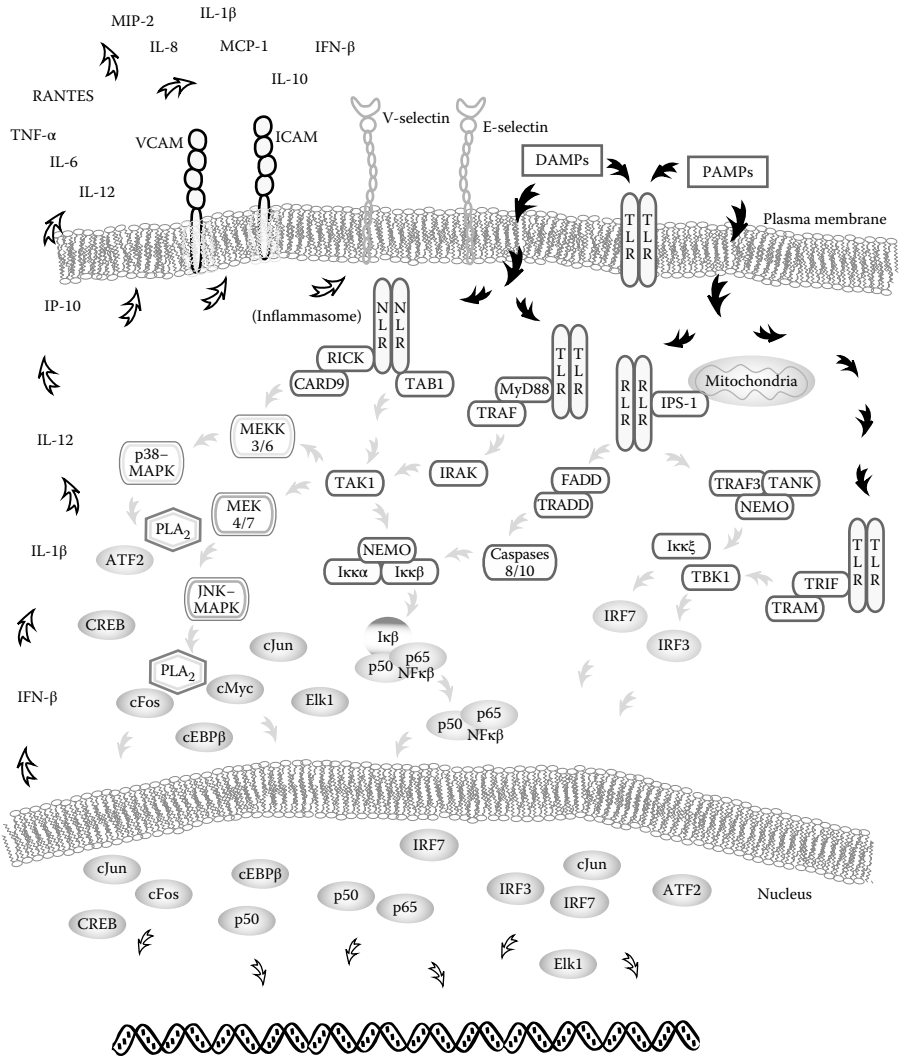

FIGURE 2.1 Activation of proinflammatory signaling by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs): binding of DAMPs and PAMPs to toll-like receptors (TLRs), RLRs, and nucleotide oligomerization domain (NOD)-like receptors (NLRs) triggers the formation of protein complexes (dark, filled arrows), which then activate signal transduction pathways to activate the transcription factors p50 and p65 (nuclear factor κ-β [NFκβ]), IRF3 and IRF7, and others (light, filled arrows). Subsequent to binding to various promoters, the proinflammatory cytokines, chemokines, and adhesion molecules are expressed (open arrows). In addition to the activation of transcription, the p38–MAPK and the JNK–MAPK also activate PLA_2 to initiate the synthesis of proinflammatory eicosanoids.

phosphorylates IκB and frees NFκβ to enter the nucleus. TAK1 can also activate the JNK–MAPK and p38–MAPK pathways by phosphorylating MEK7 and MEKK3. The activated p38–MAPK and JNK–MAPK pathways then activate a variety of transcription factors including AP-1 factors as well as NFκβ, enhancing TAK1-mediated NFκβ activation. NFκβ and the various AP-1 units then enter the nucleus and coactivate the expression of IL-6, pro-IL-1β, TNF-α, IL-8, and IP-10 with the specific cytokines produced being dependent on the array of AP-1 subunits that are coactivated along with NFκβ. The various transcription factors activated by p38–JNK include cJun, cFos, ATF2, JunB, JunD, CREB, cEBPβ, Elk1, and Iκβζ.

The TLR3–TRIF adapter protein complex activates IFN factors 3 and 7 (IRF3, IRF7), which then, along with NFκβ coactivation, stimulates the expression of IFN-β and IP-10.[108,110,111] IP-10 synthesis is included here as a component of the proinflammatory signals produced on first contact with pathogens because, even though IP-10 is normally considered to be an IFN-inducible protein, it is instructive that it is actually induced by AP-1 binding.

Because TRL4 binds both MyD88 and TRIF proteins it is capable of inducing the synthesis of IFN as well as the other cytokines, whereas TLR3 appears to be restricted to activating the synthesis of IFN and IP-10. Thus, IFN expression is a relatively modest consequence of the activation of plasma membrane–bound TLRs 1, 2, and 4–6 (by a variety of PAMPs and DAMPs) and a major response following the activation of endosomal TLRs 3 and 7–9.[101,108,110,111] Of the different inflammatory cells, dendritic cells and macrophages (which happen to be present in almost all tissues) express the greatest array of PRRs and therefore have the ability respond to almost any PAMP or DAMP. The plasmacytoid class of dendritic cells also expresses the endosomal TLRs (TLRs 3 and 7–9) to a far greater degree compared to other inflammatory cells, therefore making this class of dendritic cells a major source of IFN (outside NK and Th cell production of IFN-γ, as discussed in Section 2.3.3) resulting from a viral infection.

NLRs in the cytosol of cells that initiate an inflammatory response activate the expression of the same major proinflammatory cytokines in a manner that is similar to that observed through TLR activation and has one important additional process: the NLRP family of NLRs forms oligomer complexes along with other proteins to form an inflammasome complex. The NLRs NOD1 and NOD2, when activated, will recruit the adapter proteins RICK, TAB1, and caspase recruitment domain 9, which then complexes with TAK1 (among others) to activate NFκβ and the MAPK pathways. Again, the activation of p38 and JNK leads to the activation of an array of AP-1 subunits and the expression of IL-6, pro-IL-1β, TNF-α, and IP-10. The inflammasome then attracts and activates the enzyme caspase-1 to cleave an inactivating peptide from pro-IL-1β in order to produce active IL-1β, which is then secreted from the cell[101,106,111] (as are the other signaling molecules).

The third major family of PRRs is the cytosolic RIG-I-like receptor family, the RLRs, which bind dsRNA and uncapped 5′-triphosphate ssRNA (predominantly from RNA viruses). Some of the activated RLRs interact with IPS-1 on the outer membrane of mitochondria to form complexes with TNF receptor–associated death domain (TRADD) proteins, which then attract others to activate two separate pathways. The TRADD/TRAF3/TANK complex activates TBK1/IKKζ to phosphorylate

IRF3 and IRF7 for the subsequent coactivation of IFN expression. Other activated RLRs form complexes with IPS-1 and then with TRADD, Fas-associated death domain, and caspase-8/-10 to activate NFκβ for coactivation of IFN-β expression.

As described earlier, caspase-mediated activation of NFκβ via RLRs and activation of IRFs through TLRs also occur, indicating that additive effects are most likely to occur when more than one of the various classes of PRRs is activated. There are other points where the different signal transduction pathways for the various classes of PRRs intersect (e.g., different PRRs activate MAPKs via multiple/overlapping pathways), and this is of course very important as well. This *cross talk* between activation pathways allows for a highly graded inflammatory response that depends on the number of PAMPs associating with plasma membrane TLRs and the number of PAMPs that interact with endosomal TLRs, cytosolic NLRs, and RLRs following phagocytosis of the pathogens (or invasion of the cells by viruses).

The addition of DAMP-mediated activation of PRRs also produces an important source of possible interactions. These latter compounds are a sensor for the amount of damage caused by the pathogens and allows for an even greater inflammatory response, analogous to the concept that a weak pathogen that causes no damage requires a less intense response compared to a highly toxic pathogen that causes extensive damage. It also implies that with sufficient damage to tissues in one location the DAMPs may enter the circulation and activate some PRRs in inflammatory cells far away from the actual damage. Of course, this also holds true for infectious pathogens; there is no reason to expect that a few organisms will not be able to enter the circulation (or lymph) and then activate PRRs at sites far away from the original site of infection. This is especially true with exposures due to inhaling pathogens (such as aflatoxin or influenza virus, or many others) or ingesting them (food poisoning by *Escherichia coli* or *Vibrio cholera*, or many others) where the pathogen load may be much greater than that from a small scratch or a casual exposure and where the pathogens have easy access to a large surface area with a high rate of blood flow in the exposed tissue. The implication of this is that a minimal and highly localized inflammatory response to a very small amount of damage may be exacerbated by the appearance in that location of PAMPs and/or DAMPs that arose from an infection or a larger scale area of damage far away from that site. Interestingly, NFκβ can also be activated by hydrogen peroxide (H_2O_2)-induced phosphorylation of IκB via C-Src as well as by ataxia telangiectasia mutated (ATM) (ATM is activated in response to DNA damage).[103,108,113] The importance of these is that ROS and ROS-mediated DNA damage can also activate NFκβ, indicating that factors associated with ROS stress may also be additive to the TLR-, RLR-mediated activation process.

In addition to the synthesis and release of various cytokines in response to PRR activation, as described earlier in this section, cells such as mast cells (as well as the granulocytes basophils and eosinophils) can degranulate and release a variety of preformed compounds from intracellular granules in response to injury as well as through the binding of proteins and other small molecules to the IgE that is bound to the Fc receptors on their cell membranes. The compounds released by mast cells include histamine, several protease enzymes, TNF, heparin, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). In response to PRRs, damage, and allergens, activated mast cells also synthesize an array of cytokines (including IL-3, IL-4, IL-5, IL-6, IL-9, and IL-13). In addition to these

released compounds, several of the AA-derived proinflammatory signaling molecules are produced as well: platelet-activating factor (PAF), prostaglandin D_2 (PGD_2), PGE_2, leukotriene C_4 (LTC_4), and LTB_4.[8,105,114–116]

2.2.2 Prostaglandins, Leukotrienes, and Thromboxanes

The production of the AA-derived LTs, PGs, and TXs (as well as PAF) is not limited to mast cells. Most cells of the body can produce one or another of these important proinflammatory compounds. In general, the production of these is determined predominantly by the amount of AA released into the cell from phospholipids that form membranes of the endoplasmic reticulum, nucleus, and Golgi apparatus. The AA is cleaved from the phospholipids by phospholipase A_2 ($cPLA_2$), and the production of various lipid mediators is then dependent on the amount of the constitutive COX-1 and inducible COX-2 enzymes; amount of the 5-lipoxygenase (5-LOX) enzyme; and array of specific PG, LT, and TX synthases in the cell,[117–120] as outlined here and illustrated in Figure 2.2.

Once AA molecules are released from membrane phospholipids (predominantly from phosphatidylcholine), the AA is metabolized into prostaglandin G_2 (PGG_2) by COX-1 and COX-2. The PGG_2 is converted into PGH_2 via a peroxidase reaction (also by COX-1 and COX-2), and the PGH_2 is then converted into a variety of proinflammatory PGs by specific PG synthases. PGE_2 is synthesized by three different PG synthases that are expressed in almost all cells, the cytosolic form cPGES-1 and the two microsomal forms mPGES-1 and mPGES-2, with mPGES-1 being inducible in concert with COX-2.[119,121] PGI_2 (prostacyclin) is synthesized by an isozyme of cytochrome P450 known as prostacyclin synthase, which is predominantly expressed in vascular endothelial cells.[119,122] PGG_2 is also converted to PGD_2 by either lipocalin-type PGD_2 synthase (l-PGDS), found mainly in brain, or hematopoietic-type synthase (h-PGDS), found predominantly in mast cells and activated macrophages, platelets, and dendritic cells.[119,123,124] Because PGD_2 spontaneously dehydrates to produce PGJ_2,[119,123] a high-affinity ligand for peroxisome proliferator–activated receptor-γ (PPARγ), this PG may have important implications as an anti-inflammatory PG, as discussed in Chapter 3, Section 3.4. Thromboxane A_2 (TXA_2) synthase, a CYP5 enzyme,[125] utilizes PGG_2 as a substrate to produce TXA_2 and is mainly found in platelets and macrophages.[117]

AA is also made into 5-hydroperoxyeicosatetraenoic acid and then into leukotriene A_4 (LTA_4) by 5-LOX. LTA_4 is then converted into a variety of other LTs including LTB_4 by LTA_4 hydrolase (LTA_4H), which is expressed in activated neutrophils, mast cells, and macrophages.[117,119,126] LTC_4 is synthesized by LTC synthase, which is also expressed by neutrophils, macrophages, and mast cells.[117,119] The cellular responses to these lipid mediators are discussed in Section 2.3, whereas other anti-inflammatory lipid mediators that are produced from AA, EPA, and DHA by 12-lipoxygenase (12-LOX) and 15-lipoxygenase (15-LOX) are discussed in Section 2.3.4.

In terms of regulating the synthesis of PGs, LTs, and TXs it is the availability of AA for the various synthase enzymes that governs the production of these products, and this is determined through the activation of PLA_2, predominantly its cytosolic form ($cPLA_2$).[119] $cPLA_2$ can be activated by calcium, p38–MAPK and extracellular signal-related kinase (ERK)–MAPK, protein kinase C (PKC), or ROSs[117,127–130]

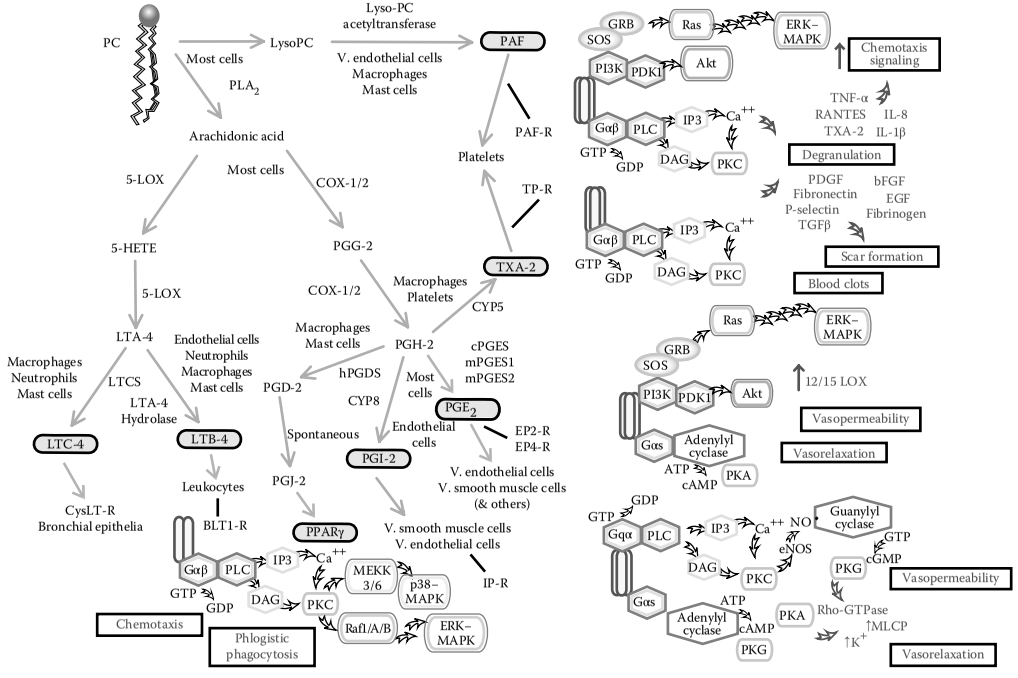

FIGURE 2.2 Eicosanoids—synthesis and cellular responses: activation of PLA_2 by mitogen-activated protein kinases (MAPKs), calcium, or reactive oxygen species (ROSs) leads to the synthesis of a variety of proinflammatory eicosanoids (shaded, rounded boxes), including PAF, TXA-4, PGE_2, PGI-2, PGJ-2, and LTB-4. On binding to their respective receptors as indicated, cellular responses (black boxes) are initiated, which result in the activation of neutrophils to move into the tissue to phagocytize pathogens; platelets degranulate to release a variety of proinflammatory molecules to enhance the activation and entry of proinflammatory cells into the region as well as growth factors to enhance healing. Vascular endothelial cells respond by reducing the integrity of VE-cadherins to enhance vasopermeability, and the vascular smooth muscle relaxes to cause vasodilation. These last effects enhance the ability of neutrophils and monocytes to leave the blood vessel and enter the tissues.

and, following activation, $cPLA_2$ binds to the endoplasmic, nuclear, and Golgi membranes. The resulting $cPLA_2$ activity usually peaks within 1 minute and declines 2 to 3 minutes after binding.[117,127–130]

Overall, PGs, LTs, and TXs are, by themselves, unstable, and they readily inactivate, usually within a few minutes. Therefore, unlike cytokine signaling, signaling by these compounds is a relatively transient and highly localized function and only with continuing activation of $cPLA_2$ will a sustained production and presence of these lipid mediators occur to initiate various inflammatory responses, conditions that certainly occur with cellular damage. It is interesting to note, however, that both AA and the unstable intermediates of AA metabolism are very stable within membrane environments and that they can be readily transferred from one cell to another during cell–cell contact,[131] indicating that activated cells can transfer both AA and AA metabolites to cells with inactive $cPLA_2$. The implication of this is that lipid signaling initiated by one activated cell (e.g., endothelial cells) could *spread* the production of these lipid mediators to other cells through direct contact (possibly to a monocyte, neutrophil, or platelet), thus bypassing the normal signal transduction pathways that activate $cPLA_2$ and enhancing and possibly prolonging the production of PGs, LTs, and TXs in the local region.

The final lipid-derived signaling molecule to be mentioned is PAF, a product that is synthesized predominantly by vascular endothelial cells and activated macrophages and mast cells. PAF is synthesized from lysophosphatidylcholine (lysoPC) through acetylation via acetyl Co-A by the enzyme lysoPC acetyltransferase.[132,133] LysoPC is the product remaining after $cPLA_2$ cleaves AA from phosphatidylcholine and, therefore, PAF synthesis is regulated through $cPLA_2$ activation (see earlier). Functions of PAF are briefly described in Section 2.3.2.

From these discussions, synthesis of the various proinflammatory cytokines and lipid mediators appears to be a highly regulated process. However, when considering that activation of $cPLA_2$ occurs via calcium-, ROS-, and MAPK-mediated events,[127–130,134,135] it seems that almost any change in cell function will result in the production of LTs, PGs, and TXs. Therefore, production of these eicosanoid compounds in cells may actually provide an exquisitely sensitive response to any form of cellular stress and inflammation-associated signaling mediated through PAMPs and DAMPs is only part of the reason it occurs. Production of these lipid mediators following normal cellular stresses in otherwise unaffected cells could therefore contribute to a preexisting local production of these same lipid mediators by cells that are responding to actual damage and/or infection.

In addition to the activation mechanisms for $cPLA_2$, the signal transduction pathways for cytokine production through both TLRs and NLRs also involve MAPK pathway activation. Because ROSs, PKC, and a wide variety of normal cell functions that are activated and/or regulated by G-protein activation processes[134] can activate MAPK pathways as well, the production of various cytokines may also be sensitive to any alterations in cell function beyond what might be called *resting homeostasis*. In a traditional view of inflammation signaling, PAMPs and DAMPs initiate the process. However, because of a significant amount of cross talk among the various activation pathways for a wide array of cellular processes that are regulated through the activation of the stress-response MAPK pathways,[134,136] the synthesis of proinflammatory cytokines also may be a component of a cellular response to stress, a response that can occur independent of any actual damage or infection (Figure 2.3). With similar

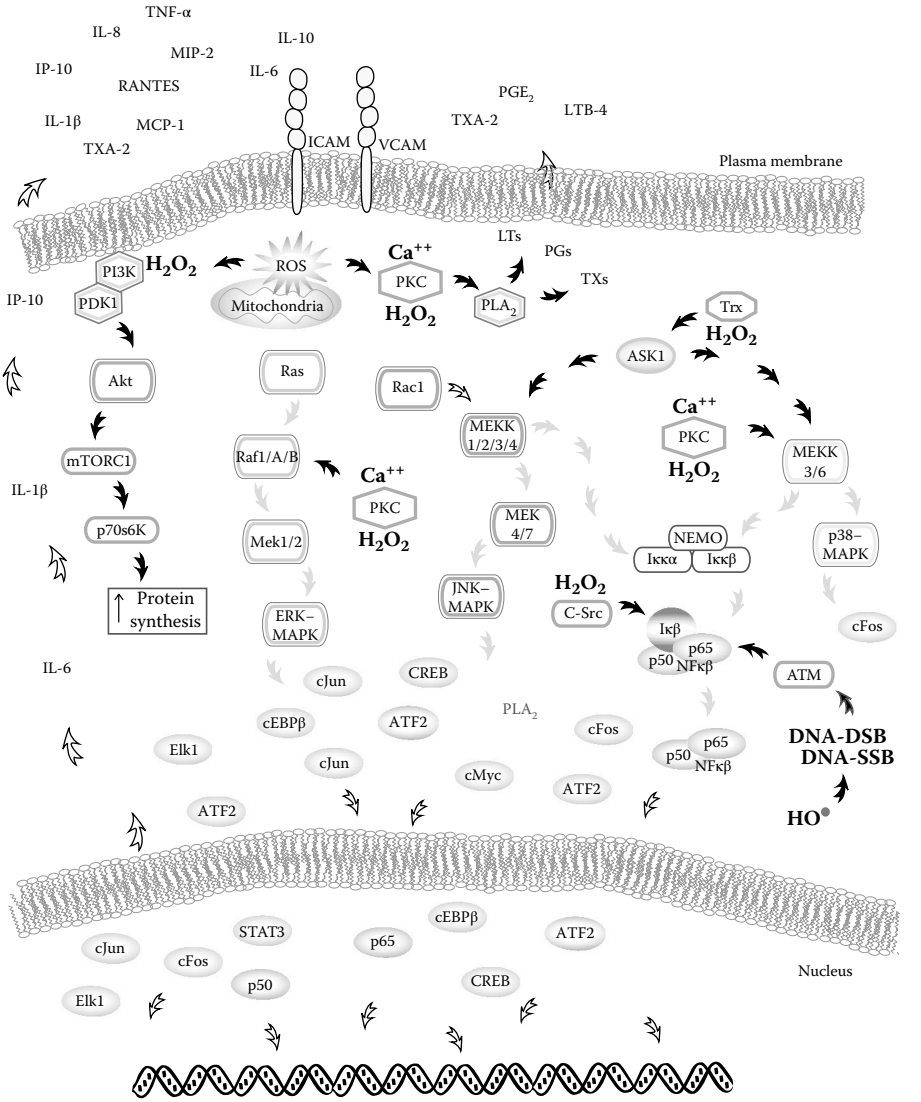

FIGURE 2.3 Stress-activated proinflammatory signaling: entry of calcium into the cytosol via normal signaling events mediated by diacyl glycerol (DAG) or through reactive oxygen species (ROS)-damaged membranes activates protein kinase C (PKC), which then may activate PLA_2, and the ERK–MAPK and p38–MAPK pathways (dark, filled arrows). At the same time, calcium-activated increases in cellular metabolism subsequently increase the production of ROSs, which may also activate PLA_2 as well as p38–MAPK and JNK–MAPK via Trx–Ask1. ROS-caused single- and double-strand breaks in DNA activate repair by ATM, which also activates nuclear factor κ-β (NFκβ), and ROS-mediated PI3K activity also enhances mTORC1 via Akt to enhance overall rates of protein synthesis (dark, filled arrows). The result of all these effects is the activation of a variety of transcription factors and the subsequent synthesis of a variety of proinflammatory molecules (open arrows) in response to cellular stress.

mechanisms of activation, one would expect LTs, PGs, TXs, and cytokines to be expressed following the same activation events, which, of course, is the case.[137,138] The production of various lipid mediators and of cytokines by human skeletal muscle as a direct result of repeated muscle contractions and the enhanced rate of metabolism necessary to support the muscle contractions,[72,73,139,140] and their production by adipocytes,[141–144] provide excellent examples of this concept. Perhaps, inflammatory signaling should be viewed as a component of a continuum of graded responses to cellular stress and not as a component of the immune system, with a full-blown inflammatory response being simply the result of the additive and synergistic effects of multiple sources of cellular stress that includes the presence of PAMPs and DAMPs.

In addition to the aforementioned proinflammatory products, there are many more cytokines and lipid-derived signaling molecules that play a role in immune functions, products such as IL-4, IL-10, IL-13, IL-15, lipoxins, resolvins, protectins, and maresins, among many others. Many of the cytokine products play different roles in regulating growth and differentiation of various immune cells or have anti-inflammatory effects and have not been discussed due to the proinflammatory focus of this section. IL-10, resolvins, lipoxins, protectins, and possibly maresins actually are anti-inflammatory molecules that play a very important role in mediating the resolution of inflammation.[145–147] These are discussed in more detail in Section 2.3.

2.3 CELLULAR RESPONSES TO PROINFLAMMATORY MOLECULES

The possible implications of proinflammatory signaling to a variety of disease issues quickly become evident when the cellular responses to proinflammatory cytokines and lipid-derived molecules are considered. A variety of cells (and tissues) are capable of responding to the proinflammatory molecules, and the specific response is governed by the expression in those cells of cellular receptors that bind the various proinflammatory molecules. The cells that initiate the production of proinflammatory molecules, in turn, also express various receptors for some of these same molecules, indicating that proinflammatory signaling molecules can have autocrine as well as paracrine and endocrine functions. Although cells of blood vessels, bone marrow, brain, liver, heart, skeletal muscle, adipose tissue, and lymphocytes all respond to inflammatory signals, the focus of this discussion is on those cells immediately involved in local inflammatory response.

2.3.1 Vascular Endothelial Cells, Vascular Smooth Muscle Cells, Neutrophils, and Monocytes

Vasodilation usually occurs within minutes of the start of a proinflammatory stress response. Of the signaling molecules discussed Section 2.2, some of the lipid-derived mediators are the predominant molecules responsible for this early response because they are synthesized immediately on stimulus, whereas the synthesis of various cytokines takes much longer; increased mRNA appears at about 30 minutes and peaks, as does expression, at about 1 hour.[148] Vasodilation is predominantly caused by the nitric oxide ($NO^{\bullet}$), PGI_2, and PGE_2 that are released by vascular endothelial and other inflammatory cells and by histamine that is released mainly by mast cells.

Of these, $NO^{\bullet}$ activates soluble guanylyl cyclase to synthesize cGMP while PGI_2 and PGE_2 increase cAMP production in vascular smooth muscle cells through the activation of the G-coupled IP and EP2/EP4 receptors, respectively. The result is the direct activation of protein kinase A by cAMP, activation of protein kinase G (PKG) by cGMP, and cross-activation of PKG by cAMP, resulting in smooth muscle relaxation and vasodilation through the activation of myosin light chain phosphorylase and the opening of large high-conductance calcium-activated potassium channels.[117,149,150]

Histamine causes increased vasopermeability as well as vasodilation through activating the H1 histamine receptors on the vascular endothelial cell membrane. H1 activation leads to a G-protein-coupled increase in phospholipase C activity, producing inositol triphosphate (IP_3), which subsequently activates calcium channels in the endoplasmic reticulum and cell membrane. The increased cytosolic calcium subsequently activates PKC through calcium-stimulated $NO^{\bullet}$ production via eNOS:PKG. The elevated $NO^{\bullet}$ contributes to vasodilation,[150,151] and the elevated calcium also triggers a calmodulin-mediated activation of myosin light chain kinase to induce the constriction of vascular epithelial cells.[152,153] Enhanced PKC and PKG activities also activate Rho-GTPases, which induce the phosphorylation of VE-cadherin, leading to the disassembly of adherens junctions and tight junctions between endothelial cells and an enhanced permeability of the blood vessels.[153] Both PAF and VEGF also stimulate enhanced vasopermeability through similar signal transduction pathways.[154,155] The end result of these vasopermeability and vasodilation effects is an increase in the entry of plasma proteins and circulating inflammatory cells (predominantly neutrophils and monocytes) into the area. The increased vasopermeability ensures that plasma proteins, neutrophils, and monocytes migrate into the tissue much more readily.

Activation of phagocytic cells that are already present in the tissue (as well as neutrophils, mast cells, and dendritic cells that are recruited into the tissue) occurs in order to enhance the destruction of pathogens. The activation process includes a complex interaction of PAMPs and/or DAMPs binding to PRRs as well as cellular responses to proinflammatory molecules and/or plasma proteins (complement)[105,156–158] in the immediate vicinity. Macrophages that are already resident in the tissues provide a good model to illustrate this process. Contact with pathogens stimulates an endocytosis response where pathogens or cellular debris are engulfed by a small section of the plasma membrane and internalized with the resulting vesicle fusing with lysosomes to create a phagolysosome. During the phagocytic response, components of necrotic or apoptotic cells and/or pathogens are destroyed by various hydrolytic lysosomal enzymes. The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme that is associated with the resulting phagolysosome generates a variety of ROSs, superoxide anions that then dismutate to form H_2O_2 and interact with the peroxide to form hydroxyl radicals. The $NO^{\bullet}$ that is produced due to eNOS activity reacts with the superoxide anions and with H_2O_2 to produce hypochlorous acid (HOCl) and a variety of reactive nitrogen species (RNSs); all of these contribute to the destruction process.

Unlike with pathogens, phagocytosis of apoptotic cells does not result in a robust activation of macrophages; this is in part because the cell membranes of apoptotic cells remain intact during the process, including when the cells split apart into smaller apoptotic bodies. This not only minimizes the release of DAMPs into the

surrounding areas but also ensures that they are destroyed in the phagolysosome before they can interact with the PRRs.[159] When a few cells die through necrotic processes, the activation and subsequent production of proinflammatory cytokines by local macrophages is minimal simply because of the presence of a limited number of necrotic cells and, therefore, a limited activation of DAMP receptors. The presence of greater numbers of necrotic cells due to a localized pathogenic infection will activate both PAMP and DAMP PRRs, and as a result the production of TNF-α, IFN-β, IL-1β, and IL-6 will be more robust. IFN-β production by activated macrophages in a small localized inflammatory response is relatively low and probably does not contribute very much to enhanced levels of IFN-β in serum; however, the presence of IFN receptors on macrophages ensures that any IFN-β production will have autocrine effects. Moderate production of IFN-β by local dendritic cells can also contribute to the activation of IFN receptors on the macrophages without significantly increasing serum levels of IFN-β. In response to IFN, iNOS is expressed at high levels in macrophages and the combination of enhanced ROS, RNS, and hypochlorite production greatly increases the efficiency of the destruction process. The increased production of highly reactive molecular species by activated macrophages (and other phagocytic cells) also has important implications for the development of chronic diseases (discussed in Section 2.4) through damaging and affecting the function of otherwise healthy cells in the immediate vicinity.

In response to proinflammatory signals produced by the PAMP/DAMP, activated tissue cells circulating neutrophils and monocytes can also be activated and stimulated to migrate into the tissues. As already mentioned, the vasodilation and increased vasopermeability response to PGI_2, $NO^{\bullet}$, PGE_2, and histamine can facilitate this process. Neutrophils and monocytes are activated to exit the circulation through the coordinated function of a variety of molecules on the vascular endothelial cells as well as the neutrophils and monocytes. In response to TNF-α, either alone or in combination with IL-6 and IL-1, vascular endothelial cells synthesize a variety of chemoattractant molecules including ICAMs, VCAMs, E-selectin and P-selection, MCP-1 (chemokine: CCL2), IL-8 (chemokine: CXCL8), and RANTES (chemokine: CCL5) through NFκβ and JNK-dependent, as well as JNK-independent, pathways.[160–170] The binding of TNF-α to its receptor initiates the formation of TRADD–TRAF complexes, which mediate the activation of NFκβ and both JNK–MAPK and p38–MAPK via TAK1 and apoptosis signal-regulating kinase 1 (ASK1), respectively. Activated IL-1 receptors form complexes with MyD88 and subsequently with IRAK and TRAF to initiate the same TAK1- and ASK1-mediated responses. Activated IL-6 receptors, on the other hand, form complexes with SOS–GRB and with Jak–STAT to activate the transcription factor STAT3 as well as the ERK–MAPK pathway through Ras. In addition, PI3K activity is also activated, which subsequently activates PDK1 and Akt, leading to the activation of mTORC1. These responses lead to the increased activation of various factors (AP-1, STAT3, and others) that are necessary for the coactivation of transcription of various proinflammatory molecules via MAPK and STAT. In addition, through the activation of mTORC1 rates of protein synthesis are enhanced, which leads to an accelerated synthesis of all proinflammatory molecules. Thus, rather than being proinflammatory in and of itself, IL-6 complements the proinflammatory effects of other cytokines (Figure 2.3).

The enhanced expression of the various adhesion molecules, cytokines, and chemokines by vascular endothelial cells then enhances the recruitment of neutrophils and monocytes into the tissue. Following the binding of E-selectin and P-selectin to sialyl-glycoprotein ligands, as well as ICAM and VCAM to the integrin ligands on neutrophil and monocyte plasma membranes, these cells start to roll slowly along the endothelial cell layer of the blood vessel. The MIP-1 and CCR2 receptors of monocytes bind with RANTES and MCP-1, and the MIP-1, CXCR1, and CXCR2 receptors of neutrophils interact with RANTES, MCP-1, and IL-8; both are *guided* to migrate through the endothelial layer at the site of the greatest concentration of these chemokines in order to enter the tissue.

LTB_4, produced by activated macrophages, mast cells, and neutrophils, is a potent chemoattractant molecule for neutrophils.[117,119] Because AA-derived signaling molecules are produced immediately on the initiation of cellular stress or damage, LTB_4 acts as the first chemoattractant that recruits neutrophils into the area through binding to G-coupled BLT1 receptors on neutrophils, initiating the activation process through increasing cytosolic calcium and the subsequent activation of MAPKs. These first-recruited neutrophils are then activated to produce various cytokines locally, which in turn enhance the recruitment and activation of additional neutrophils and monocytes through the synthesis of various chemokines. LTB_4 thus acts as an initial lipid mediator of a lipid–cytokine–chemokine cascade resulting in what might be called a full-blown inflammatory response.[119,171,172] With LTB_4 being able to initiate an inflammatory response, it is possible that any prolonged cellular stress that activates PLA_2, even without the involvement of pathogens, can promote a localized inflammatory response as well.

The activated neutrophils that migrate into the tissues at the site of the original inflammatory response phagocytize any unwanted particles and contribute to the killing of pathogens via ROS and RNS production, processes that are enhanced by the IFN-β produced by the (already) activated tissue macrophages and dendritic cells in the immediate area. Activated neutrophils also synthesize and release several cytokines and chemokines, including TNF-α and IL-1β. Macrophages that are activated through the inflammatory process also produce proinflammatory cytokines (TNF-α, IL-1α/β, and IL-6), as well as performing enhanced phagocytic functions. The end result of these vascular and immune cell responses is an increase in the number of active phagocytic cells in the local area as well as an increase in the local production of proinflammatory cytokines by the newly recruited cells. This has the effect of enhancing the local production of proinflammatory cytokines, prolonging the local inflammatory response, enhancing even more the recruitment of circulating immune cells, as well as greatly increasing the risk for ROS- and RNS-mediated damage to noninvolved cells by increasing the localized production of these reactive species.

The cytokine-mediated activation of selectin and adhesion molecule synthesis in endothelial cells is important because the activation is in part through NFκβ and JNK–MAPK-dependent as well as JNK-independent pathways. From the previous discussion, there are several possibilities for additive or synergistic cross talk between the various PRR activation pathways, cross talk that may be enhanced through stress-mediated activation of different components of these same pathways.

Because vascular endothelial cells express a variety of TLRs, NLRs, and RLRs,[173] significant interactions among those activating pathways triggered by direct interaction with PAMPs and DAMPs as well as those triggered by proinflammatory cytokines from both paracrine and autocrine sources can significantly enhance the recruitment and activation of circulating inflammatory cells compared to the effects of PAMPs and DAMPs alone (Figure 2.4). While all cells express TNF-α receptors, IL-6 receptors are expressed predominantly by hepatocytes, monocytes, T cells, and B cells. All cells, however, express gp-130, which complexes with IL-6R to form the active receptor when IL-6 binds. Interestingly, soluble IL-6R binds to gp-130 in any cell to form active IL-6R complexes, indicating that IL-6 (through transactivation events) and TNF-α (through autocrine and paracrine effects) can have effects in vascular endothelial cells.[174] These recruitment effects can be enhanced through the addition of any one of a number of non-pathogen-related stressors as well. Thus, a variety of events that activate various stress-response pathways, starting from an initial activation by PAMPs and DAMPs, can lead to an accelerating production of proinflammatory signals that leads to the recruitment of additional proinflammatory cells that also participate in proinflammatory signaling.

The final molecule to be discussed in the context of inflammation is C-reactive protein (CRP). CRP synthesis in hepatocytes is activated in response to IL-6, with an enhanced effect with coactivation by IL-1β.[175,176] Activation of transcription occurs through the binding of a variety of factors to the CRP promoter region, including STAT3, c-rel, and rel p50 (indicating the involvement of NFκβ), although there is negligible transcription activity until binding of CCAAT/enhancer binding protein (C/EBP) occurs, following activation of C/EBP through IL-6 signaling.[177] IL-6 signaling involves direct receptor-mediated activation of the Jak–STAT pathways as well as Ras-mediated activation of MAPK pathways.[178] Although details of the complex interactions of these pathways are not discussed, it is important to recognize that there are a wide variety of stress-related processes and growth factors (as discussed in Section 2.2 for the production of proinflammatory cytokines and PGs, LTs, and TXs) that also activate these same pathways.[179–181] As a result, average *nonstimulated* levels of CRP of approximately 0.8 mg/mL (to as high as 10 mg/mL) are observed in healthy volunteers, with acute-phase responses following synthesis of IL-6 and IL-1 by local inflammatory cells being as high as 500 mg/mL.[176]

Typically, CRP has been associated with complement activation. Complement refers to a family of proteins that are produced by hepatocytes and circulate freely in the blood. Although details of complement activation and function are not discussed in detail, in the classic activation process the C1q complement protein is activated by binding to the Fc fragment of antibodies, precipitating a cascade of protease-mediated reactions leading to the production of active complement protein complexes and protein products. These activated proteins then greatly enhance a local inflammatory response by acting as a chemoattractant for neutrophils and monocytes and by activating mast cells, endothelial cells, macrophages, and neutrophils (among many other cell types).[182,183] These effects are especially important in adapted immune function where the binding of antibodies to antigens can lead to a swift activation of a protective inflammatory response that destroys the infectious pathogens.

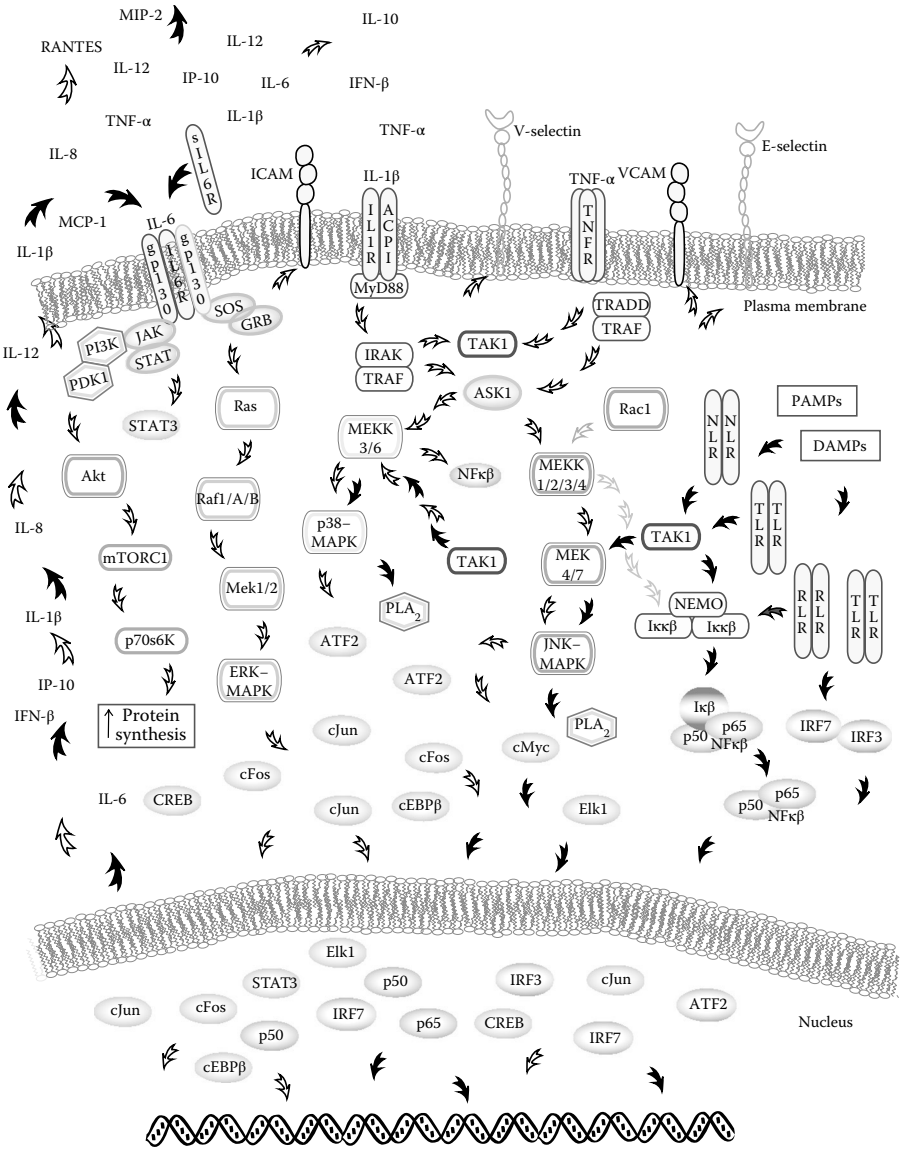

FIGURE 2.4 Acceleration of proinflammatory signaling through autocrine and paracrine signaling: the initial activation of toll-like receptors (TLRs), RLRs, and nucleotide oligomerization domain (NOD)-like receptors (NLRs) by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) leads to the synthesis of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1-β, and others (filled arrows). Autocrine binding of TNF-α and IL-1-β to their receptors mediates additional activation of MAPKs as well as p50/p65 (open arrows) via TAK1 and ASK1 to greatly enhance the expression of proinflammatory molecules, which then increases activation and chemotaxis of inflammatory cells into the region. Activation of PI3K/Akt by IL-6 (open arrows) enhances the protein synthesis in general, leading to the activation of p70s6k.

CRP can also initiate complement activation by binding to phosphocholine residues on membranes of damaged and apoptotic cells[184,185] coupled with cobinding to the C1q complement protein. But in this case where antibodies are not involved, CRP also activates the expression of several factors that inhibit further activation of the complement; therefore, it actually functions to limit the production of a highly inflammatory response caused by the later stages of the processes involved in complement activation.[175,176] This is a beneficial effect: CRP acts as a damper on a complement-mediated acceleration of inflammatory responses when it is only DAMPs originating from *normal* necrotic processes that are involved in activating macrophages and dendritic cells. This benefit is important considering the fact that an initial inflammatory response includes vasodilation/vasopermeability responses that increase the entry of circulating complement and antibodies (as well as CRP) into the local area.

From this discussion, CRP may function to ensure that a complement-mediated acceleration of inflammatory responses occurs only as a component of an adapted immune response. The initial activation of C1q, however, leads to the generation of active C3 fragments that are then involved in activating phagocytosis of the CRP-bound cells as well as in enhancing oxidative bursts following phagocytosis.[175,183] CRP also mediates an increase in the synthesis of ICAMs in endothelial cells as well as the synthesis and release of IL-1, IL-6, and TNF-α,[186–188] indicating that early-stage inflammatory responses, including ROS production, can be enhanced by circulating CRP. Thus, as with the other proinflammatory factors already discussed, an inflammatory response in one location that enhances IL-6 and IL-1 levels in serum can mediate and enhance proinflammatory processes in other locations by inducing CRP synthesis in the liver. This is why levels of CRP in the blood can be used as a biomarker of risk for inflammation-associated disease.

2.3.2 Platelets, Fibroblasts, and Macrophages: Blood Clots

In the event of vascular and tissue damage, a process to stop bleeding and permanently close and repair any wounds is necessary to prevent excessive blood loss as well as to return the damaged tissue to normal function. The processes of blood clot formation and wound healing are, of course, initiated by the cells that are involved in the inflammatory process. With respect to the contribution of inflammation to chronic diseases in general, the formation of blood clots and successful healing of damaged tissue may have minimal significance; therefore, all the details of these processes are not described here. Some of the signaling processes associated with platelet activation, blood clot formation, and wound healing are important contributing factors to various aspects of certain chronic diseases and so are discussed here.

The formation of blood clots involves the activation of platelets and the coordinated activation of various plasma proteins called clotting factors via *intrinsic* (common) and *extrinsic* (tissue factor) pathways. Platelets usually activate and adhere to disrupted endothelium by binding to exposed von Willebrand factor and/or collagen via their integrin receptors (GP1b, IX, V, IIb, and IIIa). They can also adhere to endothelial cells of nondisrupted endothelium through the binding of their GP1b integrin receptors to ICAM that is expressed on the endothelial cell membrane in response to

inflammatory cytokines.[189,190] Once activated via activation of phosphatidylinositol 3-kinase and PKC activities coupled with increased cytosolic calcium,[191,192] platelets degranulate and release a variety of proinflammatory molecules (IL-1β and TNF-α) and platelet aggregation factors (including fibrinogen, fibronectin, P-selectin, and TXA_2); growth factors (platelet-derived growth factor [PDGF], transforming growth factor β [TGFβ], epidermal growth factor, and bFGF); chemokines (RANTES, platelet factor 4, and IL-8, among others); and the coagulation factors V, XI, plasminogen activator inhibitor, plasminogen, and protein S.[189,190,193] Although the step-by-step formation of clots is not described here, the ability of platelets to bind to endothelial cells and to be activated independently of endothelial disruption is important. The cytokines and chemokines released by activated platelets provide an additional source of signaling molecules for both neutrophil and monocyte recruitment and for the synthesis of adhesive molecules by endothelial cells. To compound this process, platelets can also adhere to activated neutrophils and monocytes that are already rolling on the endothelium, further enhancing the recruitment of additional circulating inflammatory cells[189] without attaching directly to endothelial cells. Platelets attach to both neutrophils and monocytes through the binding of P-selectin (activated platelets) and ICAM-2 (*resting* platelets) expressed on platelet membranes to P-selectin glycoprotein ligand-1 and lymphocyte function–associated antigen-1 that is expressed on neutrophils and monocytes,[194] providing a vital signal amplification function for the inflammatory process, one that is independent of clot formation. Thus, interactions between platelets, macrophages, and neutrophils can amplify localized inflammatory signaling to enhance the recruitment and activation of more inflammatory cells, resulting in further amplification of the inflammatory signaling, a platelet connection to the lipid–cytokine–chemokine cascade that was described earlier.

Several issues relating to TXA_2 release by both macrophages and activated platelets are important in this platelet-mediated amplification process and deserve further discussion. As previously mentioned, activated macrophages release IL-1β, IFN-β, and TNF-α. These cytokines, through autocrine stimulation, activate COX-2 synthesis in these same macrophages (and, through paracrine stimulation, in any neutrophils that are rolling along the adjacent endothelial cells).[195–199] The increase in COX-2 activity in macrophages initially increases their production of TXA_2, which then increases the activation of platelets that may be rolling on the endothelium in the inflamed region. Platelets are activated by TXA_2 through the activation of the G-protein-coupled TP receptor, resulting in the stimulation of phosphatidylinositol 3-kinase and PKC activities and an increase in cytosolic calcium.[194] Following activation platelets degranulate and release a variety of signaling molecules, including TXA_2. The TXA_2 released by the platelets then acts in an autocrine fashion to accelerate the activation process as well as enhance the activation of other platelets in the region through paracrine effects. The activated platelets then provide additional stimuli to the already activated neutrophils and monocytes (as well as endothelial cells) via the platelet-derived cytokines and chemokines. Thus, the induction of COX-2 in activated neutrophils (as well as in macrophages) enhances the production of LTB_4, contributing further to a platelet-enhanced lipid–cytokine–chemokine cascade. However, as described in Section 2.3.4, induction of COX-2 is not as bad as is generally thought to be.

Another aspect of platelet activation that occurs independently from both endothelial disruption and TXA_2-mediated events is the activation of platelets by PAF. As described in Section 2.2.2, PAF is produced by vascular endothelial cells as well as activated macrophages, and PAF receptors are common in macrophages, neutrophils, vascular endothelial cells, and platelets. PAF activates platelets to aggregate as well as release their bioactive compounds through the activation of G-coupled PAF receptors (PAFRs) and the subsequent activation of phospholipase C, which leads to the production of IP_3 and diacyl glycerol (DAG), increasing cytosolic Ca^{2+} and activity of PKC.[200,201] PI3K is also activated by PAFRs; this can lead to SOS–GRB-mediated activation of Ras, which results in the activation of ERK–MAPK, an activity that can be enhanced by the activated PKC and results in phosphorylation of PLA_2 to further enhance the synthesis of proinflammatory eicosanoids. Thus, the ability of platelets to contribute to both the production and the amplification of inflammatory signaling is clear, and their contribution to the pathological consequences of chronic inflammation should not be underestimated.

From this discussion, it is certainly possible that a minimal activation of tissue macrophages by DAMPs released as a result of a small amount of local cellular damage could be accelerated through the autocrine induction of COX-2, a cascade aided by platelets through a variety of cellular and cytokine interactions described in this section. With an induction of COX-2 occurring within 4–6 hours of stimulation in macrophages, neutrophils, and endothelial cells,[202–204] such progressions could occur relatively quickly. In normal circumstances where responses to the various clotting and growth factors lead to complete healing, a localized inflammatory response to damage does not necessarily result in an accelerating cascade of increased signaling complete with subsequent invasion of the tissue with many additional activated neutrophils, monocytes, and macrophages. In cases where healing is incomplete, or where continuing low-level and nonnecrotic damage to cells occurs, the processes of the platelet-enhanced lipid–chemokine–cytokine cascade ensure that there will be a sustained localized production of inflammatory cytokines and chemokines, as well as growth factors and coagulation factors. The production of all these factors will last for as long as the initiating PAMPs, DAMPs, or cell stressors exist. Further, it is the prolonged production of these signaling molecules that, even at very low levels, has implications for the development of chronic diseases.

2.3.3 Wound Healing

Wound healing is also a very important consequence of an inflammatory response, when there is an open wound or a necrotic tissue damage that needs healing. However, in instances where there is only a low-level inflammatory response due to nonnecrotic sterile cellular damage, such processes can contribute to chronic disease. As with clot formation, all the details of wound healing are not discussed here; however, the important signaling processes that lead to healing are discussed.

In general, wound healing is started by the release of various signaling molecules by activated platelets, which are then responsible for stimulating the healing processes. These signaling molecules include IL-1β; TNF-α; PDGF; TGFβ; epidermal growth factor; bFGF; and the chemokines RANTES, platelet factor 4, and

IL-8 (among others). PDGF and TGFβ, as well as the various chemokines, initiate the chemotaxis of tissue macrophages, smooth muscle cells, and fibroblasts into the damaged area. PDGF also has the effect of stimulating mitogenesis of smooth muscle cells and fibroblasts, whereas TGFβ stimulates macrophages to release (among others) fibroblast growth factor and PDGF, as well as enhancing the ability of fibroblasts to deposit collagen. All of these effects result in the efficient formation of new scar and connective tissue to close the wound. Continual remodeling and maturation of the newly formed scar tissue by fibroblast activity is maintained through further production of growth factors by the macrophages and fibroblasts, as well as by vascular endothelial cells (and epidermal cells if it is a skin wound) and growth factors that include VEGF and bFGF (also produced by platelets). The latter two are very important for stimulating angiogenesis in the newly formed tissues.[205–207]

If everything goes well, a relatively strong mature scar with fully repaired and functional tissue in the formerly damaged region will result. Unfortunately, these same platelet-initiated processes occur even when the inflammation is initiated by infectious agents or toxic chemicals and there are no open wounds to close. In the case of chronic or repeated infections in the lung and liver, for example, the (in these cases, aberrant) fibrotic processes can result in the pulmonary fibrosis observed with tuberculosis infections or the cirrhosis in infectious hepatitis. Similar fibrotic changes are also observed with chronic exposures to chemical toxins such as those resulting in chronic obstructive pulmonary disease in smokers or cirrhosis of the liver in alcoholics. Although these kinds of chronic inflammatory conditions that result in pathologic fibrosis are not the focus of this chapter, these same platelet-initiated fibrotic processes are very much involved in the etiology of atherosclerosis, as described in Chapter 4, and contributory to the neurodegenerative diseases discussed in Chapter 7. In addition, the mitogenic and angiogenic effects of some of the signaling molecules readily contribute to processes associated with cancer, as do many other processes of inflammation, as described in Chapter 6.

2.3.4 Resolution of Inflammation

One final issue that needs to be dealt with is the transition from proinflammatory signaling to resolution of the initial inflammatory response in order for healing to occur. Although components of this topic might ordinarily be placed in the preceding wound healing section (Section 2.3.3), they are placed here in order to emphasize the importance of the resolution process. Obviously, without resolution inflammation can become a chronic process; the lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) made from AA, D-series resolvins made from DHA, and E-series resolvins made from EPA play an important role in the resolution process (Figure 2.5).

Details of the resolution phase of inflammation and the roles played by AA, EPA, and DHA metabolites have only recently come to light with the first descriptions of lipoxins in 1984[208] and, more recently, those of various resolvins, protectins, and maresins.[119,145–147,208–213] LXA_4 and LXB_4 can be synthesized from AA via two main dual cell processes. 15-HETE, which is synthesized by mucosal epithelial cells, vascular endothelial cells, or macrophages by 12-LOX/15-LOX, can be transferred through direct cell–cell contact to neutrophils and converted

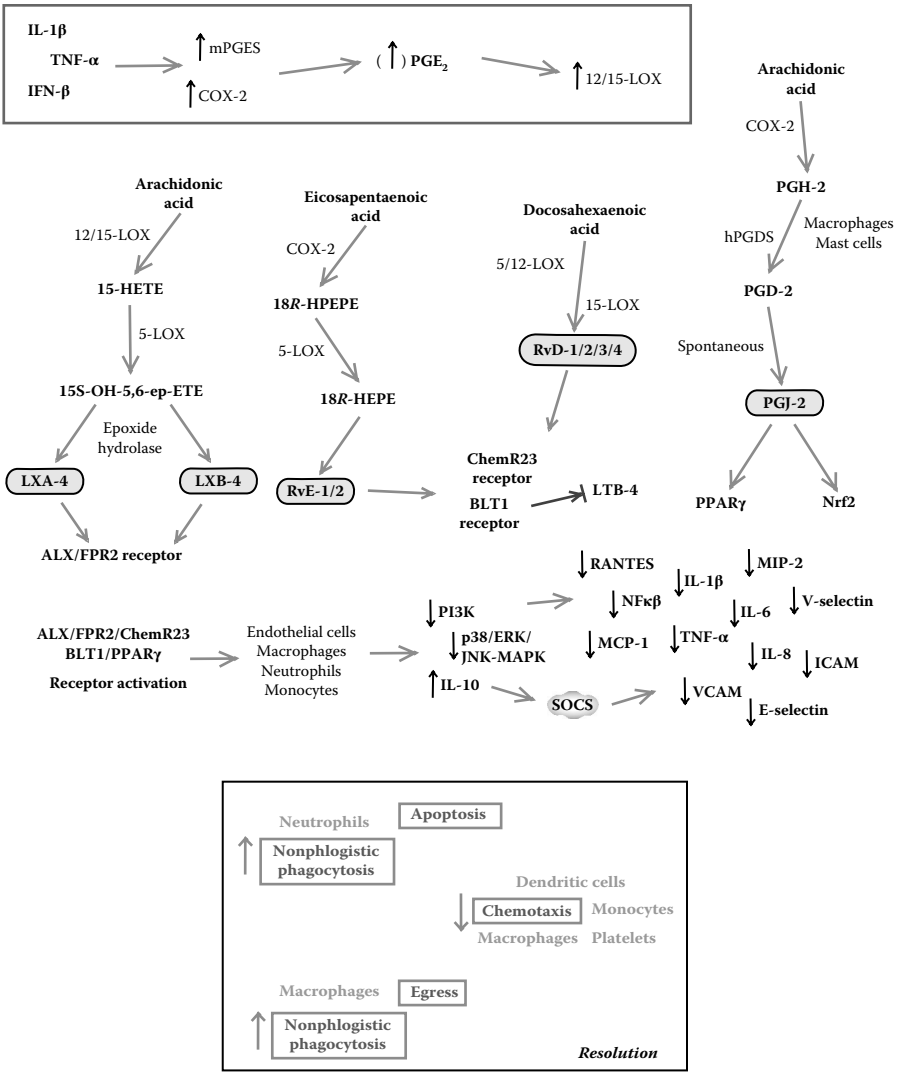

FIGURE 2.5 Resolution of inflammation: proinflammatory signaling leads to an induction of cyclooxygenase (COX)-2 and mPGES, increasing prostaglandin (PG) E-2 synthesis and subsequently resulting in an enhanced expression of 12-lipoxygenase (12-LOX) and 15-lipoxygenase (15-LOX) (upper box). Increased LOX in conjunction with COX-2 greatly enhances the synthesis of lipoxins, resolvins, and PGJ-2. Lipoxins and resolvins suppress (rounded, shaded boxes) proinflammatory signaling via inhibiting the activation of MAPKs and nuclear factor κ-β (NFκβ) through enhanced expression of suppressor of cytokine synthesis (SOCS) in all inflammatory cells while PGJ-2 (rounded, shaded box) activates apoptosis in neutrophils as well as suppresses mitogen-activated protein kinases (MAPKs) and NFκβ via activation of peroxisome proliferator–activated receptor-γ (PPARγ) and Nrf2. At the same time, macrophages phagocytize apoptotic neutrophils (without production of reactive oxygen species [ROSs]) and are stimulated to egress from the tissue, whereas chemotaxis of other inflammatory cells is inhibited, effectively ending an inflammatory response.

to 15*S*-hydroxy-5(6)-epoxy-ETE by 5-LOX in the neutrophils. The epoxide is then reduced by epoxide hydrolase to produce either LXA_4 or LXB_4. Neutrophil–platelet interactions in blood vessels can also produce lipoxins through the production of LTA_4 in neutrophils via 5-LOX activity and then LXA_4 and LXB_4 via 12-LOX/15-LOX in platelets.[147,214–216] One can easily discern that lipoxins also could be produced by neutrophil-produced LTA_4 that is made into LXA_4 by vascular endothelial cell interactions while rolling. Because of the phenomenon of the platelet activation cascade (described in Section 2.3.2), the predominant source of lipoxins during initial stages of inflammation would most likely be neutrophil–platelet interactions with the contribution of neutrophil–endothelial cell contact also. However, because of very low 12-LOX/15-LOX activity in neutrophils, the lipoxin production in these cells would be comparatively small during early phases of the inflammatory response relative to that of the proinflammatory PGs, LTs, and TXs.

Synthesis of resolvins, protectins, and maresins, of which the resolvin E1 (RvE1) and the protectin D1 (PD1) have been well characterized, also is mediated by lipoxygenase (LOX) activities. The 18*R*-hydroperoxy EPE (18*R*-HPEPE) produced by aspirin-acetylated COX-2 from EPA is converted to 5*S*-hydroperoxy 18*R*-hydroxy EPE (18*R*-HEPE) by 5-LOX, and this product can be reduced to resolvin E2 (RvE2) or further metabolized by 5-LOX to RvE1. While the initial characterization of resolvin synthesis centered on aspirin-acetylated COX-2 studies, bioactive amounts of RvEs are produced in humans in conjunction with COX-2 activity without the presence of aspirin.[147,211,214,217] The D-series resolvins (RvD1-4) are produced from DHA by a series of reactions initiated by 5-LOX,12-LOX, and/or 15-LOX, whereas PD1 synthesis from DHA is initiated through 15-LOX activity and maresin (MaR1) synthesis is initiated by 12-LOX.[147,209,214,217,218] Thus, activity of 5-LOX/12-LOX/15-LOX is central to the production of lipoxins, resolvins, protectins, and maresins.

In order for the proresolving effects of various lipoxins and resolvins to be realized, a mechanism to enhance their production is needed; in fact, multiple mechanisms exist for this. In addition to its well-known vasodilation effects, PGE_2 will also induce the synthesis of 12-LOX/15-LOX[145,219] in neutrophils and macrophages, an effect that greatly enhances the production of lipoxins through cell–cell interactions as well as by solitary neutrophils.[145,146,211,220,221] Because the production of RvDs depends on LOX activities, the induction of 12-LOX/15-LOX resulting from PGE_2 will also increase their synthesis while synthesis of E-series resolvins are enhanced because of the induction of COX-2 by the proinflammatory cytokines IL-1β, IFN-β, and TNF-α. As previously mentioned in Section 2.2.2, the synthesis of mPGES-1 is coinduced along with COX-2, resulting in a concomitant increase in PGE_2 synthesis along with its inducing effects, further enhancing the ability to synthesize these proresolving mediators.

In order to produce their effects, the various proresolving/anti-inflammatory mediators bind to their appropriate receptors and alter the function of target cells. LXA_4 (LXB_4 receptor has not yet been characterized) binds to the G-protein-coupled ALX/FPR2 receptors of neutrophils, endothelial cells, macrophages, and monocytes[214,215] and produces a variety of proresolution effects by attenuating p38–MAPK, p42/44–MAPK (ERK1/2), and PI3K activities as well as attenuating the activation and expression of NFκβ and AP1.[119,214,215,222,223] From previous discussions

on the activation of neutrophils, macrophages, endothelial cells, and dendritic cells, the implications of these effects on the proinflammatory signal transduction pathways should be profound. As expected, neutrophil chemotaxis and infiltration into the region is inhibited; apoptosis of neutrophils is enhanced; and production of ROS, RNS, and IL-8 is reduced in both neutrophils and macrophages. In macrophages, TNF-α release is blocked and TGFβ (inhibits toll-like signaling to reduce the activation of inflammatory cells) and IL-10 release is enhanced.

IL-10, through autocrine activation of the Jak–STAT pathway coupled with a prolonged STAT3 activation (activation of this pathway by IL-6 leads to a transient activation of STAT3), results in the synthesis of suppressor of cytokine synthesis (SOCS) proteins, which then inhibit the synthesis and release of IL-1α, IL-1β, IL-6, IL-8, and TNF-α.[224–226] IL-10 is also associated with the suppression of MAPK pathway and NFκβ activities, which, again, inhibits the synthesis of the acute-phase cytokines.[224–227] In addition to these IL-10 effects, macrophages are also stimulated to phagocytize apoptotic neutrophils without producing ROSs/RNSs (recall that stimulation of phagocytic activity in macrophages through the activation of PRRs is accompanied by stimulated ROS/RNS production) and to egress from the tissue.[145,214,228] This stimulation of nonphlogistic phagocytosis along with enhanced TGFβ release is an important resolution event because it helps to clear apoptotic neutrophils from the area without causing ROS-/RNS-mediated damage at the same time severely inhibiting the proinflammatory actions of macrophages and other cells in the region.

As a result of the aforementioned inducing effects of the proinflammatory mediators on LOX and COX activities, there is a progressive change in the production of proinflammatory lipid mediators over time in the inflamed region to one in which proresolution and anti-inflammatory lipid mediators predominate in the later stages of an inflammatory response. Another effect of the induced COX-2 synthesis is an increase in the production of PGD_2. The importance of this is that it spontaneously dehydrates to PGJ_2, which is a potent activator of PPARγ.[119,123,229] PPAR activation promotes neutrophil apoptosis as well as suppressing $NO^{\bullet}$ synthase and IL-1β and TNF-α release by macrophages and dendritic cells.[124,229,230] Similar to the effects described for LXA_4 binding to the ALX/FPR2 receptor, PGJ_2-mediated responses result from inhibiting TLR actions by attenuating ERK1 and p38–MAPK; by blocking AP1 activation; and through inhibiting NFκβ activation by promoting the formation of p50 homodimers, which inhibit the normal p60/p50 transactivation dimers from binding.[124,229–231] The end result of all these effects is to greatly reduce the production of both proinflammatory cytokines and lipid mediators, enhance the production of proresolution and anti-inflammatory mediators, reduce the production of damaging ROSs and RNSs, and remove neutrophils and macrophages from the area.

PGJ_2 also activates the transcription factor Nrf-2 (via modification of thiols in Keap1), resulting in the upregulation of heme oxygenase (HO)-1 as well as other electrophile-response element (ERE) (a.k.a. antioxidant-response element [ARE]) responsive genes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase (GST), and UDP-glucuronosyl transferase (UDPGT).[124,232–236] The significance of these antioxidant enzymes in protecting cells from oxidative damage (including neutrophil and macrophage-generated ROSs and

RNSs) cannot be overestimated, and these responses are discussed in more detail here. As an aside, with many of the aforementioned benefits being dependent on the induction of COX-2, the clinical use of non-aspirin COX-2 inhibitors might be brought into question.

The induction of COX-2 also increases the synthesis of E-series resolvins from EPA, whereas increases in 12-LOX/15-LOX enhance the production of D-series resolvins as well as protectins and maresins. These also have important receptor-mediated effects, with those of RvE1 and resolvin D1 (RvD1) being characterized.[147,215] RvE1 binds to the G-protein-coupled ChemR23 (a.k.a. CMKLR1) receptor as well as the BLT1 (of LTB_4 fame) receptor. Thus, RvE1 competes with LTB_4 for binding and at physiological concentrations (1 nM) RvE1 attenuates the LTB_4-dependent activation of NFκβ by approximately 40%–50% by blocking increases in Ca^{2+}.[147,215,237] The interaction of RvE1 with the ChemR23 receptor is interesting because chemerin, the peptide ligand for the same receptor, is an adipokine produced by differentiating adipocytes that has proinflammatory properties by acting as a chemoattractant (chemokine) for monocytes, macrophages, and dendritic cells. Binding of RvE1 to this receptor produces a completely different response: phosphorylation of the ribosomal S6 protein, apparently via Akt (a.k.a. PKB), is enhanced to significantly enhance nonphlogistic phagocytosis of apoptotic neutrophils by macrophages.[147,238] RvE1 can also bind to components of neutrophil membranes and through non-receptor-mediated actions to greatly reduce neutrophil chemotaxis and transmigration.[147,210,215] From these results, RvE1-mediated activities significantly enhance the resolution of inflammation in a manner similar to that of LXA_4–ALX binding. Although the RvE2 receptor has not yet been identified, there is evidence of additive effects of RvE1 and RvE2, indicating either multiple-binding effects mediated through the same receptor or the presence of a specific RvE2 receptor.[215] In any event, both RvE1 and RvE2 clearly contribute to the resolution process.

RvD1, synthesized following the induction of 12-LOX/15-LOX activities, also binds to the ALX receptor and to the GPR32 receptor. While GPR32 receptor–mediated events are not known, RvD1 binding to ALX activates phagocytic and clearance functions of macrophages very similar to those seen following the binding of LXA_4 to ALX.[147,239] The receptor-mediated events of PD1, also very poorly understood, result in reduced neutrophil infiltration and stimulated nonphlogistic phagocytosis of apoptotic neutrophils by macrophages.[147,210,215,240]

From all of the aforementioned factors, the binding of lipoxins, resolvins, protectins, and maresins to specific receptors that are present on neutrophils, macrophages, monocytes, and dendritic cells in the immediate region leads to the resolution of an inflammatory response. The generic sequence of events—pathogen-/damage-induced proinflammatory signaling → infiltration and activation of inflammatory cells → induction of various LOX/COX enzymes → anti-inflammatory/proresolution signaling → removal of inflammatory cells—has been elucidated in a variety of in vitro, in vivo, and in situ experiments that mostly relate to processes occurring in exudates or air-pouch models of inflammation. These models are highly relevant to a localized infection where the ordered process of inflammation events leading to the removal of the damaging agent followed by healing occurs. However, the significance of these resolution events to chronic disease is a little more difficult to

ascertain. In many cases of chronic disease, there is a constant production of sterile trauma that results in constant initiation (of inflammation) events followed by the constant presence of inflammatory cells and their associated signaling molecules, ROS and RNS, and on the surface resolution does not seem to be much of an option in these circumstances. When proinflammatory signaling molecules enter an area from some distal infectious (or sterile) inflammatory response, resolution also does not seem to be a local option. Even with a properly resolved inflammatory response that was initiated by an infectious event, adapted immune responses to reinfection can modify or produce inflammation-mediated events in otherwise unaffected cells, even if they are far away from the reinfection event.

2.3.5 Adaptive Immunity and Inflammation

As is well known, an adapted immune response to a particular pathogen gives us the ability to respond swiftly to a reinfection event in order to remove the pathogen before extensive damage (or death) can occur. Further, it is the innate inflammatory response that gets the ball rolling for the development of the adapted response. Once pathogens and cellular debris have been phagocytized by activated dendritic cells and macrophages, these professional antigen-presenting cells can migrate to the lymph where they interact with naive T lymphocytes (T cells) and B lymphocytes (B cells) to initiate an adaptive immune response. Once produced, the various T cell subpopulations, as well as the B cell subpopulations, circulate throughout the secondary lymphoid organs (predominantly spleen, lymph nodes, and the Peyer's patches of intestinal epithelium) via the blood with much of their time spent in the lymphoid tissues, while the long-lived plasma B cells tend to remain in the bone marrow.[241–244] Although the details of the adaptive immune processes the result in the generation of the various cell types are not a topic for this chapter, the resulting effector functions of the T and B cells that are associated with proinflammatory responses will be discussed.

An array of $CD4^+$ and $CD8^+$ T cells resulting from adaptive immune responses can respond to chemokines produced during a local inflammatory response and migrate into the area. $CD4^+$ effector T cells include the Th1, Th2, Th17, and Treg subsets.[241,245–247] Once in the area, if the effector T cells bind with the same (or structurally similar) antigens to which they were originally selected for, then they are activated to produce a variety of cytokines that are necessary for enhancing the inflammatory response as well as to initiate their effector functions. Th1 ($CD4^+$) cells express IL-2 and IFN-γ, whereas Th2 ($CD4^+$) cells express a variety of ILs, including IL-4, IL-5, IL-6, and IL-10. The different expression of cytokines relates to the different effector functions of the activated Th cells. Th1 cells are involved, in part, in activating both inflammation and cytotoxic activities through the expression of IL-2 and IFN-γ (activates macrophages) as well as lymphotoxin-α, whereas Th2 cells are potent activators of B cells (including stimulation of Ig class switching to IgE) and can downregulate macrophages through the production of IL-4 and IL-10. Thus, Th1 responses are predominantly proinflammatory and cytotoxic, whereas Th2 responses are predominantly anti-inflammatory. Not coincidently, a variety of autoimmune diseases are associated with Th1 overactivation without appropriate activation of anti-inflammatory Th2 responses.[246–249] The

more recently described Th17 and Treg cells also are associated with autoimmune diseases and inflammation. Th17 cells express the chemokine receptor CCR6, which binds the CCL20 produced by cells associated with Peyer's patches and lymphoid follicles as well as by inflamed intestinal epithelium, joints, and central nervous system. The Th17 cells produce the proinflammatory cytokines IL-17, TNF-α, and lymphotoxin-β. IL-17 is proinflammatory because it stimulates the synthesis and release of a variety of proinflammatory cytokines (IL-1, IL-6, and TNF-α) through the activation of NFκβ in a variety of cells, including endothelial cells, macrophages, and fibroblasts.[250,251] On the other hand, Treg cells are associated with regulating and inhibiting autoimmune responses and autoimmune conditions have recently been associated with enhanced Th17 and reduced Treg cell function.[245,246,252–256]

Cytotoxic T cells (Tc cells—$CD8^+$) also respond to various chemokines produced during an inflammatory response and home in to the inflamed tissue. Here, they are activated when they come in contact with and bind to the antigen. Activated Tc cells secrete perforins to create pores in the membranes of antigen-containing cells through which they release granules that contain enzymes, which then induce apoptosis in the target cells by activating caspases. They can also activate apoptosis by directly binding to surface-bound Fas molecules on the target cells. In addition to contact-mediated mechanisms of killing, Tc cells also release the proinflammatory cytokines IFN-γ, TNF-α, and TNF-β.[241,257–259] Finally, similar to the Th1/Th2 dichotomy, $CCR7^-$ and $CCR7^+$ memory T cells have differing functions. The $CCR7^-$ memory cells circulate and migrate into inflamed areas, whereas the $CCR7^+$ memory T cells home in to the lymph because of the differing expression of chemokine receptors. It is the $CCR7^-$ memory T cells that produce IFN-γ, resulting in a proinflammatory effect by further activating macrophages.[246,260,261]

The proinflammatory effects of cytokine release by the various subsets of Th cells (as part of their effector functions) may be relevant as a component of risk for inflammation-associated chronic diseases, especially in the event of recurrent infections. In addition, there is a subset of memory T cells that tends to circulate through the tissues in which the original antigen exposure occurred.[262–264] This enhances the likelihood of proinflammatory effects in those tissues susceptible to multiple exposures over time, for example, tissues such as skin, oral and intestinal mucosa, and lung. Although the effector functions resulting from an adapted immune response limit the pathological consequences of a second infection through efficient removal of the pathogen, they can also increase the probability that a local inflammation response due to non-infection-related trauma can be exacerbated through circulating IFN-γ, or even locally produced cytokines in the event *stray* pathogens enter an already inflamed area.

Unlike the various subsets of T cells, B cells do not actually appear to have much to do with acute inflammation, most likely because if they are not already in the secondary lymphoid tissues they will migrate to those locations in response to the chemokines SDF-1, SLC, CKβ-11, and BLC.[241,247,265] B cells do, however, appear to play a role in the development of autoimmune disease as a consequence of their ability to act as antigen-presenting cells for T lymphocytes (B cells are able to activate T cells even at very low titers of antigen,[265] as would be expected for self-antigens),

as well as the ability of some subsets of memory B cells to regulate the activation of T cells.[246,265,266]

The last lymphocyte family to be considered is that of NK cells. NK cells are typically identified with innate immunity, although recent evidence indicates that NK cells not only interact reciprocally with dendritic cells through (paracrine) cytokine release during the initiation of adaptive responses but some subsets also appear to have memory functions.[267–271] NK cells migrate into sites of inflammation in response to various chemokines, where they interact with a variety of ligands on target cells (such as CD16, NK cell protein 30 [NKp30], NKp44, and NKp46, among others), ligands commonly seen on cells that are infected with viruses or damaged. The cells that do not express sufficient class I MHC molecules to inhibit the killing function of NK cells are then lysed through secreted perforins and *granzymes* (similar to Tc cells). Cells that do not produce sufficient class I MHC also tend to be those that are transformed or have undergone some form of damaging stress. Thus, NK cells provide the vital function of eliminating what essentially are unhealthy and poorly functioning cells.[16,18,241]

Although details of the roles that NK cells play in developing adaptive immunity and (NK cell) memory functions are beyond the scope of this chapter, NK cells can and do play a role in inflammation. In addition to their cytolytic activity, activated NK cells synthesize and release the proinflammatory cytokines IFN-γ and TNF-α as well as various chemokines, including CCL2, CCL3, CCL4, and CCL5.[267,271–273] The release of chemokines by activated NK cells attracts a variety of inflammatory cells to the area, including macrophages and dendritic cells. The end result of activating NK cells is therefore an increased inflammatory response with an increase in killing of invading pathogens via activated macrophage phagocytosis and ROS production as well as NK-mediated destruction of infected cells. Although all this results in a more efficient removal of pathogens, it also means an increased likelihood of damage to the otherwise healthy cells in the immediate area of the infection as well as a possible increase in the localized production of various growth factors. The implications of peripheral damage may be minimal in terms of a single infection-mediated activation of NK cell activity. The ramifications may be much more severe, however, when repeat viral infections are considered, especially as NK cells retain some memory functions and will more readily home into areas of previous infections.[270,273]

2.4 CONTRIBUTIONS OF INFLAMMATORY PROCESSES TO CHRONIC DISEASE

The mechanisms through which inflammatory responses may contribute to chronic disease have been mentioned as part of the summary discussions throughout the various sections in this chapter. In this section, the general risk-enhancing effects of inflammation are discussed, whereas the application of these mechanisms to specific diseases is discussed in detail in Chapters 3 through 8. At its simplest, inflammatory responses contribute to disease through two main mechanisms: the first is through the effects of various ROSs, RNSs, and other active molecular species produced by activated neutrophils, mast cells, and macrophages as a means to kill or destroy

pathogens. The second is through the generation of signaling molecules that can alter the function of noninflammatory cells as well as those specifically involved in inflammatory response.

2.4.1 Reactive Molecular Species

As already described in Section 2.3.1, the generation of various ROSs and RNSs occurs in the phagolysosomes of phagocytes as a result of the activities of NADPH oxidase and nitric oxide synthase. It is these reactive molecules produced by enzymes that contribute to disease, as first described by McCord, Halliwell, Beckman, and others.[274–281] Single electrons released by NADPH oxidase get picked up by molecular oxygen to create the superoxide anion radical: $O_2^{\bullet-}$. The superoxide anions cannot cross cellular membranes due to their charge, but they do react with one another to produce peroxide and oxygen: $O_2^{\bullet-} + O_2^{\bullet-} + 2H_2O \rightarrow H_2O_2 + O_2 + 2OH^-$. The peroxide is very stable and readily diffuses through cellular membranes to enter the extracellular space as well as into and through the membranes of surrounding cells. The nitric oxide (an uncharged radical) that is produced by activated phagocytes also readily diffuses through cellular membranes. Most $NO^\bullet$ produced within the phagolysosome will react with $O_2^{\bullet-}$ to produce peroxynitrite: $ONOO^-$ (a highly reactive nonradical oxidant); some of the $NO^\bullet$ will diffuse through membranes, out of the cell, and into adjacent cells. Although H_2O_2 and $NO^\bullet$ are not highly toxic alone, it is their reactions with $O_2^{\bullet-}$ and carbon dioxide (CO_2) that are highly relevant because of the resulting production of extremely reactive products.

The $ONOO^-$ produced from the $NO + O_2^{\bullet-}$ reaction is highly reactive with CO_2, producing carbonate radicals ($CO_3^{\bullet-}$) and nitrogen dioxide radicals ($NO_2^\bullet$). There is certainly no shortage of $CO_3^{\bullet-}$ because of the high rate of production of $NO^\bullet$ during inflammatory responses and the easy availability of CO_2 and $O_2^{\bullet-}$ in tissues because of normal cellular respiration. Thus, DNA, proteins, and phospholipids in cells within the immediate region of the activated phagocytes are all susceptible to attack by both the reactive carbonate radicals and the moderately reactive nitrogen dioxide radicals.[281–283] The $ONOO^-$ can also react with transition metals in proteins such as SOD, myeloperoxidase, aconitase, zinc-containing transcription factors, heme, and others to produce a nitrating species, which results in the production of a variety of nitration reactions with almost any nearby amino acids,[274,276,281] most of which result in inactivation of the proteins. As might be expected, nitration products are highly associated with chronic inflammation and a variety of chronic diseases, including atherosclerosis, Alzheimer's, cancer, and Parkinson's.[276,281,284–288] Thus, inflammation-associated nitric oxide production is a significant source of RNS that leads to chemical damage in cells that otherwise would not have occurred. While the affected cells have an antioxidant capacity due to their own SOD, glutathione reductase (GSR), GPX, and CAT enzymes, the additional H_2O_2 and $NO^\bullet$ from extracellular sources simply overwhelms the endogenous antioxidant capacity to produce additional damage and an enhanced risk for chronic disease.

HOCl is another oxidant produced by activated neutrophils that can diffuse through cell membranes and cause damage to cells. Neutrophils (and macrophages) secrete the enzyme myeloperoxidase, which uses the extracellular H_2O_2 and chloride

ion to produce HOCl.[289–293] Because HOCl is produced extracellularly prior to diffusing into adjacent cells, this oxidizer can react with a variety of compounds in extracellular spaces as well, including lipids. This reaction is relatively slow, however, rendering it relevant mainly in cases of a prolonged exposure of extracellular lipids to HOCl, such as in atherosclerotic plaque. Once inside a cell, HOCl can oxidize a variety of cellular components, including DNA, RNA, proteins, and lipids.[289–293] However, HOCl reacts most strongly with thiol residues of proteins,[290] indicating that this oxidant might function predominantly as a modifier of the cellular redox state (discussed here).

The H_2O_2 produced by the phagolysosomes and by the plasma membrane–bound NADPH oxidases is a significant source of ROSs for cells within the inflammatory region. Hydroxyl radicals, probably the most aggressive oxidizing ROS known, are produced from the reaction between $O_2^{\bullet-}$ and H_2O_2 ($O_2^{\bullet-} + H_2O_2 \rightarrow OH + HO^- + O_2$). As with NO•, H_2O_2 is sufficiently stable for it to diffuse across membranes and throughout the entire cell where it reacts with locally produced $O_2^{\bullet-}$; the resulting •OH will then oxidize any molecule within the immediate vicinity. Because of the continuous production of $O_2^{\bullet-}$ from normal respiration and the dismutation of $O_2^{\bullet-}$ to H_2O_2, the production of •OH and subsequent damage to proteins, DNA, and phospholipids is inevitable. Even without inflammation, this damage is common and considered normal. However, when additional H_2O_2 enters cells from inflammation-related events and ultimately overwhelms their antioxidant capacity the additional damage caused by the ROS can significantly alter cell functions. It is the alterations in cell function due to excessive inflammation-associated ROS damage that contributes (in part) to greater risks for chronic diseases.[274–281,294,295]

2.4.2 Reactive Molecular Species and Cellular Signaling

Inactivation of proteins, formation of DNA adducts, and peroxidation of lipids are well-known consequences of endogenous ROS- and RNS-mediated reactions and are a major reason for the constant synthesis activities in all cells; the damaged structures simply must either be replaced or be repaired in order to minimize the inefficiencies produced. Transcription is a highly regulated activity, and increased rates of protein synthesis in response to enhanced rates of damage can only occur following alterations in the signal transduction pathways that regulate transcription. In the case of increasing amounts of oxidative damage due to additional inflammation-produced ROSs/RNSs, however, increased synthesis and repair activities are not necessarily a direct response to the increased damage. In fact, cells have evolved the interesting ability to use the damaging agent itself as a signaling device to activate repair and synthesis. As is now well known, ROSs, RNSs, HOCl, and other oxidizing agents indirectly modify the activity of signal transduction pathways by oxidizing the *redox sensor* proteins thioredoxin (Trx), peroxiredoxin (Prx), and glutaredoxin. These redox sensor proteins affect the function of specific enzymes of the pathways or factors that then activate the pathways, depending on their oxidation state.[295–302] Although all the details of these forms of transcription regulation are not the major focus of this chapter, some general issues are discussed here.

A reduction in cellular glutathione (GSH) concentration through a sustained–GPX-mediated reduction of H_2O_2 leads to decreased GPX activity and increased cellular H_2O_2 and ultimately to increased oxidation of the different redox sensor proteins. H_2O_2 directly oxidizes the susceptible cysteines of reduced Prx and Trx, resulting in the formation of disulfides on the proteins and H_2O from the peroxide. For this reason, both Trx and Prx have been considered to be antioxidant enzymes. One role for Trx is to form an inhibitory complex with the ASK1 protein, which disassociates on Trx oxidation, allowing ASK1 to then activate the JNK and p38–MAPK pathways with the resulting pattern of protein expression depending on the degree of Trx oxidation by H_2O_2. The antioxidant function of Trx can also be an important component of its regulatory function. For example, cytosolic NFκβ can be activated by H_2O_2 and the peroxidase activity of Trx reduces the H_2O_2 to water, thereby reducing the activation of NFκβ. Because of the heterogeneous distribution of these redox sensors throughout a cell, a given redox state in one location of a cell produces a differing pattern of protein expression than the same redox state in another location of the cell. At this point, it is instructive to mention that the activation of NADPH oxidases at the plasma membrane (NOX-1) is one of the immediate effects when cytokines bind to their G-protein receptors (a component of activating the formation of phagolysosomes in phagocytes, as previously discussed in Section 2.3.1). This creates a transient and highly localized production of H_2O_2, which then alters the oxidation state of Trx in the immediate vicinity and, subsequently, the activity of signal transduction proteins (again, only in the immediate vicinity). The result is a pattern of transcription activation that is highly specific to the degree of cellular oxidation at one specific location. One can imagine that as the concentration of a specific cytokine increases, there will be an increase in the duration, amount, and spatial distribution of H_2O_2 production, which then slightly changes the pattern of transcription activation. Add to this the additional localized productions of H_2O_2 by different cytokine receptors (at different physical locations on the membrane) and the complexity of the various possible interactions provides an explanation for the differing responses to different cytokines in spite of the fact that they utilize common NFκβ and MAPK pathways.[302–304]

Interestingly, Prx can form dimers with specific regulator proteins such as JNK, c-Abl, and c-Myc to attenuate their activation. The activation of certain transcription factors also appears to be dependent, in part, on the redox state of Trx and Prx, including Jun/Fos (AP-1) and heat shock factor 1.[298,302,303,305] All of these activation events at the transcription factor and signal transduction level are important when one considers that a highly localized and transient production of ROSs is a common component of receptor-mediated regulation of signal transduction pathways. Because global changes in the redox state of a cell can occur as a result of excess ROS entry into cells due to phagocytic responses, the ability of inflammatory responses to alter a highly regulated process to cause large changes in signal transduction activity is profound. Of course, many of the cellular responses to global changes in ROS-mediated activation of signal transduction pathways provide important protection effects through induction of a variety of antioxidant enzymes and an increased capacity for repair and replacement of damaged proteins. On the other hand, with prolonged and nontransient activation of some of these pathways, JNK,

AP-1, and c-Abl, for example, cell division can be stimulated and tumorigenesis starts to become a distinct risk.

One additional issue related to the redox-based regulation of these pathways is the activity and cellular distribution of antioxidant enzymes, which serve to quench the various ROSs. As with redox sensors and oxidants, antioxidant enzymes such as SOD, CAT, and GPX alter the local concentrations of ROSs and therefore contribute to the optimal regulation of signal transduction pathways in the cell.[295–303,305] As with redox sensors, the cellular distribution of antioxidant enzymes is heterogeneous, resulting in a very complex, yet highly regulated (through both redox-sensing and antioxidant enzyme systems) system of control. This does raise the interesting possibility that nutritional insufficiencies and the resulting inability to synthesize optimal amounts of these antioxidant enzymes might interfere with what would otherwise be normal regulation of cell signaling.

The inflammation-associated peroxidation of phospholipids resulting from the additional supply of oxidants also may lead to alterations in cellular function through mechanisms that are independent of protein and DNA damage and alterations in transcription caused by these oxidants. The unsaturated fatty acids on phospholipids are highly susceptible to attack by hydroxyl radicals, and the resulting lipid peroxidation makes the membrane leakier to various charged molecules such as K^+ and Ca^{2+} that normally diffuse across the membrane very poorly.[305] The importance of calcium in intracellular signaling process cannot be overemphasized.

Highly localized transient increases in calcium throughout the cell regulate a large variety of cellular functions, with the duration, concentration, and spatial distribution of the calcium spikes being the key determining factors in the resulting cellular effects.[306–313] The entry of calcium into cells is a highly regulated process with a variety of endoplasmic membrane– and plasma membrane–bound calcium channels being involved, including voltage-gated calcium channels; DAG-regulated calcium channels; inositol-1,4,5-3P receptor (I3PR); and ryanodine receptors. Opening and closing of the various calcium channels is normally controlled by a wide array of cytokine, paracrine, and autocrine factors, as well as being modified by ROSs, NOSs, and calcium. Once in the cytosol, calcium exerts its regulatory effects by binding to a large variety of proteins, including calcium-dependent protein kinases (PKC), calmodulin, PLA_2, calcineurin, adenylate cyclase, phosphorylase kinase, and a variety of metabolic enzymes in the cytosol and mitochondria. Through the activation of PKC and calmodulin, a variety of Rho-GTPases and Ras are activated, leading to phosphorylation and activation of p38–MAPK, JNK–MAPK, ERK1/2–MAPK, and Jak–STAT pathways. If sufficient calcium enters the cell, then mitochondrial function will be compromised, lysosomal activity will be activated, and the cell will die through either necrosis or apoptosis depending on the degree of mitochondrial disruption. If nonfatal amounts of calcium enter through damaged membranes, then extensive activation of the MAPK and STAT pathways can lead to the protective effects of increased synthesis, repair, and antioxidant function; if chronic, a risk for hypertrophy/mitosis and tumorigenesis due to the proliferative effects of prolonged activation of MAPKs is the result. With respect to the last comment, lipid peroxidation due to excessive ROS production results in the production of ketones, aldehydes, malondialdehyde, acrolein, 4-hydroxy-2-nonenal, and crotonaldehyde from

the peroxidized fatty acids. All of these peroxidation products can damage other lipids, proteins, as well as DNA,[314–316] significantly disrupting cellular function and enhancing mutagenesis, thereby increasing the risk for cancers.

2.4.3 Inflammation-Associated Cytokines and Growth Factors and Cellular Signaling

The last major mechanism through which inflammatory processes contribute to disease that we discuss is through the signaling process itself. The various cytokines and growth factors produced by activated inflammatory cells initiate processes that are necessary for repair of wounds and replacement of the cells that die as a result of the pathogenic or endogenous trauma. The variety of growth factors released by activated platelets, macrophages, fibroblasts, and endothelial cells, including VEGF, PDGF, bFGF, EGF, and TGFβ, are necessary for normal scar formation and wound healing. In essence, the effects of these growth factors are to activate resident fibroblasts to produce scar tissue to close the wound and to proliferate and differentiate into functional tissue cells to replace dead cells. Unfortunately, these same processes can result in the pathological scar formation that occurs in the liver as a result of the chronic inflammation associated with alcohol abuse or infection with hepatitis C. Chronic inflammation also increases the risk for tumorigenesis, which helps explain the increased incidence of hepatocellular cancer in drinking alcoholics and those infected with hepatitis B and C.[317]

Following extensive tissue damage, endothelial cells produce VEGF and bFGF (along with a variety of other factors) for a long period. One effect is angiogenesis in the newly regenerating tissue through activating a growth and proliferative response in fibroblasts and smooth muscle cells. With unresolved inflammation, the production of growth factors also provides exactly the right mix of signaling molecules that can lead to tumorigenesis because of their effects on any adult stem cells that might be in the region. Although the presence of fibroblasts in tissue (as well as the recruitment of more through inflammation-mediated chemotaxis) is more than sufficient for the repair of relatively small regions of tissue damage, more extensive damage (and the resulting prolonged signaling) will include the recruitment and activation of adult stem cells in the area to proliferate and differentiate into functional tissue cells. Although this is necessary for closing and healing extensive wounds, adult stem cells can also be activated to proliferate with prolonged inflammatory signaling and without any overt wounding present,[318,319] again, leading to a greater risk for tumorigenesis.

The proinflammatory cytokines also bind to receptors on nonimmune/inflammatory cells in the vicinity and affect their function. Of the proinflammatory cytokines, IL-1β, TNF-α, and IL-6 are of the greatest interest because of the near ubiquitous expression of their receptors and the detrimental effects of chronic Il-1, IL-6, and TNF-α exposure on cellular function. The concept is best illustrated by the syndrome cachexia. Cachexia is mostly recognized as a wasting syndrome associated with end-stage cancer, but it also occurs with other chronic immune disorders. It is characterized by abnormal cellular metabolism, increased metabolic production of ROSs, insulin resistance, muscle and adipose tissue wasting, and extreme fatigue

and is known to be a chronic inflammatory syndrome associated with the severe metabolic and immune stresses of the underlying disease conditions.[320–324] Cachexia is characterized by moderate to high levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α, and IFN in serum as well as the proteolysis inducing factor, with each of the cytokines being directly involved in the pathogenesis of the syndrome. With the recognition that inflammatory signaling is of paramount importance to the pathology of this syndrome, and that metabolic disturbances, insulin resistance, and fatigue are common components of chronic inflammation, diabetes, and metabolic syndrome, it is certainly tempting to consider that cachexia is nothing more than the extreme end of a continuum of systemic inflammatory disorders: chronic metabolic disturbances → metabolic syndrome → diabetes → cachexia.

To briefly summarize, proinflammatory signaling processes can lead to enhanced phagocytic activities, which can result in excessive ROS- and oxidant-mediated damage to membrane lipids, proteins, and DNA in adjacent cells. These types of damage can affect the already ROS-/calcium-altered signal transduction and regulatory pathways in detrimental ways. Add to these interactive effects the effects of proinflammatory cytokines and it is clear that unresolved inflammation can have profound effects on cellular function and subsequent risk for chronic disease. As discussed in detail throughout this book, these risks for chronic disease by inflammatory processes can be enhanced through nutritional insufficiencies, weight gain, and inactivity.

2.5 ATTENUATION OF INFLAMMATORY PROCESSES THROUGH DIET

2.5.1 General Nutrition

The possible array of cellular dysfunctions caused by excess damage to individual cellular components, or to alterations in signaling pathways due to ROSs–calcium interactions caused by inflammatory responses, is nearly infinite; however, in terms of reducing inflammation-associated risk for chronic disease there appear to be only a few relevant issues. The first is that entry of a variety of inflammation-produced oxidants into adjacent cells provides a constant source of damaging agents. These are primarily H_2O_2, HOCl, and $NO^{\bullet}$, of which H_2O_2 and $NO^{\bullet}$ react with locally produced CO_2 and $O_2^{-\bullet}$ to produce the damaging oxidants $CO_3^{-\bullet}$, $NO_2^{\bullet}$, and $^{\bullet}OH$. Damage through oxidant-mediated reactions is a major source of cellular dysfunctions; therefore, optimizing the antioxidant function of cells should be a major focus for prevention. This concept needs to be tempered with the fact that it is the normal, metabolically produced $O_2^{-\bullet}$ (and CO_2) in the tissue cells that reacts with the inflammatory cell-produced oxidants and that metabolic regulation of ROSs through the expression of antioxidant enzymes does play a vital role in a wide array of normal cellular functions. Severely reducing endogenous ROSs through supplementation with antioxidant compounds should therefore produce cellular dysfunctions at some level and would be contraindicated. Exercise studies provide interesting examples of this. The well-known increase in mitochondrial biogenesis and insulin sensitivity in skeletal muscle caused by exercise in humans can, in some cases, be prevented by

supplementation with vitamin C (1 g) and vitamin E (400 IU).[325] In a variety of other studies, antioxidant supplementation has been observed to reduce aerobic performance, reduce training-induced increase in VO_2 max, and reduce maximum muscle force.[326–329] Thus, optimizing antioxidant function should really mean maintaining optimal cellular antioxidant enzyme function and redox sensing function to ensure appropriate regulation of transcription activities. This should be achieved through eating a sufficient array of foods to provide the recommended dietary allowance (RDA) or adequate intake (AI) amounts of all nutrients. This may be especially relevant in light of the fact that although epidemiological evidence clearly indicates a decreasing risk for chronic diseases with an increasing intake of antioxidant nutrients and compounds in foods, clinical trials testing the effects of antioxidant supplementation on chronic diseases such as atherosclerosis and a variety of cancers rarely provide evidence of a positive benefit.[330] In fact, negative effects such as increased deaths due to lung cancer with β-carotene or with vitamin C or E supplements and increased stomach cancer with α-tocopherol and/or β-carotene have been observed.[331–337] These results might have been expected had the importance of regulating cellular redox states at the microenvironment level been considered; these concepts were, of course, not known at the time that any of the clinical trials were designed.

The concept of using supplements for prevention in a general population deserves a little discussion at this point. The concept of using supplements was developed on the basis of a large array of epidemiology-based research studies on diet and risk for disease that revealed an inverse association between antioxidant intake and risk for a variety of chronic diseases. A common conclusion of these studies was that antioxidant supplements should therefore reduce risk for the diseases as well, a conclusion that, on the basis of negative results from several recent large clinical trials, was probably inaccurate. Possibly, the observed associations between individual dietary antioxidants and preventive effects in epidemiological studies were more likely due to the wide range of nutritional and dietary statuses of the subjects. For instance, intakes of fruits and vegetables observed in American diets are traditionally low with less than 10% of Americans actually eating the recommended minimum number of servings of fruits and vegetables.[338] This likely means that a majority of Americans consume insufficient amounts of many nutrients and beneficial phytochemicals because fruits and vegetables are by far the main food sources for these compounds. In fact, only about 50% of Americans meet their RDA intake recommendations for vitamin C.[339] Actually, the estimated average requirement (EAR) is the proper reference value for groups (obviously producing a lower percentage of potential insufficiencies), and according to the latest National Health and Nutrition Examination Survey (NHANES) data (2003–2006) intakes for vitamins C, D, and E are below the EAR for 25%, 70%, and 60% of Americans, respectively, and below the EAR for zinc (~8%), iron, copper, and selenium (~5% to 6%), as well as calcium (38%) and magnesium (45%).[340] These specific vitamins and minerals were chosen to demonstrate the extremely poor dietary choices of Americans (insufficiencies of other nutrients also are common). They were chosen because they are antioxidants, components of antioxidant enzymes, or directly involved in calcium homeostasis, illustrating a potentially very important point. The point is as follows: while highly

useful statistical techniques are used to control for a variety of possible confounders in epidemiological studies, such as exercise history, ethanol intake, financial status, ethnicity, age, gender, and so on (and certainly not all relevant lifestyle factors are controlled for in each study), it is nearly impossible to test for and then control for each of the wide variety of existing nutritional insufficiencies. Because of the wide array of nutritional insufficiencies found in many of the populations studied, it is much more likely that the general epidemiological associations between antioxidant nutrient intake and prevention of disease are largely a reflection of a decreasing degree of nutritional/dietary insufficiency with increasing fruit/vegetable intake (correcting many different nutritional insufficiencies) and not necessarily a specific preventive effect of any individual antioxidant nutrient or compound. Although this does not mean that supplements are not useful to correct dietary insufficiencies and deficiencies, it does raise doubts whether supplementing beyond normal cellular requirements has any additional beneficial effects beyond that of normal nutrient function. In fact, the effect could be detrimental; the previously mentioned negative results of clinical trials with antioxidant supplements certainly attest to this. In a similar vein, nutritional supplements, other than correcting nutritional insufficiencies, have little to no positive effects on athletic performance either.[341,342] This and the previously mentioned prevention of an exercise-induced mitochondrial biogenesis by the antioxidant vitamins C and E should certainly raise serious doubts about any potential use of antioxidant supplements beyond that of ensuring normal cellular nutritional requirements. For these reasons, the starting point of a dietary approach to reducing risk for disease through attenuating inflammation-associated events is a diet that provides RDA/AI amounts of all the necessary nutrients and not one that includes supplements. Considering the fact that a majority of Americans consume insufficient quantities of many different nutrients through less-than-ideal food selections, moderately large changes in diet are more than likely needed just to accomplish this.

2.5.2 Cellular Signaling

The second major area for reducing risk through attenuating inflammation is cellular signaling, which may represent what could be the single most effective area for overall risk reduction other than optimizing nutritional status. On the basis of the signaling processes that lead to the activation of various components of an inflammatory response, one general concept of cellular signaling emerges: the signal transduction pathways for activating the synthesis of proinflammatory cytokines converge on the p38–MAPK, JNK–MAPK, and NFκβ pathways, leading to the activation of a large array of transcription factors. The anti-inflammatory effects of IL-10, lipoxin, resolvin, and protectin all function by inhibiting these same pathways, providing a clear indication of how to reduce proinflammatory signaling. Blocking the pathways would, of course, prevent an appropriate inflammatory response and cause severe problems in the event one is necessary; therefore, only a dampening of inflammatory responses to minimize the risk of an excessive inflammatory response is prudent.

A related signaling issue involves the various PGs, LTs, TXs, lipoxins, resolvins, protectins, and maresins. The lipid mediators made from AA tend to be

proinflammatory in nature, whereas those made from DHA and EPA tend to be either anti-inflammatory or proresolving in their effects on cell function. Again, they function through receptor-mediated effects that then alter the same signal transduction pathways. The production of anti-inflammatory lipoxins from AA complicates this issue (as discussed here); so it is not quite as simple as merely changing the availabilities of AA, EPA, and DHA by manipulating diet. On the other hand, attenuating absolute rates of synthesis of all the lipid mediators will occur primarily through dampening the activation of PLA_2, a relevant concept where initiating an inappropriate inflammatory response is a major issue. This will, not coincidently, also occur through giving appropriate attention to maintaining proper calcium/antioxidant (and nutrient) status and through attenuating stress-mediated activation of PKC and MAPK pathways.

2.5.2.1 Linoleic Acid, Linolenic Acid, Arachidonic Acid, Eicosapentaenoic Acid, and Docosahexaenoic Acid

An association between consumption of dietary DHA and EPA and reduced risk for inflammation-associated chronic disease has been known for many years, and these ω-3 fatty acids can be an important factor in helping to suppress inappropriate proinflammatory reactions, for two basic reasons. First, they are commonly known to compete with AA for incorporation into membrane phospholipids, which leads to a reduced production of PGE_2 and LTB_4 and an enhanced production of the (~1000-fold) less inflammatory 3-series PGs (PGE_3, PGA_3, and PGD_3), TXA_3/TXB_3, and LTB_5. More importantly, they are essential precursors for the D- and E-series resolvins and for protectin. From a clinical standpoint, in a variety of epidemiology-based studies consumption of these ω-3 fatty acids from marine sources appears to be strongly associated with lowered markers of inflammation (predominantly CRP) as well as a lowered risk for a variety of chronic diseases. While there is certainly sufficient biological evidence for the mechanisms of their resolving and anti-inflammatory functions; neither one, however, is an essential fatty acid.

As a nutrition issue, the essential fatty acids are linoleic acid (LA) (ω-6) with AIs of 12 and 17 g/day for adult women and men, respectively, and α-linolenic acid (ALA) (ω-3) with AIs of 1.1 and 1.6 g/day for adult women and men, respectively.[343] Of these fatty acids, LA is synthesized into AA (also ω-6) and ALA is synthesized into EPA and DHA. Although the AI standards are designed (only) to prevent an essential fatty acid deficiency and, therefore, are properly considered to be adequate and not excessive, there has been a lot of recent attention on the idea of reducing the AI recommendations for LA intake and increasing the recommendations for ALA intake (to reduce inflammatory and enhance anti-inflammatory effects by reducing AA and increasing EPA and DHA levels in vivo). For such a recommendation to actually be beneficial, a simple change in substrate supply (reducing LA and increasing ALA) for AA, EPA, and DHA synthesis would need to have a greater ultimate effect on inflammatory signaling than the combined effects of all the regulatory mechanisms that govern the synthesis of AA, EPA, and DHA, and the synthesis of lipid mediators, as well as those of the regulatory mechanisms that are altered through their (activated) receptors. From an enzymology standpoint, this is simply not logical.

In a series of meta-analyses of human clinical trials where LA consumption was manipulated to determine the effect on various risks for coronary artery disease (CAD), no enhanced inflammatory functions were observed; in fact, higher LA intakes were actually associated with an anti-inflammatory effect (lower CRP), along with a lowered LDL cholesterol and a higher HDL to LDL ratio.[344,345] The observed anti-inflammatory effects of higher LA ingestion are consistent with the anti-inflammatory effects of lipoxins, which, of course, are made from AA. The implication is that greater availability of AA for lipoxin synthesis due to greater intake of LA is important. In reality, however, AA synthesis is very tightly regulated and large changes in LA intake above AI amounts do not substantially change AA content of platelets and erythrocytes.[346] Although this does not rule out the possibility that small changes in the AA content of cells can be beneficial, it is more likely that LA-enhanced diets alter biomarkers of inflammation through mechanisms that are independent of AA-based signaling. Because of an apparent anti-inflammatory effect of additional dietary LA, and the fact that the effect is more than likely due to effects other than those following eicosanoid signaling, it would be difficult to justify reducing the AI recommendations for LA on the grounds that lower AA will reduce inflammatory signaling, per se.

The nutrient-based story for ALA is a bit less clear but centers around a similar concept. Conversion rates of ALA to EPA are low with reported efficiencies anywhere from less than 1% to as high as 8% in men and from approximately 8% to as high as 21% in women, with the conversion rate observed in women consistently being 2.5× that of men, whereas conversion to DHA is much less than 1% for both.[347,348] Because of the inefficient conversion to EPA and DHA, as well as the fact that ALA is a small minority fatty acid in most commonly available vegetable oils (<2% for all, except for ~50% in flax oil) and there is 9–10 g ALA/100 g of walnuts or butternuts[349] (the two major ALA-containing non-oil foods), an awful lot of calories from oil or oily nuts would need to be consumed just to get the biological availability of a single gram of EPA (and of far less DHA); this is certainly not a prudent concept if obesity (or nutritional status) is any consideration. Assuming an average conversion of ALA to EPA of around 4% and 10% for men and women, respectively, about 25 g of ALA for men or 10 g of ALA for women would need to be consumed to produce a 1 g EPA benefit. Of course, substituting ALA-rich foods for ALA-poor foods to boost intake without increasing calories is possible. It takes about 50 g of walnuts (along with 300–350 kcal) to get 5 g of ALA, and a tablespoon of flax oil (only ~130 kcal) provides about 7 g of ALA; the taste, however, is, well, an acquired one and not really practical (or available) for everyone. These diet/calorie issues compare to 45 or 90 kcal from a 5 or 10 g ALA supplement, probably a major reason why most clinical trials use supplements and not whole foods. Even combining supplements with dietary changes to produce a substantial increase in ALA intake still requires a moderately large dietary modification as well as reliance on single food sources, not necessarily a good dietary approach. For example, if half the dietary intake of lipid comes from walnuts and the diet is supplemented with walnut oil and flax oil, an increase in daily ALA intake of approximately 10 g will result.[350] Such dietary changes to produce a potential 1 g EPA benefit are not very practical.

In addition to basic dietary considerations of practicality, the biological effects of ALA supplementation also need to be considered. EPA is preferentially incorporated into the membrane phospholipids of circulatory cells (platelets, neutrophils, and monocytes, certainly where you want them to be) and in clinical trials using ALA supplements (doses ranged from a minimum of ~5 g/day to as high as 26 g/day); EPA content was either unaltered or increased up to more than 300%, with inconsistent increases even at intakes at or slightly above 10 g/day[44,344,347,348,351,352] and with little to no effect on DHA content. Thus, substantial increases in ALA intakes to a 10+ g/day range for what might be a negligible increase in EPA content of platelets, neutrophils, and monocytes and an inconsistently positive effect on risk for disease[44,46] are simply not practical, nor are they recommended. This does not mean, however, that high-ALA foods should not be recommended as a component of a healthy diet; in fact, this would be prudent simply because only about 70% of adults in the United States meet the 1.2–1.7 g/day recommendation. In addition, simply switching from low-ALA oils to higher ALA oils for cooking is associated with a reduction in risk for CAD.[353] Whether the seemingly beneficial effect of increased ALA intake in such studies is related to an increase in EPA/DHA synthesis is impossible to tell. With inconsistent changes in cellular EPA/DHA at high levels of ALA supplementation and little to no indication that any change results in faster resolution, it is doubtful. However, it certainly is possible that the switch is simply correcting an underlying ALA insufficiency. Thus, there is little justification for increasing the nutritional recommendation for ALA.

Fish oils that contain preformed EPA and DHA are the only realistic choice for a diet-based approach to modify EPA and DHA availability in order to avoid large-scale dietary changes and efficiently correct a possible insufficiency in ALA intake. The basic questions become the following: how much EPA/DHA intake is necessary to have a beneficial effect? Further, what is the minimal amount of fish to eat in order to get an effective dose? Of the studies reviewed,[37–59] an intake in the range of 0.25–3 g/day of EPA reduces the risk for a variety of chronic diseases (especially CAD), whereas intakes in the higher ranges (2–4 g/day) reduce markers of inflammation and platelet adhesiveness, with the majority of the protective effect against cardiac deaths occurring in the lowest range (250–500 mg) of additional EPA intake. As might be expected, the studies that indicate a reduction in risk at the lowest intakes already have very low initial EPA/DHA (and ALA) consumption levels and along with that a high risk for CAD death. Based on many of these studies, the American Heart Association (AHA) recommends either an intake from supplements of approximately 1 g/day of a mix of both EPA and EPA or a weekly consumption of at least two servings of fish. Two 6 oz. servings of fish (salmon, ~1.7–4.5 g EPA/DHA/serving; herring, ~1.7 g EPA/DHA/serving) each week will provide a sufficient intake of these ω-3s to average well within the lowest beneficial range (with very little risk of exposure to mercury, polychlorinated biphenyls, and other toxic contaminants).[354] This is a practical recommendation because it will provide the greatest risk reduction benefit with the least drastic change in diet. The AHA's recommendation for 1 g/day when using supplements is most likely because this is the dose in the most commonly available fish oil or EPA/DHA supplements and (similar to fish consumption) most of the risk reduction for cardiac death also occurs at this lower level of EPA/DHA

supplementation. Because the anti-inflammatory effects in the supplementation studies are observed at EPA intakes in the 3 to 4 g range, although the greatest increase in protection is achieved at intakes of 1 g or lower, it might appear that the majority of the benefits of EPA are most likely achieved through mechanisms that are not related to EPA-based lipid mediators and inflammatory signaling per se. This might be, however, because the type of data collected in many of the studies is highly limiting. Using CRP as a marker for inflammation is limiting because it is synthesized by the liver in response to IL-6 and not because of a local production of extremely short-lived resolvins and protectins from EPA elsewhere in the body. A lack of a change in a serum marker for inflammation does not necessarily indicate a lack of a local anti-inflammatory effect in areas of low-grade inflammation. In addition, EPA (and DHA) is known to be a PPARγ agonist[355–357] and PPAR activation results in the inhibition of p38–MAPK, ERK–MAPK, and NFκβ-mediated proinflammatory signaling. Again, from a CAD standpoint (a very small and highly localized area of low-grade inflammatory response) it is doubtful that a systemic effect could be produced by a local activation of PPARs, but a local anti-inflammatory response would certainly occur. Whether the cardioprotective effects of low-dose EPA are due to PPAR-mediated effects, resolvin-mediated effects, correcting an underlying EPA/ALA insufficiency, or all three are unclear, but the protective effects do appear to be consistent.

For all the aforementioned reasons, the dietary recommendations specifically related to LA, ALA, EPA, and DHA are to include two servings of high-EPA/DHA fish each week and to eat at least one serving of a high-ALA food each day to ensure adequate intake of ALA, EPA, and DHA. There is little justification to be concerned about LA intakes simply because these are already relatively high in the American population and consuming even higher amounts are not proinflammatory. When this recommendation is considered in concert with the one recommending a proper diet that includes RDA/AI amounts of all other appropriate nutrients, the variety of cellular control mechanisms that depend on an adequate supply of nutrients should ensure that the signaling processes initiated by the lipid mediators will function optimally.

2.5.2.2 Proinflammatory Signal Transduction

The ability to ensure appropriate control of an inflammatory response also is very important. Whereas activation of the NFκβ-, JNK–MAPK-, and p38–MAPK-associated signal transduction pathways is the key to inducing synthesis of the proinflammatory signals, suppressing these is central to anti-inflammatory and proresolution functions. Ideally, an appropriate array of cellular responses to the presence of pathogens, oxidizing agents, and cellular damage lead to an inflammatory response. This is then followed by a series of resolution events when the pathogens and/or damaging agents are removed. For the most part, this ordered process works very well, especially in the case of infectious agents. However, in the face of constant cellular trauma, especially that resulting from unavoidable environmental or endogenously produced toxic molecules, or constant physical trauma, an ordered resolution of inflammation is not going to happen. This is simply because all of the initiating DAMPs and other cellular stresses are continually present, resulting in a continuous

production of proinflammatory signals, leading to continuous low-level inflammation. A solution to the dilemma that unavoidable chronic trauma leads to unavoidable inflammation-associated responses is to dampen the signaling processes that lead to proinflammatory signaling.

These desired effects can be accomplished by eating foods that contain compounds that can dampen the NFκβ, JNK–MAPK, and p38–MAPK signaling processes. This will produce a reduction in proinflammatory signaling in response to a given (chronic) initiating event. This reduction in signaling would, at the same time, be additive to any resolving mechanisms that are occurring (resulting from the chronic induction of COX-2 and mPGES1) because resolution effects are mediated through the same mechanisms. A large variety of phenolic compounds are capable of doing just that, and it may be no surprise that these compounds are found predominantly in fruits, vegetables, whole grains, teas, wine, and many other plant-based foods.

Common food sources[358] that contain phenolic compounds (total phenolics listed high to low) are listed in Table 2.1.

From perusing the listed sources of phenolics, it may be no coincidence that the spices and foods in the high-end range for content of phenolic compounds are more common in the Mediterranean diet, whereas those in the low end of content are more common in the American diet. The reference to the Mediterranean diet, of course, is because of the well-known reduction in incidence of chronic diseases in cultures that follow the traditional diet compared to the westernized American diet.[67,359] A seemingly similar dichotomy of choice is apparent with respect to alcoholic beverages, with wines (reds, ~200 mg/100 mL; rosés, ~80 mg/100 mL; white, ~30 mg/100 mL) being more commonly consumed with the Mediterranean diet and beers (~12–25 mg/100 mL) being more commonly consumed in the United States. Very large variations in the consumption of specific phenolic-rich foods between individuals and large variations in the content of phenolics in individual foods (depending on season, storage, and preparation) result in large variations in intake between individuals in the consumption of individual phenolics, varying by more than 100-fold. On the other hand, because phenolics are common to so many foods the average total consumption of phenolics is in the 0.5–2 g/day range with about 66% coming from flavonoids and stilbenoids and about 33% from phenolic acids. Most of the variability between individuals depends on the differences in consumption of flavonoids in vegetables, cooking oils, and fruits (including juices) and of phenolic acids in coffee, tea, and chocolate,[360,361] with the traditional Mediterranean diet being on the high end (>1.3 g/day) and the American experience tending to be on the lower end (<0.5 g/day) of phenolic consumption.[362,363]

Although many of the phenolic compounds found in the aforementioned foods have antioxidant properties, it is far more likely that their preventive properties are due to their ability to modify components of signal transduction pathways. As an antioxidant only, they would be susceptible to the same mechanistic limitations in prevention that other antioxidant supplements might have, as suggested at the beginning of Section 2.5.1 and by others,[361] that is, overwhelming a cell with antioxidants interferes with the regulation of cellular redox states at the microenvironment level. In addition, because of a bioavailability of 2%–20% and a half-life of anywhere

TABLE 2.1
Phenolic Content of Some Common Foods

	Herbs—Spices
>5000 mg/100 g	Cloves, Ceylon cinnamon, dried pot marjoram, dried spearmint, dried wild marjoram oregano
>3000 mg/100 g	Dried summer savory, dried sweet basil, dried sweet bay, dried marjoram, capers
>1000 mg/100 g	Dried common sage, caraway, dried rosemary, dried coriander, dried turmeric, dried cumin, nutmeg, dried winter savory, dried common thyme, star anise, dried parsley, dried dill, curry, black pepper
	Dried Fruits
~1000 mg/100 g	Prunes, raisins, figs
	Fruits
>500 mg/100 g	Black elderberry, black chokeberry, skunk currant, black raspberry, black currant, Canada blueberry, gooseberry
>200 mg/100 g	Plum, lowbush blueberry, orange, American cranberry, sour cherry, strawberry, peach
>50 mg/100 g	Grapes, apple, kiwi, banana, pineapple, mango, pear, star fruit, guava
	Nuts
>1000 mg/100 g	Chestnut, pecan, walnut, pistachio
>200 mg/100 g	Hazelnut, almond, Brazil nut, cashew
>50 mg/100 g	Macadamia nut, dehulled almond, dehulled peanut
	Beans
>500 mg/100 g	Raw whole adzuki bean, raw whole lentils, raw dehulled black bean, raw whole bean, raw whole broad bean
10–150 mg/100 g	Raw whole white bean, raw whole dried pea, raw whole climbing bean, raw whole lima bean
	Vegetables
>500 mg/100 g	Swiss chard
>200 mg/100 g	Raw dandelion, red cabbage, raw green bean, chili pepper, spinach, sweet pepper, raw Brussels sprouts, broccoli, cauliflower
<100 mg/100 g	Raw green cabbage, sauerkraut, tomato, squash, zucchini, lettuce, onion, carrot, asparagus, sweet potato, potato, celery
	Oils
~500 mg/100 g	Peanut oil
~20 mg/100 g	Virgin/refined olive oil
~20 mg/100 g	Canola/rape
~2 mg/100 g	Sesame oil
	Grains
700+ mg/100 g	Whole-wheat buckwheat flour, wheat germ
~180 mg/100 g	Whole-grain hard wheat, corn flour
~70–100 mg/100 g	Whole-grain common flour, oat flour, corn flour, rice flour

from 1 to 2 hours[360,361] for most flavonoids, antioxidant effects per se are likely to be minimal at best and relatively transient in nature. For example, curcumin (from turmeric), a more effective antioxidant than either resveratrol or quercetin, can function as an antioxidant in serum at concentrations of 1–50 μM in vivo, a level that requires the consumption of more than 8 g/day to reach the 1+ μM level.[364] With a maximum observed dietary consumption of curcumin of approximately 1.5 g/day in some Asian communities[365] (higher than the dietary consumption of resveratrol or quercetin), it is doubtful that the antioxidant properties of curcumin (or other single phenolics) significantly contribute to a diet-based protective effect. This does not rule out the possibility that high-dose supplements might achieve a sufficient concentration in vivo to have antioxidant properties but does make it unlikely that the epidemiological associations between dietary consumption of phenolics and prevention are due specifically to their antioxidant effects.

As modifiers of specific components of signal transduction pathways, however, the transient effects of dietary phenolics in vivo should be a benefit. This would certainly avoid constant alterations of regulatory pathways that might prove to be highly problematic, as indicated by the high risk for cancers when mutations render various components of the MAPK pathways constitutively active. In addition, because the molecular effects of most phenolics are targeted to a very narrow range of proteins, they are unlikely to have global detrimental effects on cell function. Large doses of individual phenolics from supplements do not have such a favorable metabolic profile and if present in large quantities for a prolonged time adverse effects might occur, such as the hepatotoxicity and altered reproductive function described in case studies in which individuals consumed supplements containing polyphenol-enriched tea and soy isoflavones.[361]

In terms of diet-based risk reduction, it is also likely that phenolics do not necessarily work in isolation; rather, it is the additive and synergistic effects of different phenolics that may account for their apparent overall benefit. From the results of clinical trials, somewhat inconsistent positive effects have been observed for single phytochemicals and for phenolic extracts from single sources or single foods. Using 0.5–9 g/day of green tea extracts in a supplement produced no change in CRP or IL-6 in diabetic patients, an effect (or rather, lack thereof) also seen with 166 mg quercetin plus 133 mg vitamin C in arthritis patients.[366–368] Similar negative results have been observed with drinking up to 900 mL/day of black tea and green tea, 800 mg of pomegranate extract, or diets rich in berries and apples.[369–372] On the other hand, anthocyanin from blueberries (300 mg/day; equivalent to ~1 cup or ~150 g blueberries), 280 g cherries/day (equivalent to ~1.5 cups; also high in anthocyanin), grape seed extract (600 mg/day), and concentrated grape juice supplements (100 mL; 640 mg total polyphenols) have been observed to reduce CRP and/or MCP-1 in serum.[373–376] Obviously, in some instances the differing results could be due to the differences in the total doses used. In other cases, they might be due to the different arrays of phenolics supplied with the different foods or supplements, or simply due to the fact that some studies used nondiseased normal subjects while many others have used diabetics, cancer patients, HIV-infected individuals, or other *medically challenged* subjects, each with their own possible differences. Overall, it is difficult to determine from clinical studies whether there is a consistent anti-inflammatory

effect from any individual phenolic or phenolic mixture–based supplement simply because there have not been multiple trials performed with all likely phenolics in large heterogeneous groups of subjects (and, because there are thousands of different phenolic compounds, this would actually be impossible). Based on somewhat limited evidence, however, some whole food–based phenolic mixtures or extracts from whole foods, such as grape, blueberry, and cherry sources, do appear to have a beneficial anti-inflammatory effect at concentrations that can be achieved from dietary consumption of these fruits.

Translating this information directly into a dietary recommendation, however, could be awkward. In spite of the apparent success of some supplements or single food sources of phenolics, mixes of different phenolics from several food sources are more likely to be relevant for a preventive diet. This is simply because reasonably large servings of single foods have been found necessary to produce a beneficial effect in clinical trials, and it is doubtful that such servings of the same foods are an everyday occurrence for very large numbers of people in the general population. With a 1.5–2 cup recommendation for fruit consumption (teenagers through adults; U.S. Department of Agriculture), consuming 1.5 cups of cherries each day to get a *clinical dose* of anthocyanins would mean the entire day's fruit recommendation would come from a single fruit, a concept that is not consistent with current recommendations for consuming a variety of fruits and vegetables in order to meet requirements for all nutrients. It is also much more likely that the observed benefits of phenolics in epidemiological studies are due to the additive and possibly synergistic effects that many different phenolics coming from many different foods can have on cell function, specifically signal transduction pathways.

Because of the technical nature of the methodologies, research on the effects of various phenolics on signal transduction pathways is pretty much limited to in vitro or ex vivo studies of human or mammalian cells. Some of the more commonly known phenolics that have been studied extensively for their ability to modify signal transduction pathways include *flavonoids* (food sources listed from high to low): quercetin (black elderberry, dark chocolate, oregano, and capers and some in vinegar, tomatoes, shallots, and red onions), apigenin (extra virgin olive oil [EVOO], Welsh onion, Italian oregano, and pistachios), epigallocatechin-3-gallate (EGCG) (green tea, oolong tea, black tea, pecan, hazelnut, pistachio, and banana), theaflavin (black tea), genistein (soy products), anthocyanins (blueberries, strawberries, cherries, and common black beans), and kaempferol (capers, cloves, black tea, broccoli, apples, cherry tomatoes, and onions); *phenolic acids*: ellagic acid (chestnut, Japanese walnut, walnut, blackberry, black raspberry, and pomegranate juice), capsaicin (hot peppers, Hungarian peppers, and sweet peppers), curcumin (turmeric and curry powder), and caffeic acids (coffee); and *stilbenoids*: resveratrol (red wine, red/black grapes, lingonberry, European cranberry, vinegar, and roasted peanuts); among many others.[359,364,377–388]

In both cell culture and animal models, various inflammatory-response proteins have been reported to be affected by treatment with phenolic compounds. The synthesis of a variety of proinflammatory signaling molecules, including IL-6, IL-1β, TNF-α, and IFN, has been observed to be suppressed by quercetin, apigenin, genistein, green tea polyphenols, luteolin, anthocyanins, and curcumin. In response to a reduction in synthesis of these signaling molecules, a reduction in expression of

iNOS, COX-2, ICAM, and MCP-1 would be expected. Of course, these changes have also been observed with the same phenolics.[364,377,380,384,386] Thus, phenolics that are found in many common consumed foods are capable of suppressing inflammatory signaling, a suppression of signaling that can be mediated at several different points along an activation → signal transduction → expression pathway.

From the standpoint of initiating synthesis of inflammatory cytokines, it is the activation of PRRs by PAMPs and DAMPs that initiates the process, and expression is then mediated through the activation of the NFκβ and MAPK signaling pathways. Recent experiments have revealed that activation of TLR4 can be suppressed by curcumin and sulforaphane (cruciferous vegetables) and NOD1/2 activation can be inhibited by curcumin. In addition, resveratrol, EGCG, luteolin, and quercetin all directly inhibit TBK1 (necessary for IFN synthesis), whereas resveratrol inhibits TRIF to suppress both the MyD88 and the non-MyD88 pathways of TLR signaling, resulting in a suppression of both NFκβ- and MAPK-mediated responses.[380]

Activation of NFκβ signaling also can be suppressed through a variety of mechanisms including inhibition of IKK activity, which then suppresses Iκβ release and its subsequent degradation; this prevents free NFκβ from binding in the nucleus. EGCG from green tea and theaflavins from black tea inhibit NFκβ signaling through this mechanism, whereas quercetin, curcumin, kaempferol, and lycopene can inhibit NFκβ signaling by suppressing nuclear translocation of p50/p65. Suppression of NFκβ signaling has also been observed with apigenin, morin (strawberries), anthocyanins, and procyanidins (peaches, plums, apples, and nectarines) through a variety of related mechanisms, including suppression of phosphorylation of p65 and of DNA binding of NFκβ, although in many studies the specific molecular mechanism of inhibition is not always clear.[364,377,384,386] Regardless of the exact mechanism, subsequent synthesis of IL-1, IL-6, TNF-α, and IFN is reduced.

Because MAPK pathways are activated via PRRs and are integral to activating the expression of proinflammatory cytokines, these pathways are prime targets for suppressing inflammatory signaling by phenolics as well. Apigenin, luteolin, quercetin, resveratrol, EGCG, and kaempferol can suppress ERK1/2–MAPK, JNK1/2–MAPK, and p38–MAPK pathways. Cyanidins can suppress ERK–MAPK activation, and EGCG has been observed to inhibit the activation of ERK–MAPK as well. In addition to suppressing the different MAPK pathways, curcumin has also been observed to inhibit PKC, a calcium-activated enzyme that can activate various components of the MAPK (and other) pathways.[364,377,384,386] From these observed effects, phenolic compounds exert their suppression activities through a variety of different mechanisms that suppress the activation of MAPK and NFκβ pathways. Although not detailed here, different cell types used in different studies tend to yield differing results. Some cell types, such as respiratory epithelial cells, exhibit the suppression of all MAPK pathways as well as NFκβ pathways by single phenolics. In activated macrophages, only one or two pathways are inhibited by the same phenolics, with similar variations in response observed for a variety of different phenolics.[364,376,377,384,386] The differences in effect indicate that different cells have different susceptibilities to inhibition by different phenolics, and because of this it is doubtful that single phenolic supplements will have the desired overall preventive effects. In addition, large doses of single or single-source phenolics can produce a prolonged

suppression of signaling or even a toxic effect; hence, a dietary recommendation designed to produce a suppression of proinflammatory signaling should include a variety of fruit and vegetable sources.

The suppressive effects on proinflammatory signaling by phenolics are not limited to NFκβ and the various MAPK pathways; a variety of phenolics also appear to affect the production of lipid mediators. EGCG, quercetin, and kaempferol have been observed to inhibit COX-1 and COX-2 and LOX with subsequent decreases in PGE_2 production in a variety of cell culture studies. Phenolics from EVOO also have the same properties. Tyrosol, hydroxytyrosol, apigenin, luteolin, and oleuropein compounds are found in EVOO, and all have been documented to inhibit the production of PGE_2, LTB_4, and TXA_2.[377,389,390] While luteolin and apigenin reduce the production of proinflammatory PGs, TXs, and LTs by suppressing the synthesis of COX-2 (by suppressing NFκβ and MAPKs, as discussed earlier), the other phenolic compounds suppress signaling by directly inhibiting COX or LOX. Regardless of the mechanism, however, in many clinical trials using virgin olive oil and EVOO in the 25–50 g/day range (certainly a dietary possibility if olive oil is the only oil used in cooking), olive oil is documented to have consistent anti-inflammatory effects. Further, the extra virgin version appears to have these beneficial effects at a lower range of intake, which is more than likely because of the much higher content of these phenolics in EVOO.[377,391,392] Then, from a dietary perspective recommending the consumption of approximately 25 g of extra virgin oil to get an array of anti-inflammatory phenolics when the caloric cost is approximately 225 kcal (or about 10% of total caloric intake) is neither harmful nor impractical. Such a recommendation also happens to be well within acceptable dietary guidelines.

In addition to the anti-inflammatory benefits obtained through the suppression of signaling discussed earlier, an increased expression of various antioxidant and redox-control enzymes would also be highly beneficial. Increasing synthesis of SOD, CAT, GPX, and GSR would be relevant for increasing protection from additional ROSs/RNSs, whereas enhanced synthesis of thioredoxin and peroxiredoxin would enhance the ability to maintain appropriate regulatory control of redox status.

The role of SOD is to catalyze the reaction $O_2^{\bullet-} + O_2^{\bullet-} \rightarrow H_2O_2 + O_2$ while CAT catalyzes the reaction $H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$ at rates far faster than the spontaneous dismutation rates that can occur in vivo. GSR and GPX work in tandem with GSH to eliminate H_2O_2 through the GPX-catalyzed reaction $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$. The GSR then reduces the GSSG back to 2 GSH using NADPH as the electron donor. The benefit of these enzyme-catalyzed reactions is that they minimize the concentrations of both $O_2^{\bullet-}$ and H_2O_2, which in turn minimizes the production of the highly reactive •OH. Of the eight known forms of GPX, GPX1 in the cytosol of mammalian cells reduces free H_2O_2, whereas GPX4 associates with cellular membranes and reduces lipid hydroperoxides. In concert, these antioxidant systems ultimately reduce damage to proteins, lipids, and DNA caused by ROSs.[278,280] Of the three isozymes of SOD, the cytosolic copper–zinc form of SOD (CuZn SOD—*SOD*: cytosol, nucleus, and mitochondrial membrane) and the extracellular copper–zinc form (EC SOD—*SOD*) appear to be less inducible than the manganese form (Mn SOD—*SOD*: mitochondrial matrix), even though NFκβ response elements are found in the promoter region of all three sod genes. This is

most likely due to the fact that the sod2 promoter contains AP-1 as well as ARE binding elements.[393] An increase in SOD activities mediated through NFκβ is a logical response to proinflammatory signaling in order to protect cells from the inevitable increase in ROS production.

Maintaining the ability to regulate redox status at the microenvironment level is very important for ensuring proper control of signal transduction pathways. This occurs through the redox-sensing enzyme systems as well as through endogenous antioxidant enzyme activity. With this in mind, compounds that can enhance the synthesis of a variety of antioxidant enzymes through Nrf2 activation will protect cells against the damaging effects of additional inflammation-produced oxidants. Increasing antioxidant enzyme capacity should also reduce the ability of incoming oxidants to disturb redox-based regulation of signal transduction pathways. An induction of both thioredoxin and peroxiredoxin through Nrf2 activation will certainly contribute to ensuring proper regulatory control. The expression of CAT, GSR, thioredoxin, peroxiredoxin, HO-1, and glutamate cysteine ligase (GCL) (rate limiting for GSH synthesis) as well as other protective enzymes is also induced through Nrf2 activation.[383,394–396] In addition, a variety of endogenous regulatory factors are capable of activating Nrf2, including PGJ_2; PKC; MAPKs (ERK, p38, and JNK); PI3K/PKB; and even Ca^{2+} and ROSs through PKC- and redox-mediated regulation of MAPKs, respectively.[382,395] Thus, activating the expression of a variety of protective enzymes through the activation of Nrf2 is a more general response to cell stress than increasing only SOD through specific proinflammatory stimuli. It is through these stress-response genes that polyphenols can function to enhance protection from inflammation-produced ROSs as well as a variety of other damaging agents. The induction of enzymes such as GST, UDPGT, and NADPH:quinone oxidoreductase-1 is much more applicable to the prevention of cancer and will be discussed in Chapter 6, Sections 6.3.3.3 and 6.3.5.2.

A number of phenolics have been documented to activate Nrf2 binding to ARE and induce the expression of antioxidant and other protective proteins. Quercetin, resveratrol, diallyl sulfide, *s*-allyl cysteine, lycopene, EGCG, curcumin, and sulforaphane have all been documented to activate Nrf2 and to induce the synthesis of Nrf2/ARE-inducible proteins, including GST, GSH, HO-1, SOD, CAT, GCL, thioredoxin, peroxiredoxin, and UDPGT.[397–410]

The different phenolics appear to act at different points within the (albeit brief) Nrf2 signaling pathway. Nrf2 normally forms complexes with Keap1 in the cytosol, which not only prevents it from entering the nucleus but also may target it for degradation. Phosphorylation of Nrf2 by protein kinases can disassociate Nrf2 from the complex to allow it to migrate into the nucleus and bind to ARE transactivation binding sites.[383,411] Some phenolics activate protein kinases such as PKC, PKB, ERK–MAPK, JNK–MAPK, and p38–MAPK, which in turn can phosphorylate Nrf2, whereas others appear to covalently modify susceptible thiols on Keap1. Modification of susceptible thiols on Keap1 by phytochemicals reduces ubiquitination (and subsequent degradation) of Nrf2 while stabilizing the Keap1–Nrf2 complex; this allows newly synthesized Nrf2 to remain unbound to Keap1 and enter the nucleus. These results indicate that modification of Keap1 (see later) as well as activity of various components of the MAPK pathways can activate Nrf2 and lead to the expression of the array of protective

enzymes. They also raise the conundrum of how a variety of phenolics can suppress the activation of the MAPK pathways to produce an anti-inflammatory effect while others activate p38–MAPK, ERK–MAPK, and JNK–MAPK themselves to produce a protective effect. Because this has not been specifically addressed in the research literature, conjecture will have to suffice: inhibiting the ability of the pathways to be activated by DAMPs, PAMPs, and stress should dampen the proinflammatory response to reduce the risk of an inappropriately intense response regardless of the initiator. In spite of the fact that these dampening effects would be transient, there would be a general overall reduction in the proinflammatory response. On the other hand, transiently activating the specific MAPKs may be sufficient to activate the Nrf2–Keap1 complexes to disassociate and enhance binding to ARE without them being sufficiently active to initiate a stress-induced proinflammatory response. This, of course, remains to be determined.

One potential issue with many of these cell culture–based studies is that relatively large concentrations of phenolics are used (10–50 μM in most cases) in order to ensure that measureable differences can be seen after several hours or a day (or more) of treatment. This might make it difficult to extrapolate the data to humans because consumption of up to 10 g/day (or more) of the individual phenolics is necessary to produce such a concentration in vivo, an amount unlikely to be obtained from anything but the most extreme diet. They do reveal, however, that mixtures of phenolics can alter the activity of various signal transduction pathways to affect the expression of many different proteins.

The precise mechanism through which the phenolics work and at what step or steps of the signal transduction–expression pathway they have their effect are extremely difficult to determine from these endpoint studies. In many respects, it is simply impractical to measure the activation/oxidation state of every single kinase involved in a signal transduction pathway, as well as the binding of various relevant transcription factors to the promoter regions of the relevant genes, in addition to determining the expression of all the relevant proteins in a single experiment with one phenolic. Further, including all the complex interactions between the different pathways just elevates the impractical to the seemingly impossible (or absurd). Because phenolics happen to be metabolized fairly quickly, it is clear that their effects also will be relatively transient. Small, transient changes in activity of the different components of the signaling pathways are extremely difficult to measure. In spite of this, it is through studies that are designed to determine the time course of location-specific alterations in each of the pathways that the specific mechanisms through which the various phenolics produce their protective effects would be revealed.

Earlier in Section 2.5.2.2, it was stated that phenolics were unlikely to have an effect based on their antioxidant properties because of a requirement for micromolar concentrations of phenolics to act as antioxidant scavengers in serum. Most antioxidant function tests are based on the oxidation of target molecules in solution in vitro or in biological fluids and require moderately large concentrations of phenolics to measure the antioxidant effect. This is compounded by the fact that nutrient antioxidants are not only more effective antioxidants but also typically as much as two to three orders of magnitude greater in concentration in vivo (e.g., 50–100 μM for vitamin C) than the tested polyphenols; there simply needs to be a relatively

high concentration of phenolics in order to observe an antioxidant effect above background. This does not rule out small, transient antioxidant effects in vivo. It just means that they may be very difficult to measure; their relative contribution to total antioxidant function would still, however, be very low (less than 1% to 2%). Thus, the antioxidant abilities of phenolics may not be their most relevant feature.

Because $2O_2^{\bullet-}$ spontaneously dismutates extremely quickly into the more stable H_2O_2 and active SOD catalyzes the same reaction, H_2O_2 is the predominant nucleophilic biological oxidant. Antioxidant molecules (and antioxidant enzymes such as CAT) can, of course, reduce the peroxide to water (or rather water and oxygen). The ability of flavonoid phenolics to scavenge hydroxyl radicals, peroxyl radicals, and peroxynitrite radicals is predominantly conferred through the electron (and hydrogen)-donating capabilities of the hydroxyl groups on the B-ring.[412] However, because these properties are unlikely to provide the major protective function in vivo other properties must exist. With catechol-type polyphenols, it is far more likely that autoxidation of the hydroxyl groups occurs, promoting a rearrangement of electrons within the ring to produce a quinone, which then participates in conjugation reactions with susceptible thiols such as those on Keap1.[413,414] As already mentioned, a wide array of phenolics can covalently modify the susceptible thiols in Keap1, leading to reduced ubiquitination and degradation of Nrf2 and subsequent entry of Nrf2 into the nucleus to bind to ARE/ERE to produce its characteristic protective effects. In addition, the quinone structure can be easily reduced and H_2O_2 is produced during the redox resonance between the two states.[395,413] H_2O_2 is a potent and common oxidant for thiols, which leads to dissociation of the Keap1–Nrf2 complex and subsequent Nrf2–ARE binding in the nucleus. Thus, it is the prooxidant and autoxidizing character of dietary phenolics that gives them their protective effects and not their antioxidant properties. With easily oxidizable thiols in Prx and Trx one could imagine that H_2O_2 generated by phenolics can exert their effects there as well, creating small, localized changes in the redox state that transiently affect components of signal transduction pathways. The transient nature of these phenolic-mediated oxidation events produces the protection effects, effects that are mediated through essentially the same signaling pathways that when continuously activated lead to detrimental effects on cell function.

Before moving on to exercise-based prevention, this is a good place for a brief discussion on HO-1 mentioned earlier in this section. The constitutive enzyme HO-2 and the inducible enzyme HO-1 catalyze the rate-limiting step in heme degradation to produce free iron (which immediately stimulates ferritin synthesis), bilirubin, and carbon monoxide (CO), with the constitutive form being expressed almost universally (with much more in spleen, liver, and bone marrow) and the inducible HO-1 being at very low to undetectable levels.[415,416] HO is well known as being necessary for scavenging iron, so it can be recycled into new heme proteins (hemoglobin, myoglobin, respiratory chain proteins, and cytochrome P450 enzymes, among many others). As a component of a stress response, the HO-1 enzyme is highly inducible through Nfr2/ARE activation; inducing this enzyme would make sense considering the potential for oxidative damage to inactivate heme proteins (among many others) and produce a requirement for an additional capacity for scavenging iron. As a consequence of increased HO-1 activity CO production is enhanced, and this

molecule appears to be important in inflammatory responses. In cell culture models, macrophages with induced HO-1 release less TNF-α in response to LPS than noninduced cells, an effect that can be produced with externally supplied CO in noninduced macrophages. This indicates that CO is the suppressing molecule in this model. In similar models, HO-1 induction also inhibits the synthesis of IL-1, IL-6, ICAM, VCAM, and E-selectin.[416–420] In all these studies, CO appears to affect one or another component of the different MAPK pathways to decrease proinflammatory responses to inflammatory stimuli, in a manner reminiscent of the redox effects on these pathways; because CO does not bind to any of the proteins, it appears to affect the activity of the pathways through upstream events. In any event, as a component of a generalized stress response in cells, induction of HO-1 appears to have anti-inflammatory effects.

With these concepts in mind, the previous interpretation of prevention-based studies must still stand: it is the additive and synergistic effects from an array of phenolics that are more than likely responsible for their observed protective effects on inflammation and disease as illustrated in Figure 2.6. Any dietary recommendations should therefore take into account the phenolic content of many different foods and attempt to formulate a recommendation based on both the content of phenolics and their mechanisms of action.

2.6 ATTENUATION OF INFLAMMATORY PROCESSES THROUGH EXERCISE

An exercise-induced enhancement in the production of ROSs in skeletal muscle has been recognized for many years with the enhanced ROS production being thought to be the source of muscle damage.[421,422] Many athletes took to heart the message and started consuming antioxidants, hoping for a benefit; of course, the previously documented ill effects of supplementation on performance were largely unknown at the time. Most likely this was simply a result of the entrenched idea that radicals are bad because they cause damage and that the more recent recognition that ROSs are vital regulators of signal transduction pathways was not well recognized by scientists until the past decade. Much of the earlier data on inflammatory signaling focused on damage-associated inflammatory cytokines produced following downhill running, unaccustomed strenuous exercise, or exhaustive exercise (exercise models that tend to produce some degree of muscle damage and often result in delayed-onset muscle soreness). As might be expected, increases in proinflammatory cytokines, including IL-1β, IL-6, IL-8, TNF-α, CRP, as well as creatine kinase activity in serum (a common marker for muscle damage) and markers of ROSs, have been identified in serum under these conditions. In addition, there is a tendency for an increase in soluble TNF receptors as well as in soluble IL-1 receptor and soluble IL-1 receptor antagonist.[70,423–426] The enhanced synthesis of various cytokines in response to the highly stressful exercises simply reinforced the idea that it was ROS that caused the damage and that a proinflammatory response resulted from the damage.

With moderately strenuous concentric exercise, there is also a production of cytokines; however, the responses are not the same. Damaging or exhaustive exercise leads to elevated TNF-α and IL-1β that can last for 24 hours or more; with more

prolonged nonexhaustive exercise, the increase in IL-1 and TNF-α does not occur, whereas the increases in IL-6, IL-10, sIL-1ra, sIL-1r, and sTNFr are far more pronounced and transient in comparison to those of exhaustive exercise.[68,70,72,426,427] The anti-inflammatory effects of IL-6 are realized through the IL-6-mediated induction of sIL-1r, sIL-1ra, and IL-10. IL-10 suppresses NFκβ and MAPKs, increases SOCS signaling, and stimulates nonphlogistic phagocytosis. There is a large increase in soluble receptors for both IL-1 and TNF; they exert their anti-inflammatory effects by binding to circulating IL-1α, IL-1β, and TNF-α while sIL-1ra attenuates the ability of circulating IL-1α and IL-1β to bind to cellular receptors, thus suppressing proinflammatory cellular responses. A moderate-intensity cycling workload that can elevate IL-6 levels is approximately 50% of the maximum watts of power output, which is approximately a 6 MET level of intensity, a minimum level in which significant increases in IL-6 occur with 1 hour of exercise. It is also important to note that changes in IL-6 are not observed with exercise that utilizes small muscle groups, such as the upper arms, indicating that large muscle–group activities of a minimum intensity of 6 METs, sustained for approximately 1 hour, might be essential for the IL-6-mediated anti-inflammatory effects of exercise.[73,428,429]

As discussed previously, TNF-α activates the synthesis of IL-6 as part of a coordinated inflammatory response. A crucial difference with regard to exercise is that the production of IL-6 by muscles occurs without being activated by TNF-α. This means that the exponential (and transient) increase in IL-6 is independent of TNF-α signaling and illustrates that metabolic stress can activate signaling pathways for cytokine expression, just not for all of them. There clearly is a difference between the activation profile of cytokines due to metabolic stress and that due to activation through an elevation in DAMPs (as would be expected from damaging or exhaustive exercise) or PAMPs. This reinforces the concept that even though the various cytokines are synthesized in response to the activation of transcription factors via oxidant-mediated activation of the MAPK pathways and NFκβ pathways, the presence or absence of PAMPs and/or DAMPs makes a significant difference in the pattern of activation, resulting in different patterns of cytokine expression. In response to the highly elevated IL-6, the other anti-inflammatory molecules are subsequently expressed, with the degree of increase in expression of all anti-inflammatory molecules being dependent on the intensity and duration of exercise and the amount of muscle mass activated by the exercise. Thus, prolonged, moderately stressful exercise is anti-inflammatory while damage and pathogenic stress is proinflammatory, even though the same signaling pathways are involved.

Another issue relating to IL-6 release from skeletal muscle is the association between this cytokine and glycogen content of muscle and liver. IL-6 strongly activates glycogenolysis in liver, an effect that is greatly enhanced through increased IL-6 release from glycogen-depleted exercising muscles in comparison with nondepleted muscles.[73,425,430,431] Activation of glycogen breakdown and glucose release by the liver via muscle-derived IL-6 is a major reason why IL-6 is considered by some to be a *glucoregulatory* cytokine. The concept raises some interesting interpretations with respect to IL-6 release from muscle and how this might relate to exercise-based anti-inflammatory effects. For instance, if glycogen levels need to decline

to produce an IL-6 response, this will not happen during short-term low-intensity physical activity and especially not if the low-intensity activity is performed in a fed or an immediate postprandial state. Thus, recommendations for physical activity to be an active anti-inflammatory agent through induction of IL-6 must be for a sufficient duration and intensity to provoke an IL-6 response. Unfortunately, clinical evidence of a consistent reduction in inflammation signaling by exercise is in short supply.

In many studies relating to inflammatory risk for chronic disease, CRP has been used as the marker of choice because of its ease of measurement as well as its documented inverse association with risk for chronic disease. Because the potential use of exercise as an anti-inflammatory agent is a relatively recent development, only a moderate number of studies that delineate the forms of exercise that can reduce CRP have been conducted. In various trials, the results that have been obtained range from no association between exercise and CRP to significant reductions in CRP due to exercise.[69,432–440] In these studies, however, it is the longer duration exercise of at least 45 min/day at a frequency of 5 day/week or more that appears to produce a chronic reduction in CRP; in studies using 30-minute durations of exercise or resistance training, CRP was largely unchanged. These results are somewhat consistent with an earlier analysis of NHANES III data that indicated a dose effect on elevated CRP with odds ratios of 0.98, 0.85, and 0.53 for light, moderate, and vigorous exercise, respectively.[441] Interestingly, in some of the exercise-intervention studies in which there were consistent reductions in CRP, there was also a reduction in adiposity, not necessarily an unexpected result with the addition of 45–60 minutes of exercise on most days of the week. The weight-loss effects are consistent enough that it is difficult to disassociate the effects of weight loss per se from those of exercise on reducing CRP. With IL-6 synthesis by myocytes being related to intensity, duration, and glycogen content, it is possible that low glycogen levels that would be associated with weight loss may contribute to the exercise effect. Obviously, more studies are required to sort this out. It may even be possible that simply performing exercise in a fasted state as opposed to a postprandial state may produce an elevated IL-6 response at the same exercise load without actually being in a negative calorie balance; this is sort of like being able to have your cake and eat it too.

In addition to the anti-inflammatory effects of exercise through mediation by IL-6, an increase in antioxidant enzyme activities is also relevant from a prevention standpoint. Today, it is well recognized in the research literature that the transient alterations in free radical production in skeletal muscle as a result of the metabolic stress of exercise are responsible for the anti-inflammatory response as well as the hypertrophy and mitochondrial biogenesis adaptations observed with different types of repeated exercise.[296,326,422,442,443] H_2O_2, the major oxidant species produced that can affect susceptible thiols, transiently activates the different susceptible components of the MAPK pathways, various protein kinases, as well as Nrf2 to produce large, transient increases in antioxidant, phase II, and redox enzymes. As discussed in Section 2.5.2.1, an increase in antioxidant enzymes can be an important factor in ensuring appropriate regulatory control over the

signaling pathways. Further, in terms of prevention of chronic diseases in general, the induction of antioxidant, phase II, and redox enzymes occurs not only in the exercised muscles but also in other tissues, including the heart, liver, lung, and brain.[296,444–449] As with the cytoprotective effect of polyphenols, these adaptations to exercise also appear to be due to oxidant-mediated activation of Keap1 with subsequent nuclear binding of Nrf2.[450–454] Thus, sufficiently stressful exercise provokes a global anti-inflammatory and preventive effect throughout most tissues of the body.

However, these protective effects of exercise must be viewed as a relatively short-term effect. What this means is as follows: any alterations in signaling due to exercise are destined to be transient simply because of the nature of exercise; you just cannot do it for 20 hours each day. Because the levels of ROSs in the muscles would be expected to return close to baseline within a few minutes, the activation effects will last only as long as the actual exercise. With a half-life of only 90 minutes for IL-6, the cytokine profile in response to nondamaging/nonexhaustive exercise will decrease back to baseline within a few hours after the stop of exercise, as is observed. The cytoprotective effects of the antioxidant/regulatory enzymes also cannot be expected to last beyond the life of the proteins. With an approximate half-life (the time for half of newly synthesized proteins to be degraded) of approximately 10 minutes, approximately 5 hours, approximately 30 hours, 24–48 hours, and approximately 20 hours for CuZn SOD, Mn SOD, CAT, GPX, and thioredoxin reductase, respectively,[455–458] the protective effects of enzyme induction do not last very long either. Thus, the exercise must be repeated on a daily or near daily basis if these cytoprotective and anti-inflammatory effects are to be realized on a long-term basis.

One last aspect of exercise that is just gaining a lot more interest is, well, the lack of it. Inactivity has been recognized for its strong relationship to increased risk for chronic disease for well over a decade.[459–465] Only recently, however, has an understanding of how inactivity results in increased risk for disease been developed. As discussed earlier, the transient increase in oxidants resulting from exercise induces a global protective response. In contrast, physical inactivity produces a chronic low-level increase in radical production that leads to muscle damage, atrophy, and insulin resistance in the inactive muscles.[466–469] From the latest 2005–2008 NHANES analyses, 33% of Americans report that they do not participate in physical activity at all and of the 76% who claim to be active approximately 50% do not meet the minimum activity guidelines.[470–475] Thus, the majority of Americans may be under chronic metabolic stress due to inactivity (in addition to nutrient insufficiencies). Possible reasons for such a large increase in risk for chronic disease due to inactivity could include the insulin resistance that accompanies inactivity. In addition, as observed in some of the studies, an increase in abdominal fat at the expense of subcutaneous fat occurs with inactivity. As is discussed in more detail in Chapters 3 and 5, abdominal adipose tissue is a significant source of proinflammatory cytokines, which are a profound risk factor for many chronic diseases. Thus, sufficiently stressful exercise provokes a global anti-inflammatory and preventive effect throughout most tissues of the body as illustrated in Figure 2.6.

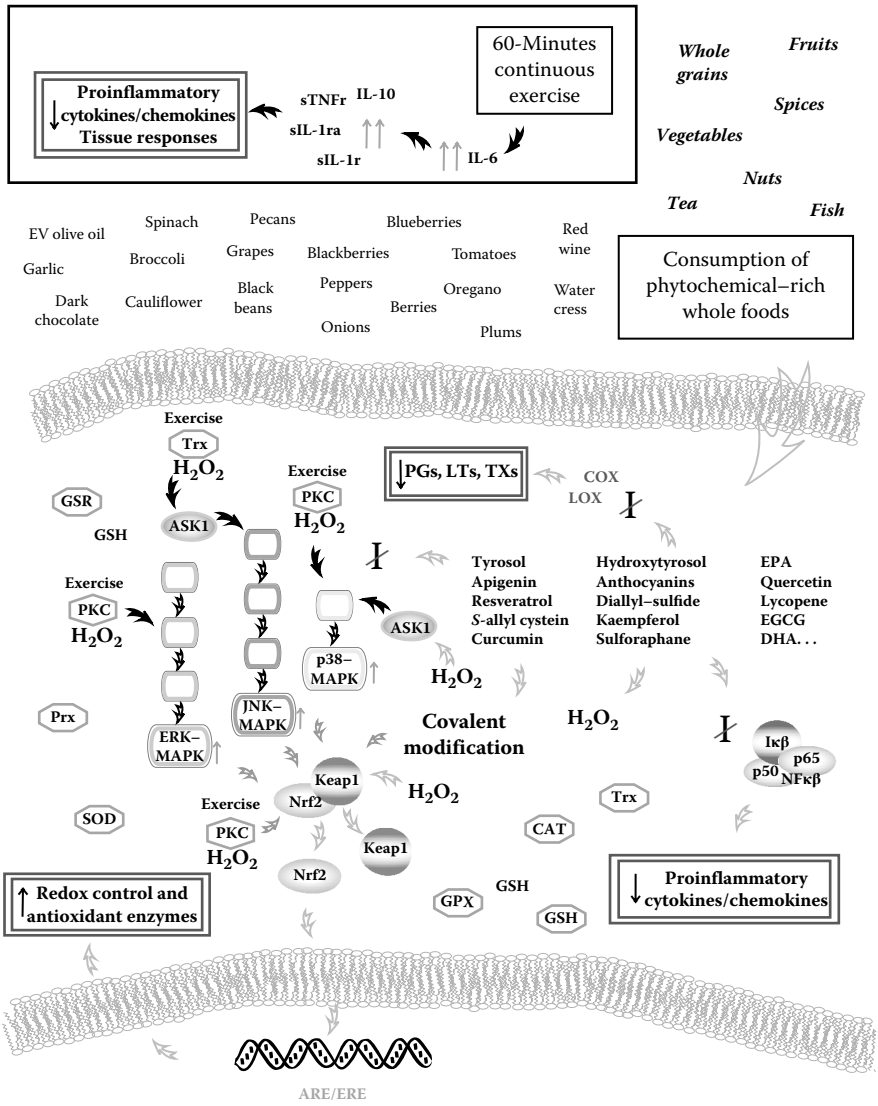

FIGURE 2.6 Attenuation of inflammation by exercise and phytochemicals: transient production of oxidants through exercise in muscle cells activates mitogen-activated protein kinase (MAPK) pathways sufficiently to lead to the synthesis of large amounts of interleukin (IL)-6, which produces a systemic reduction in the synthesis of proinflammatory cytokines and chemokines as well as in cellular responses to these. Various phytochemicals moderately inhibit the activation of MAPK pathways as well as activate nuclear factor κ-β (NFκβ) to dampen the activation of proinflammatory responses, whereas the transient production of oxidants from phytochemicals activates Nrf2, which, in addition to the covalent reactions of phytochemicals with Nrf2/Keap1, leads to the activation of antioxidant-response element (ARE)-/electrophile-response element (ERE)-mediated promotion events that increase the synthesis of a variety of redox-control and antioxidant enzymes to enhance redox control and further dampen proinflammatory responses.

2.7 SUMMARY AND RECOMMENDATIONS

2.7.1 Inflammation

In the traditional inflammatory model, inflammation in a component of the immune system occurs as a result of pathogenic infections that produce a variety of intracellular and extracellular stimuli, which are capable of activating a proinflammatory signaling response. A sustained low-level activation by these stimuli of the various MAPK, NFκβ, and IFN pathways mediates the activation of a wide array of transcription factors and the subsequent synthesis of proinflammatory cytokines. These pathways are activated via a variety of TLRs, NLRs, and RLRs through the binding of these receptors with a large array of PAMPs and DAMPs. Over the last decades, it has become increasingly apparent that in addition to pathogenic stimuli increased cellular levels of calcium and ROS production resulting from either sterile damage or metabolic stress will also directly activate these same pathways through a variety of protein kinases and PI3K-mediated effects. The activation profile of these MAPK, NFκβ, and IFN pathways depends on the subcellular locations and relative concentrations of ROSs and calcium and on their additive and synergistic effects on different components of these pathways. The various proinflammatory PGs, LTs, and TXs also are produced as a result of the same calcium- and ROS-mediated events.

Thus, an inflammatory response is not necessarily a part of the immune system per se but rather a component of a normal continuum of graded responses to cellular stress. At moderate levels of metabolic stress, tissue perfusion will be enhanced via a temporary vasodilation response, allowing appropriate metabolic responses to the stressor to occur and returning the cell to homeostasis. As the level and duration of cellular and tissue stress increases, so does the cellular signaling response, to the point where in the continuing presence of stressors the cellular responses will include new synthesis of various antioxidant and redox regulation enzymes and may include tissue remodeling (hypertrophy) with sufficient intensity or duration of the stress. In the event of cellular damage, the stress-response signaling will intensify through the activation of PRRs by various DAMPs, increasing the possibility for recruiting macrophages, platelets, dendritic cells, and fibroblasts into the tissue. If the array of stressors includes pathogens, then additional inflammatory signaling is activated by various PAMPs to enhance the recruitment of inflammatory cells into the tissue, and the pathogens are then destroyed through a combination of enhanced ROS/RNS production and phagocytic activity by macrophages, dendritic cells, and neutrophils. The macrophages and dendritic cells can also participate in initiating an adapted immune response against the offending pathogens through interactions with various cells of the (adaptive) immune system. In the event of more extensive tissue damage (with or without pathogens), the continuing production of inflammatory signaling will activate fibroblasts, platelets, and monocytes, as well as any adult stem cells in the area to coordinate the closing of any wounds with blood clots and the replacement of destroyed tissue with new tissue cells and scar tissue. At the same time, the induction of COX-2, 12-LOX, and 15-LOX resulting from the initial proinflammatory cytokine response will start a shift in the production of lipid mediators from proinflammatory PGs, TXs, and LTs to anti-inflammatory PGJ_2, lipoxins, resolvins, and protectins. These compounds produce their anti-inflammatory

effects through suppressing MAPK, NFκβ, PI3K, and IFN signaling; activating Nrf2; stimulating IL-10 release from macrophages; inducing apoptosis of neutrophils; promoting nonphlogistic phagocytosis by macrophages and their egress from the area; and inhibiting the synthesis of various chemokines.

Unfortunately, some components of the inflammatory response are capable of producing detrimental effects on surrounding healthy cells. Proinflammatory cytokines can alter cellular signaling to produce insulin resistance and enhance ROS production, leading to increased subcellular damage. The ROSs and RNSs produced by inflammatory cells can also diffuse into otherwise healthy cells to cause additional damage to proteins, lipids, and DNA. The excess ROSs can also interfere with normal signaling processes to disrupt the regulation of protein synthesis. In the case of properly resolved short-term inflammation, these effects are temporary and the damage is easily repaired.

In cases where the initiating stressor is relatively mild and constant, the normal cellular response to the continual presence of the stressor is to continually produce low-level amounts of proinflammatory signals, amounts that are insufficient to produce a full-blown inflammatory response. In such cases of unresolved low-level inflammation signaling that continues indefinitely, these effects can lead to progressive insulin resistance, cellular damage, enhanced fibrosis, inappropriate cell proliferation, and mutagenesis, all of which contribute to a variety of chronic diseases. Further, multiple short-term exposures to inflammatory responses due to repeated infections with the same pathogens (or chronic unresolved infections) in the same location can produce a similar array of damaging events.

The various anti-inflammatory and resolving molecules function by dampening the activation of the various MAPK pathways, NFκβ, and various protein kinases to suppress proinflammatory signaling as well as activate Nrf2/ARE to enhance the synthesis of a variety of antioxidant and redox system enzymes. The net effect of these changes is to greatly enhance a cell's ability to avoid the detrimental effects of ROSs as well as to maintain tighter regulatory control of signaling pathways through the endogenous regulation of redox states throughout the cell.

2.7.2 Prevention of Inflammation

Nutritional status is an important determinant in the ability to handle metabolic and other cellular stressors. Because a majority of Americans consume insufficient amounts of fruits and vegetables, most also have an insufficient intake of one or more of the nutrients that are essential for maintaining antioxidant and signaling control. Common nutrient insufficiencies among Americans include the minerals calcium, magnesium, iron, copper, selenium, and zinc and the vitamins C, D, and E. As a result of these dietary insufficiencies, a majority of Americans are under constant low-level metabolic stress and, therefore, in a constant low-level proinflammatory state. Thus, a primary recommendation to reduce inflammation-associated risks is to consume a nutritionally adequate diet to ensure that signaling, metabolic, and antioxidant cell functions are controlled appropriately. For convenience, the *MyPlate* approach (www.choosemyplate.gov), which includes standardized serving

sizes, does provide an appropriate and nutritionally adequate model from which to start. In spite of some controversies related to the saturated fat content of the dairy recommendation, dairy products are a commonly available food that is high in calcium and currently comprise the primary source for the majority of calcium for most Americans; therefore, the current *MyPlate* dietary recommendations for three servings of dairy each day are considered acceptable. For overall nutrition, following these general recommendations will result in an adequate intake of all nutrients. In order to optimize the anti-inflammatory benefits, however, recommendations that go beyond adequate nutrient intake need to be made.

In terms of essential fatty acids, in spite of recent controversies in the academic literature and the lay press, there appears to be little evidence for worrying about moderate to high consumption of LA. In fact, there appear to be some anti-inflammatory benefits from the higher levels of consumption of this essential fatty acid. The consumption of ALA, on the other hand, does appear to be below the recommended minimum for a sizeable minority of Americans. However, because it is relatively simple to ensure an adequate supply of EPA and DHA by consuming two servings of fish each week, rather than making extreme modifications to the diet in order to increase ALA consumption, this becomes the recommendation. It has the added advantage of ensuring that any additional anti-inflammatory benefits of excess EPA and DHA, such as suppression of proinflammatory PG, LT, and TX signaling and activation of PPARs, will be realized.

Regular consumption of a specific array of foods that are high in (different) phenolic content from fruits, vegetables, nuts, seeds, and whole grains is also recommended. Many phenolic compounds have beneficial effects on regulation mechanisms of cell signaling and, therefore, taking advantage of these effects is essential to developing an anti-inflammatory diet. TLR and NLR signaling pathways, as well as NFκβ and the various MAPK signaling pathways, are inhibited by a wide array of phytochemicals. Through suppressing these proinflammatory signaling processes, the production of proinflammatory cytokines and prostanoids is inhibited, resulting in blunted inflammatory responses. In addition to these anti-inflammatory effects, phytochemicals can induce the synthesis of SODs, CAT, GPX, GSR, Trx, and Prx, all of which enhance the ability of cells to maintain appropriate regulatory control of redox status as well as to withstand the detrimental effects of ROSs. These latter effects are apparently mediated by affecting the regulation of Nrf2 by Keap1; ultimately enhancing the activation of the ARE/ERE regulatory element by Nrf2.

The difficulty in developing a dietary recommendation is that although individual phenolics and selected high-phenolic foods have been tested in limited short-term dietary experiments, there are no well-controlled clinical trials that actually test the long-term anti-inflammatory effects of diets that incorporate a variety of phenolic-rich foods. In spite of this, the apparent protection from inflammation-associated chronic diseases associated with phenolic intake in the 1 to 2 g range as observed for Mediterranean diets is used as a guideline.

As discussed in Section 2.5.2.2, the effective half-life of most phenolics is in the 1- to 2-hour range due to absorption and metabolic factors, resulting in a potential effect on signaling over a similar time frame. These effects are therefore highly transient; as a result, the protective effects cannot be expected to last beyond the lifetime

of the expressed proteins. Thus, for an anti-inflammatory benefit from high-phenolic foods to be realized they must be eaten on a regular basis. The basic recommendations for 2.5 cups of fruits and 3 cups of vegetables every day is an appropriate starting point to ensure nutritional requirements. As essential components of a phytochemical-rich diet, the nuts/seeds and beans/peas categories have been separated from the general protein foods or vegetable foods categories as described in the *MyPlate* approach. They are placed in their own food category in order to emphasize their importance and to ensure their consumption.

Certain common beverages such as tea, coffee, and red wine also contain beneficial phytochemicals, including EGCG, theaflavins, caffeic acids, and resveratrol. EGCG and theaflavins are commonly consumed in the form of black and green teas, whereas caffeic acids are usually obtained from drinking coffee. Because there has been little to no research to directly compare the anti-inflammatory effects of EGCG and theaflavins to those of caffeic acids, there is currently little justification to recommend tea over coffee. Because recommending alcohol consumption may be contraindicated for some, wine is not a recommended beverage. This does not mean resveratrol cannot be obtained because it is found in grapes, cranberries, and other specific foods that are recommended in Section 2.7.3.

When it comes to exercise, for an anti-inflammatory effect to be realized the best evidence so far indicates that a large increase in IL-6 to enhance serum levels of IL-10, sIL-1ra, sIL1r, and sTNFr (without a concomitant increase in IL-1β or TNF-α) is necessary. This is usually observed after 60 minutes or more of whole-body exercise at an intensity ≥ 6 METS. IL-10 is associated with suppressed NFκβ and MAPK signaling, whereas sIL-1ra, sIL1r, and sTNFr reduce the effects of circulating IL-1α/β and TNF-α.

The benefits of reducing proinflammatory signaling and cellular responses to these signals specifically relate to the prevention of chronic diseases. By reducing inflammation, risks for cellular dysfunctions due to insulin resistance; enhanced damage to DNA, lipids, and proteins through ROS-/RNS-mediated damage; altered signaling pathways that lead to unscheduled cell proliferation; and enhanced fibrosis can be minimized. These will in turn reduce risk for cancer, neurodegenerative diseases, atherosclerosis, osteoporosis, and diabetes.

2.7.3 Dietary Recommendations

In order to optimize the anti-inflammatory effects of dietary components, a variety of foods need to be consumed regularly in sufficiently large quantities. The selections for specific fruits, grains, oils, and vegetables were made on the basis of phytochemical content in order to maximize both variety and total consumption of these beneficial compounds, whereas the minimum serving selection in the meat category is based on the EPA/DHA content. The caloric content of these recommendations for fruits, vegetables, grains, oils, and meats will range from approximately 1500–1700 kcal, depending on the specific selections of individual foods. With the addition of 3 servings of dairy to maintain calcium intake, the total daily caloric intake for these recommendations will range from approximately 1760–1960 kcal. Because there is little evidence that moderate amounts of individual fats, proteins, or carbohydrates

make a significant difference in inflammatory mechanisms, the remaining selections for food servings that are necessary to satisfy caloric needs are considered discretionary, provided there is no excess consumption of calories that results in a gain in adiposity. The specific foods that make up the anti-inflammatory recommendations are as follows:

Fruits—minimum of 2.5 cups each day with at least three different selections from each category each week:
- A. Elderberries, blueberries, pomegranates, blackberries, raspberries, Saskatoon berries, black currants, raisins, figs or prunes.
- B. Plums, oranges, grapefruit, lemons, cantaloupe, cherries, cranberries, red/black grapes, or apples.
- C. Kiwi, bananas, pineapples, mango, pears, star fruit, guava, strawberries, peaches, or any other fruit.

Vegetables—minimum of 3 cups each day with at least three different selections from each category each week:
- A. Chili peppers, broccoli, water cress, garden cress, spinach, arugula, Brussels sprouts.
- B. Cauliflower, tomato, Romaine lettuce, green beans, sweet peppers, red cabbage.
- C. Onion, garlic, celery, green cabbage, asparagus, Swiss chard.
- D. Squash, zucchini, pumpkin, sweet potatoes, carrots, potato.

Nuts and seeds—minimum of one 0.25-cup serving each day with a minimum of two different selections from each category each week:
- A. Chestnuts, pecans, walnuts, pistachios.
- B. Hazelnut, almonds, Brazil nuts, cashews, Macadamia nuts, or peanuts.

Beans and peas—minimum of one 1-cup servings each day with a minimum of two different 1-cup selections from each category each week:
- A. Black beans, adzuki beans, kidney beans, peas/pea pods, lima bean.
- B. Lentils, pinto beans, chili beans, chick peas, navy beans.

Whole grains and cereals—minimum five 1 oz. servings of whole grains each day with a minimum of one selection from each category each week:
- A. Buckwheat, wheat, bulgur.
- B. Oats, corn, barley.
- C. Wild rice, rice.

Oils—used for cooking, salads, and dipping—approximately 2 tbsp/day.
- A. EVOO.

Meats—minimum of two servings of high-EPA/-DHA fish each week.

Beverages—although water is the recommended beverage for fluid replacement, we acknowledge that a lot of coffee and tea is consumed all over the world for a variety of reasons. Although there is a lot of information on the preventive mechanisms associated with EGCG from green tea, there is actually little data to directly support the consumption of green tea over black tea, or over coffee, so no specific recommendation for either is made; both contain beneficial phenolics.

2.7.4 Exercise Recommendations

In order to obtain an optimal anti-inflammatory effect from physical activity, the recommendations are for whole-body, vigorous activities that are 6 METS or greater in intensity and carried out for approximately 1 hour each day for 3–5 days each week, with 3 days each week as the minimum recommended frequency. These activities include jogging, running, cycling at 10–12 mph or greater, swimming, and cross-country skiing.[476]

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3 Diabetes

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CONTENTS

3.1 INTRODUCTION

Diabetes mellitus is a disease diagnosed by fasting hyperglycemia that results from defects in insulin secretion, insulin action, or both.[1] Impact of diabetes mellitus on the economy of the United States is overwhelming as an estimated 25.8 million people or 8.3% of the population have diabetes mellitus.[2] This incidence of the disease has doubled since 1980. As diabetes is the leading cause of new renal failure, blindness, and nontraumatic lower-limb amputations, as well as a major contributor to heart disease, stroke, and complications of pregnancy, it is not surprising that costs were estimated in 2007 at $174 billion.[3] The adverse effects of the twin epidemics of obesity and diabetes are expected to rise, unless we develop effective therapeutic and preventive strategies. This chapter reviews the development and pathophysiology of diabetes and discusses therapeutic and preventive value of diet and exercise against this disease.

Types of diabetes are defined by etiology. Simplistically, hyperglycemia is caused by a mismatch in glucose appearance, from gut or liver gluconeogenesis, and glucose disposal, due to insufficient insulin action or decrease in end-organ glucose metabolism. The current etiological classification of diabetes mellitus by the American Diabetes Association (ADA) specifies 51 types of diabetes mellitus in addition to "others."[1] For simplicity, diabetes can be divided into four main categories: "Type 1," caused by autoimmune mechanisms that result in pancreatic β-cell destruction; "Type 2," which has a metabolic origin that results in insulin resistance and defective insulin secretion; "Gestational," which also has a metabolic origin resulting in impaired insulin function and release; and "Other" forms with a variety of possible causes including genetic, infectious, neoplastic, pharmaceutical, hormonal, and trauma also leading to impaired insulin release and function or defective glucose metabolism. By far, the etiologies of diabetes most familiar to clinicians are autoimmune-mediated destruction of pancreatic β cells or type 1 diabetes (T1DM), which currently represents approximately 5%–10% of cases, and metabolically mediated resistance to insulin action and dysfunction of glucose regulation or type 2 diabetes mellitus (T2DM), which represents approximately 90%–95% of cases. Although gestational diabetes mellitus (GDM) is metabolically mediated and similar to T2DM, its usual transient nature and differences in definition and aggressiveness of treatment assign it a separate category.

The recent explosion in the prevalence of diabetes is associated with similar rise in obesity and associated T2DM. This rise coincides with increased availability of food and the readiness of industrialized nations to adopt a sedentary lifestyle. If one considers that we evolved to accommodate scarcity of food and relatively short life span, it is no surprise that we have a thrifty genotype[4] and naturally carry hedonistic appetites for calorically dense food and a natural tendency to avoid physical activity that is not essential to survival and continuation of the species.

Hyperglycemia defines diabetes mellitus. Specific definitions based on fasting glucose (>7 mM; 126 mg/dL) or standardized 75-g oral glucose tolerance test (2-hour value >11.1 mM; 200 mg/dL) are well established. Diabetes can also be diagnosed in a patient with symptoms of hyperglycemia and random plasma glucose greater than 11.1 mM (200 mg/dL) or glycated hemoglobin (HbA_{1c}) >6.5%. A state of glucose intolerance or impaired fasting glucose associated with insulin resistance and visceral obesity often precedes T2DM. As this state of prediabetes is often reversible, it has now become a treatment target. Summary of definitions of prediabetes and diabetes including GDM are found in Table 3.1.

3.1.1 Physiology of Glucose Metabolism

Diabetes is a complex disease that involves abnormal insulin metabolism and action. Proper regulation of insulin metabolism and action is essential for the maintenance of normal glucose levels and whole-body insulin response. In a healthy insulin-sensitive individual, the rise in portal vein glucose concentrations following a meal stimulates release of insulin from pancreatic β cells that generally reside centrally in the islet. Insulin flows toward the periphery of the islet where it suppresses glucagon secretion of pancreatic α-cells.

TABLE 3.1
Criteria for Diagnosis of Diabetes, GDM, and Prediabetes

Type	Criteria
Diabetes mellitus	Fasting (>8 hours) plasma glucose >7.0 mM (126 mg/dL)
	Or
	75 g oral glucose tolerance test after fast (>8 hours) with 2-hour plasma glucose >11.1 mM (200 mg/dL)
	Or
	Symptomatic hyperglycemia with random plasma glucose >11.1 mM (200 mg/dL)
	Or
	Glycated hemoglobin (HbA_{1c}) >6.5%
Gestational diabetes mellitus	75 g oral glucose tolerance test after fast (>8 hours) between 24 and 28 weeks gestation
	Fasting plasma glucose >5.1 mM (92 mg/dL)
	1-hour plasma glucose >10 mM (180 mg/dL)
	2-hour plasma glucose >8.5 mM (153 mg/dL)
Prediabetes	Impaired fasting glucose
	Fasting plasma glucose 5.6–6.9 mM (100 to 125 mg/dL)
	Or
	Impaired glucose tolerance
	2-hour plasma glucose in 75 g oral glucose tolerance test of 7.8–11 mM (140–199 mg/dL)

Source: American Diabetes Association, *Diabetes Care*, 2012, S64–S71.

Insulin flows through the portal vein to suppress hepatic glucose production and increase glycogen synthesis. It also leads to clearance of approximately one-third of an oral glucose load following an overnight fast.[5]

In the peripheral circulation, insulin increases glucose uptake by skeletal muscle and white adipose tissue, thereby decreasing circulating glucose to sustain normal glucose levels. Additionally, insulin exerts an antilipolytic effect in white adipose tissue to increase fat storage in adipocytes and to decrease lipolysis and circulating plasma free fatty acid (FFA) levels.[6] Decrease in circulating FFA further suppresses liver gluconeogenesis and enhances muscle glucose disposal.

Skeletal muscle comprises about 55% of body mass and, in healthy individuals, accounts for disposal of approximately two-thirds of an oral glucose meal following an overnight fast.[5] Glucose uptake in myocytes and adipocytes is mediated through solute carrier family 2 (facilitated glucose transporter) member 4 (SLC2A4), formerly known as GLUT4 or insulin-regulated glucose transporter. In response to insulin or muscle contraction in vitro, these transporters are translocated to the plasma membrane for cellular uptake of circulating glucose.[7] In addition to insulin, exercise is a main contributor to GLUT4 transport to the plasma membrane, and hence, the effect of insulin and exercise on glucose disposal is more than additive.[8,9]

Insulin action begins with insulin binding to its receptor, a plasma membrane protein that undergoes phosphorylation to trigger the involvement of a cascade of signaling pathways[10] that mediate the pleiotropic effects of insulin. While insulin action is mediated by these signaling events, it is regulated by the amount of circulating insulin, which is in turn determined by insulin secretion from β cells and clearance, an event that occurs mostly in liver and to a smaller extent in kidney.[11] Abnormal insulin metabolism and action can cause insulin resistance and diabetes.

In the postabsorptive or fasting state, about 70% of glucose disposal takes place in insulin-independent tissues with uptake by brain responsible for approximately 50% of total hepatic glucose production. As mentioned in the preceding discussion, skeletal muscle contraction will increase SLC2A4 (GLUT4) translocation and increase muscle uptake of glucose in an insulin-independent manner that is not impaired by an insulin resistance state.[12]

3.1.2 Effects of Exercise on Glucose Metabolism

With exercise, a complex homeostatic interplay between the central nervous system, sympathetic nervous system, and endocrine systems maintains blood glucose in a narrow range. Although a detailed treatment is beyond the scope of this chapter, knowledge of some of the normal physiology is important for the proper use of exercise as a therapeutic modality.

With initiation of exercise, sympathetic nervous system activity suppresses insulin secretion and increases adipocyte production of FFA and liver gluconeogenesis and glycogenolysis. A small drop in blood sugar increases these effects. These mechanisms generally preclude hypoglycemia, even in prolonged exercise. With cessation of exercise, sympathetic nervous system activity and glucose counter-regulation persist for a few minutes, and transient rebound hyperglycemia can occur.

Contracting muscles translocate SLC2A4 (GLUT4) to the surface to increase glucose transport and oxidative metabolism. Local factors are recruited to increase opening of capillary beds to enhance delivery of glucose and residual insulin to muscle. This can potentially explain the more than additive increase in glucose uptake when exercise is added to insulin.[8,9]

With increased workload, glucose oxidation increases as FFA oxidation decreases. With prolonged duration, there is a shift from liver and muscle glycogenolysis to liver gluconeogenesis and increased oxidation of FFA.

After exercise, there is a prolonged period of increased glucose uptake to replenish glucose stores in liver and skeletal muscle. This residual increase in glucose uptake is especially marked in the first 1 or 2 hours and then disappears over the next 20–72 hours depending on food consumption and the level of upregulation of glycogen synthase.[13–15] This period of relatively insulin-independent glucose uptake by liver and muscle represents an opportunity for energy substrates derived from food to go to liver and muscle, but not fat.

Sensitivity to insulin action disappears on average by 30 hours following exercise, and most of the effect of exercise is due to the last bout of exercise. Exercise training, that is regularly repeated bouts of exercise, not only induces physiological changes to allow more intense exercise (and therefore more intense effects on insulin sensitivity), but also creates structural changes of increased muscle mass, decreased fat mass, increased capillary area, and decreased capillary basal membrane thickness to modestly improve insulin sensitivity even after the effects of acute exercise have waned.[16,17]

3.2 ETIOLOGY OF DIABETES

3.2.1 Type 1 Diabetes

T1DM is characterized by deficiency of insulin due to autoimmune damage to pancreatic β cells. Historically, T1DM was referred to as juvenile diabetes, but with recognition that adults are also susceptible to autoimmune-mediated diabetes mellitus and that T2DM now occurs frequently in children, this descriptive term has been abandoned. Because uncontrolled T2DM leads to severe insulin deficiency that requires insulin therapy, the term "insulin-dependent diabetes mellitus" should also no longer be used to refer to T1DM.

Autoimmune markers for risk of T1DM include islet cells antibodies, insulin autoantibodies, and antibodies against neuroendocrine antigens, glutamic acid decarboxylase (GAD_{65}), and tyrosine phosphatase–like protein islet antigen-2 (IA-2 and IA-2 β). One or more of these autoantibodies are present in more than 95% of newly diagnosed T1DM patients, and they present variably during the prodrome of islet autoimmunity long before the clinical onset. Two or more of these autoantibodies have a high predictive value of T1DM.[18]

The severity of T1DM depends on the rate and extent of the loss of β-cell mass, which is higher in children and adolescents than adults. With older age, the autoimmune response is generally less complete, and in some cases, residual function of β cells enables patients to manage without exogenous insulin for several years and in particular if good diet and exercise are followed.[19] When very little or no secretion of

insulin occurs, as determined by low or undetectable levels of C-peptide in plasma, the patient develops an absolute need for exogenous insulin to survive.

T1DM patients can be at risk to develop other autoimmune diseases as part of an autoimmune polyglandular syndrome. Hypothyroidism is the most common autoimmune disorder associated with diabetes and affects 17%–30% of patients with T1DM.[20] Other autoimmune diseases that can occur in autoimmune polyglandular syndromes include Addison's disease, autoimmune hepatitis, celiac disease, Graves' disease, Hashimoto's thyroiditis, myasthenia gravis, pernicious anemia, and vitiligo. With risk for the development of autoimmune polyglandular syndrome, the ADA recommends antithyroid antibody testing and consideration of antitissue transglutaminase testing at diagnosis.[21]

3.2.2 Type 2 Diabetes

T2DM was previously referred to as noninsulin-dependent diabetes mellitus and adult-onset or maturity-onset diabetes mellitus. As insulin deficiency often develops due to metabolic destruction or disabling of β cells, and as the condition now occurs in preadolescent children, these obsolete terms should be avoided.

T2DM is characterized by resistance to insulin action and abnormal insulin secretion that is represented phenotypically by fasting hyperinsulinemia, impaired glucose tolerance, and reduced glucose disposal rate during glucose clamps.[22] Initially, insulin secretion increases to compensate for peripheral insulin resistance. As the burden on β cells increases, especially with uncontrolled diet and physical inactivity,[23] insulin secretion progressively declines resulting in progressive insulin deficiency, hyperglycemia, and eventually T2DM.

Hence, the etiology of T2DM relates to factors that lead to compromised response to insulin. This etiology involves a complex interplay among genetic, metabolic, and environmental factors.[24] Although environmental factors such as persistent overnutrition might be necessary to phenotypic expression of T2DM, these are insufficient without preexisting genetic determinant.

Patients with T2DM suffer disproportionately from atherosclerosis and related macrovascular complications (stroke and heart disease),[25] and they show a twofold to fourfold rise in mortality related to cardiovascular disease (CVD). Five-year risk of myocardial infarction in patients with T2DM without known cardiac disease is similar to that of nondiabetic patients with prior myocardial infarction.[26] In this context, a joint statement from the ADA and the American Heart Association recommends structured programs that emphasize lifestyle changes, which include reducing fat and total energy intake with increased regular physical activity to produce long-term weight loss.[27]

3.2.2.1 Genetic Factors

Although there are clear family clusters of T2DM, it does not separate in Mendelian fashion and is considered a polygenic disease. In some populations, gene polymorphisms in the insulin receptor and one of its most characterized substrates, the insulin receptor substrate 1 (IRS-1) are two to three times more common in patients with T2DM,[28] although studies of other populations have not confirmed this association.[29]

More recently, genome-wide association studies and linkage analysis have been used.[30] Meta-analysis of genome-wide association studies of hyperglycemia or insulin resistance in nondiabetic patients identified 19 genetic loci, most of which were variably associated with insulin processing, secretion, and action.[31] The diversity of mechanisms potentially affected by these loci only serves to emphasize the polygenic nature of glucose control.

3.2.2.2 Environmental Factors

Environmental factors commonly associated with T2DM include overeating, high-fat diet, physical inactivity, and stress.[32–37] Prolonged high-fat intake reduces tissue responsiveness to insulin and glucose-induced insulin secretion. This results in insufficient compensatory insulin secretion and subsequently T2DM.[23] Physical inactivity has also been associated with decreased activity in muscle oxidative enzymes[38] and insulin resistance. Importantly, as little as 7 days of exercise reverses decreased oxidative enzymes and restores insulin sensitivity.[39,40]

Stress-related neuromodulation of metabolism can also lead to pathologically positive energy balance (hyperphagia and reduced energy expenditure).[41] We know, for example, high cortisol or abnormal sex hormones levels[42] can cause a gradual onset of hyperglycemia and hyperinsulinemia.[43] Sleep deprivation, another common stressor, causes insulin resistance and abnormal intravenous glucose tolerance.[44] In this context, there is no surprise to find that prevalence of sleep apnea among patients with T2DM is 86%[45] and that severity of sleep apnea independently predicts poor glucose control.[46]

3.2.3 Prediabetes and Metabolic Syndrome

Prediabetes (IFG and IGT) and T2DM are components of the metabolic syndrome, previously known as insulin resistance syndrome, central obesity syndrome, syndrome X, and others.[47] Metabolic syndrome is characterized clinically by increased abdominal obesity and bodily fat content, dyslipidemia (with low HDL cholesterol) and hypertension; together representing a cluster of cardiovascular risk factors.[48]

3.2.3.1 Metabolic Syndrome

Metabolic syndrome is defined by risk factors for atherosclerosis, stroke, and myocardial infarct. Several outcome data show that insulin resistance precedes atherosclerosis[49] and trumps hyperglycemia as a risk factor.[50] However, the mechanistic link has not been well elucidated, in part because insulin resistance is not a monolithic entity, but rather genetically, biochemically, and pathophysiologically heterogeneous, brought about by different mechanisms in different individuals. Regardless of its initial mechanism, insulin resistance is associated with a host of additional abnormalities, key among which are those involving lipid metabolism and visceral obesity, an inflammatory state that plays a critical role in hastening T2DM, atherosclerosis, and CVD. With diet and exercise ameliorating insulin response, treatment of insulin resistance with these modalities promises to deliver effective preventive measures against the progression of metabolic intertwined diseases. Visceral obesity is also associated with activation of inflammatory pathways in fat and associated macrophages, liver, and vascular cells, among others.[51,52]

Insulin resistance, a key factor in the etiology of metabolic diseases, is commonly heralded by hyperinsulinemia, which reflects a need for higher than normal levels of insulin to elicit a normal response.[53] Insulin resistance is associated with a number of related metabolic disorders including visceral obesity, glucose intolerance, T2DM, fatty liver disease, dyslipidemia, and hypertension.[42,43,54–56] In spite of remarkable advances in defining novel molecules involved in insulin signaling, the molecular events that result in the development of insulin resistance in humans are not fully identified.[57] However, insulin resistance is commonly attributed to defects in insulin signaling,[58] in particular at the level of IRS and IRS-associated PI3 kinase.[59–61] Moreover, there is growing evidence that defects in insulin secretion and clearance also contribute significantly to the pathogenesis of insulin resistance.[47,62]

3.2.3.2 Visceral Obesity and Insulin Resistance

The majority of type 2 diabetic patients are obese[63] and have elevated plasma FFA levels[64] that represent an independent risk factor for glucose intolerance and progression to T2DM.[65,66] In obesity, visceral fat becomes less sensitive to the antilipolytic effect of insulin,[67] thus supplying higher levels of FFA to liver, skeletal muscle, and vasculature.[68] Excessive fat intake exaggerates the release of fatty acids from the adipose tissue and their redistribution to other tissues, leading to systemic insulin resistance.[69–73] Increased liver fatty acid transport causes insulin resistance by promoting gluconeogenesis,[74,75] fatty acid β-oxidation, and lipogenesis,[76,77] in addition to inhibiting insulin-mediated suppression of glycogenolysis[78] and impairing insulin clearance.[79] Increased fatty acid transport into skeletal muscle competes with glucose oxidation and causes insulin resistance.[80]

Continuous supply of fatty acids to these extra-adipocytic tissues eventually leads to incomplete oxidation, fat accumulation, and lipotoxicity.[81] Increased ectopic intracellular content of lipid and of their metabolites contributes to insulin resistance[63] by activating signaling pathways, which impair insulin signaling directly (by impairing PI3 kinase activity[82]) or indirectly, through engaging inflammatory signals such as activating the c-Jun N-terminal kinase (JNK) pathway and increasing secretion of TNF-α,[83] which deactivates IRS proteins.[84–86]

There is growing evidence that visceral obesity and insulin resistance are chronic subacute inflammatory states that implicate elevation in fat-derived cytokines (adipokines),[87–90] such as TNF-α[91,92] in humans[93,94] and rodents.[95–100] In addition to increased release of adipokines, visceral obesity is also associated with increased release of resistin[101] and decreased adiponectin,[102] both of which play a significant role in promoting insulin resistance.

3.2.3.3 Altered Insulin Metabolism and Insulin Resistance

Although hyperinsulinemia, a cardinal feature of the metabolic syndrome, reflects an increase in insulin secretion to compensate for peripheral insulin resistance,[103] considerable data support the alternative view that hyperinsulinemia can result from impaired insulin extraction,[104] which mostly occurs in liver and to a lower extent in kidney.[11] The cause-effect relationship between hyperinsulinemia and insulin resistance is complex.

Peripheral insulin resistance causes secondary compensatory hyperinsulinemia, resulting from increased insulin secretion from β cells. But chronic hyperinsulinemia, caused by impaired hepatic insulin clearance, can worsen insulin resistance by downregulating insulin receptors in peripheral tissues and causing de novo lipogenesis in liver. This, in turn, results in increased lipid distribution to the white adipose tissue for storage and consequently visceral adiposity develops.

Considerable evidence in humans supports the view that impaired hepatic insulin extraction causes chronic hyperinsulinemia in obesity.[104] Our studies on the role of the carcinoembryonic-related cell adhesion molecule 1 (CEACAM1) in insulin clearance provide more convincing evidence that hyperinsulinemia causes insulin resistance. CEACAM1 promotes receptor-mediated insulin endocytosis and degradation, a process that underlies insulin clearance.[105] Consistent with liver being the main site of endogenous insulin clearance, L-SACC1 mice with liver-specific inactivation of CEACAM1 and mice with global *Ceacam1* null mutation (*Cc1*$^{-/-}$) develop chronically elevated levels of insulin resulting from impaired insulin clearance. The chronic hyperinsulinemic state in these mice causes whole-body insulin resistance and increases lipogenesis in liver, followed by lipid redistribution to the white adipose tissue and subsequently visceral obesity.[106–109]

The similarity in the phenotype of L-SACC1 and *Cc1*$^{-/-}$ mice emphasizes the role of hepatic insulin clearance in regulating insulin response in extra-hepatic tissues and assigns a major role for CEACAM1 in this process. In partial support of this notion, CEACAM1 levels are markedly reduced in rats selectively bred for low aerobic running capacity (low-capacity runners [LCR]) that phenocopy the metabolic syndrome,[110] including liver steatosis,[111,112] and in obese humans who have fatty liver disease in the absence or presence of T2DM.[113]

3.3 EFFECTS OF DIET AND EXERCISE ON INSULIN RESISTANCE

In the absence of exercise, dietary composition and restriction play a significant role in ameliorating altered fat and insulin metabolism, critical parameters in the pathogenesis of insulin resistance, fatty liver disease, T2DM, and their cardiovascular comorbidities.

3.3.1 Dietary Composition

The notion that dietary changes affect predominantly hepatic metabolism is bolstered by the fact that the liver plays a major role in regulating dietary fat metabolism. In addition to being a major site for glucose conversion into fatty acids, more than 50% of dietary fat can be transported to liver through chylomicrons. Excessive fat intake and fatty acid transport to liver cause insulin resistance by promoting fatty acid β-oxidation and lipogenesis[76,77] and gluconeogenesis,[74,75] in addition to inhibiting insulin-mediated suppression of glycogenolysis.[78] It can also impair insulin clearance.[79] Thus, it is not at all surprising that reduced fat intake can decrease liver fat mass and reverse the metabolic abnormalities associated with hepatic steatosis. A low-fat/low-saturated fat/low glycemic diet for 4 weeks markedly reduces liver fat mass in older subjects.[114] Moreover, the type of dietary fat has a significant effect.

A monounsaturated fat diet for 8 weeks causes significant loss of hepatic steatosis in patients with T2DM when compared to patients who fed an isoenergetic carbohydrate/fiber-enriched diet, independently of an aerobic training program.[115]

3.3.2 Caloric Restriction

Caloric restriction reverses most of metabolic abnormalities, including hepatic steatosis and insulin metabolism, more strongly than exercise alone, in rats bred for LCR.[112] Similarly, a calorically restricted diet with low glycemic index for 16 weeks markedly decreases serum insulin concentrations and waist circumference in insulin-resistant obese adolescents.[116] The lowering effect of caloric restriction in man on hepatic steatosis is supported by a 6-month study, which demonstrated that caloric restriction reduces liver triglyceride and hepatic steatosis, improves hepatic histology, and reverses insulin resistance with a 10% loss of body weight.[117] While these studies did not investigate the effect of caloric restriction on insulin metabolism (clearance), they serve to emphasize the beneficial therapeutic role of caloric restriction on lipid metabolism in liver.

3.3.3 Effects of Exercise on Insulin Resistance

A number of biological mechanisms may contribute to an increase in insulin sensitivity and improvement in glucose homeostasis in response to exercise training. Exercise can cause both structural and biochemical changes in skeletal muscle. Structural changes include elevation in fiber size, fiber transformation, and an increase in capillary density with a subsequent increase in blood flow.[23,55] Biochemical changes include, but are not limited to, increased insulin signaling and changes in the activity of enzymes associated with enhanced glucose and lipid metabolism.

3.3.4 Exercise and Glucose Metabolism

During exercise, glucose and fat serve as fuel for the working skeletal muscle. Hence, several adaptive changes occur in an exercised muscle in several steps of glucose and fat metabolism. Glucose metabolism is initiated by its cellular uptake through SLC2A4 (GLUT-4), followed by its conversion into glucose-6-phosphate, which may then undergo glycolysis to generate ATP for energy or be incorporated into glycogen by stimulating glycogen synthase enzymatic activity.[118]

Insulin resistance and T2DM are frequently associated with alterations in this process, including reduced glucose transport. Exercise training increases capacity for glucose transport into the cell,[119] through increase in glucose transporter content,[120] and enhanced insulin signaling through the PI3 kinase/Akt pathway that leads to translocation of SLC2A4 (GLUT-4) to the membrane for glucose uptake.[119,121] Moreover, insulin resistance is associated with reduction in the muscle oxidative and respiratory capacity due to a decrease in the number of mitochondria and key oxidative enzymes such as citrate synthase.[122] Endurance training increases mitochondrial number[123] and protein expression and activity of citrate synthase in skeletal muscle,[124,125] leading to improved glucose tolerance.

Insulin resistance is also associated with reduced glycogen synthesis,[126–128] resulting from impaired insulin-induced stimulation of glycogen synthase activity.[128] During exercise, glycogen stores serve as an energy source for contractile activity. Hence, its synthesis is upregulated to replete the storage and meet demand imposed by the exercised muscle. This is mediated by elevating both insulin-stimulated glycolysis and glycogen synthesis during exercise.[118,129]

3.3.5 Exercise and Lipid Metabolism

Ectopic fat accumulation in the liver and skeletal muscle is strongly associated with the development of insulin resistance in T2DM.[130–132] Fat is synthesized primarily in the liver and the adipose tissue, a tissue that is better positioned to store fat in response to insulin. In an insulin resistance state and hyperinsulinemia, hepatic fat production and redistribution to the adipose tissue is increased, and fatty acids are mobilized from the adipose tissue. Plasma fatty acids are then cleared in skeletal muscle by β-oxidation[133] and in liver predominantly by undergoing esterification.[68,134]

During exercise, β-oxidation increases in skeletal muscle, the major site of energy expenditure.[135] This is regulated by stimulating 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK), through a high AMP:ATP ratio, thereby, reducing production of malonyl CoA, an inhibitor of carnitine palmitoyltransferase-1 that mediates translocation of long chain fatty acids into the mitochondria for oxidation.[136,137] Thus, exercise increases β-oxidation, particularly in skeletal muscle.

This mechanism contributes to enhanced insulin sensitivity in patients with metabolic syndrome, as shown by several studies, in particular in response to a program that incorporates aerobic interval training (90% of highest measured heart rate), three times a week for 16 weeks.[138] This program has been shown to be superior to a continuous moderate exercise program (70% of highest measured heart rate) in terms of reversing endothelial dysfunction, insulin resistance, and fat metabolism in patients with metabolic syndrome.[138]

Similarly, subjecting LCR rats to high-intensity interval endurance training for 8 weeks effectively reduces markers of cardiovascular risk, such as endothelial dysfunction and hypertension, fat mass, and glucose intolerance.[139] While this program reduces fat accumulation in skeletal muscle and adipose tissue, it does not substantially reduce hepatic triglyceride and insulin metabolism. This could be due to the need for the liver to produce and contribute to providing triglycerides (energy) to the exercised skeletal muscle to meet increasing demand. It can also suggest that endurance training does not suffice to reverse all metabolic abnormalities, especially those pertaining to the liver, which appears to be more responsive to dietary changes and caloric changes.[139]

3.4 TREATMENT OF DIABETES WITH EXERCISE AND DIET

Although caloric restriction and exercise training can independently restore the altered metabolic state associated with insulin resistance, their combined therapy appears to provide a more effective treatment to fully restore metabolic regulation by insulin. In the following part of this chapter, we will translate this knowledge to patient care.

Before the past few decades, caloric restriction and physical activity were the norm for most of the world. Food was scarce and significant physical activity was necessary to seek food, shelter, and continuation of the species. In this context, diabetes mellitus was relatively rare and limited primarily to T1DM, for which there was no effective treatment. Although dietary caloric restriction therapy was widely adopted at the beginning of the past century, the natural progression of this fatal wasting disease was not changed. The advent of insulin therapy has allowed survival, and diet and exercise are now commonly recommended adjunct therapies to maintain glucose control, insulin sensitivity, and functional capacity.

The explosion in incidence of diabetes follows our ability in the industrial era to adopt a sedentary lifestyle in the face of excess energy intake. Although the relationship between caloric intake and obesity in T2DM was recognized early, only recently has it been recognized that physically inactive lifestyle initiates maladaptation to promote T2DM and other chronic disease.[140]

3.4.1 Requirement for a Clinical Team in Diabetes Self-Management and Lifestyle

Management of diabetes requires a capable patient and a multidisciplinary team of diabetes professionals to provide medical oversight, pharmacological therapy, hypoglycemia training, nutritional therapy, exercise, and education in self-management and psychosocial care. Diabetes self-management and education, when provided by a program that meets national standards, is reimbursed as part of the Medicare program, Indian Health Service, and most health insurance plans. Medical nutrition therapy is usually reimbursed separately.

3.4.2 Goals for Glycemic Control in Adults with Diabetes Mellitus

Most physicians and patients are familiar with the use of goals based on HbA_{1c}. There has been some misconception that goals for HbA_{1c} should be rigid and universally applied, and therefore confusion about the nature of the difference between the American Association of Clinical Endocrinology recommendation of 6.5%[141] and the ADA recommendation of 7.0% as goals for maximum HbA_{1c}. In fact both sets of guidelines acknowledge that more or less stringent glycemic goals may be appropriate for individual patients, and the ADA specifically recommends a customized range from 6.5% to 8.0% as reasonable goals depending on specific circumstances such as safety, age, proclivity to complications, and residual insulin secretory capacity.[21]

For nonpregnant adults, the ADA recommends maximal preprandial capillary plasma glucose of 3.9–7.2 mM (70–130 mg/dL) and postprandial capillary plasma glucose of 10 mM (180 mg/dL).[21]

For pregnant women with preexisting T1DM or T2DM, a recent consensus statement[142] recommends premeal, bedtime, and nocturnal glucose range of 3.3–5.4 mM (60–99 mg/dL), peak postprandial glucose range of 5.4–7.1 mM (100–129 mg/dL), and HbA_{1c}<6.0%.

For pregnant women with GDM, recommendations from the 5th International Workshop-Conference on Gestational Diabetes[143] include maximal preprandial capillary

blood glucose of 5.3 mM (95 mg/dL) and either 1-hour postprandial maximal glucose of 7.8 mM (140 mg/dL) or 2-hour postmeal maximal glucose of 6.7 mM (120 mg/dL).

3.4.3 General Principles for Use of Exercise in Therapy for Diabetes Mellitus

As the effects of exercise on muscle glucose disposal and insulin sensitivity wane significantly after 2 hours and become insignificant by 30 hours,[13–15] we generally recommend daily exercise with the bulk of exercise preceding the major meal by 1 hour or 2 hours. For patients with adequate time to exercise, we recommend exercise or physical activity to precede both morning and evening meals. This differs somewhat from current guidelines for standard of care that is based on recommendations of 150 min/week of moderate intensity or 75 min/week of vigorous physical activity in three to four sessions per week.[21,144]

Guidelines[21,144] recommend use of both aerobic and resistance exercise. This recommendation has general support and seems reasonable. Aerobic exercise (continuous exercise below threshold for lactate production) represents an efficient means toward caloric expenditure. In addition, aerobic exercise of more than 20 minutes duration and moderate intensity predominantly uses FFA stores and can be especially effective to reduce body fat, especially if paired with caloric restriction. On the other hand, whole-body glucose disposal also correlates with muscle mass. Although muscle sensitivity to insulin of weight lifters is no better than that of sedentary subjects, whole-body insulin sensitivity and glucose disposal were nearly identical to that of marathon runners with muscle highly sensitive to insulin.[145]

In this context, we generally recommend a combination of exercise: balance, stretching, and postural therapy for safety; aerobic activity to efficiently oxidize calories and to improve muscle sensitivity to insulin; and resistance training to maintain or increase muscle mass and to maintain joint stability. We suggest lighter exercise in the morning and more intense exercise in the evening. This not only reduces risk for hypoglycemia in those taking insulin following overnight fast but also acknowledges circadian rhythm of performance, with power output being generally better in the late afternoon or evening.[146] To avoid overuse injury, we usually recommend that the exercise program should vary daily and that specific exercises be repeated only every 3 days.

3.4.4 Can One Exercise Too Much or the Wrong Way?

This question was addressed 20 years ago by two studies. In the first, Kirwan et al.[147] found eccentric exercise caused marked muscle soreness and significantly elevated creatine kinase levels 48 hours after exercise (273 ± 73, 92 ± 27, 87 ± 25 IU/L for the eccentric, concentric, and control conditions, respectively) 48 hours after exercise. Sensitivity to insulin action was reduced by about 40% after eccentric exercise (3.47 ± 0.51 mg/kg/min) compared to concentric exercise (5.55 ± 0.94 mg/kg/min) or control conditions (5.48 ± 1.0 mg/kg/min).

The morning after the 1991 Helsinki marathon, we found whole-body insulin sensitivity (insulin-stimulated glucose disposal) as measured by 19 concurrent

euglycemic clamps was "paradoxically" decreased by 12% in spite of glycogen depletion.[148] Insulin resistance following eccentric or unusually long or traumatic exercise is believed to be due to a combination of metabolic factors including elevated fatty acid oxidation and muscle damage.

3.4.5 Avoidance of Adverse Events Related to Hypoglycemia

New drugs for treatment of diabetes mellitus that minimize risk of hypoglycemia allow aggressive nutritional therapy and exercise. We believe these medications should be preferred therapy. Once insulin or sulfonylureas are used, a patient is obligated to aggressive glucose testing and constant modulation of caloric intake and expenditure to avoid hypoglycemia. In spite of this significant limitation, sulfonylureas continue to be used quite aggressively in the United States, presumably due more to reasons of therapeutic inertia or short-term economics than medical science.

Once hypoglycemic agents are used, hypoglycemia becomes a limiting and dangerous factor in treatment.[149,150] In patients who use hypoglycemic agents, we have found the ability to analyze trends of interstitial fluid glucose in real time with continuous glucose monitor technology to be an invaluable tool for patients prescribed diet and exercise. In this context, we have tested a feed-forward neural network model for prediction of glucose in patients requiring insulin to predict future blood glucose (75-minute horizon) on the basis of continuous glucose monitor values, insulin dose, nutritional intake, and physical activity.[151] As technology surrounding continuous glucose monitoring improves, we believe that cost analysis will demonstrate improved therapeutic effectiveness of diet and exercise through use of this technology with subsequent reduction in hypoglycemia, hyperglycemia, risk of cardiac events, and small vessel disease. In this context, we take issue with guidelines for clinical care or insurance reimbursement that emphasize early use of sulfonylureas or severely limit access of patients who require insulin to technology of continuous glucose monitoring.

3.4.6 Recommendations for Exercise in the Context of Complications of Diabetes

Complications of diabetes can in turn complicate recommendations for diet and exercise. Cardiovascular complications are especially prevalent in T2DM, which as noted in Section 3.2.2, is considered a risk factor equivalent to coronary artery disease. In spite of this, current recommendations state that routine screening for coronary artery disease is not recommended, as it does not improve outcomes as long as risk factors are treated.[21,144,152] These guidelines recommend cardiac testing only in the face of cardiac symptoms or abnormal resting electrocardiogram.[21,144,152] Providers are reminded, of course, to use clinical judgment in this area. From a practical perspective, if there is a question as to a patient's ability to safely exercise, then exercise testing should be undertaken.

Autonomic neuropathy due to microvascular disease can increase the risk of exercise-related adverse events through postural hypotension, impaired thermoregulation, and decreased cardiac responsiveness to exercise. Autonomic neuropathy can also cause gastroparesis. As gastric emptying is the limiting factor for glucose

absorption, gastroparesis can predispose to hypoglycemia, especially if gastric emptying times are variable and unpredictable. Gastric emptying can be further slowed by commonly used medications such as antidepressants as well as hot or cold food and fat content. Pupillary dysfunction in addition to retinopathy can impair vision and increased risk of accident.

Peripheral neuropathy can increase risk for inadvertent skin and skeletal injury. Skeletal injury often leads to Charcot's joint distraction. Prevention remains critical, and the patient should use proper footwear, examination of feet, and coaching to avoid injury.

Although physical activity can acutely increase urinary protein excretion, there is no evidence that exercise increases the rate of progression of diabetic kidney disease. At this time, there is no need for specific exercise restrictions for patients with diabetic kidney disease.

3.4.7 General Recommendations for Timing, Quality, and Quantity of Diet

As with exercise (see Section 3.4.3), diet should be prescribed by time, quantity, and quality to match caloric expenditure and nutritional requirements for micronutrients. Again, we note the rapidly waning residual effects of exercise on insulin sensitivity and glucose disposal to muscle and liver,[13–15] and we therefore recommend the bulk of daily calories be eaten within 2 hours of finishing exercise. In this context, we avoid discussion with patients of the importance of breakfast, use of multiple small meals, or avoidance of evening meals, which are recommendations generally supported only by epidemiological evidence and short studies in sedentary populations. The ADA Standards of Medical Care[21] also omits these topics from general recommendations for dietary care of diabetes mellitus, which are summarized in Table 3.2.

TABLE 3.2
General Recommendations for Nutritional Therapy from American Diabetes Association

- Weight loss is recommended for overweight or obese individuals
- Weight loss diets may be effective for up to 2 years
- Weight loss diets can restrict either carbohydrate, fat, or specific fats (Mediterranean diet)
- Physical activity is important to weight loss
- Physical activity is especially important to maintenance of weight loss
- Carbohydrates should be monitored
- Saturated fat should be less than 7% of calories
- Intake of *trans* fat should be minimized
- Alcohol should be limited to one drink/day for women and two drinks/day for men
- Routine supplementation with vitamins E and C and carotene is not advised.
- Diet should meet patient preferences, metabolic goals, and current recommended daily allowance for all micronutrients

Source: American Diabetes Association, *Diabetes Care*, 2012, S11–S63.

Caloric content of the diet is usually aimed at weight maintenance in athletic patients with desired muscle and fat mass and weight loss or gain in others. Malnourishment is rare in the United States, and there has been no demonstration that incorporation of recommended daily allowance or dietary reference intake for micronutrients has clinical benefit. Nonetheless, misinformation and the nutraceutical industry have made education of patients on micronutrient intake an oft complicated and time-consuming affair.

Although relatively simple physiological principles might be involved, variables of patient preference, physical activity, basal metabolic rate, mood, educational background, and motivation can complicate dietary prescription. Many patients come armed with misperceptions and misinformation promoted by various media on all aspects of dietary therapy. We have found that use of a full-time professional in diabetes care with background in medical nutrition therapy and exercise seems mandatory. As noted, Medicare and the Indian Health Services reimburse medical nutritional therapy for patients with diabetes through specific legislation and rules.

3.5 DIET AND EXERCISE IN PREVENTION AND TREATMENT OF DIABETES MELLITUS

The incidence of T1DM is increasing worldwide.[153] Whether this increase in incidence is related to a higher incidence of preclinical autoimmunity or faster progression to diabetes after development of autoimmunity is not clear. Rise in T1DM has stimulated speculation that insulin resistance and obesity might modulate autoimmunity. A recent meta-analysis supports the association between childhood obesity and subsequent T1DM.[154] At least one paper has proposed a mechanism by which exercise might modulate preclinical autoimmunity.[155] In this context, exercise and diet might prove important in reduction of risk for development of T1DM and other autoimmune diseases.

3.5.1 Diet and Exercise in Treatment of T1DM

Hypoglycemia is the limiting factor in management of T1DM.[149] To date, nutritional interventions for patients with T1DM are essentially limited to recommendations to match carbohydrate content of meals and snacks with insulin therapy and exercise. As any serious athlete with diabetes will attest, this is no mean task, especially when one considers the 30–40-minute biological half-life of insulin action and the immediately synergistic effect to lower blood glucose of insulin and exercise.[8,9] Successful therapy and lifestyle intervention in this group require an intelligent and willing patient supported by a full team of professional diabetes educators and clinicians.

To avoid hypoglycemia with exercise, long-acting insulin should generally be avoided in patients with T1DM who exercise regularly and maintain insulin sensitivity. Pumps have been especially useful to patients with T1DM. They can be removed before exercise, and waning levels of short-acting insulin present little risk of hypoglycemia and are usually sufficient to prevent ketosis, even in prolonged exercise. The ability to analyze trends of interstitial fluid glucose in real time with continuous glucose monitor technology is an invaluable tool for patients with T1DM who exercise.[156] Although there is a delay of 10–15 minutes between changes in blood and interstitial fluid glucose content, software-enabled analysis of trends and appropriate alarms provide additional safety.

Given longer life, obesity-related disease has not surprisingly become a problem for patients with T1DM.[157] Weight loss requires a careful match among caloric intake, caloric expenditure, and insulin dose. This usually requires intense glucose monitoring. More detailed recommendations pertinent to weight loss can be found in Section 3.5.2.

Patients with T1DM can find it difficult to strike a balance between insufficient insulin and hyperglycemia on one hand and too much insulin and hypoglycemia on the other hand. We have always been impressed with the minimal requirements, often in the range of less than 12 units/day, required by professional or competitive amateur athletes with T1DM. Tests of blood glucose should be undertaken before, during, and after exercise. Although hyperglycemia per se does not require postponement of exercise, hyperglycemia in the context of ketosis can worsen with exercise.[158] Adequate treatment and retesting should be undertaken.

It should be remembered that athletes with T1DM do not automatically enjoy improved insulin sensitivity. We previously reported that in 11 athletes with T1DM, insulin sensitivity measured across the forearm as arteriovenous difference, by euglycemic clamp, was not improved when compared with 12 more sedentary patients with T1DM.[159] These athletes suffered higher HbA_{1c} ($8.4 \pm 0.4\%$ vs. $7.2 \pm 0.2\%$), took 20% less insulin, and had higher fatty acid oxidation and lower glycogen synthase activity. We therein concluded that better education of athletes with T1DM was needed to maintain good control and insulin sensitivity.

ADA guidelines recommend consumption of additional carbohydrate if pre-exercise glucose levels are less than 5.5 mM (100 mg/dL). The patient should undertake subsequent testing during exercise with frequency dependent on how rapidly blood glucose has dropped, current insulin or sulfonylurea dose, and intensity of exercise.

3.5.2 Diet and Exercise in Prevention of T2DM

Impaired fasting glucose and impaired glucose tolerance (prediabetes) are generally associated with insulin resistance and metabolic syndrome. These conditions are generally considered to be a consequence of modern lifestyle of low physical activity and consumption of excessive calories. Therefore, it follows that lifestyle changes that involve increase in physical activity and decrease in caloric intake might reverse this condition or at least prevent progression to T2DM. To date, there have been four prospective randomized studies to demonstrate that use of exercise and diet in patients with impaired fasting glucose and/or impaired glucose tolerance will prevent onset of T2DM.

The first of these trials, the Da Qing trial, was published with a 6-year follow-up in 1997.[160] This was the only study of this kind to directly compare effects of diet, exercise, and exercise plus diet. When compared to the control group, all three interventions were equally effective, with reduction in the incidence of diabetes at 6 years by more than 50%. Data can be found in Table 3.3. The Finnish Diabetes Prevention Study (FDPS),[161] Indian Diabetes Prevention Program (IDPP),[162] and United States Diabetes Prevention Program (USDPP)[163] all followed and demonstrated similar reductions in the development of T2DM. The USDPP was memorable in that it was closed early for ethical reasons after only 2.6 years of follow-up. Results from these studies are summarized in Table 3.3.

TABLE 3.3
Prospective Intervention Trials to Prevent Diabetes in Populations with Impaired Glucose Tolerance

			Incidence of Development of Diabetes (%)				Reduction in Diabetes (%)		
	n	Duration (Years)	Control	Diet	Exercise	Diet and Exercise	Diet	Exercise	Diet and Exercise
Da Qing DPS[160]	577	6.0	68	44[a]	41[a]	46[a]	55[a]	59[a]	51[a]
Finnish DPS[161]	400	3.2	23			11[a]			58[a]
U.S. DPPT[163]	3234	2.8	29[b]			14[b]			58[a]
India IDPP[162]	531	2.5	55[b]			39[b]			28[a]

[a] Different from control group.
[b] Estimated cumulative incidence to 3 years.

In the USDPP,[163] lifestyle intervention was found twice as effective in preventing diabetes as therapy with metformin, a biguanide suppressor of gluconeogenesis. Interestingly, the IDPP[162] demonstrated no additional effect of metformin on top of exercise and diet. The reduction in incidence of T2DM with lifestyle modification was virtually identical to lifestyle modification plus metformin therapy. Superiority of nondrug approaches to drug-based approaches in prevention of T2DM was confirmed in recent meta-analysis of 10 randomized, controlled trials ($n = 23,152$) of more than 100 patients and more than 1 year in duration.[164] A separate meta-analysis of 16 studies of patients with metabolic syndrome with interventions of at least 24 weeks also suggests, using mixed-treatment comparison methods, that lifestyle interventions were more probably more effective than pharmacological interventions (87%).[165] In that study, the odds of metabolic syndrome reversal by lifestyle were 3.8 (2.5–5.9) and by pharmacological intervention 1.6 (1.0–2.4).

Long-term follow-up of 20 years in the Da Qing trial,[166] 7 years in the FDPS[167] and 5.7 years in the USDPP Outcomes Study,[168] demonstrated continued reduction in cumulative incidence of diabetes of 43%, 43%, and 34% respectively. The lifestyle treatment group in the USDPP Outcomes Study continued to demonstrate twice the reduction as the group treated with metformin.

Retrospective meta-analysis of these studies was undertaken to examine whether a specific intervention was more effective.[169] Studies variably used low-fat, high-protein, or Mediterranean diets. All seemed equally effective. They calculated that maintenance of weight loss requires regular exercise with additional expenditure of 2000 kcal/week. They estimated it would be necessary to treat 6.4 (5.0–8.4) patients with prediabetes to prevent or delay just one case of diabetes through lifestyle intervention.

In sum, these studies are encouraging as they demonstrate that exercise and diet are inexpensive and effective therapies to prevent progression of prediabetic states to T2DM and that their effectiveness can be maintained beyond a period of intensive education and intervention. On the other hand, we note that 80% of the patients in the Da Qing trial developed T2DM by the 20-year follow-up.[166] Although we acknowledge that those 20 years were a period of rapid industrialization in this area, and that the group treated with exercise and diet had lower incidence of diabetes than the control group, long-term intervention to prevent T2DM has room for new ideas and improvement.

3.5.3 Diet and Exercise in Treatment of T2DM

The Look AHEAD (Action for Health in Diabetes) trial, designed to prevent complications in obese individuals with T2DM, has been the only one large prospective randomized controlled trial of lifestyle modification in patients with T2DM.[170] This trial, which was planned for up to 11 years of follow-up, recently underwent a midcourse correction when a much lower-than-expected rate of cardiovascular complications was found in the first 2 years, to expand both the definition of primary end point and follow-up of participants.[171]

Four-year follow-up of approximately 4800 participants was recently reported,[172] and patients with intensive lifestyle intervention (ILI) had greater improvements in treadmill fitness, systolic and diastolic blood pressure, and HbA_{1c}, than a group assigned to more routine diabetes self-management and education (DSE) program. Weight loss was maintained in the ILI group at a somewhat disappointing 4.7% after initial weight loss of 8.6% in year 1. Meanwhile, success of both interventions is suggested by maintenance of weight loss (–1.1% in the DSE group) and occurrence of only one cardiovascular event among over 5,000 participants in the course of 4 years.

In addition, 1-year follow-up of erectile function using the International Index of Erectile Function demonstrated a trend toward improvements in the ILI group than the DSE group ($p = .06$).[173] Also, at 1 year, and in the face of significant weight loss, women in the ILI group had improvement in incidence, although not resolution, of urinary incontinence.[174] Each kilogram of weight loss was associated with a 3% reduction in odds ratio for development of urinary incontinence.

ILI and increased physical activity were not detrimental to joint disease, and in fact the pain improved in this group at 1 year.[175,176] We await follow-up data to determine whether this is simply due to weight loss or other factors.

T2DM is a risk factor for osteoporosis. Bone density declines with weight loss, and not surprisingly the ILI group demonstrated decrease bone density at 1 year.[176] Again, significance is not certain as bone biopsy and quality of bone are not known. We await long-term follow-up.

The Sleep AHEAD component of the Look AHEAD trial found more than 86% of obese patients with T2DM have obstructive sleep apnea ($n = 306$, mean apnea–hypopnea index $= 20.5 \pm 16.8$ respiratory events/hour).[45] This treatable condition causes insulin resistance, hypertension, weight gain, and glucose intolerance. The condition is often curable with weight loss and exercise. One-year follow-up of this subgroup demonstrated weight loss of 10.8 kg in the ILI group and 0.6 kg weight loss

in the DSE group.[177] The ILI had 20% decrease in AHI while the DSE group actually suffered 20% increase in AHI. A 13.6% of ILI participants had remission of obstructive sleep apnea. Greater weight loss was associated with greater improvements in sleep apnea. Although this study found a trend to improved HbA_{1c} ($p = 0.08$), quantitation of the effects of diet and exercise mediated through improved obstructive sleep apnea on cardiovascular risk factors and metabolic control have not been determined by a randomized, controlled trial. Sleep apnea is an independent risk factor for worsening glycemic control in patients with T2DM. It may also be an independent risk factor for complication of diabetic nephropathy[178] and coronary artery disease.[179–181] Future clinical research in diabetes needs to address and control for sleep apnea.

3.5.4 Diet and Exercise and Prevention of Gestational Diabetes Mellitus

As morbidity associated with pregnancy in overweight and obese patients becomes more apparent, a more aggressive approach has been taken to prevent GDM. This is especially important as GDM is not usually diagnosed until at least the 24th week of gestation. This late date makes intervention short and perhaps less effective once a patient develops diabetes mellitus.

Unfortunately, results to date of randomized controlled trials of exercise and diet have not been as encouraging as hoped for on the basis of epidemiological studies. In part, this may reflect understandable reluctance of investigators and institutional review boards to jump into studies of effects of stressful exercise on sedentary and pregnant patients. The time to initiate exercise and diet is before pregnancy, but few young women have the luxury of this level of planning.

In this context, a very recent Norwegian study of 702 women[182] declared "A 12-week exercise program performed during the second trimester does not prevent gestational diabetes in healthy pregnant women." Like many studies, this one followed recommendations for three bouts of 45 minutes of low-impact exercise each week. Another study published this year of 855 women echoed these results with demonstration that a 12-week standard exercise program provided no evidence for prevention of gestational diabetes or improvement in insulin resistance.[183] Although this study did aim for a moderate-to-high intensity activity 3 days/week, it suffered a dropout rate of 45%. A recent meta-analysis of three randomized controlled trials with moderate risk of bias found no significant difference in incidence of GDM between women receiving additional exercise intervention and routine care.[184] Another group undertook meta-analysis of 13 randomized and 6 nonrandomized trials of lifestyle intervention in obese pregnant women. Although lifestyle intervention reduced maternal pregnancy weight (–2.2 kg) and produced a minimal trend toward reduction in prevalence of gestational diabetes (odds ratio 0.8 with 95% confidence interval 0.6–1.1), there was no clear difference in outcomes of cesarean delivery, large for gestational age, birth weight, or macrosomia. All studies were statistically of low-to-medium quality.

In summary, data available to date suggest that exercise and diet as currently prescribed are insufficient to prevent GDM in overweight and obese women and that future studies of lifestyle intervention to prevent GDM should include daily exercise, diet designed to match the exercise and requirements of the pregnancy, and intervention at an earlier gestational age.

3.5.5 Diet and Exercise in Treatment of Gestational Diabetes Mellitus

Obesity and GDM are associated with adverse outcomes in pregnancy including macrosomia, cesarean section, hypertension, preeclampsia, and eclampsia. Slightly different recommendations have evolved for goals for glucose control in pregnant women with GDM and pregnant women with preexisting diabetes mellitus. These recommendations are in Section 3.4.2.

Medical nutritional therapy has been the cornerstone of treatment for GDM.[143] Although the Institute of Medicine report of 1990 had recommendations for weight gain with pregnancy in patients who are obese and for patients who are underweight, there are no published guidelines for weight gain in women with GDM. The situation is further complicated as diagnosis of GDM is usually delayed until weeks 24–28, at which time many patients have reached or exceeded their weight gain target.

In workshop recommendations,[143] consensus recommendations for physical activity are addressed in a mere four sentences. Physical activity of 30 min/day is recommended without recommendation for intensity or caloric expenditure aside from comment that 10 minutes of brisk walking or arm exercises while seated in a chair for 10 minutes after each meal accomplishes the stated goal. Meta-analysis of four trials based on these recommendations as carried out in 114 pregnant women found no effect of exercise on perinatal outcomes, pregnancy complications, or maternal morbidity. The authors concluded that there is insufficient evidence to recommend or advise against enrollment of pregnant women with GDM in exercise programs. It should be noted that by necessity of diagnosis, all studies started in the third trimester and duration was only 6 weeks. The authors of the study also commented that they were unable to find any studies with exercise intervention in pregnant women with T1DM or T2DM. Again, sparsity of data for use of exercise and diet in GDM or pregnant women with other types of diabetes mellitus precludes specific recommendations.

3.6 CONCLUSIONS

Consistent consumption of surplus calories and proclivity to an available sedentary lifestyle have caused an epidemic of metabolically mediated insulin resistance, T2DM, and associated morbidity. This metabolic milieu might also be associated with the rise in autoimmune-mediated T1DM. More certain is the observation that more patients with T1DM have become obese and insulin resistant as they live longer in the current environment.

As consumption of surplus calories and low levels of physical activity have created the metabolic problems of obesity, insulin resistance, and T2DM, it seems reasonable that therapy with diet and exercise can reverse these problems. A large body of work both in the laboratory and in the clinic confirms that diet and exercise have a role in the prevention of T2DM and modification of risk factors in T2DM. Unfortunately, the same cannot be said for the role of exercise and prevention or treatment of GDM or diabetes during pregnancy.

Specific guidelines from the American College of Sports Medicine[144] and the ADA on use of medical nutrition[21,185] and exercise[21,144] in the treatment of diabetes are a good starting point. Ultimately, these guidelines address nutritional therapy and exercise therapy as separate modalities. To optimize therapy, we need integrated

guidelines that allow a more physiologically rational integration of diet and exercise. If, for example, insulin-sensitizing effects of exercise persist at relatively high levels for only an hour or two after exercise, then meals should be prescribed to follow exercise in the physiologically natural rhythm of exertion, followed by eating and sleep.

We also suffer from lack of information on how best to deliver our prescription for diet and exercise to the person who will carry it out—the patient. The future might include an exercise station equipped with monitors for continuous glucose measurement, caloric consumption, substrate utilization, blood pressure, and sympathetic nervous system activity, as well as software to use these data to recommend appropriate and immediate adjustment in exercise, diet, and medication. For now, however, most patients receive prescription of diet, exercise, and medications from separate individuals with little integration of instructions that sometimes conflict and often overwhelm.

In this context, Table 3.4 provides a sample of 10 recommendations that summarize the science and clinical guidelines for an average patient with diabetes mellitus. Recommended calories are personalized on the basis of calculation or measurement of basal metabolic rate, estimate of caloric expenditure of planned daily exercise, with calories added or subtracted to accommodate needs for weight gain or loss. Recommendations can be expanded or contracted as needed.

In the end, the patient will have to integrate his/her prescription into a life already complicated by demands of survival—food, shelter, and continuation of the species. To deny ourselves food and to do physical work without reward are not natural after puberty. Health-care providers must arm themselves with enough information to convince the patient of the importance of diabetes and exercise or to convince third-party payers to reward the patient directly for healthy changes in lifestyle.

TABLE 3.4
Sample of Practical Recommendations for Diet and Exercise for Nonpregnant Adults with Diabetes Mellitus

- Aim for _____ calories daily to include 80 g of protein (320 calories)
- Eat sufficient fiber and water to avoid constipation
- Eat the bulk of calories within 2 hours of finishing exercise
- Try light exercise such as yoga in the morning and heavier exercise such as weight lifting in the evening
- Walking on flat ground can be hard on the knees. (Walking is not the best exercise if you are overweight.) Uphill treadmill walking and exercise cycles are easier on the knees
- Avoid daily repetition of the same exercise to avoid injury
- Respect your orthopedic or cardiac limitations
- Stop and call the physician for chest discomfort. Go to an emergency department for chest discomfort of greater than 5 minutes
- If needed, get help from your physical therapist or physical medicine physician to design a pain-free program and exercises to stabilize your joints
- If you are on insulin,
  - Test glucose before exercise and every 30 minutes during exercise and as needed
  - Test 20 minutes after exercise
  - Remember, the effects of insulin and glucose are more than additive
  - Use a snack if glucose is less than 100 mg/dL or dropping quickly

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4 Atherosclerosis

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CONTENTS

4.1 INTRODUCTION

Coronary heart disease (CHD) and its primary cause, atherosclerosis, are the leading causes of mortality in developed countries and the number one killer of both men and women in the United States. Every year since 1919, CHD was ranked as the number one killer in the United States; in 2008, CHD accounted for approximately 32% of all deaths with direct and total costs being estimated to be $273 and $444 billion, respectively.[1–4] Interestingly, there has been an almost 50% decline in mortality from heart disease between the 1960s and the 1990s, a decline that has continued through the last decade with the latest reported decline in age-adjusted mortality of approximately 6.7% to 6.0% between 2006 and 2010.[5,6] The decline in mortality is due in part to better control of most of the traditional risk factors, that is, reduced smoking rates and better pharmaceutical control of hypertension, diabetes, and the dyslipidemias.[6–9] In addition to controlling risk factors, various technologies for treating CHD patients have undergone tremendous improvements over the same time frame and account for as much as one-half of the total decline in mortality.[10–13] However, the traditional risk factor obesity has increased in prevalence, and risk behaviors such as physical activity and dietary patterns have been difficult to improve given current trends in technology, automation, and food production.

From the series of American Heart Association (AHA) updates of heart disease and stroke statistics from 2007 through 2012 (and others), there appears to have been a slight decline in overall prevalence among whites (6.6% to 6.4%) and Hispanics (6.0% to 5.2%), but a moderate increase among blacks (5.2%–6.2%) has been observed. As is expected, the prevalence increases with age from a low of 1.2% for 18–44 years to 7.1% for 45–64 years and 19.8% above 65 years, with males incurring a higher prevalence than females prior to menopause. In this same time frame, there has been a relatively large decline in the incidence of CHD in 35- to 44-year-old men from approximately 7% to approximately 3%, whereas incidence among 85- to 94-year-old men has remained essentially the same.[3,7,14–19] Interestingly, these statistical updates also reveal that there has been a consistent reduction in the prevalence of traditional risk factors in the population over this same time, especially among younger persons. This younger age group has been on the receiving end of intensive public campaigns against smoking; against eating high-fat diets; and for getting checked and treated for hypertension, hyperglycemia/diabetes, and hyperlipidemias throughout most of their lives. The observed reduction in prevalence of these factors probably demonstrates some success for the public health education initiatives. Both physicians and patients are certainly far more aware of the importance of managing the traditional risk factors. The lack of change in incidence among the older population is more than likely a reflection of many years of poor lifestyle habits throughout most of their early lives prior to the public health initiatives and might indicate that attention to risks later in life cannot significantly alter the progression of well-developed disease.

Two other observations from these statistical updates are probably the most important from the perspective of this chapter. The first is that the percentage of Americans who participate in any physical activity has steadily declined among all ethnic groups since the 1990s; in the 2012 update, 33% of adults reported no activity at all with

half of all adults not meeting either the aerobic or the strength guidelines. As is well known, self-reports of physical activity tend to be exaggerated, with men and women reporting about 44% and 138% greater activity, respectively, than they actually perform.[20] Because the majority of surveys on activity, diet, and health issues rely on self-reported data, such discrepancies are common and it certainly is possible that the actual increases in inactivity-based risks published may be much worse than reported. The second observation is with respect to diet. There has also been a steady increase in the reported consumption of total calories, 22% for women and 10% for men, between 1970 and 2005 and a huge increase in obesity rates during the same period. As with physical activity, self-reported dietary intake of calories is often underreported by between 15% and 25% nonobese controls compared to actual measurements using the double-labeled water technique, with obese and diet-resistant groups underreporting intake from 38% to as high as 59%.[21] Thus, the actual increase in caloric intake between 1970 and 2005 may be higher than the reported 10% (male) and 22% (female). The prevalence of obesity in men increased from 12.2% in 1971–1974 to 20.5% and 33.3% in 1994–1999 and 2005–2008, respectively. In women, the prevalence increased from 16.8% to 26.0% and to 36.2% in the same time periods. Obviously, a decline in physical activity combined with an increase in caloric intake contributes to weight gain and increase in obesity rates. Although small declines in total fat and protein intake and an increase in carbohydrate intake have occurred over the same time frame, diet has changed very little overall in terms of fruit, vegetable, and whole-grain consumption and the average American still consumes far less than the minimum recommended servings of each. On the basis of these data, the overall reduction in mortality from atherosclerosis to date is mostly due to a combination of better surgical treatment of symptomatic atherosclerosis and more effective pharmaceutical management of hypertension, hyperglycemia, and hyperlipidemias.

While factors associated with both diet and exercise impact many chronic diseases, including atherosclerosis, none of these factors has changed in a beneficial direction over the last four decades. One possible contributor is an emphasis on the importance of medical management of the traditional CHD risks while minimizing the impact of diet and exercise. For example, who has not seen the commercial on American television for a particular cholesterol-lowering drug that starts with a phrase something along the following lines: "If diet and exercise are not enough to lower your cholesterol… you need ___"? The implication of such public advertising is that cholesterol is the major cause of CHD risk and that proper exercise and diet have no real protective effect other than lowering cholesterol. Although many in the medical and health professions have decidedly different ideas on this compared to the pharmaceutical companies who are trying to sell a product, the negative effect on public opinion is still the same. In a related area of public discourse, isolated culprits such as high-fructose corn syrup (HFCS) and refined sugar have currently got significant blame for the rising obesity rates, all the while overlooking the fact that these diseases are multifactorial. As with the mistaken belief that a single food component can be the major reason for the protective effects of a diet, blaming a single dietary component for obesity and chronic diseases is open to the same level of criticism. In addition to the somewhat one-sided publicity, perhaps there is also a little bit of a general misunderstanding on just how important proper diet and exercise are in reducing risk for CHD.

4.1.1 Exercise, Diet, and Atherosclerosis

In the research literature, physical activity and exercise training have consistently been observed to be a factor in reducing risk for atherosclerosis even though the mechanisms of the protective effect have not been well described. Inverse associations have been observed between physical activity and both CHD and CHD mortality.[22,23] Relative risks (RRs) for CHD appear to be between 1.5 and 3 times higher for inactive persons compared to active,[24–27] and physical activity is comparable to conventional risk factors in the ability to predict risk.[28,29] Results of meta-analyses of available clinical studies, as well as reports from the Council on Clinical Cardiology of the AHA, clearly support the effects of physical activity and exercise on CHD risk reduction in both men and women, including those with heart failure and peripheral arterial disease.[30–33]

Among active persons, the level of fitness also appears to be associated with risk. High levels of cardiorespiratory fitness, as measured by VO_2 max, are associated with a slower progression of existing atherosclerosis and with decreased mortality in both women and men.[34–38] In a meta-analysis of studies on fitness level and CHD risk, the RR for CHD events for the least fit subjects was 1.56 and for the mid-level fit individuals it was 1.40 in comparison to those with the highest level of fitness.[30] In a similar manner, reductions in RR of between approximately 20% and approximately 45% between most fit and least fit subgroups are commonly seen with the degree of reduction being directly related to the weekly levels of physical activity.[39] Interestingly, in the Health Professionals' Follow-Up Study, men who trained with weights for at least 30 min/week also had a 25% reduction in CHD risk.[40] This effect was independent of the general reduction in risk afforded by other forms of exercise, and they suggested this might be associated with the ability of strength training to enhance glycemic control. They also observed a reduction in risk with moderate- to low-intensity exercise that appeared to be associated with improvements in lipid profile, with additional reductions in RR occurring with more intense and longer duration exercise. These additional RR reductions appeared to be due to mechanisms other than changes in lipids or glucose levels. Although not known at the time, the additional protection observed was most likely afforded by the anti-inflammatory effects of longer term moderate-intensity exercise.

The association between diet and CHD was also investigated throughout the 1900s, although the vast majority of research focused on dietary components and their effects on serum cholesterol throughout much of the century.[41] A variety of feeding studies as well as clinical and epidemiological studies support the concept that saturated fat and dietary cholesterol increase serum cholesterol and are associated with CHD mortality.[42–45] Keys' Seven Countries Study examined risk factors for CHD in over 12,000 men, and both the average population intake of saturated fat and the changes in average serum cholesterol levels were found to be strongly related to CHD mortality rates. The Framingham Heart Study and Multiple Risk Factor Intervention Trial also emphasized the relationship between serum cholesterol, especially low-density lipoprotein cholesterol (LDL-CHOL), and CHD.[46–48] In comparing the U.S. experience with that of China, fat intake was less than 0.5 that in the United States, fiber intake was 3 times higher than that in the United States,

animal protein intake was approximately 10% of the U.S. intake, and serum total cholesterol (total-CHOL) was 127 mg/dL in rural China versus 203 mg/dL in the United States for adults aged 20–74 years. Mortality due to CHD was 16.7-fold greater for U.S. men and 5.6-fold greater for U.S. women than for their Chinese counterparts. Although results such as these further cemented the idea that high-fat diets both raised serum cholesterol and were correlated with CHD, an interesting conclusion from this study that probably merited much more attention was that there was no evidence of a threshold beyond which further benefits did not accrue with increasing proportions of plant-based foods in the diet.[49] Migration studies have also provided compelling evidence for the relationship between saturated fat intake and CHD, whereas others such as the Nurses' Health Survey provided clear documentation that saturated fat intake is associated with CHD and that intake of trans-fatty acids also increases LDL-CHOL.[50–52] These early data have been confirmed by the more recent cohort studies. The Nurses' Health Study reported that saturated and trans-fatty acids are associated with an increased risk for CHD. Interestingly, intakes of flavonols were also independent contributors in explaining population differences in CHD mortality rates, suggesting that low-density lipoprotein (LDL) oxidation may also be critical to the progression of atherosclerosis.[53]

All this evidence of associations between diet, serum cholesterol, and CHD risk must be qualified, however, by the fact that they are based on overall associations observed in populations and that these associations do not demonstrate cause. When these same values of cholesterol or fat consumption and serum cholesterol are correlated within individuals or compared between individuals who have CHD and those who do not, the associations do not hold up. In a variety of comparison studies (Framingham, Tecumseh, Honolulu, and Puerto Rican men), essentially no associations were observed between serum cholesterol and dietary consumption of either fat or cholesterol and differences in serum cholesterol between patients and nonpatients were minimal.[44] This does not mean that no association is possible between cholesterol and CHD; rather, this illustrates the difficulties in interpreting general associations observed from population-based data as causal.

Other components of diet also have garnered some attention, most notably the type of carbohydrate that has been associated with CHD risk. Refined carbohydrates are highly processed, resulting in the removal of some or most of the fiber, vitamins, minerals, phytonutrients, and essential fatty acids. Consumption of refined carbohydrates in comparison with whole-grain sources is associated with increased risk for CHD;[54,55] this is possibly related to the increased glycemic load of these types of carbohydrates.[56] Increased fiber consumption also is inversely related to both CHD and all-cause mortality; it also lowers LDL-CHOL levels and improves insulin sensitivity.[57–61] However, the direct effects of fiber per se on these parameters are very difficult to determine because it is the consumption of fruits and vegetables that provides most of the fiber and in many studies it is the consumption of fruits and vegetables (along with their phytochemicals) that is associated with a decreased CHD risk.[62–64] Additionally, while moderate consumption of protein is associated with a reduced risk of CHD, lower protein diets tend to be higher in fruit and vegetable intake.[65]

Other diet-related risks also reveal the potentially protective aspects of a healthy diet. In the Nurses' Health Study cohort, in which 84,129 women aged 30–55 years

were enrolled and followed up for 14 years,[66] a healthy lifestyle was defined as not smoking, consuming at least half a drink of alcoholic beverage per day, engaging in moderate to vigorous physical activity for >30 min/day, and a body mass index <25 kg/m^2. The study also defined a healthy diet as one that included components such as cereal fiber, marine *n*-3 fatty acids, folate, low trans-fatty acids, and low glycemic load foods. Adherence to these factors correlated inversely with 14-year CHD incidence. Others have noted that 82% of CHD events could be prevented by a combination of physical activity and diet, providing evidence for an additive or a synergistic effect.[66] Comparing Mediterranean-type diets to the American diet reveals that consumption of vegetables, fruit, legumes, whole grains, fish, and poultry is associated with a decreased risk of CHD, whereas the typical American diet patterns with higher intakes of red and processed meats, refined grains, sweets/desserts, high-fat dairy products, and low fruit and vegetable intake are associated with increased risks for CHD, risks that are independent of other risk factors.[67,68]

From the various studies conducted over the last 40 years or more, it is clear that performing regular physical exercise and consuming a proper diet are associated with a reduction in risk for CHD. Although such associations are important, they do not define how these lifestyle risk factors affect mechanisms of cause. Even though there are many clinical studies that demonstrate that various dietary and exercise treatments reduce risk factors for CHD, factors such as serum lipids and cholesterol, such studies address only a variable that is correlated with disease and not a mechanism of cause. In addition to a lack of attention to mechanisms of cause, none of the aforementioned studies conducted nutritional assessments to determine the nutritional status of their subjects. With the majority of Americans consuming insufficient amounts of one or more of the essential nutrients, there is sufficient cause to suspect that nutritional insufficiencies and deficiencies are serious confounders that are not addressed in these studies.[69–71] The only way to understand why any of these observed associations might exist is through a thorough understanding of the molecular etiology of atherosclerosis. From that point, the mechanisms through which dietary components and exercise alter the causal mechanisms will reveal how these lifestyle variables reduce risk for atherosclerosis.

4.2 MOLECULAR ETIOLOGY OF ATHEROSCLEROSIS

4.2.1 Stress Response or Adaptation

Although macrophages were recognized to be components of atherosclerotic lesions as early as the 1930s, the predominant focus of CHD research in the early 1980s was on lipoproteins and cholesterol; lipid-transport function and metabolism; and how various pharmaceutical, dietary, and other lifestyle factors could affect serum cholesterol.[41] This was in spite of the fact that the response-to-injury hypothesis for the pathogenesis of atherosclerosis was first published by Russell Ross in 1973, a hypothesis that was developed further in 1986 and expanded again in 1993 and 1999 into what has become the currently accepted inflammatory model for the etiology of atherosclerosis.[72–75] Even though the major emphasis of most CHD research through the 1980s was still on factors associated with serum cholesterol, a plethora of new information on

the cell biology of inflammatory responses made it possible to recognize the complex relationships between the multiple factors that alter cell function and the subsequent development of inflammation-associated cellular responses and their association with atherosclerosis.[41] Since that time, a massive amount of information has been developed to implicate inflammation-associated mechanisms that are initiated in various cells within the vascular wall as the cause. These mechanisms are in response to shear- and pressure-associated hemodynamic forces in addition to oxidized lipids, reactive oxygen species (ROSs) from various exogenous and endogenous sources, and glucose and fructose, implicating all of them as components of cause.[41,75–89] In addition to these factors, both innate and adapted inflammatory processes that follow viral and bacterial infections have been proposed to contribute to the process.[75,76,90–97]

An important concept relating to the mechanisms of cause is whether the inflammatory mechanisms that ultimately lead to CHD are a response to abnormal tissue damage and stresses or a component of an adaptive response to normal cellular stresses. The distinction is a conceptual one because if CHD (in the context of disease processes) is a result of normal adaptive functions initiated by ongoing (normal) cellular stresses then it is simply not preventable; it is only modifiable. What this means is as follows: although the mechanisms of the disease cannot be completely stopped, they might be attenuated to the point that symptomatic disease does not occur. From a tissue damage perspective, endothelial cells of the arterial circulatory system are on the receiving end of potentially damage-inducing agents. For example, molecules with accessible carbonyls, such as the aldehyde of open-chain glucose and the ketone of open-chain fructose, can form a Schiff base with available amino groups on membrane proteins and amino-phospholipids and initiate the formation of advanced glycation end products (AGEs).[87,98,99] These products are involved in causing cellular damage by enhancing rates of lipid oxidation in membrane phospholipids and in lipoproteins, as well as activating proinflammatory signaling pathways by interacting with AGE receptors (RAGEs) on vascular endothelial cells, macrophages, and smooth muscle cells.[84,85,100,101] As a result, glucose and fructose have been implicated as important components of atherosclerotic risk because they can lead to membrane damage, lipoprotein oxidation, and proinflammatory responses, processes that certainly help explain the greater risks for atherosclerosis in diabetics.

Atherosclerotic plaque, however, does not form equally in all locations throughout the arterial circulation, even though arterial concentrations of glucose and fructose are essentially the same everywhere. In atherosclerotic patients, the plaque forms predominantly in the wall of arteries at bifurcations, including immediately distal to or at the leading edge of the bifurcations themselves, on the lee side of branch points. The plaque also forms on the inside curve of sharply curved stretches, especially in the aorta and coronary arteries, carotid arteries, and the femoral and kidney vasculature.[83,102–104] It is relevant to consider that the distribution throughout the arterial system of all major risk factors such as glucose, fructose, hypertension, cholesterol, and various lipoproteins and any prooxidant molecules originating from the environment is essentially the same everywhere. With an equal distribution of damaging agents, there must be nondamaging factors involved in initiating inflammation-associated responses to produce such large differences in atherosclerotic plaque formation at different vascular locations.

The major factors that differ at the different locations of the arterial system are those of blood pressure and blood flow hemodynamics. The obvious difference in pressure is the gradient in blood pressure that exists from the aorta through to the capillaries in order to ensure circulatory flow, and it happens that the atherosclerosis-prone areas also tend to be in the zones of higher pressure. The tiny localized changes in vessel diameter that constantly regulate local pressures and flow characteristics in different regions of specific blood vessels are not always so obvious. As requirements for local flow change, vasoconstriction or vasodilation responses through the regulation of smooth muscle contraction occur at the local level. With changes in physical forces at these local sites, alterations in strength of the vessel wall might be necessary in order to handle the unusual stresses. Structural differences in vessels also contribute to differences in local hemodynamics; the presence of various arterial branch points, bifurcates, and sharply curved sections contributes to differences in shear forces and to the extent of laminar/turbulent flow throughout the vasculature. A much lower (relative to immediately adjacent areas) average shear stress that oscillates in response to the cardiac cycle, and in some cases includes turbulent flow, is characteristic of these locations. The arterial walls adapt to these low oscillating shear forces and turbulent flow, and to increases in tensile stress (stretching due to pressure), through a localized hypertrophy response, and the thickened vascular structure is characterized by a greater abundance of smooth muscle cells and elastic fibers.[82,83,102,103,105,106] The additional smooth muscle cells and connective fibers are necessary to strengthen and modify the shape of that specific region of the artery to more efficiently respond to hemodynamic conditions and to ultimately ensure adequate blood flow. It is these hypertrophied regions that also happen to be the major sites of atherosclerotic plaque development, indicating that this adaptive response is fundamental to the CHD process.

Because the thickening of arterial wall does not occur in regions of high laminar shear forces, it most likely represents a response to low shear forces. This is logical because low shear would indicate low flow and the hypertrophy response is an attempt to enhance flow through the artery by slightly narrowing the diameter (and increasing the pressure) at that point. In response to increased tensile strain due to higher blood pressures, hypertrophy of the arterial wall also occurs.[103] This increase in strength of the artery reduces pressure-induced stretching and distortions in blood vessel shape that would disrupt laminar flow and induce turbulent flow immediately distal to that location, potentially compromising flow. Thus low shear, turbulent flow, and increased tensile forces are logical stimuli for initiating changes in arterial structure in order to ensure adequate perfusion of downstream tissues. One could imagine that from around the second trimester of pregnancy and throughout growth to late childhood the constant growth of the heart and vascular system would produce constant demands for continual arterial remodeling and angiogenesis in order to handle the changing growth, pressure, and flow demands of the growing individual. Results of many cadaver studies demonstrate that an initial thickening at the susceptible sites is evident by the late stage of fetal development and at a few months following birth, with the extent of the remodeling gradually increasing throughout growth to produce arterial thickening in a pattern of locations that matches the pattern of atherosclerosis-prone areas.[103,107] In many cases

fatty lesions appear at these same sites in the late stage of fetal development and very early childhood, and then they progress throughout childhood to become a lot more extensive around the time of puberty. This demonstrates that what happens in utero and soon after birth may play a role in the development of atherosclerosis. It is relevant that some of the cadaver studies were conducted prior to the 1970s because these early studies indicate that fatty lesions were common in children long before our current diabetes and obesity "epidemics" and other potential risks ever became significant population-wide issues. Thus, the initiating CHD processes appear to be the result of an adaptive stress response to mechanical signals during periods of fast growth that are *interpreted* as insufficient blood flow and not to damage per se. The development of CHD in adults might therefore be a really unfortunate side effect of a mechanical stress response that is designed to ensure adequate perfusion of the heart. Of course, this does not mean that inflammation-associated mechanisms cannot add to the disease process because they certainly do. It is no coincidence that these hypertrophied areas have approximately a threefold increase in the number of tissue macrophages within the intima compared to the intima of other locations with higher and constant shear forces, making them far more sensitive to any subsequent damage or infection-associated proinflammatory stimuli.

Although such a concept may seem unreasonable, it is certainly logical from an evolutionary standpoint. A localized adaptive response to hemodynamics that guarantees adequate blood flow for the organism is a highly useful phenomenon when it comes to ensuring the demands of growth and development of a 10 g organism at approximately 10 weeks gestation to a 40+ kg adolescent individual. Even though the side effect of the response is a gradual buildup of plaque in susceptible locations of the vasculature that might ultimately be fatal, it happens slow enough to guarantee that the individual survives more than long enough to produce progenies and raise them to independence. Thus, a potentially fatal adaptive response that guarantees short-term survival of the individual ultimately has no detrimental effect on species survival.

4.2.2 Adaptation

Although every detail of the activation processes are not the major focus of this chapter, a summary of the important concepts is described. If low oscillating shear forces are to activate a hypertrophy response within the arteries, then mechanoreceptor complexes in the various vascular-associated cells (endothelial cells, smooth muscle cells, platelets, fibroblasts, and tissue macrophages) would be necessary. A variety of surface proteins are capable of responding to mechanical stimulation, including the heparin sulfate proteoglycan components of the glycocalyx, caveolae, G-protein-coupled receptors, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, phosphatidylinositol 3 (PI3)-kinase, calcium channels, potassium channels, vascular endothelial growth factor (VEGF) receptors, platelet-derived growth factor (PDGF) receptors, platelet endothelial cell adhesion molecules, integrins, intracellular junctional proteins, and primary cilia.[108–120] Of these, most are cell surface proteins that are involved in some form of cellular regulatory function, whereas the integrins and junctional proteins are more structural in that they ensure

cell–cell and cell–extracellular matrix connections. All have the ability to respond to changes in their three-dimensional structure from stretch, torsion, and tension forces that are caused by shear forces and by transluminal pressures. Proteins associated with the proteoglycan component of the glycocalyx, such as the syndecans, have an extracellular glycosaminoglycan component that extends into the glycocalyx as well as a cytosolic component that is physically linked to both G-protein receptors and actin filaments. These syndecans ensure that mechanical forces experienced by the cell surface are efficiently transduced to both regulatory and cytoskeletal structures within the cell. The integrins and junctional proteins ensure that the mechanical forces on the surface of cells are transduced to other cells and to structures they are in contact with, including the smooth muscle cells adjacent to the extracellular matrix. Thus, there is an extensive network of structures that not only responds to physical forces and ensures that the forces are transduced throughout the arterial wall but also can activate a variety of regulatory proteins that then affect cell function.

Repeated stretching/relaxation of the ion channels will allow the entry of calcium and potassium into the cell while stimulation of primary cilia on the surface membrane of endothelial cells activates the opening of cilia-specific as well as polycystin-1 (cilia-associated protein)-activated calcium channels. The entry of calcium stimulates a variety of cellular effects, including the activation of protein kinase C (PKC) and eNOS, with the resulting NO• activating protein kinase G (PKG) as well as inducing vasodilation.[121,122] The elevated calcium also activates myosin light chain kinase to induce constriction of the vascular epithelial cells[123,124] while the enhanced PKC and PKG activities activate Rho-GTPases, which interact to induce the phosphorylation of VE-cadherin. This leads to the disassembly of adherens junctions and tight junctions between endothelial cells and an enhanced permeability of the blood vessels.[124] Although these responses are classically associated with damage-associated inflammatory responses, they also occur in response to shear stress that is independent of any damage. The increased cytosolic calcium also will activate phospholipase A_2 (PLA_2), calcineurin, adenylate cyclase, phosphorylase kinase, and Ras, leading to the activation of the p38–mitogen-activated protein kinase (MAPK), JNK–MAPK, extracellular signal-related kinase (ERK)–MAPK, and nuclear factor κ-β (NFκβ) pathways as well as PI3 kinase (PI3K).[113,125–132] As a result of activation of these pathways, expression of the angiogenesis factors PDGF, VEGF, and endothelin-1 as well as monocyte chemoattractant molecule-1 (MCP-1) is enhanced. Mechanical strain of VEGF and PDGF receptors also activates them in a manner independent of ligand binding, which adds to the growth factor–mediated increase in smooth muscle cell proliferation and fibrin deposition that ultimately enhances the strength of the vascular wall at the specific site of high shear stress.[108,110,133] The disassembly of the cell–cell connections between endothelial cells and their proliferation that occurs in response to autocrine stimulation are events that contribute to the hypertrophy/angiogenesis responses that would be essential for the growth of arteries and heart. Thus, mechanical activation of various cellular structures activates a hypertrophy response that utilizes the same stress-response pathways that occur with inflammation-associated angiogenesis. A response that reiterates the general concept that, as described in Chapter 2, inflammation-associated responses are not necessarily a component of the (innate) immune system but rather are a series of

cellular responses that make up a generalized stress response that can be activated by mechanical or metabolic stresses, or by either sterile damage or pathogen-induced damage. While the desired hypertrophy and angiogenesis responses occur following a mechanical stress-induced stress response, the signaling responses to mechanical stress unfortunately include the same signaling pathways that are involved in what is commonly known as an inflammatory response.

There is, however, a conundrum built into the aforementioned process; if shear stress induces a hypertrophy response, then logic might dictate that the hypertrophied areas should exist at locations of high shear stress and not where low (and oscillating) shear exists. To address this, comparing cellular responses to constant high shear stress with those in response to low oscillating shear forces reveals why low oscillating shear is hypertrophic/angiogenic (and atherogenic) while constant high laminar shear is antiatherogenic.[82,105,109–111,113,134–140] From these studies, there is a consistent pattern of activation of Ras and the various MAPK and NFκβ pathways by mechanical shear stress that leads to increased expression of PDGF, VEGF, MCP-1, vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1, E-selectin, tumor necrosis factor (TNF)-α, interleukin (IL)-1, cyclooxygenase (COX)-2, and interferon-γ (INFγ) as well as other molecules that are associated with inflammatory responses (see Chapter 2 for details on these). A major difference between the two shear conditions is that with constant high laminar shear stress the responses are only temporary. Over a period of several seconds to hours, there is a downregulation of Ras and MAPK pathway activities and MCP-1 synthesis via an increased synthesis of Iκβ and activation of Nrf2, both of which inhibit the activation of NFκβ. In addition, a sustained expression of eNOS, COX-2, extracellular superoxide dismutase (eSOD; CuZn-SOD), cytosolic SOD (cSOD; CuZn-SOD), mitochondrial SOD (Mn-SOD), and the transcription factors heme oxygenase (HO)-1 and Kruppel-like factor 2 (KLF2) occurs. The enhanced synthesis of eNOS ensures that the blood vessel has an enhanced capacity for synthesizing NO• for essential vasodilation responses, whereas the increased synthesis of Iκβ and activation of Nrf2 ensures that NO•-mediated activation of protein kinase B (PKB) does not contribute to a continued progression into a full-blown inflammatory stress response. With the resulting suppression of NFκβ and the MAPK pathways, the subsequent expressions of PDGF, VEGF, MCP-1, vascular endothelial adhesion molecule, intercellular adhesion molecule, E-selectin, TNF-α, IL-1, and INFγ are reduced to the original nonactivated levels and in some cases much lower. Increased expression of the transcription factor KLF2 leads to alterations in the expression of a variety of proteins that are involved in regulating the basic phenotype of endothelial cells, including a reduction in the expression of both chemotactic ligands and their receptors, resulting in decreased activation and attraction of inflammatory cells in the immediate vicinity into the vessel wall. Other KLF2-responsive proteins are involved in the development of the overall morphology of cells such that they elongate and realign their longitudinal axis along the axis of flow. When aligned with the flow, the shear stresses on the cell are reduced and, therefore, the activation of the mechanosensitive structures is attenuated as well. In addition, primary cilia disassemble in response to prolonged shear, which eliminates a mechanosensitive activator of calcium entry.

From this discussion, many of the alterations in stress responses observed are reminiscent of those that occur during the resolution phase of an inflammatory response (described in detail in Chapter 2). The suppression of NFκβ through the activation of Nrf2, along with an induction of COX-2 following chronic shear, also is observed during resolution. Although the effects of chronic shear on factors directly associated with resolution have not yet been delineated, it would certainly be a useful area of study in order to develop a more complete mechanistic understanding of the apparent anti-inflammatory effects of chronic shear stress. From the observed sustained induction of COX-2, a variety of cellular effects would be expected. One of the enzymes that synthesize prostaglandin E_2 (PGE_2), the mPGE-1 form, is highly coinduced along with COX-2.[141,142] This would result in an increased localized production of PGE_2. Induction of PGE_2 will induce synthesis of 12/15-lipoxygenase (12/15-LOX) in endothelial cells as well as in any neutrophils or macrophages in the immediate region, resulting in an enhanced production of lipoxins (lipoxin A_4 and lipoxin B_4) from arachidonic acid (AA).[143–147] Along with the lipoxins, resolvins and protectins are essential for the resolution phase with the E-series resolvins being produced from eicosapentaenoic acid (EPA) via a combination of COX-2 and constitutive 5-LOX activities.[147–150] The D-series resolvins and the protectins are synthesized from docosahexaenoic acid (DHA) via a combination of 5/12/15-LOX activities.[148–152] These resolution-activating ligands then bind to receptors on neutrophils, endothelial cells, macrophages, and monocytes[150,153] to produce their proresolution effects by attenuating p38–MAPK, ERK–MAPK, and PI3K activities and by attenuating activation and expression of NFκβ and AP-1.[141,150,153–155] COX-2 also produces prostaglandin D_2, which spontaneously dehydrates to prostaglandin J_2 (PGJ_2) and activates peroxisome proliferator–activated receptor-γ (PPARγ).[141,156,157] The increased binding of PGJ_2 to peroxisome proliferator–activated receptor (PPAR) would then reduce IL-1β and TNF-α release by macrophages and dendritic cells, inhibit toll-like receptor (TLR) signaling, and inhibit NFκβ activation.[157–160] Because Nrf2 activation has already been observed with chronic high shear stress, and PGJ_2 also can activate this factor, other resolution-associated adaptations to chronic high shear may occur. Activation of Nrf2 stimulates an upregulation of HO-1 as well as electrophile-response element (ERE) -responsive gene products such as glutathione peroxidase (GPX), SOD, catalase (CAT), glutathione-*S*-transferase, and UDP-glucuronosyl transferase (UDP-GT).[159,161–165] From these, it is tantalizing to hypothesize that what appears to be an adaptive response to chronic high laminar shear is actually the ultimate expression of a chronic resolution-phase phenotype.

The endothelial cells in blood vessel walls therefore respond to chronic high laminar flow in such a manner that they are less affected by shear stress because of changes in cellular morphology and they also are far less responsive to any stress because their stress-response signaling pathways are inhibited. Unfortunately, under low oscillating shear stress the cells in that localized region retain the ability to continue the mechanically induced stress response for as long as the interrupted flow cycles remain and none of the protective responses occur.

Thus, atherosclerosis is caused by inflammation-associated mechanisms that are initiated by mechanical shear stresses and then exacerbated by the various proinflammatory mechanisms associated with risk factors such as hypertension, obesity, hyperglycemia, smoking, inactivity, poor diet, and dyslipidemia.

4.2.3 Stress-Response Events in Atherosclerosis

As described in Section 4.2.2, the mechanical stimulation by oscillating shear forces activates the stress-response activities of the p38–MAPK, JNK–MAPK, ERK–MAPK, and NFκβ pathways as well as PI3K.[113,125–132] Each of these stress-response actions comprises mechanisms that are classically associated with an inflammatory stress response and are described in detail in Chapter 2. Following the activation of these pathways, a variety of cellular responses occur, including (as listed in Section 4.2.2) increased expression of PDGF, VEGF, MCP-1, VCAM, ICAM, E-selectin, TNF-α, IL-1, and INFγ as well as increased PLA_2 and adenylate cyclase activities.[82,105,109–111,113,134–138]

Activation of PLA_2 enhances the availability of AA, leading to the production of PGE_2, prostaglandin I_2 (PGI_2), thromboxane (TX) A_4, leukotriene (LT) A_4, and platelet-activating factor (PAF) through COX and LOX activities and the various prostaglandin (PG), LT, and TX synthase activities.[84,141,166–168] The increase in PGI_2 and PGE_2 causes localized vasodilation via the activation of G-coupled IP and EP2/EP4 receptors.[121,166,169] Although the localized vasodilation effects may not be contributing factors to the atherosclerotic process, the activation of the G-protein itself and the subsequent activation of PKG are certainly important from a signaling standpoint. An increase in vasopermeability occurs following the disassembly of adherens junctions and tight junctions, which, along with the increased expression of MCP-1, VCAM, ICAM, and E-selectin, leads to the attraction and entry of monocytes into the local area. The vasopermeability and attraction of monocytes are then responsible for the approximately threefold greater concentration of tissue macrophages in areas of low oscillating shear forces observed in infants and young children, as already mentioned.

The processes that lead to the formation of plaque are, of course, the fundamental processes of atherosclerosis. It stands to reason that as long as the minimal processes that actively produce it continue to occur, progression to an atherosclerotic state would be the ultimate result. It does appear that mechanically induced stress response is minimally necessary to initiate the production of the first observable lesion in the development of atherosclerosis: the type I fatty lesion; the presence of this lesion in a fetus attests to that.[103,107] The subsequent progression of type I lesions to type II through to the more clinically relevant type IV and potentially fatal type V and type VI lesions is then dependent on a continuation of the same initiating signaling processes as well as an acceleration of these processes by a variety of factors that are commonly associated with classical risks for the production of the advanced lesions,[41,72–89] with the possible inclusion of factors associated with infection.[75,76,90–97]

The initial development of plaque is largely a consequence of macrophages *consuming* lipids and their conversion into foam cells, thus creating the initial fatty streaks. The uptake of lipids by macrophages depends on scavenger receptor–mediated endocytosis of LDLs, with the major scavenger receptors that are highly relevant for atherosclerosis being the class A and CD36 forms.[170–174] Both of these receptors are activated by oxidized and acetylated LDL proteins as well as oxidized cholesterol (CHOL-Ox), and they account for the majority of lipid uptake by macrophages. Both scavenger receptors also recognize a variety of bacteria,

microbial lipopolysaccharides and diacylglycerides, apoptotic cells, and AGEs, indicating that the major scavenger receptors responsible for lipid uptake and conversion of macrophages to foam cells are pattern recognition receptors (PRRs). Activation of CD36 by oxidized LDL and of oxidized CHOL leads to uptake of lipids and subsequent foam cell formation and also leads to JNK–MAPK activation of a variety of AP-1 subunits, activities essential for activating the synthesis of proinflammatory cytokines in macrophages.[174–176] Oxidized LDL also activates TLR4 and TLR6 while AGE-LDL activates TLR4, leading to the activation of JNK–MAPK, p38–MAPK, AP-1 subunits, and NFκβ, resulting in increased TNF, IL-1, and IL-6 synthesis.[177,178] Thus, uptake of oxidized and glycated LDL and CHOL by scavenger receptors on macrophages activates an inflammatory stress response through signaling pathways that are commonly activated by pathogens and damage and adds to the already existing mechanical activation of these signaling pathways. The presence of CD36 in endothelial cells and in smooth muscle cells ensures that these cells also participate in the production of proinflammatory signaling molecules through the same mechanism.[170,176,179]

The major mechanism missing from the aforementioned stress responses is a process that can enhance ROS-mediated oxidation of LDL and of CHOL. Further, it is the mechanical stress–mediated activation of G-proteins and their associated NADPH oxidase activity, as well as the direct activation of NADPH oxidase by the same mechanical stress, that provides the necessary mechanism.[109,110,113,114,117] The immediate effect of stimulating G-proteins is the activation of their NADPH oxidase component on the cell membrane. Further, whether activated by mechanical stress or via G-protein stimulation, the oxidase generates superoxide ions outside the plasma membrane. The superoxide anions react with one another to produce peroxide and oxygen $O_2^{\bullet-} + O_2^{\bullet-} \rightarrow H_2O_2 + O_2$, where the peroxide can either diffuse into the cell or react with other superoxide ions to produce hydroxyl radicals, $O_2^{\bullet-} + H_2O_2 \rightarrow {\bullet}OH + HO^- + O_2$. The resulting •OH can then oxidize any LDL or CHOL (or any other molecules) in the immediate vicinity. The $O_2^{\bullet-}$ produced also can react with NO• to produce damaging nitroxide radicals, enhancing oxidative damage to LDL. In contrast to low oscillating shear, the increased expression of eSOD observed with chronic laminar shear stress ensures that activation of the NADPH oxidases does not necessarily result in enhanced ROS-mediated damage.

The last remaining issue is how to increase the entry of LDL into the vascular wall at the site of mechanical stress. This is accomplished through the mechanical stress response that enhances PKC, PKG, and Rho-GTPase activities that ultimately lead to the disassembly of adherens junctions and tight junctions between endothelial cells and enhances the permeability of blood vessels. While under noninflammatory conditions it is predominantly the smaller LDL particles that can penetrate the endothelial layer of the vessel wall to gain access to the tissue cells, the increased vasopermeability ensures that additional LDL particles can penetrate the layer in larger amounts to ultimately increase the concentration of LDL particles in the local region of the vessel wall. Thus, low oscillating mechanical shear stress activates all the requisite mechanisms that are necessary for the formation of foam cells: greater local concentration of tissue macrophages (via mechanical stress–induced inflammatory stress response), greater localized concentration of lipoproteins (via

increased vasopermeability), and greater production of extracellular ROSs (via enhanced NADPH oxidase activity). Further, because the uptake of oxidized LDL/CHOL is mediated by scavenger PRRs, the mechanical stress–induced inflammatory responses are enhanced via PRR-mediated inflammatory signaling.

Because nonoxidized LDL proteins, cholesterol, and other lipids are not recognized by these scavenger receptors, it follows that cholesterol per se cannot be an initiating cause of the CHD processes. When oxidized LDL (LDL-Ox) proteins activate the scavenger receptors, uptake of the lipids that are part of the proteins is coincidental, not causal. Even when CHOL-Ox activates the scavenger receptors, the cholesterol itself can be viewed as a somewhat passive component of the process; but for the oxidizing process, it would not happen. Thus, oxygen radical mechanisms are essential to initiating events that lead to foam cell production, not cholesterol per se. LDL-CHOL may not be the direct initiating cause; it is, however, a risk factor. Increased levels of LDL-CHOL in serum obviously provide increased entry into the immediate area and therefore a greater numbers of targets for LDL and CHOL oxidation, thus explaining the general trend for an increase in risk as LDL-CHOL levels increase. At the same time, LDL-CHOL is one among many integrated risk factors, all of which affect one or more of the cellular mechanisms that are activated by mechanical stress. Thus, the large variations in associations between LDL-CHOL and CHD that are observed among different socioeconomic groups, cultures, and countries are logical.[43,180–183] One of the more well-known differences is that originally reported by Serge Renaud[183] between France and the United States, with mean cholesterol levels being approximately 15% greater in the French and CHD deaths per 100,000 being approximately 37% of those in the United States. The greater levels of cholesterol in the serum of the French with much lower mortality due to CHD is impossible to explain if cholesterol is the cause of CHD. In light of current information, there is no paradox because cholesterol does not cause CHD; there are, however, different arrays of risk factors that directly affect the mechanisms that occur in response to those of the proximate cause, and an excess of one can be outweighed by a paucity of others.

From these discussions, intermittent low shear stress can be viewed as the proximate cause that initiates CHD, whereas inflammatory signaling, along with the normal scavenging function of macrophages, mediates the various cellular responses that comprise the processes of the disease. The risk factors of smoking, dyslipidemias, high blood pressure, obesity, infections, hyperglycemia, inadequate diet, and inadequate exercise are then viewed as accessories to the same disease processes (Figure 4.1). Further, the following does bear repeating: the role of these risk factors in the CHD process should not be underestimated because while oscillating low shear stress may be sufficient for the formation of type I and type II fatty lesions these do not necessarily progress into the more clinically relevant type IV–VI lesions.[41,72–89,107,184,185]

Progression to more dangerous lesions demands continuing inflammation-associated processes beyond the formation of foam cells. Further, the expression of ICAM on vascular endothelial cells in response to the mechanical stress is an important consideration because platelets bind to the ICAM that is expressed on endothelial cells.[186,187] This binding is independent of vascular damage or of exposure to von Willebrand factors or collagen proteins. On binding to ICAM, platelets degranulate and release the platelet aggregation factors fibrinogen, fibronectin, P-selectin, and

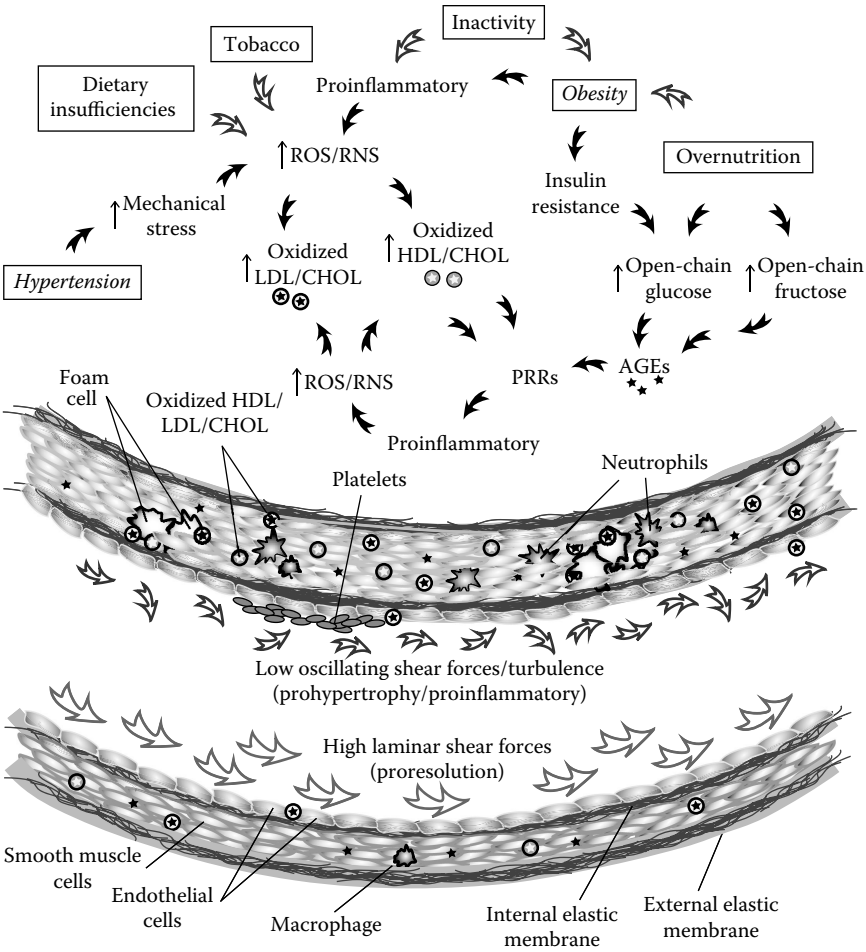

FIGURE 4.1 Proximate causes and risks for coronary heart disease: the activation of proinflammatory and prohypertrophy signaling by mechanical disturbances of vascular cells due to low oscillating shear forces and turbulent flow creates a sensitive proinflammatory environment within the cells located at bifurcates and inside the curves of arteries. The initial infiltration of inflammatory cells contributes to the hypertrophy–adaptation response to ensure adequate downstream flow, as well as to the formation of type I and II fatty lesions. Poor diet, overeating, and inadequate physical activity lead to poor redox control in cells, increased formation of advanced glycation end products (AGEs), increased oxidation of high-density lipoprotein (HDL)/low-density lipoprotein (LDL)/cholesterol (CHOL), increased blood pressure, and greatly enhanced pattern recognition receptor (PRR) activation with subsequent proinflammatory signaling, greatly increasing the risk for progression to the potentially fatal type V and VI lesions.

TXA_2, as well as PDGF; transforming growth factor β (TGFβ); epidermal growth factor; basic fibroblast growth factor; and the chemokines RANTES, platelet factor 4, and IL-8 (among many others).[186–188] These factors are very important for the formation of blood clots as well as for attracting fibroblasts, smooth muscle cells, and additional tissue macrophages into the immediate area for wound healing and

angiogenesis; other cells such as dendritic cells, mast cells, and T cells also can be attracted to the immediate region by the same chemokines. All these factors are responsible for the expansion of the asymptomatic type I, II, and III lesions into the more advanced type IV, V, and VI atherosclerotic lesions that extend into the vascular lumen, lesions that are characterized by extensive smooth muscle cell proliferation as well as the presence of fibroblasts, dendritic cells, mast cells, macrophages, T cells, neutrophils, aggregated platelets, and increased fibrosis in addition to more numerous foam cells.[90,92,184,188,189] In this context, the proinflammatory role of the CD36 PRRs in the vascular endothelial cells (and the smooth muscle cells as well) is an important consideration in the atherosclerotic process. The endothelial cells not only provide a source of proinflammatory signals that is initiated by CHOL-Ox, a source that is in addition to the sources produced by macrophages and oscillating shear stress, but also provide a direct mechanistic link to platelet activation and the subsequent acceleration of events that are necessary for the formation of advanced atherosclerotic lesions. This is important because activated platelets appear to be an essential requirement for the development of the symptomatic lesions. Continued expansion of the lesions mediated by the expanding repertoire of local inflammatory cells will not only increase the size of the lesions but also enhance their instability through increased production of CD40 from the platelets, which activates an increased expression of matrix metalloproteinases (MMPs) by macrophages, endothelial cells, and smooth muscle cells. Any resident mast cells will secrete cathepsin G, which, along with the MMPs, can degrade collagen, VE-cadherin, and fibronectin, contributing further to the degradation of the matrix and greatly increasing the risk for rupture of the plaque.[188]

From this viewpoint, primary prevention of CHD requires an emphasis on the central mechanisms of cause rather than on any single risk factor. It also suggests that dealing with all of the sources of risk is necessary because diminishing one risk does not change the fact that many other risk factors also contribute to the same mechanisms of cause. Prevention efforts must treat the disease process as a tightly interconnected network of biological mechanisms that all have significant contributions to the primary process and not focus on one or two isolated biological mechanisms to the exclusion of all others. Although research has been clear on this for many years, our clinical practices have unfortunately not caught up, which is an unfortunate phenomenon described by Claude Lenfent in 2003, who observed that in spite of the wide availability of published reports describing the unequivocal success of new therapies for the treatment of CHD, their universal adoption in the clinic within 10 and even 15 years is simply not seen.[190]

4.2.4 Coronary Heart Disease Risks

In keeping with the focus on prevention that is based on the inflammatory mechanisms of the proximate cause, the molecular contributions of each of the risk factors that enhance the mechanical stress–induced CHD processes are described. In essence, anything that enhances ROS production, causes damage, or activates scavenger or other PRRs in vascular cells will either additively or synergistically enhance the production of foam cells and the progression from type I to type VI lesions. At the same time, any inflammatory signaling molecules that enter the local area from

the circulation will also enhance these processes. In addition, conditions that lead to the inability to adequately regulate the stress responses can exacerbate the mechanisms of cause.

4.2.4.1 Low-Density Lipoprotein Cholesterol (and High-Density Lipoprotein)

As already discussed in detail in Section 4.2.3, LDL-CHOL levels in the blood provide a source of risk, predominantly through passive diffusion from the blood through the endothelial layer into the intima. Once the LDL-CHOL particles are localized to the immediate vicinity of the endothelial cells, smooth muscle cells, and macrophages, they are susceptible to attack by ROSs produced by metabolic and regulatory processes that are associated with normal cell functions. They are also susceptible to attack by any circulating oxidants that originate from exogenous sources such as food, contaminated air and water, tobacco smoke, or even some medications. LDL-Ox and CHOL-Ox are recognized by scavenger PRR receptors on the macrophages, smooth muscle cells, and endothelial cells, and on their uptake by the receptors proinflammatory signal transduction pathways are activated. Thus oxidation events are critical to the development of foam cells as well as to enhanced inflammatory signaling, and procedures that limit oxidative damage to LDL/CHOL will have profound effects on mechanisms of risk for CHD that are independent of LDL/CHOL levels.

There are a number of variables that can affect lipoprotein levels in the blood as well as in the tissues such as the genetic polymorphisms of LDL (and high-density lipoprotein [HDL]) receptors in the liver and in peripheral tissues, affecting cellular uptake. Genetic and metabolic factors associated with the synthesis of various apoproteins in the liver (and intestinal epithelia) and the subsequent packaging of different lipids (triglycerides, phospholipids, cholesterol) into the various classes of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein, LDL, and HDL also contribute to LDL/HDL-associated risks, but all of these are beyond the scope of this chapter. This is not to mean that these are not biologically relevant issues; however, changing genes is not yet a practical part of prevention and the bottom line of the LDL/CHOL-associated risk is still how much LDL/CHOL is available for oxidation. As described later, treatments that lower LDL/CHOL levels in the blood also tend to lower the risk for atherosclerosis, although their effectiveness is not assured especially in the face of other risks.

Even though pharmacology-based prevention is not a topic for this book, the use of statins (HMG-CoA reductase inhibitors) that lower serum cholesterol has yielded some interesting results. Statins are well documented to lower rates of CHD progression as well as reduce risks for cardiac events such as heart attack, sudden cardiac death, and stroke in treated groups by as much as 25%–40%, while LDL-CHOL is typically reduced between 25% and 35%.[191–194] Of interest though is the fact that in spite of the observed associations between lowered cholesterol and CHD events the lowered cholesterol may not be the mechanism behind the lowered risk. Relatively recent evidence has revealed that statins exert a profound anti-inflammatory effect through induction of eNOS, induction of KLF2, lipoxin synthesis by COX-2, PPARα-mediated repression of NFκβ, a reduced expression of MCP-1 and PAF, and lowering of C-reactive protein (CRP) levels by as much

as 36%.[195,196] The reason why this is relevant is that with cholestyramine therapy (a bile-acid binding resin to enhance cholesterol loss) similar reductions in serum cholesterol are observed.[197–200] Cholestyramine therapy is also associated with a reduction in the rate of progression of early atherosclerotic lesions to more advanced lesions. However, unlike statins, similar significant reductions in fatal cardiac events are not seen. More than likely, it is the additional anti-inflammatory effects of the statins that are responsible for the reduction in fatal events and not the cholesterol-lowering effects per se. One could argue that reducing LDL/CHOL might reduce the rates of foam cell formation to attenuate the progression of early lesions to the more extensive type V or VI lesions; but without reducing inflammatory responses to attenuate the destabilization of existing lesions, fatal events associated with the formation of thrombi will still occur, even in the smaller lesions. And there are plenty of risk factors that enhance inflammatory signaling in spite of lower LDL/CHOL.

The role of HDL in increasing the risk for atherosclerosis is discussed here only briefly. HDL levels in the blood are well known to be inversely related to risk for CHD. The protective effects are thought to be due to the presence of paroxonases and apoA-I, which reduce lipid peroxides to inhibit LDL oxidation, and the ability of HDL to promote the efflux of cholesterol from foam cells and other tissue cells and transport them to the liver.[194,201–204] HDLs are also known to attenuate local inflammation by inhibiting the formation of chemokines by vascular epithelial cells and the expression of proinflammatory cytokines by macrophages.

Such results have inspired the development of HDL-raising drugs such as Torcetrapib, Anacetrapib, and Dalcetrapib (cholesterol ester transport protein [CETP] inhibitors; CETP is associated with the transport of cholesterol from HDL to LDL, enhancing risk for CHD).[205–210] Unfortunately, as was seen with Torcetrapib, although HDLs increased and LDLs decreased, there were increases in mortality due to cardiac events and the trial was stopped due to the increase in risk. Although preliminary phase I and II trials with Anacetrapib and Dalcetrapib indicate that they do not have the same toxicity as Torcetrapib, the results are too preliminary to know if there are any actual benefits to taking these drugs. These trials, in light of the recent study by Voight and others,[211] wherein genetic mechanisms that raise HDL are not associated with a reduction in risk for cardiac events raise doubts about the (exclusively) preventive effects of HDL. This last issue deserves comment. While the pharmaceutical-based studies are based on the HDL-raising effects of the drugs, the drugs themselves inhibit an enzyme that can transfer cholesterol to LDL from HDL. This is a function of HDL that contributes to risk for CHD by increasing the cholesterol content of LDL and therefore increasing the probability of LDL-CHOL oxidation. In addition, both native and oxidized HDLs bind to CD36 scavenger receptors on both macrophages and platelets, initiating a proinflammatory (and proatherogenic) response.[212–215] Thus, HDL can participate in proatherogenic actions.

The ability of HDL to inhibit LDL oxidation is not a consistent effect; Navab and others have reported that no antioxidant effects were observed when using cocultures of human aortic endothelial cells and HDL fractions from their patients, even in patients with very high levels of HDL.[216–220] On the other hand, the ability of HDL to protect against LDL oxidation can be improved through diet plus exercise interventions. Roberts and others[221,222] have noted decreases in MPO, lipid

hydroperoxides, and HDL while an increase in protection against LDL oxidation per milligram of HDL was observed. They also documented a reduction in serum-stimulated production of $O_2^{\bullet-}$ and peroxides. With such results, wherein lower HDL levels occur at the same time as enhanced protection from LDL oxidation by the HDLs, it is clear that the concept of high HDL levels being preventive based on population-based correlations is highly problematic. It is apparent that the physical properties of HDL particles are the relevant issue and not the level in serum and that the physical properties are responsive to lifestyle alterations. Because a clear protective role for raising serum HDL levels is ambiguous, various factors associated with raising serum levels of HDL as a preventive procedure will neither be discussed nor be recommended. When the physical properties of the protective forms of HDL have been elucidated and serum levels of protective HDL can be easily differentiated from those of nonprotective HDL, they (the protective forms of HDL) can become a biomarker of risk reduction.

4.2.4.2 Diabetes and Insulin Resistance

Details of the molecular etiology of diabetes are discussed in Chapters 3, so they are not detailed here. However, this condition is an important risk for CHD because overall levels of glucose in the blood will be increased. Normally, serum glucose is under very tight control through the effects of the hormones insulin (glucose uptake by insulin-responsive cells), glucagon (glycogenolysis to enhance glucose release to the blood), and cortisol (increase rates of gluconeogenesis), and under conditions of stress both epinephrine and IL-6 can enhance rates of glycogenolysis. The overall integrated effect of these hormones that are critical for glycemic control is to maintain levels somewhere within the normal fasting range of 70–100 mg/mL (4–6 mM) and somewhat less than 180 mg/mL (~10 mM) following meals. With insulin resistance, levels of glucose in the blood remain higher than normal, and as a result glycation reactions caused by glucose are increased.

As previously mentioned, the accessible carbonyls of the aldehyde of open-chain glucose and the ketone of open-chain fructose can form a Schiff base with available amino groups on DNA, phospholipids, and proteins. The Schiff bases can rearrange to produce Amadori (glucose) and Heyn's (fructose) products, which are the basis for the formation of dicarbonyls such as 3-deoxyglucosone, glyoxal, and methylglyoxal, which then form a variety of cross-linked advanced glycation end products such as pentosidine, carboxymethylethanolamine (CME), and *N*-(carboxymethyl)lysine (CML).[87,98,99,101,223–226] From a kinetics standpoint, the formation of the freely reversible Schiff bases with open-chain glucose/fructose occurs in minutes following second-order kinetics (rate of formation decreases in proportion to the square of the glucose concentration, i.e., very quickly), whereas rearrangement to the more stable Amadori and Heyn's products takes from weeks to months to occur. In addition, the half-life of the Schiff bases is approximately 2.5 hours (hemoglobin in serum under physiological conditions); the concentration of the Schiff bases is essentially in equilibrium with serum levels of glucose. The cross-linked AGEs occur throughout all cells and tissues, and this appears to be a normal process associated with aging, indicating that these reactions occur with normal serum glucose levels but can be accelerated under high glucose loads. When these cross-links occur in vascular tissues,

they are associated with decreased compliance and increased stiffness, properties that are responsible, in part, for the increased peripheral resistance and hypertension usually observed in diabetics. In addition, such cross-links could be hypothesized to enhance the efficiency at which mechanical stresses are transduced through the vascular wall and therefore enhance oscillating shear stress responses. Both of these effects (hypertension and increased mechanical transduction) would effectively enhance risk for proinflammatory signaling through mechanical stress and may be an area worth more research.

In addition to the mechanical stress issues, AGEs can bind with RAGEs on vascular endothelial cells, macrophages, and smooth muscle cells.[84,85,100,101] Similar to scavenger receptors, RAGEs also are PRRs, in this case ones that recognize various proteins that are released by damaged cells (DAMPs). Activation of RAGE triggers the activation of PKC and NFκβ as well as the expression of all the expected proinflammatory signaling molecules. Just to make things even more interesting, Amadori products can initiate lipid peroxidation of LDL apoproteins, the lipid fraction of LDLs, and the fatty acids of phospholipids in both membrane and LDL lipids.[98,227] Thus, the formation of Amadori and Heyn's products can enhance mechanisms that directly contribute to the CHD process. In addition, the peroxidation of membrane phospholipids will enhance calcium leaking into the cell, which could then lead to additional calcium-mediated activation of $cPLA_2$ activity, further increasing proinflammatory eicosanoid signaling.

It is important to note here that the formation of glyoxal, CML, and CME also can occur as a result of peroxidation reactions with polyunsaturated fatty acids (in the absence of glucose) at rates comparable to, and in some cases greater, than those from glucose and that the formation of AGEs also occurs in foods as a result of cooking.[87,99,101,223,224] This is relevant with respect to studies using AGEs as markers for diabetic risk because there are multiple and indistinguishable sources of AGE-producing reactions; they are not only derived from glucose or fructose. This does not mean that uncontrolled glucose is not a contributory factor to atherosclerosis through the production of AGEs but rather that the potential contributions of glucose levels per se must be considered in light of the fact that there are non-glucose-mediated reactions that result in the same mechanisms that contribute to the CHD process.

One last issue to discuss is that of carbohydrates and fructose. There is a lot of interest in dietary fructose as a major cause of obesity and chronic disease. This interest probably stems from the large increase in intake of HFCS since 1970 that coincides with a large increase in obesity over the same time frame. In order to help clarify some issues as they relate to CHD, a brief analysis of several of the important issues, as recently reported, is warranted.[87,228] The predominant use of HFCS is as a sweetening agent in the same manner that sucrose is used as a sweetening agent; HFCS is used in manufactured foodstuffs (such as soft drinks) and rarely as a sweetener in the home. Use of HFCS as a commercial sweetener has steadily increased over the last five decades; simply because it is much less expensive to use than sucrose, the use of sucrose as a commercial sweetener has declined to an identical degree over the same time. This means that the contribution of fructose to total carbohydrate consumption relative to that of glucose has not changed at all; only the total amount has changed. From the recent 1999–2004 National Health and

Nutrition Examination Survey (NHANES) data in comparison with the 1977–1978 data, the intake of HFCS as a sweetener has increased by approximately 42%, which is essentially the same increase as that reported for total carbohydrate intake (41%) during the same period.

The most commonly used formulation of HFCS is 55% fructose and 45% glucose, a mixture that is essentially the same as that found in sucrose. Thus, the approximately 42% increase in fructose consumption is not due to the intake of pure fructose but rather the consumption of a formulation that is nearly indistinguishable from sucrose in metabolic terms. It makes no difference from where the fructose and the glucose come from, whether eaten in the form of sucrose or HFCS, because only the free forms are available in the blood for metabolism; therefore, the source has no effect. Because the liver metabolizes fructose very efficiently, serum levels of fructose tend to be much lower than those of glucose, with values for fructose in healthy subjects being approximately 8 μM. With over 500× more glucose in serum, it is certainly plausible that glucose might be the most important by far to deal with as a risk factor. However, this might not be the appropriate parameter for comparison. Fructose is far more likely than glucose to be in the open-chain configuration, and the subsequent rate of forming dicarbonyls is much greater. Overall, from in vivo data the formation of AGEs from fructose amounts to 10%–20% of the total AGEs, a pretty impressive amount considering that serum levels of fructose are far lower than those of glucose. Thus, the AGE-related risks for CHD from fructose are not trivial, but it should be kept in mind that they are still a small minority of the total risk when AGE formation from fructose is compared to glucose. One last consideration is that of the kinetics of Schiff base and Amadori product formation. With the normal postprandial fall in the concentration of glucose in serum (in those with normal insulin sensitivity), the decline to normal serum glucose within an hour or two of eating will automatically reduce the steady-state formation of Schiff bases to normal as well. Because the rearrangement process occurs over many weeks and months, and requires maintaining Schiff-base levels to do so, hourly and daily variations in serum glucose that ultimately return to normal will have minimal effect on enhancing the rate of AGE formation. On the other hand, a sustained elevation of serum glucose above normal over many months, regardless of the daily fluctuations due to eating behaviors, will enhance the formation of AGEs. Thus, based on the kinetics of AGE formation, an enhanced rate of AGE formation depends on serum levels of glucose that are constantly elevated above normal; transient changes due to the consumption of high-GI/-GL foods or meals would have minimal effect. In the final analysis, it is the lowest serum glucose levels that are sustained during our traditional nighttime fast that define the highest glucose (or any carbohydrate) levels that are relevant for AGE-related risk.

The ultimate concept of diabetes and AGE-related risks is, of course, insulin sensitivity and the resulting increase in serum glucose that follows increasing insulin resistance (fructose does not depend on insulin for absorption). From several recent reviews, there is a consistent association between consumption of sweetened soft drinks, obesity, and insulin resistance. However, these associations are commonly seen in population-based studies, whereas in clinical trials there is little evidence that sucrose or glucose consumption has a direct causal effect on insulin resistance

unless the carbohydrates are part of an *excess* calorie load.[229–237] Supplementing diets with pure fructose does appear to increase risk for insulin resistance and enhance abdominal adiposity, with no evidence that dietary glycemic index is related to the development of insulin resistance.[238] These results do indicate that consuming a high-fructose diet might confer additional risk. However, because consumption of HFCS-sweetened foods does not produce a higher dietary fructose consumption in comparison with sucrose-sweetened foods and because pure fructose is a rarely available commodity for public consumption, high-fructose diets such as those using fructose supplements might not be easy to generalize to the population. Thus, from currently available evidence it appears that with weight gain there is a relatively consistent association between increasing carbohydrate consumption and insulin resistance, leading to the conclusion that insulin resistance is more likely an issue directly associated with chronic weight gain than it is of carbohydrate consumption per se. This does not mean, however, that insulin sensitivity returns with weight loss.

This leads to one final issue. If carbohydrates per se are not responsible for the developing insulin resistance, then something else has to be. In many studies, the evidence indicates that inactivity is a major component of the problem.[239–246] From a variety of detraining studies using normal daily activities to bed rest studies, inactivity may lead to insulin resistance within days. In addition, insulin resistance can be reversed through physical activity. This increase in insulin sensitivity with exercise training (both endurance and resistance training) also occurs in obese subjects, both with and without weight loss, and it can quickly return to an insensitive state on cessation of the activities.[247–252] Because physical activity can increase insulin sensitivity in the obese, it follows that obesity per se is not necessarily the cause of insulin resistance and that physical activity is extremely important in the ability to maintain glucose homeostasis. In light of the large increase in physical inactivity since the 1970s, it is possible that this is a major contributor to the current type 2 diabetes epidemic, whereas the increase in calorie consumption coupled with the inactivity since then has accelerated the development of the current obesity epidemic. With this model, inactivity leads to insulin resistance that then results in enhanced Amadori-/AGE-mediated inflammatory signaling, which contributes to risk for atherosclerosis; the state of being obese per se might then be a relatively passive bystander in the context of this process.

4.2.4.3 Metabolic Syndrome and Obesity

Metabolic syndrome and obesity are very tightly associated and are commonly thought to result from a combination of poor diet and lack of physical activity.[253–255] Metabolic syndrome includes abdominal obesity, hypertension, insulin resistance, and dyslipidemia and is associated with increasing risk for many chronic diseases, including atherosclerosis. The major issue as a risk for CHD, however, is how these conditions enhance risk.

The increased risks associated with elevated lipids, hypertension, and insulin resistance have already been addressed in Sections 4.2.1, 4.2.4.1, and 4.2.4.2, which leaves abdominal obesity (and obesity in general) as the major issue to briefly review. There is a highly complex interplay of many factors that ultimately lead to the development of obesity and subsequent risk for chronic diseases, with much of

the risk being associated with insulin resistance and the resulting increase in glycation events. In addition, obesity is highly associated with a *proinflammatory state* in which proinflammatory cytokines, along with other adipokines, originate from the adipose tissue itself.[256–261] The adipokines include adiponectin, leptin, and visfatin as well as the inflammation-associated TNF-α, IL-6, IL-1β, and MCP-1. Whereas some of these adipokines are synthesized by stromal cells and macrophages that are resident in the adipose tissue, many are expressed by adipocytes as well. As discussed in Chapter 2, the chemokine MCP-1 enhances macrophage infiltration into the adipose tissue, which then adds to the local production of proinflammatory cytokines. The entry of these proinflammatory cytokines derived from the adipose tissue into the circulation then exacerbates any localized inflammatory responses elsewhere, including those associated with the development of atherosclerotic plaque. Thus, inflammatory cytokines that originate in the adipose tissue may comprise the major source of obesity-associated risks.

The development of the proinflammatory state is initiated as a result of the demand for increased adipocyte hypertrophy and for adipogenesis from preadipocyte fibroblasts during periods of net caloric excess.[262–268] In response to excess fatty acid uptake, adipocytes increase the rates of triglyceride synthesis, a process that also requires increased GLUT4-mediated glucose uptake for the necessary production of glycerol-3-phosphate. With prolonged periods of net caloric excess, endoplasmic reticulum dysfunction or endoplasmic reticulum stress occurs. This condition is associated with activation of JNK, increased production of TNF-α, and release of free fatty acids (FFAs) by adipocytes in addition to the hallmark accumulation of improperly folded and newly synthesized proteins that have been unfolded. As a result of autocrine activation of TNF receptors, an array of proinflammatory cytokines are released, leading to the recruitment and activation of preadipocytes (fibroblast lineage) and monocytes (which differentiate into macrophages), as well as triggering the shift of resident macrophages from an anti-inflammatory phenotype to a proinflammatory phenotype, thus greatly enhancing the production of proinflammatory cytokines with the concomitant production of PGs and other proinflammatory lipid mediators. Chronic release of TNF-α and FFAs to the circulation leads to mitochondrial dysfunction and insulin resistance in both adipocytes and skeletal muscle. Increased insulin resistance in adipocytes contributes to their compromised ability to synthesize additional triglycerides (TGs), more than likely enhancing endoplasmic reticulum dysfunction and leading to increased fatty acid levels for inflammatory signaling. PGs are a necessary component for activating the differentiation of preadipocytes into functional adipocytes, in part, through their interaction with PPARγ2.[256,264,269] Thus, it appears that prolonged TG synthesis activities resulting from caloric overload lead to hypertrophy and hyperplasia of adipocytes as well as insulin resistance, all of which are associated with the production of proinflammatory signaling molecules. In addition to these, the processes of angiogenesis are initiated, possibly in association with the localized development of hypoxia due to the adipocyte hyperplasia. As a result, additional cytokines and growth factors that are necessary for angiogenesis are produced (see Chapter 2 for details), which can then enter the circulation to affect other tissues as well. Although the order of events adipocyte hypertrophy → endoplasmic reticulum dysfunction → inflammatory

signaling → insulin resistance → adipocyte hyperplasia → adipose angiogenesis has been observed in some animal models of obesity-associated insulin resistance, it has not been confirmed in humans, but both hypertrophy and hyperplasia have been observed in subcutaneous fat. Abdominal fat, though, is more proinflammatory than subcutaneous fat with a preferential infiltration by macrophages and is known to be a more substantial risk associated with CHD as well.[270–272]

Interestingly, it is possible to be both obese and display a normal metabolic profile, referred to as metabolically normal obese (MNO) with approximately 11%–36% of obese individuals (depending on the study) displaying this, a phenotype that includes normal insulin sensitivity.[273–278] Paradoxically, the opposite is true as well with a similar prevalence of an obesity-associated metabolic phenotype observed in lean people.[276] In addition to normal insulin sensitivity, these MNO individuals display normal levels of inflammatory markers in both serum and tissue biopsies as well as evidence of no additional risk for cardiovascular disease or cancer. As a relatively new area of study, very little is known about all the lifestyle variables of these MNO individuals, although in one study the lack of additional risk for CHD was directly attributable to enhanced cardiorespiratory fitness, which is suggestive of the importance of physical activity to this phenotype.[273] On the basis of these studies, in addition to those documenting a return to insulin sensitivity with exercise in the obese (without weight loss), it is apparent that the lack of any physical activity is a primary driver for the increased risk for disease in the obese rather than obesity per se. Clearly, more studies in the obese that include an analysis of physical activity variables along with appropriate biomarkers of effect are needed. It is also clear that inadequate nutrition plays an important role in exacerbating inflammatory disease; therefore, nutrient status and a variety of dietary variables also need to be included. From the molecular and clinical data, it is apparent that gaining weight coupled with remaining inactive is a major cause of excess risk for CHD and simply being overweight while all other risk factors are normal is not.

4.2.4.4 Infection

As alluded to in Section 4.1, inflammatory processes that follow viral and bacterial infections may enhance the inflammatory mechanisms that are integral to the CHD process. In a variety of population-based studies, chronic infections with a variety of organisms including herpesvirus (cytomegalovirus), hepatitis A, *Chlamydia pneumonia*, *Porphyromonas gingivalis*, and *Helicobacter pylori* have been documented to be associated with risk for atherosclerosis independently of other risk factors.[75,76,90–97,279] In the majority of cases, it appears that chronic infections of tissues that are distant from the coronary or carotid arteries (e.g., pulmonary infections and hepatitis) are the ones that produce the risk and the evidence for risk is often elevated serum cytokines or CRP. This indicates that it is circulating inflammatory cytokines that are most likely the predominant component of the increased risk; the additional proinflammatory signals simply add to the locally produced proinflammatory effects of the intermittent low shear forces. The difficulty with such studies is that circulating proinflammatory markers can have origins that are completely independent of an infection (see Section 4.2.4.3).

In addition to circulating cytokines that are associated with infections, a localized infection within the artery wall appears to occur with a variety of pathogens being observed in atherosclerotic plaque.[280–285] In many cases, the identified pathogens are the same as those that cause chronic infections elsewhere, with organisms that cause pulmonary and periodontal infections appearing to be more common than others. It is of interest that in the clinical studies that analyze the specific associations between the content of pathogens in plaque and the severity of the disease either small associations or no associations are actually observed. This does not mean that a local infection with pathogens cannot initiate additional inflammatory responses to increase risk for CHD, but it does raise the possibility that the presence of pathogens in plaque is more likely coincidental with total pathogen burden. Further, in support of the passive association concept, a recent meta-analysis of 13 different clinical trials of antibiotic treatment for secondary prevention of CHD revealed no effect and in another recent study the associations with atherosclerosis and infection were dependent on smoking status and not infection alone.[279,286] With a majority of lesions being positive for pathogen DNA, it is safe to conclude that CHD risks may be influenced by such pathogen exposures. On the other hand, if infections were a major risk for atherosclerosis then one would expect chronic antibiotic therapy to attenuate the risk. Thus, although circulating proinflammatory cytokines can certainly contribute to CHD risk, those arising from infections are likely to be a relatively minor component in comparison with the other sources of risk. Prevention strategies for atherosclerosis that relate to infection are therefore limited to the standard advice for proper personal hygiene, proper cooking and handling of foods, and appropriate precautions to avoid infectious diseases.

4.2.4.5 Cigarette Smoking

Smoking has been known for many years to be a significant risk factor for CHD, and quitting smoking significantly reduces risk for CHD.[287–290] However, because smoking cessation is not part of a dietary or exercise approach to reducing risk for atherosclerosis, smoking cessation is not discussed here. Smoking is included here because a brief discussion on possible mechanisms of risk enhancement by smoking is quite revealing.

Formation of platelet thrombi is acutely enhanced by smoking, an effect that occurs within a few minutes of smoking and occurs with both mainstream and sidestream (environmental) smoke.[291,292] The activation process is associated with the prooxidant effects of many of the components in cigarette smoke and can be inhibited with dietary vitamin C.[291,293–296] These prooxidant components also cause damage to endothelial cells and can oxidize LDL and LDL-CHOL. Thus, the prooxidant and subsequent proinflammatory effects of cigarette smoke appear to be the major factor in the smoking-enhanced risk for CHD and the predominant reason why risks for CHD decline very quickly on cessation of smoking. These illustrate that any increase in exposure to prooxidant molecules, either exogenous or endogenous, will enhance risk for CHD. In addition, the ability to handle excess prooxidants from any source would be very important in reducing risk; the ability of vitamin C supplements to reduce much of the smoking-associated risk attests to this.

4.2.4.6 Nutritional Status/Redox Control

Several important issues relating to nutrient status are highly relevant to the proinflammatory stress responses that are causal. It has been estimated that only 10% of Americans eat the recommended minimum number of servings of fruits and vegetables.[69] Intakes for vitamins C, D, and E are below the estimated average requirement (EAR) for 25%, 70%, and 60% of Americans, respectively, and below the EAR for zinc (~8%), iron, copper, and selenium (~5% to 6%), as well as calcium (38%) and magnesium (45%).[71] Some of these specific vitamins are antioxidants, while other vitamins and minerals are components of antioxidant enzymes or redox sensor proteins or directly involved in calcium homeostasis. This apparent large prevalence of insufficient intake of these particular nutrients in the United States directly enhances risk for CHD through a less than optimal ability to control antioxidant and redox statuses of cells.

As discussed in Section 4.2.3 and in detail in Chapter 2, the initiation of an inflammatory stress response can be mediated through the activation of both PLA_2 and the various MAPK pathways, leading to the downstream activation of NFκβ and the ultimate production of proinflammatory signaling. The regulation of these signal transduction pathways is highly dependent on the redox status of the cell. Oxidizing agents can modify the activity of signal transduction pathways by oxidizing redox sensor proteins such as thioredoxin (Trx) and peroxiredoxin (Prx).[297–304] For example, activation of Jun/Fos (AP-1) can occur through a Trx-mediated activation of apoptosis signal-regulating kinase 1, which then activates the JNK–MAPK and p38–MAPK pathways and subsequently Jun, Fos, and a variety of other transcription factors.[299,304–306] The antioxidant enzymes SOD, CAT, and GPX alter the local concentrations of ROSs and therefore contribute to the optimal regulation of signal transduction pathways in the cell.[297–306] Any nutritional insufficiency that contributes to a nonoptimal antioxidant function can then compromise redox control of the signal transduction pathways and lead to enhanced inflammatory signaling. With such a large majority of Americans eating insufficient amounts of the aforementioned nutrients, it is clear that redox control and antioxidant status of cells may be compromised and may become a significant source of risk. Perhaps, this is the place to suggest that LDL-CHOL (and HDL cholesterol [HDL-CHOL] as well) is actually a component of an extracellular redox sensor network.

The role of the cellular redox sensing proteins as well as the antioxidant enzymes in regulating signal transduction pathways is an important component of the cellular stress response. As discussed in Chapter 2, increasing ROS stress within a cell may lead to damage and orchestrating a stress response that can ultimately lead to increased repair activities is an important survival mechanism. Using agents of damage (ROSs and other oxidants) as agents to activate a stress response is both logical and highly efficient; it takes time for the processes of repair to be increased, so the earlier in the damaging process that they are initiated, the more likely the cell will survive. The various redox sensor proteins within cells provide such a mechanism. In the circulation, however, it may be LDL-CHOL that provides the same redox sensor function. It is the ability of CHOL-Ox (and oxidized LDL/HDL as well) to bind to scavenger receptors of macrophages, endothelial cells, and smooth muscle cells and

initiate proinflammatory signaling that would be the mechanism through which this circulatory redox sensing system might work. In an ideal situation, such a (low-level) systemic response might be additive to the local cellular stress responses and thereby might enhance the local tissue responses that ultimately lead to repair. Such a system would simply prime all tissues for a local stress response when circulating oxidants are present and perhaps lead to a heightened stress and subsequent repair response in those tissues that suffer actual damage; lung and liver in the case of environmental exposures, for example. Unfortunately, such a system could have negative implications for those very few tissues that are under chronic proinflammatory stimuli, tissues such as the inner curves and sharp bends of arteries. The negative effects may even become fatal when they are seriously exacerbated by poor cellular redox control coupled with enhanced physical stress and chemical damage due to damaging environmental exposures, poor diet, and improper physical activity. The concept that LDL/HDL-CHOL may be a component of a circulatory redox sensor system remains to be tested.

4.3 ETIOLOGY OF PREVENTION

Managing risk for CHD through attempting to modify its major risk factors has been a common theme of CHD prevention over the last five decades. Declines in mortality due to CHD over the same time period has been due predominantly to pharmaceutical control of hypertension, diabetes, and the dyslipidemias[6–9] and to improved treatment of existing CHD patients,[10–13] with a lower prevalence of smoking also being an important contributor. Although the decline in mortality is important, it has done little to reduce the actual impact of the disease and CHD remains the number one cause of death in the United States.[1–4] A major reason for this is that primary prevention has not focused on the actual mechanisms of cause. To be consistent with the general theme of this book, if you do not have an effect on mechanisms of cause you will not change the development of disease. With an additional focus on attenuating the mechanisms of cause, rather than a sole focus on factors associated with risk, substantially better preventive effects should be realized. This does not mean that attention to risk factors is not important but rather that prevention should focus primarily on the actual mechanisms of cause with a secondary focus on associated factors. With inflammatory mechanisms being recognized as the primary cause of CHD, the target for a preventive focus is clear.

4.3.1 Exercise

There are several basic factors that may contribute to an exercise-based risk reduction of atherosclerosis: exercise can be used as a calorie burner to modify adiposity, reduce blood pressure, prevent insulin resistance, enhance antioxidant status in order to reduce oxidative damage to lipids, attenuate inflammatory signaling, and alter serum lipids. Of these, attenuating proinflammatory signaling, reducing ROS-mediated oxidation of LDL/HDL/CHOL, and preventing insulin sensitivity may provide the greatest benefits overall as they appear to address the greater of the population-wide risks. Although currently a common component of clinical

recommendations, altering serum lipids through exercise may not be a major contributor to risk reduction for most. This is discussed first.

4.3.1.1 Serum Lipids

A number of population and cross-sectional studies relating to exercise and serum lipids reveal that physically active individuals tend to have a more favorable lipid profile.[307–315] However, difficulties in controlling for individual differences in diet and nutritional status, body composition, and the amount/intensity of exercise make definitive conclusions of a consistent association between increased physical activity and normalization of serum lipids difficult. In spite of these limitations, the cross-sectional data reveal that there may be a consistent effect of reducing serum TG by 8–20 mg/dL when physical activity approximates an additional expenditure of 1500–2000 kcal/week (equivalent to ~15–20 mi.), with an additional decline of up to 8 mg/dL with an additional approximately 10 mi./week.[316–320] Such activity levels can be attained by most otherwise healthy people. In addition, although such data might be informative of existing differences between groups of people, they do not really address the effects of exercise on these parameters in individuals and therefore cannot be the sole basis of decisions regarding the preventive benefits of exercise. Clinical trials would provide much more meaningful information.

As a means to reduce risks associated with serum lipids, a variety of exercise protocols have been utilized to induce favorable changes. For example, a normalization of moderately elevated TG levels and VLDL patterns have been observed after a variety of daily walking or jogging programs.[321,322] Similar reductions in total-CHOL and LDL-CHOL of 4%–20% have been observed with a variety of exercise protocols that range from 30 minutes of cycling at moderate intensity three times a week to continuous running of up to 45 minutes for 5 days/week.[319,322–328] Although such changes in lipid-based risk appear to be beneficial, a serious drawback of many exercise studies is a lack of proper controls. When randomized controls are used the exercise effects tend to be much less in comparison with changes observed in controls, and in many cases the differences are nonsignificant.[329–336] From these and a variety of other studies, moderate reductions of 4%–7% in total-CHOL and LDL-CHOL in both men and women have been observed in approximately 25% of the studies with the endurance exercise–mediated effect being observed predominantly in subjects who were initially inactive.[319] Thus, as a general concept, moderate physical activity has a small effect on serum cholesterol, whereas the effect of moderate physical activity on TG levels are often greater, possibly stemming from the metabolic demands of the activity. There is little evidence for consistent favorable reductions in total-CHOL or LDL-CHOL at these levels of physical activity.

Because serum lipids are favorably changed by either a relatively small amount (<10%) or not at all, altering this source of risk cannot be the major reason for the general and consistent decline in risk for CHD that is observed as a result of repeated exercise. Perhaps, this is one reason for some to not promote exercise as a preventive measure against CHD; exercise does not consistently reduce LDL-CHOL. Resistance and endurance exercise can, however, enhance insulin sensitivity in both normal and overweight/obese individuals with insulin resistance. This is an important protective effect for CHD that is independent of serum lipids and

one that has very often been overlooked in population studies on exercise, lipids, and CHD. Possibly, another reason why exercise may be discounted as an effective means to prevent CHD may be that the protective benefits of exercise are simply not well known.

4.3.1.2 Antioxidant/Redox Status

Another preventive effect of exercise that is independent of serum lipids relates to several antioxidant enzymes, an area of considerable importance to cellular redox control and its role in ensuring a controlled inflammatory stress response. Exercise enhances eSOD, cSOD, and GPX levels in skeletal muscle as well as in cardiac muscle, aorta, and coronary arteries, an effect that appears to be mediated through Nrf2/ARE signaling and that disappears soon after the cessation of exercise.[337–343] Enhanced GPX and cSOD would enhance control of the cellular redox state and, at the same time, an induction of eSOD would ensure that extracellular superoxide anions will be reduced to attenuate the oxidation of extracellular LDL and LDL-CHOL. These exercise effects would be in addition to the *adaptation* responses to chronic high shear stress discussed in Section 4.2.2, and they occur in all tissues examined, including liver, lung, and brain.[297,344–349] Although a systematic examination of the effects of exercise on all the redox regulatory systems in the arterial vasculature has not been conducted, the results of such studies might be highly instructive. Because CAT, glutathione reductase, Trx, Prx, HO-1, and glutamate cysteine ligase (GCL) are induced through Nrf2/ARE activation, positive results would be expected.[350–353]

The benefits of an increased antioxidant status would be a reduction in oxidative damage to both cellular components and HDL/LDL/CHOL. As a result, inflammatory signaling resulting from oxidative damage to cells or through the activation of scavenger receptors by oxidized HDL/LDL/CHOL will be reduced. As might be expected, the degree of LDL oxidation and the ability to activate macrophages are highly associated with the antioxidant status of the tissues, and treatment with dietary components that are known to activate Nrf2 also reduce the cell-mediated oxidation of LDL/CHOL.[354–357] Exercise interventions, especially when combined with dietary modifications, also are known to enhance antioxidant and redox enzymes, reduce LDL oxidation and other markers of oxidative stress, and reduce risk for CHD.[358–364] It is interesting to note that in studies that focus on antioxidant enzyme activities significant enhancements of activity occur following 60 minutes or more of moderate-intensity exercise and also following 20–30 minutes of continuous high-intensity exercise.[340,365–367] Although activation status of Nrf2 is not determined in the (human) intervention studies, it is tantalizing to hypothesize that this is the mechanism that mediates, at least in part, the positive effects.

4.3.1.3 Inflammatory Signaling

In addition to optimizing control of cellular redox states, the other major protective effect of exercise is on attenuating inflammatory signaling. The sustained production of the proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α and CRP, as well as an increase in creatine kinase activity in serum (a common marker for muscle damage) and markers of ROSs in response to damaging or exhaustive exercise, has

been well known for many years.[368–372] Unfortunately, such data have perpetuated the concept that exercise is a source of damaging ROSs and inflammatory signaling. With nonexhaustive exercise that is continuous for 1 hour (or more), however, the increase in IL-1 and TNF-α does not occur; at the same time, IL-6, IL-10, soluble IL-1 receptor antagonist (sIL-1ra), soluble IL-1 receptor (sIL-1r), and soluble TNF receptor (sTNFr) increase to a much greater degree. The IL-6 increase can be as much as 100-fold, and it is the large initial rise in IL-6 that then induces an increase in the other anti-inflammatory molecules, all of which return to baseline several hours after the stop of exercise.[371–376] Even at 50% of the maximum watts of power output (cycling, an approximate 6 MET level of intensity), significant increases in IL-6 are observed with 1 hour of cycling exercise. It is also important to note that changes in IL-6 are not observed with exercise that utilizes small muscle groups, such as the upper arms, indicating that large-muscle-group activities of a minimum intensity of approximately 6 METs that are sustained for approximately 1 hour are essential for these anti-inflammatory effects of exercise.[376–378] This does not mean that higher intensity activity carried out for less than 60 minutes will not produce anti-inflammatory cytokine responses; it just remains to be seen in properly conducted experiments.

The importance of the differences between exhaustive and nonexhaustive exercise cannot be overemphasized. The large induction of IL-6 without a concomitant production of TNF-α is the major reason for the anti-inflammatory effects of prolonged exercise. The large increases in sIL-1r and sTNFr exert an anti-inflammatory effect by binding to circulating IL-1α, IL-1β, and TNF-α and the sIL-1ra prevents circulating IL-1α and IL-1β from binding to their cellular receptors, thus suppressing cellular responses to circulating proinflammatory cytokines. These effects will reduce the proatherosclerotic effects of circulating proinflammatory cytokines that originate from inflamed tissues or from abdominal adipocytes. It may also be possible for these soluble receptors and receptor antagonists to attenuate autocrine and paracrine signaling within inflamed tissues. Because TNF-α is involved in producing insulin resistance, the development of insulin resistance also might be blunted as a result of the enhanced sTNFr.

IL-10 exerts its anti-inflammatory effects through suppressing the production of the proinflammatory cytokines. IL-10 activates the Jak–STAT pathway along with prolonged STAT3 activation to result in the synthesis of suppressor of cytokine synthesis proteins, inhibiting the synthesis of IL-1α, IL-1β, IL-6, IL-8, and TNF-α.[379–381] IL-10 also suppresses MAPK and NFκβ signaling, which in turn attenuates the synthesis of acute-phase cytokines.[379–382] Thus, there is a profound anti-inflammatory effect for moderate-intensity endurance-type exercise, which reduces inflammatory mechanisms at play within the arterial vessel walls. The results of these exercise-induced anti-inflammatory effects are also evident in CHD patients and high-risk individuals where reductions in CRP and enhanced eNOS activity are observed.[383–386]

The effects of exercise on CRP are instructive because they tend to equate well with those on IL-6 induction. Associations between exercise and reductions in CRP have been observed in several clinical trials, whereas in others no associations were evident.[387–396] Of these, the longer duration exercise periods of 45–60 min/day were more consistently associated with a chronic reduction in CRP; in studies that used

durations of exercise of 30 minutes, levels of CRP were unchanged. These results are similar to an earlier observed dose effect between exercise and risk for elevated CRP with odds ratios of 0.98, 0.85, and 0.53 for light, moderate, and vigorous exercise (NHANES III).[397] Interestingly, in these studies the weight-loss effects of the longer duration exercise were sufficiently consistent that it was difficult to disassociate the effects of weight loss per se from those of the exercise. Because the exercise induction of IL-6 expression is related to lowered glycogen content, a lower glycogen level that might be associated with losing weight may actually be additive to the exercise effect and therefore be contributory, not confounding.

The anti-inflammatory effects of prolonged exercise are important because they help to explain the previously discussed data in which subjects with high levels of fitness (as measured by VO_2 max) have a lower risk for CHD compared to active people with lower levels of fitness. Lower intensity and duration activity would still contribute to lower risks through enhancing insulin sensitivity and increasing the efficiency of redox control. Only with longer duration activities that greatly enhance IL-6 levels do subsequent anti-inflammatory benefits accrue. Hence, this is the type of activity that is recommended for an optimal reduction in risk for CHD.

In summary, exercise can exert a variety of effects that greatly reduce risk for atherosclerosis by directly affecting the inflammatory mechanisms of cause. These effects are in addition to those mediated by increasing insulin sensitivity and enhancing cellular redox control. However, these effects are transient and the inductive mechanisms last only as long as the exercise-induced levels of regulatory molecules and cytokines remain elevated and the benefits of the induced enzymes remain only as long as the proteins exist. With half-lives from approximately 10 minutes for eSOD to less than 1 or 2 days for GPX and Trx reductase, the protective effects of enzyme induction do not last very long.[398–401] The exercise must therefore be repeated nearly daily if these cytoprotective and anti-inflammatory effects are to be realized on a long-term basis.

4.3.1.4 Insulin Sensitivity

As described in Section 4.2.4.2, lack of physical activity is associated with the development of insulin resistance, possibly because of a proinflammatory environment in muscle cells produced by inactivity.[239–246,402] An increase in local production of ROSs due to inactivity mediates a stress-mediated induction of TNF-α synthesis that in turn enhances phosphorylation of insulin receptor substrate (IRS)-1 on lysine to enhance its degradation as well as impair its ability to complex with p85, thus reducing insulin signaling. Because of the known effects of increased serum glucose on the development of AGE and subsequent inflammatory signaling, the increased insulin resistance resulting from inactivity contributes to risk for atherosclerosis. The obvious way to reduce risk would then be to increase levels of activity. Most interestingly, the insulin resistance appears to be reversed through almost any kind of physical activity. Both endurance training and resistance training enhance insulin sensitivity, and the effect of an acute exercise bout appears to last approximately 24 hours. In addition, enhanced insulin sensitivity occurs even in obese subjects whether they lose weight or not, although it returns very quickly to an insensitive state on cessation of the activity.[247–252,403–406] Further, where

measured, decreases in glycosylated hemoglobin were also observed in association with the increased insulin sensitivity; in some cases, the amount of decrease was correlated with the intensity of the exercise training and was greater with resistance training plus endurance training in comparison with either one alone. The intensity issue, however, appeared to be associated with 3-day/week programs and not those that utilized daily activity. Possibly, this means that alternate-day activities need to be of greater intensity and volume compared to daily activities. These are important results because they indicate that risks for atherosclerosis that arise from insulin resistance can be prevented with most any kind of regular physical activity and that markers of damage from elevated glucose can be reduced. They also reveal that these effects are temporary and, therefore, the higher intensity activity must be repeated at least on an alternate-day schedule to ensure a relatively constant degree of insulin sensitivity.

There are two main issues with respect to the effects of exercise on glucose transport. The first is an enhanced glucose transport due to mechanisms relating to muscle contraction during an acute bout of exercise and an increased sensitivity to insulin that follows for a short time after the activity. As a result of muscle contraction, two events occur with respect to glucose transport, via insulin-independent mechanisms.[407–410] Translocation of the GLUT4 transporter to the membrane to activate glucose transport in skeletal muscle is dependent on Rab-GTP. The inhibitor protein AS160 is a Rab-GTPase, which hydrolyzes Rab-GTP to Rab-GDP to prevent GLUT4 translocation. Another putative Rab-GTPase is TBC1D1; although both can be phosphorylated by AMP-associated protein kinase (AMP-PK), AS160 has multiple sites for phosphorylation by Akt, rendering it the protein through which insulin activates glucose transport (insulin → IR/IRS → PI3K → PI3,4,5-TP:PDK1 → Akt → AS160 pathway). Interestingly, both inhibitor proteins also have a calmodulin-binding site, which would make both of them sensitive to calcium-mediated effects. Because the AMP to ATP ratio increases with increasing intensity of calcium-activated muscle contraction, it is an attractive model that muscle contraction can inactivate AS160 and TBC1D1 via calmodulin and AMP-PK-mediated mechanisms. Increased phosphorylation of both occurs during muscle contraction, but only AS160 remains phosphorylated for several hours after the exercise. Thus, both proteins appear to be involved in an exercise-stimulated glucose transport, although only the AS160 protein would contribute to a sustained insulin sensitivity following the exercise period because it remains phosphorylated, in some cases, for 3–17 hours post exercise.

The second issue relates to an increase in capacity to respond to insulin with repeated exercise. In many studies, both resistance training and endurance training result in an increase in GLUT4 content, whereas an insulin-stimulated PI3K:IRS activity or increase in IRS-1 content has been inconsistently reported.[411–416] Increases in the content of IRS may increase PI3K-mediated signaling by increasing the efficiency of IRS binding to IR to activate PI3K at a given insulin concentration. Increasing GLUT4 content would also increase insulin sensitivity by enhancing the amount of GLUT4 translocation at a given concentration of insulin. The mechanism of the reported increases in expression of these proteins has been associated with an increased activation and expression of PGC-1α, and it is known that downregulation

of PGC-1α along with a decrease in PI3K activity is observed in skeletal muscles of subjects with type 2 diabetes.[411,414,416,417] PGC-1α is known to be involved in the increased mitochondrial biogenesis that is observed following exercise training, and it is activated to enter the nucleus and bind to the promoter regions of several genes following activation by either p38–MAPK, silent information regulator (SIR) T1, SIRT3, or AMP-PK. Of these, both AMP-PK and SIRT1/2 are activated when NAD^+ to NADH ratios and AMP to ATP ratios increase in tandem. AMP-PK phosphorylates PGC-1α to activate it, whereas SIRT1 deacetylates it. In addition, AMP-PK can activate p38–MAPK, which in turn can activate PGC-1α. Thus, PGC-1α activation is exquisitely sensitive to both metabolic stress and any other stressors that activate p38–MAPK. This transcription factor was originally described as a coactivator for the nuclear receptor PPARγ coactivator-1α and is known to act as a coactivator (along with other transcription factors) for a variety of proteins, including GLUT4 and PGC-1α. It is not known, however, to be involved in the expression of IRS-1. Interestingly, the promoter of IRS-1 contains multiple binding sites for the estrogen receptor (ER) and skeletal muscles contain both ER-α and ER-β; in addition, PGC-1α can act as a coactivator of ER-mediated transcription.[418–422] Further, phosphorylation of ERs by a variety of exercise-activated kinases, including PKC, ERK–MAPK, p38–MAPK, and Akt, enhances the binding of ER to its coactivators to enhance ER-mediated expression.[423–425] Thus, it may be possible that a coordinated activation of PGC-1α and ER will also enhance the expression of IRS-1 (among many other proteins) and that inconsistencies in enhanced IRS-1 expression might be explained in part by differences in age and ER and PCG-1α expression in subjects in different studies.

Although an increased expression of GLUT4 and other proteins involved in the insulin signal transduction pathway is a topic requiring intense investigation and there is still much to be learned, it does appear to be involved, at least in part, in the increase in insulin sensitivity observed with exercise training. However, this does not rule out that an increase in AS160 phosphorylation from an acute bout of exercise is not involved. As long as the acute periods of exercise are repeated multiple times each week, the temporary increase in insulin sensitivity due to this mechanism (lasting for as long as 17 hours in one study) would surely be additive to any longer term effects due to the increased synthesis of the proteins involved in signal transduction and glucose transport. The important message is that only with repeated exercise, resistance, endurance, or a combination of both, are these changes seen. The risk-reduction effects of exercise are summarized and illustrated in Figure 4.2.

4.3.2 DIET

As with exercise, there are several basic concepts in the role of diet in reducing risk for CHD: diet as a means to reduce hypertension, control adipose tissue, maintain insulin sensitivity and maintain proper nutrient status, alter dyslipidemias, and attenuate inflammatory signaling. These risk-reduction effects of diet are summarized and illustrated in Figure 4.3. Dietary issues relating to nutrient status and inflammatory signaling are extensively discussed in Chapter 2 (Section 2.5.1); but they are summarized here, as are the issues relating to antioxidant status/redox control and serum lipids.

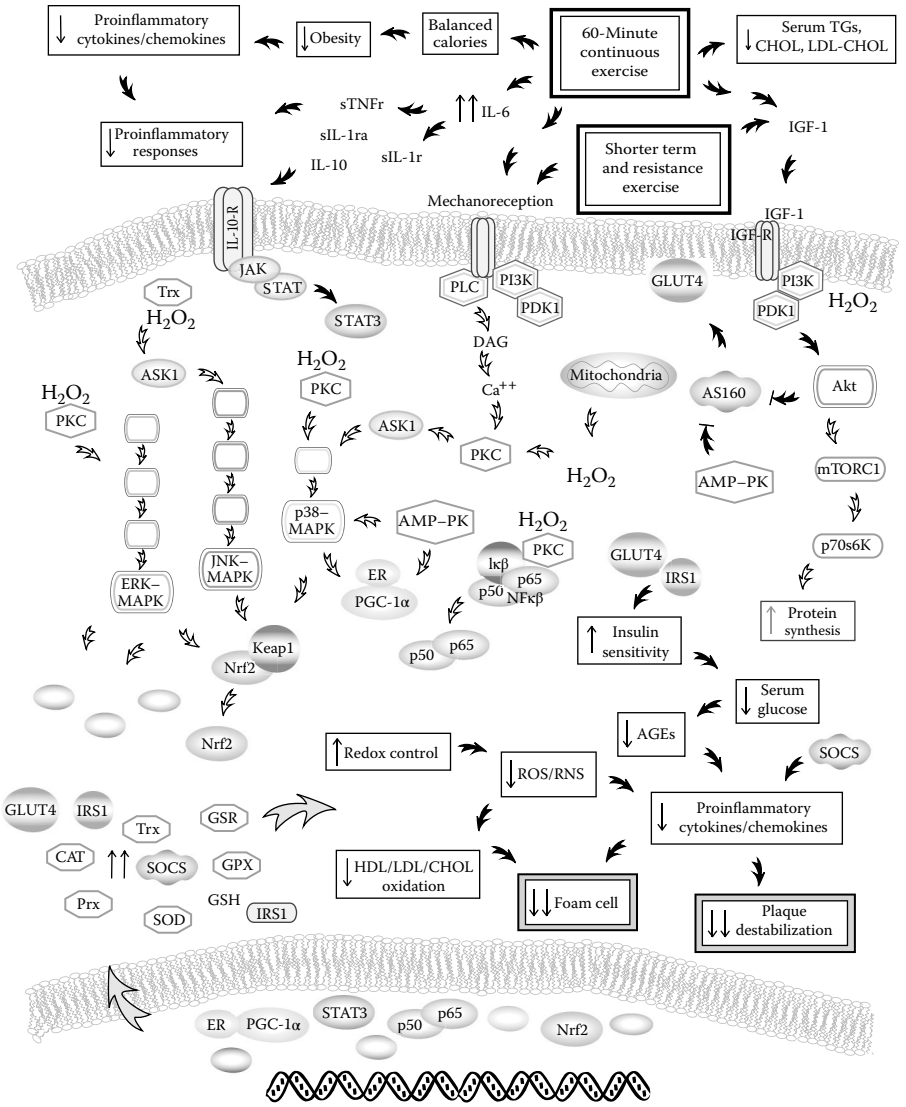

FIGURE 4.2 Reductions in mechanisms of risk for atherosclerosis by exercise: exercise stimulates the release of insulin-like growth factor (IGF)-1 from muscle and activates various mechanoreceptors, leading to the transient activation of phosphatidylinositol 3-kinase (PI3K), PLC, AMP-associated protein kinase (AMP-PK), and the mitogen-activated protein kinases (MAPKs) as well as transient increases in cellular hydrogen peroxide. Subsequent activation of Nrf2, p50/p65, PGC-1α, estrogen receptor, and many other transcription factors enhances expression of superoxide dismutase (SOD), catalase (CAT), peroxiredoxin (Prx), thioredoxin (Trx), glutathione peroxidase (GPX), GSR, GLUT4, and IRS1. Longer term exercise increases the release of interleukin (IL)-6 from muscle, activating the expression of soluble tumor necrosis factor receptor (sTNFr), soluble IL-1 receptor (sIL-1r), soluble IL-1 receptor antagonist (sIL-1ra) and suppressor of cytokine synthesis (SOCS) via IL-10–STAT3.

4.3.2.1 Nutrient Status

A relatively large minority (and in some cases a majority) of Americans consume insufficient amounts of individual nutrients, including nutrients that are important antioxidants or components of redox enzymes and coenzymes.[69–71] This is likely due to the fact that over 90% of Americans consume less than the minimum recommended number of servings of both fruits and vegetables, a factor in the commonly observed associations between low fruit/vegetable intake and risk for chronic disease in population-based studies. In the past, a frequent interpretation of these associations was that low fruit intake meant low antioxidant intake and that antioxidant supplements would then be useful to reduce risk. Unfortunately, in clinical trials no clear protective benefit of antioxidant supplements has been observed and in many instances negative effects such as increased deaths due to lung cancer with β-carotene or with vitamin C or E supplements and increased stomach cancer with α-tocopherol and/or β-carotene have been observed.[426–433] A more likely interpretation of these population associations is that different degrees of nutritional deficiencies are responsible for the associations and not antioxidant deficiencies. In addition to the lack of a protective effect on human disease, antioxidant supplements appear to have little to no positive effect on athletic performance either and, in some studies, have been observed to prevent exercise-induced mitochondrial biogenesis and reduce both aerobic performance and maximum muscle force.[434–440]

Using antioxidant supplements may overwhelm cells and potentially interfere with the regulation of redox states at the microenvironment level. As discussed in detail in Chapter 2, the metabolic regulation of ROSs through the production of $O_2^{\bullet-}$ and H_2O_2 at specific cellular locations, as well as the location-specific expression of antioxidant enzymes, plays a vital role in a wide array of normal cellular functions.[441] Severely reducing endogenous ROSs through supplementation with antioxidant compounds is therefore contraindicated.

For these reasons, the starting point for a dietary approach to reducing risk for CHD is a diet that provides recommended daily allowance (RDA)/adequate intake (AI) amounts of all the necessary nutrients rather than one that includes supplements. For convenience, the new *MyPlate* approach (www.choosemyplate.gov), which includes standardized serving sizes, provides an appropriate model from which to start; however, it must be modified to meet the additional anti-inflammatory effects that are necessary for optimal CHD prevention (see Section 4.4.2).

4.3.2.2 Serum Lipids

Modifying diet to alter the parameters that are associated with serum cholesterol and subsequent risk for CHD has been a common theme in CHD prevention over the years, and various beneficial changes have been observed in both patients and the nonpatient general population. Using diets that are characterized as low in fat, saturated fat, and cholesterol (typically <20% calories as fat, <200 mg cholesterol, with >50% unsaturated fats) while emphasizing increased consumption of fruits and vegetables have induced changes in serum lipids that are similar to those observed with lovastatin.[442–445] Approximately 30% reductions in total-CHOL and LDL-CHOL along with smaller reductions in TG were seen with both statins and dietary

changes in the studies from Jenkins' group, whereas smaller changes due to diet were observed in the DASH and OSLO studies. Strict dietary studies such as these and others have their drawbacks when it comes to dietary recommendations for fat and cholesterol content per se; in some cases they were too small to detect significant effects on mortality, and in others no reductions in mortality due to CHD were actually seen.[446–448] Although it is possible that the larger studies simply were not carried out for a sufficiently long time to observe a significant effect, the modest changes in serum lipids that have been observed with these dietary studies coupled with the general lack of effect on CHD mortality indicate that, changing serum lipids without addressing mechanisms of inflammation does not significantly alter mortality.

While changing total fat and cholesterol through diet alone appears to have little effect on risk, addressing multiple risks at once does appear to provide preventive benefits. In the Stanford Coronary Risk Intervention Project, low-fat, low-cholesterol diets were prescribed along with an endurance exercise training program. Significant reductions were observed for LDL-CHOL, TG, and body weight and exercise capacity was enhanced while achieving a 47% reduction in the rate of narrowing of diseased coronary artery segments.[449] Similar results have been seen with the Lifestyle Heart Trial where not only did the experimental groups exhibit a relative reduction in the diameter of stenoses of 7.9% compared with a 27.7% increase in diameter in the control group but also was the RR for a cardiac event 2.7 in the controls.[450,451] The Pritikin residential lifestyle intervention utilizes a high-fiber, low-fat diet with high fruit and vegetable consumption and 45–60 minutes of aerobic exercise with additional flexibility and resistance exercises. Results from this program are similar, with experimental groups reducing total-CHOL, LDL-CHOL, and TG by approximately 25% along with reductions in body weight as well as in the requirement for coronary bypass surgery as treatment for CHD.[452,453] From these results, it is difficult to determine how much of the effects were due to the changes in diet and how much were due to the extensive exercise activities. However, because of the multiple mechanisms through which exercise can reduce risk it is much more likely that the risk reduction is due to the additive effects of exercise in the diet plus exercise protocols.

As discussed earlier, oxidative damage to LDL and LDL-CHOL is an integral component of risk for CHD and larger LDL particles are more resistant to oxidation. Interestingly, using a diet plus exercise approach to reducing risk reduces the oxidizability of LDL.[361,454–456] A reduction in LDL oxidation of up to 27% was observed in non-CHD patients, whereas a reduction of 15% was observed in CHD patients. Similar results have been observed in postmenopausal women as well.[457] These results are interesting because they indicate another mechanism through which a diet plus exercise effect can reduce risk for CHD, a reduction in the oxidizability of LDL-CHOL.

4.3.2.3 Inflammation

Lifestyle modification also has been demonstrated to contribute to reduced inflammation. CRP can be used as a marker for the general inflammatory status of a person. When considered in conjunction with total-CHOL in plasma, CRP + total-CHOL

serves as a better predictor of CHD risk than total-CHOL alone, is a stronger predictor of cardiac events than LDL-CHOL, and appears to add prognostic information for all degrees of metabolic syndrome.[458,459] This is, of course, logical because inflammatory mechanisms are central to the cause of atherosclerosis. In diet plus exercise studies such as the Pritikin protocol, declines of 45% in CRP and similar decreases in amyloid A and sICAM have been observed, as are reductions in platelet aggregation, formation of TXs, and formation of plasminogen activator inhibitor.[460–462] These results indicate a general suppression of inflammation by the combined treatments. Long-term weight-loss programs in the form of lifestyle modification in obese women also significantly reduce CRP, P-selectin, and ICAM-1.[463,464] Furthermore, in the aforementioned diet-only studies by Jenkins and others, CRP decreased by 28% in the diet group and 33% in the lovastatin group, suggesting that the ability of diet to reduce CRP is comparable to that of statin therapy. While reductions in CRP and adipose-derived cytokines are associated with weight loss in obese individuals, the Jenkins studies did not produce weight loss but still displayed a reduction in inflammatory status. In a recent short-term diet plus exercise study by Izadpanah and others[465] in overweight/obese children where there was a small change in weight, significant reductions of 20%–40% in proinflammatory and adipose-derived cytokines were observed as well. These results indicate that weight reduction is poorly correlated with the anti-inflammatory effects of diet/exercise, further supporting the concept that obesity per se is not always a high-risk phenotype.

4.3.2.3.1 Lipid Mediators of Inflammatory Signaling

As discussed in detail in Chapter 2, the mechanisms responsible for the observed reductions in inflammation by dietary components in the studies mentioned in Section 4.3.2.3 are most likely related to a combination of enhancing nutrient status by increasing fruit and vegetable consumption and of the anti-inflammatory effects of various phytochemicals and fatty acids. The effects of nutrient status on inflammatory signaling have already been discussed, whereas those of phytochemicals and other components of a healthy diet follow. The major targets for reducing inflammatory risk are the signal transduction pathways that lead to the synthesis of proinflammatory cytokines and those that lead to the activation of PLA_2, which then leads to the production of proinflammatory lipid mediators.

From the diet plus exercise studies where reductions in CRP are commonly seen, an enhanced availability of EPA and DHA from the increased intake of α-linolenic acid (ALA) (an ω-3 fatty acid from nuts, seeds, and some vegetable oils) has been cited as one of the reasons for an anti-inflammatory effect. EPA and DHA are made from ALA and are commonly known to compete with AA for incorporation into membrane phospholipids. Once they are in the membrane phospholipids, they would reduce the production of PGE_2 and LTB_4 and enhance the production of the much less inflammatory PGs, TXs, and LTs. As discussed later, however, this may not be a major mechanism of protection.

As described previously in Section 4.2.2, the mechanical activation of plasma membrane–bound calcium channels allows the entry of calcium into the endothelial cells. The increased cytosolic calcium will ultimately activate PLA_2, Ras, and PKC, with the activated Ras and PKC enhancing the activation of PLA_2 (an effect

also produced by ROSs).[166,466–469] Once AA molecules are released from membrane phospholipids, they are metabolized into prostaglandin G_2 (PGG_2) by COX-1 and COX-2. The PGG_2 is converted into prostaglandin H_2 (PGH_2) and then into a variety of proinflammatory PGs and TXs by specific synthases.[141,156,159,167,470] AA also is made into the proinflammatory LTs (including LTB_4) by 5-LOX in concert with other synthases.[141,166,168] Thus, the mechanical activation of proteins in endothelial cells by intermittent shear results in the synthesis of proinflammatory lipid mediators. Dampening the production of these molecules will then have the effect of reducing proinflammatory effects. Through competing with AA for incorporation into membrane phospholipids, additional availability of DHA and EPA will lead to an enhanced production of the less-inflammatory 3-series PGs (PGE_3, PGA_3, and PGD_3), TXA_3/TXB_3, and LTB_5 by the same enzymes that modify AA. In addition to this indirect anti-inflammatory effect, as precursors for the synthesis of resolvins and protectins, DHA and EPA also might have a direct anti-inflammatory effect.

For these effects to occur, there must be some dietary or nutritional issues that are not optimal in the general population, issues that are corrected by the commonly used preventive diets. As a nutritional issue, it is the essential fatty acids linoleic acid (LA, an ω-6 fatty acid) and ALA (an ω-3 fatty acid) that are important. Because LA is made into AA while ALA is synthesized into EPA and DHA, many have proposed the idea of reducing LA intake and increasing ALA intake through dietary modification.[471] Such recommendations are hypothesized to reduce inflammatory and enhance anti-inflammatory effects by reducing AA and increasing EPA and DHA levels in vivo. They are also associated with an antiarrhythmic effect and, as discussed later, these effects may be the predominant protective effect of increasing ALA intake from nuts and oils, whereas different mechanisms may be responsible for the protective benefits of EPA/DHA from fish.[472–474]

In a series of meta-analyses of human clinical trials where LA consumption was manipulated, the higher LA intakes were associated with an anti-inflammatory effect (lower CRP), along with a lowered LDL-CHOL cholesterol and a higher HDL to LDL ratio.[475,476] Enhanced LA intake also does not substantially change the AA content of platelets and erythrocytes.[477] These diet-induced changes are certainly associated with a reduction in risk for CHD and make it difficult to justify reducing the intake of LA on the grounds that lower AA will reduce inflammatory signaling, per se.

Greatly increasing ALA intake to reduce inflammatory signaling also is suspect. Conversion rates of ALA to EPA are only 1%–8% in men and approximately 8%–21% in women; conversion to DHA is much less than 1% for both.[478,479] ALA is also a small-minority fatty acid in available vegetable oils (<2% except for ~50% in flax oil), and there is only 9 to 10 g of ALA per 100 g of walnuts or butternuts[480] (the two major ALA-containing non-oil foods). Because of the inefficient conversion to EPA and DHA, excessive calories from oil or oily nuts would need to be consumed to get the biological availability of a single gram of EPA (and of far less DHA). Even substituting high-ALA foods for low-ALA foods is impractical; 25 g of ALA for men and 10 g of ALA for women would have to be consumed just to produce an approximate 1 g benefit of EPA. Half of the dietary intake of lipid would have to come from walnuts and be supplemented with walnut oil and flax oil in order to obtain an increase in the

daily ALA intake of approximately 10 g without substantially increasing calories.[481] Such extensive dietary changes are not very easy for most.

Even in clinical trials using ALA supplements, the EPA content of platelets, neutrophils, and monocytes was either unaltered or inconsistently increased to greater than or equal to 300% at ALA intakes above 10 g/day, with little to no effect observed on DHA content.[475,478,479,482–484] Thus, the far smaller increases in ALA consumption that are typical of the preventive diets discussed earlier are unlikely to be a source of additional EPA and DHA to achieve an effective competition with AA and a substantial anti-inflammatory effect through this mechanism. Recommending substantial increases in ALA intakes to a greater than or equal to 10 g/day range for what is an inconsistent increase in EPA content in membranes, along with an inconsistently positive effect on risk for disease, is not practical.[484,485] This does not mean that high-ALA foods should not be recommended as a component of a preventive diet. Such a recommendation is appropriate because only about 70% of adults in the United States meet the AI for ALA and simply switching from low-ALA oils to higher ALA oils for cooking has been associated with a reduction in risk for CHD.[486] Because clinical trials have revealed essentially no increase in EPA/DHA incorporation into cellular membranes with moderate diet-based increases in ALA consumption, it is more likely that these diets improve risk for CHD, in part, by helping to correct underlying ALA insufficiencies along with correcting other nutrient insufficiencies.

This does not mean that altering the dietary intake of EPA and DHA (as opposed to ALA intake) does not provide any benefit. Consuming fish that already contains EPA and DHA is a highly practical choice to modify EPA and DHA availability, a practice that not only avoids large-scale dietary changes but also efficiently corrects any possible insufficiency in EPA/DHA due to a low ALA intake. Based on results from a wide array of studies, there appears to be a consistent reduction in risk for CHD at EPA intakes of 0.25–3 g/day, whereas intakes of 2–4 g/day reduce markers of inflammation and platelet adhesiveness.[484,485,487–496] Interestingly, the majority of the protective effects against cardiac deaths occur in the 250–500 mg range of additional EPA intake. In studies that indicate a reduction in risk at the lowest intakes, subjects already had very low EPA/DHA (and ALA) consumption to begin with as well as a high risk for coronary artery disease death. From a practical standpoint, two 6-oz. servings of fatty fish (e.g., salmon or herring) a week will provide a sufficient intake of EPA and DHA to average well within the lowest beneficial range as well as minimize the risk of exposure to mercury, polychlorinated biphenyls, and other toxic contaminants.[497] The recommendation of the AHA for a weekly consumption of at least two servings of fish or approximately 1 g/day of a mix of both EPA and DHA in a supplement is certainly reasonable.

Although anti-inflammatory effects of EPA/DHA supplementation are observed only at higher levels of intake (>3 g/day), the recommendation for a lower intake is not necessarily inappropriate. A limitation of the studies that document a reduction in systemic inflammation with higher doses of EPA/DHA is that CRP is most often used as a marker for inflammation. Both EPA and DHA are known to be PPAR agonists,[498–500] and PPAR activation results in an inhibition of NFκβ-mediated pro-inflammatory signaling. This may, in fact, be the reason for the protective effect of diets that contain higher amounts of ALA but do not actually increase EPA incorporation into cellular membranes. For these reasons, the dietary recommendations that

are specifically related to LA, ALA, EPA, and DHA include consuming two servings of fish high in EPA/DHA each week and at least one serving of a high-ALA food each day to ensure adequate ALA/EPA/DHA intake.

Altering the intake of ALA, EPA, and DHA is not the only way to suppress proinflammatory signaling by lipid mediators. A variety of phytochemical components that are commonly found in fruits and vegetables are known to attenuate the production of the proinflammatory lipid mediators. Epigallocatechin-3-gallate (EGCG), quercetin, and kaempferol inhibit COX-1, COX-2, and LOX activities with subsequent decreases in PGE_2 production. Phenolics from extra virgin olive oil, tyrosol, hydroxytyrosol, apigenin, and luteolin, and several oleuropein compounds also inhibit the production of PGE_2, LTB_4, and TXA_2.[501–503] In clinical trials using virgin and extra virgin olive oils in the 25–50 g/day range, the extra virgin version consistently provides anti-inflammatory effects at the lower range of intake.[502,504,505] From a dietary perspective, recommending the consumption of approximately 25 g of extra virgin oil at a caloric cost of approximately 225 kcal (or about 10% of total caloric intake) is neither harmful nor impractical, especially when extra virgin olive oil is substituted for other cooking oils.

4.3.2.3.2 Cytokine Mediators of Inflammatory Signaling

As discussed in Section 4.2.1, the constant activation of proinflammatory signaling by low, intermittent shear stress is the proximate cause of CHD, whereas constantly enhancing these responses through additional activation mechanisms results in the progression of initial fatty lesions to the more deadly type V and VI lesions. Activation of the NFκβ-, MAPK-, and p38/JNK-associated signal transduction pathways is essential to inducing the synthesis of proinflammatory signals, and suppressing these pathways is central to the proresolution process. Thus, in order to attenuate the unavoidable proinflammatory response to these mechanical stresses, proinflammatory signaling processes simply need to be attenuated. These desired effects can be accomplished by eating foods that contain phenolic compounds that inhibit the NFκβ-, MAPK-, and p38/JNK-associated pathways.

There are a large variety of phenolic compounds that are capable of doing just this, and these compounds are predominantly found in fruits, vegetables, whole grains, teas, wine, and many other plant-based foods.[506] Foods that contain clinically relevant amounts of phenolic compounds include various herbs and spices (e.g., cloves, Ceylon cinnamon, dried pot marjoram, dried spearmint, dried wild marjoram oregano, dried summer savory, dried sweet basil, dried sweet bay, dried marjoram, and capers), dried fruits (e.g., prunes, raisins, and figs), fruits (e.g., black elderberry, black chokeberry, skunk currant, black raspberry, black currant, Canada blueberry, and gooseberry), nuts (e.g., chestnut, pecan, walnut, pistachio, hazelnut, almond, Brazil nut, and cashew), beans (e.g., adzuki bean, lentils, black bean, and broad bean), vegetables (e.g., Swiss chard, dandelion, red cabbage, green bean, chili pepper, spinach, sweet pepper, raw Brussels sprouts, broccoli, and cauliflower), oils (e.g., peanut oil, extra virgin olive oil, canola/rape, and sesame), and whole grains (e.g., buckwheat and wheat flour, wheat germ, oat flour, corn flour, and rice flour).

Although many of the phenolic compounds found in the aforementioned foods have antioxidant properties, their preventive properties are likely due to their ability

to modify components of signal transduction pathways. This is because with a half-life of only 1 to 2 hours and levels of consumption (at the highest levels documented) that do not come within 20% of that necessary to provide antioxidant function in vivo, it is doubtful that their antioxidant properties contribute significantly to a diet-based protective effect.[441,507–509]

Some of the more commonly known phenolics that have been studied for their ability to modify signal transduction pathways include the flavonoids quercetin, apigenin, EGCG, theaflavin, genistein, anthocyanins, and kaempferol; the phenolic acids ellagic acid, capsaicin, and curcumin; and the stilbenoid resveratrol.[352,502,508,510–519] In both cell culture and animal models, the syntheses of IL-6, IL-1β, TNF-α, and interferons have been observed to be suppressed by these compounds. In response to the reduction in synthesis of these signaling molecules, a reduced expression of iNOS, COX-2, ICAM, and MCP-1 has also been observed with the same phenolics.[502,508,513,516,518]

In a related area, the activation of PRRs is an essential component of NFκβ and MAPK-mediated inflammatory signaling in response to infection and damage. As recently reviewed,[513] experiments have revealed that the activation of TLR4 can be suppressed by curcumin and sulforaphane (cruciferous vegetables) and nucleotide oligomerization domain (NOD) 1/2 activation can be inhibited by curcumin. Resveratrol, EGCG, luteolin, and quercetin can directly inhibit TBK1, whereas resveratrol also inhibits TRIFF to suppress both the MyD88 and the non-MyD88 pathways of TLR signaling.[513] These results are important because they will reduce the contribution of infections to the CHD process.

Because activation of NFκβ is essential to proinflammatory signaling, its suppression provides an important target for attenuating inflammatory signaling. Activation of NFκβ signaling can also be suppressed through a variety of mechanisms including inhibition of IKK activity, which then suppresses Iκβ release and its subsequent degradation. This prevents free NFκβ from binding in the nucleus. EGCG and theaflavins inhibit NFκβ signaling in this way, and quercetin, curcumin, kaempferol, and lycopene inhibit NFκβ signaling by suppressing nuclear translocation of p50/p65. Apigenin, morin, anthocyanins, and procyanidins also suppress NFκβ signaling through a variety of related mechanisms, including inhibition of phosphorylation of p65 and DNA binding of NFκβ.[502,508,516,518]

The various MAPK pathways also are important targets for suppressing inflammatory signaling, and various phenolics are known to do this as well. Apigenin, luteolin, quercetin, resveratrol, EGCG, and kaempferol suppress ERK1/2–MAPK, JNK1/2–MAPK, and p38–MAPK pathways, whereas the cyanidins suppress ERK activation. EGCG has been observed to inhibit ERK–MAPK and, in addition to suppressing the MAPK pathways, curcumin inhibits PKC, a calcium-activated enzyme that can activate various components of the various MAPKs.[502,508,516,518]

All these effects on different aspects of NFκβ and MAPK signaling have been tested in a variety of different cell types. Some cell types exhibit suppression of all MAPK pathways as well as NFκβ pathways by single phenolics, whereas in others only one or two pathways are inhibited by the same phenolic; similar differences in responses are observed for different phenolics as well.[502,508,516,518,520] Thus, it is doubtful that any single-phenolic supplement will have the desired overall preventive

effects. The dietary recommendation to produce the suppression of all proinflammatory signaling pathways therefore includes a large variety of fruit and vegetable sources in order to maximize the consumption of the many different phenolics.

4.3.2.3.3 Redox Control

Preventive effects of phenolics are not limited to attenuating inflammatory signaling. An increased expression of various antioxidant and redox control enzymes also enhances redox control. SOD, CAT, GPX, and glutathione reductase are all highly relevant for increasing protection from additional ROSs/RNSs in addition to integrating with Trx and Prx to ensure appropriate control of redox status.

The role of SOD and CAT is to promote the dismutation of superoxide anions and peroxide to O_2 and H_2O at rates that are much faster than spontaneous rates of dismutation. GSR and GPX also work with glutathione to eliminate H_2O_2. The benefit of these reactions is that they minimize both $O_2^{\bullet-}$ and H_2O_2, which in turn minimizes the production of •OH. In concert, these antioxidant systems ultimately reduce damage to LDL-CHOL as well as to proteins, lipids, and DNA caused by ROSs.[521,522]

It is highly instructive that activation of Nrf2 and its subsequent interaction with ARE increase the synthesis of a variety of protective enzymes, including Mn-SOD, CAT, glutathione reductase, Trx, Prx, HO-1, and GCL.[350–353] A variety of endogenous factors are capable of activating Nrf2, including PGJ_2, PKC, MAPKs, and PI3K/PKB, in response to a variety of stresses that enhance cytosolic calcium and ROSs.[351,515] It is through the Nrf2/ARE stress-response genes that polyphenols also function to enhance protection from inflammation-produced ROSs as well as to enhance control of cellular redox states.

A number of phenolics have been documented to activate Nrf2/ARE binding to DNA and induce the expression of antioxidant, redox control, and other protective enzymes. Quercetin, resveratrol, diallyl sulfide, *S*-allyl cysteine, lycopene, EGCG, curcumin, and sulforaphane have all been documented to activate Nrf2/ARE and induce synthesis of GST, GSH, HO-1, SOD, CAT, GCL, Trx, Prx, and UDP-GT.[523–536] Nrf2 normally forms complexes with Keap1 in the cytosol, which prevents it from entering the nucleus and targets it for degradation. Oxidation of thiol residues on Keap1 or phosphorylation of NRF2 will disassociate it from the complex to allow it to enter the nucleus and bind to the ARE-binding sites.[352] From induction studies, an array of phenolics alters the oxidation state of susceptible thiols on Keap1, whereas others activate protein kinases, which then phosphorylate Nrf2. Phenolic phytochemicals also can form conjugates with susceptible thiols on Keap1, which then reduces the degradation of Nrf2 to enhance its binding to the ARE/ERE response element.[351,537] These results indicate that dietary polyphenols modify the oxidation or phosphorylation state of regulatory proteins such as Keap1 and various components of the MAPK pathways to activate Nrf2/ARE and the expression of a large array of protective enzymes.

It is also worth noting that HO-1 (along with the constitutive HO-2) catalyzes the rate-limiting step in heme degradation to produce free iron, bilirubin, and CO.[538,539] The HO-1 enzyme is highly inducible through Nfr2/ARE activation; inducing this enzyme would make sense considering the potential for oxidative damage to inactivate heme proteins and produce a requirement for an additional capacity for scavenging the iron. As a consequence of increased HO-1 activity, CO production is

enhanced, and this molecule appears to be important in inflammatory responses. Macrophages with induced HO-1 release less TNF-α and, in similar cell culture models, HO-1 inhibits the synthesis of IL-1, IL-6, ICAM, VCAM, and E-selectin.[539–543] While the chemistry of the effect is unknown, CO appears to affect the activity of MAPK pathways through upstream events.

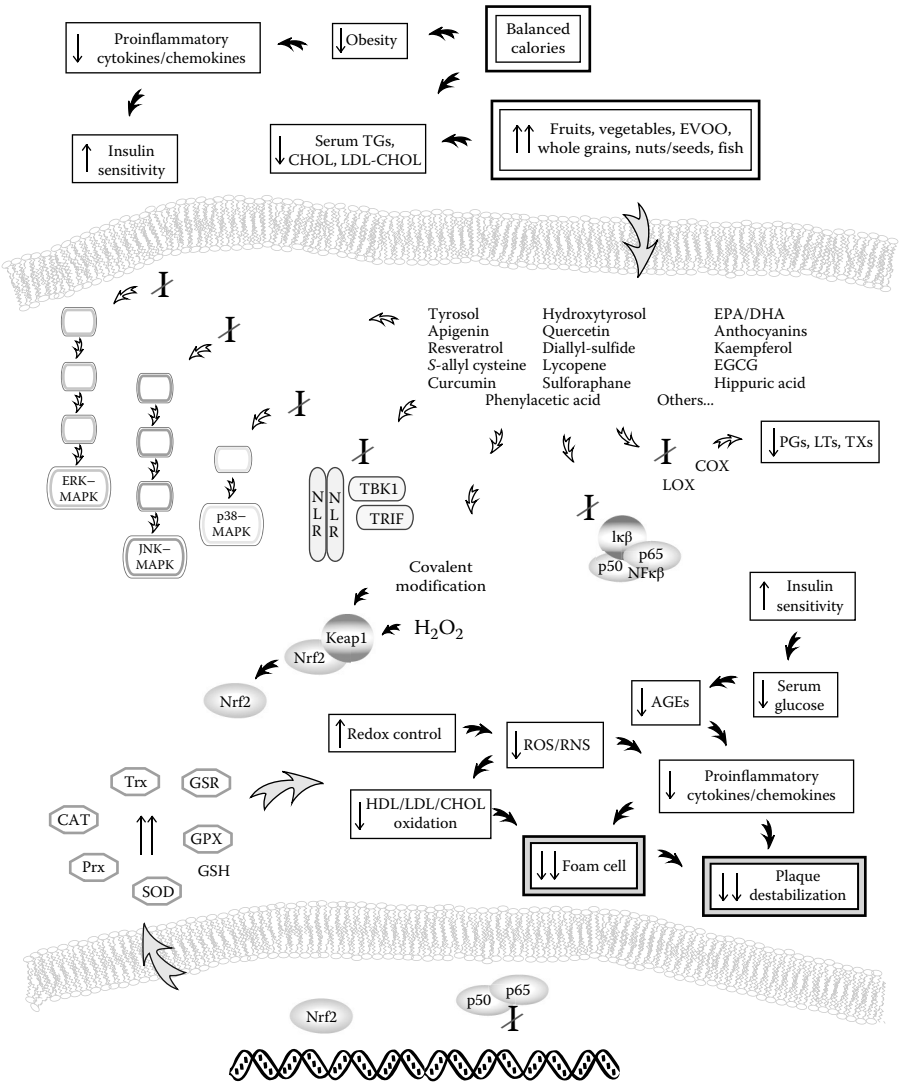

FIGURE 4.3 Reduction in mechanisms of risk for atherosclerosis by diet: maintaining body weight through diet and increasing the consumption of fruits, vegetables, and fish reduce proinflammatory signaling through suppressing the activation of mitogen-activated protein kinases (MAPKs), nuclear factor κ-β (NFκβ), cyclooxygenase (COX), and lipoxygenase (LOX) as well as inducing the synthesis of antioxidant and redox control enzymes via Nrf2. EVOO refers to extra virgin olive oil.

From all of these studies, it is apparent that the additive and synergistic effects of an array of phenolics are responsible for the observed protective effects on inflammation and CHD. Therefore, dietary recommendations include specific recommendations for a wide variety of different foods with moderate to high phenolic content to produce a diet that maximizes the preventive effects.

4.4 SUMMARY AND RECOMMENDATIONS

4.4.1 Etiology of Coronary Heart Disease

CHD, caused by atherosclerosis, is an inflammatory disease. The apparent proximate cause of the initiating inflammatory events is an intermittent low shear mechanical stress, which occurs primarily at the outside walls of bifurcates, the leading edges of the bifurcates themselves, and the inside curve of curved stretches of arteries. The intermittent mechanical stress activates a nonresolving inflammatory stress response via the entry of calcium into the cell through mechanical stress–activated calcium channels and the mechanical activation of G-protein-coupled receptors, NADPH oxidase, and PI3K. The subsequent activation of PLA_2, and PKC, along with PI3K leads to the activation of various components of NFκβ and the different MAPK pathways, resulting in the production of proinflammatory lipid mediators and cytokines. This then leads to the production of chemoattractant factors and a breakdown of various adhesion proteins to more easily allow the entry of monocytes, which differentiate into macrophages, as well as various lipoproteins (and eventually the entry of other inflammatory cells) into the vessel wall. At the same time, the activated G-coupled proteins and NADPH oxidases lead to an enhanced production of extracellular ROSs. The ROSs oxidize LDL and LDL-CHOL, which then bind to class A and CD36 scavenger receptors (PRRs) on macrophages, endothelial cells, and smooth muscle cells and activate their uptake by these cells. With continued uptake of oxidized lipids by the macrophages, they eventually become lipid-laden foam cells that form the primary type I and type II fatty lesions. The activation of PRRs by LDL-Ox and CHOL-Ox enhances proinflammatory signaling in the vessel wall through the activation of TLRs, adding to the proinflammatory stress responses to the intermittent mechanical forces.

When these localized inflammatory responses are enhanced through a variety of endogenous and exogenous factors, continued expansion of the lesions mediated by the expanding repertoire of local inflammatory cells and platelets will increase the size of the lesions and enhance their instability through increased production of CD40 from platelets. The resulting increased expression of MMPs by endothelial and smooth muscle cells along with cathepsin G will degrade the collagen, VE-cadherin, and fibronectin within the plaque. With continuing aggressive inflammatory responses, the lesions will progressively deteriorate and ultimately break off as a thrombus and block a smaller vessel, causing heart attack or stroke.

The role that the various risk factors play in the disease process is to accelerate the local mechanical shear–induced inflammatory stress response. Over the last several decades, due to changes in dietary patterns and activity levels, there has been a consistent increase in adiposity, resulting in expanding adipose tissue

production of proinflammatory cytokines. These circulating cytokines exacerbate the local responses in the arterial vessel wall. In individuals who are very inactive, this chronic low-level proinflammatory signaling also contributes to the development of insulin resistance. With insulin resistance, the serum levels of glucose rise substantially, which leads to the formation of Amadori products. These products rearrange to form various AGEs, which then bind to RAGEs. RAGEs are PRRs that recognize damaged proteins and, as such, they also activate proinflammatory signaling pathways, contributing additional risk for CHD. AGEs can also initiate lipid peroxidation events and in this way contribute to enhancing LDL and LDL-CHOL oxidation. Hypertension increases the mechanical stretch of the vessel walls and contributes to the mechanical stress response in order to enhance a localized hypertrophy response. Although this response may be necessary to maintain appropriate regulation of blood flow in the face of increased pressures, it does add to the localized inflammatory stress responses. In addition to these factors, a large majority of Americans have an insufficient consumption of vitamins and minerals that are directly involved in antioxidant function and redox regulation and therefore do not have optimal control of either cellular redox function or antioxidant function. These nutritional insufficiencies lead to increased oxidation of LDL and cholesterol as well as to exaggerated inflammatory responses, again, enhancing the CHD processes.

In order to maximally reduce risk for atherosclerosis, attenuating the various mechanisms of cause is necessary. Because inflammatory stress responses are the primary cause of CHD, inflammatory signaling is a primary target for preventive measures. The first preventive measure would be to correct nutritional insufficiencies by following a diet that provides sufficient amounts of all the required nutrients in order to optimize redox and antioxidant control. This will minimize the likelihood of occurrence of inappropriately large inflammatory responses to low-intensity stressors. Then, the diet concept must be modified to include the consumption of only a sufficient amount of calories to maintain body weight in order to avoid inflammatory responses due to increasing adiposity. In addition to this, the diet must also include an array of foods (fruits, nuts, and vegetables) that have a high phytochemical content in order to dampen inflammatory signaling and reduce inflammatory responses to unavoidable stressors. By adding sufficient physical exercise, insulin resistance can be minimized while additional anti-inflammatory effects can be obtained.

4.4.2 Dietary Recommendations for Prevention of Coronary Heart Disease

Because CHD is an inflammatory disease, the anti-inflammatory diet and exercise protocols presented in Chapter 2 become the preventive measures for CHD as well. The diet recommendations are designed to provide the greatest anti-inflammatory effects possible while ensuring compliance with current nutritional recommendations, as well as being practical. The specific foods that make up the recommendation for an anti-inflammatory diet are chosen based on their known anti-inflammatory effects. The recommendations depart from the *MyPlate* approach by expanding the food categories to include a 0.25-cup serving of nuts and seeds as well as

a 1-cup serving of beans and lentils as individual categories in order to emphasize phytochemical intake. In addition, the consumption of high-phytochemical foods is increased at the expense of grains and cereals by increasing the number of fruit servings to 3 cups/day from 2 and the number of vegetable servings to 4 cups/day from 3. The addition of a minimum of two servings of fish each week provides additional anti-inflammatory effects through increased EPA/DHA consumption. The reader will note that the caloric content of these recommendations will approximate 1500–1700 kcal, which is somewhat less than the average daily requirements for both men and women. Because the consumption of moderate amounts of individual sources of proteins, carbohydrates, or fats has little to no negative impact on CHD, the remaining calorie requirements can be obtained from whole foods at the individual's discretion, provided, of course, that no excess calories are consumed that will result in an increase in adiposity.

4.4.3 Physical Activity Recommendations for Prevention of Coronary Heart Disease

For exercise, it is apparent that different forms of exercise provide risk reduction effects through at least three basic mechanisms. Frequent short periods of low- to moderate-intensity endurance exercise and/or resistance exercise enhance insulin sensitivity and reduce risk for CHD by minimizing serum levels of glucose. Longer term periods of strenuous physical activity for 20–30 minutes appear to enhance antioxidant enzyme function, which would enhance redox control and reduce risk by dampening proinflammatory signaling. Longer term exercise of approximately 60 minutes that is of vigorous intensity (at a minimum of 6 METs) and utilizes large muscle groups provides anti-inflammatory effects due to large increases in IL-6, sIL-1ra, sIL-1r, and sTNFr while maintaining insulin sensitivity and enhancing antioxidant and redox enzyme function in most tissues, an effect that reduces ROS damage to a variety of cell components as well as to LDL/HDL/CHOL. In addition, considering the difficulties in delineating the protective effects of exercise from those of weight loss in many studies where daily exercise periods exceed 45–60 minutes in duration, a confounder that occurs far less frequently with shorter periods of activity, it appears that the longer periods of exercise at moderate intensity may be associated with reducing obesity-related risks as well.

Thus in order to obtain optimal reduction in risks for CHD through the anti-inflammatory, enhanced antioxidant/redox control, weight maintenance, and insulin-sensitizing effects of exercise, the American College of Sports Medicine and the AHA recommendations for adolescents that includes vigorous activities that are 6 METs or greater in intensity and carried out for a minimum of 1 hour for 5 days each week are recommended here. These activities include jogging, running, cycling at 10–12 mph or greater, swimming, cross-country skiing, or similar vigorous activities.[544]

Although not providing optimum effects, reductions in risk can also be obtained from shorter duration (20–30 minutes) and higher intensity activities performed three to five times each week. Such activities include high-intensity running, cycling, swimming, and skiing and would maintain insulin resistance and enhance redox control.

Lower levels of protection might be provided through several short periods of moderate-intensity activities performed daily, such as fast walking, yard maintenance, leisurely bicycling, volleyball, baseball, skating, or canoeing, and through resistance-type weight-lifting and muscle-endurance training activities by maintaining insulin sensitivity.

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5 Osteoporosis

David W. Dodington and Wendy E. Ward

CONTENTS

5.1 INTRODUCTION

Osteoporosis is characterized as a thinning of skeletal tissue that occurs due to a substantial loss of hydroxyapatite crystals and bone matrix proteins. This phenomenon results in a weakened skeleton that is more prone to debilitating fragility fractures—the ultimate outcome that lifestyle strategies including diet and exercise aim to prevent. Osteoporosis is defined as "a skeletal disorder characterized by compromised bone strength, predisposing to an increased risk of fracture."[1] The emphasis is on compromised bone strength, and recognition that bone strength is altered by both the quantity and quality of a skeletal site. Bone quantity is routinely measured clinically as bone mineral density (BMD) by dual-energy X-ray absorptiometry, whereas bone quality is more challenging to assess but includes consideration of how mineral and matrix proteins are integrated to result in structurally

strong bone. Sophisticated imaging using quantitative computed tomography and magnetic resonance imaging are proving useful in more fully understanding how changes in bone structure and mineral content relate to risk of fragility fracture. In animal models, it is possible to directly measure the strength, specifically the maximum load a specific skeletal site can withstand before fracture, with a materials testing system.

The International Osteoporosis Foundation reports that "worldwide, an osteoporotic fracture is estimated to occur every 3 seconds, a vertebra fracture every 22 seconds"—with a total of 200 million women suffering from osteoporosis throughout the world.[2] Men are also susceptible to osteoporosis albeit at lower risk than women. Genetics is a major risk factor for developing osteoporosis. Other risk factors include lifestyle—namely nutrition and physical activity—and since they are modifiable, it is especially important to consider their potential impact. Poor nutrition and sedentary lifestyle are independently linked to poorer bone health throughout the life cycle. Although osteoporosis is often viewed as a disease of aging, bone health is in fact a lifelong process. At any stage of the life cycle, the amount of mineral in the skeleton and skeletal strength is an accumulation of what has occurred in utero through to the present age.[3] As such, it can be argued that osteoporosis is a pediatric disease with geriatric consequences. Working from this premise fits well with the focus of this book, namely that a disease starts because of cellular dysfunction early in life and that actual symptoms of the disease state often do not appear until much later in the life cycle. However, most research investigating effects of whole foods and food components on bone health has focused on adult populations and using animal models that mimic aging, many of which have used ovariectomized (OVX) rodent models.

5.2 MOLECULAR ETIOLOGY OF OSTEOPOROSIS

5.2.1 Mechanisms of Bone Loss

Within the context of bone health and prevention of osteoporosis, symptoms are often silent until fragility fracture occurs—the ultimate endpoint that prevention and treatment strategies aim to avoid. Osteoporosis, or a heightened risk of fracture, occurs when the rate of bone formation and the rate of bone resorption are not balanced. Essentially, bone formation may be reduced, bone resorption may be elevated, or both situations may occur. The resulting loss of mineral, matrix, and overall bone microarchitecture leads to a fragile skeleton. There are three main cell types found in bone: the osteoblast, the osteocyte, and the osteoclast. Osteoblasts are the bone-forming cells that are derived from mesenchymal stem cells (MSCs). They are responsible for producing and mineralizing the bone matrix. As osteoblasts become isolated in the bone matrix, they become osteocytes, which cease to produce osteoid and mineralized matrix. Osteoclasts are derived from the monocyte lineage, which is derived from hematopoietic stem cells. Osteoclasts are, therefore, related to other inflammatory cell types such as macrophages. Osteoclasts are responsible for the degradation of bone tissue through release of acids and proteases.

Thus, balanced activity of osteoblasts and osteoclasts is essential to maintaining a healthy skeleton. Further discussion of the molecular regulation of osteoblasts and osteoclasts and their interconnected activities are extensively covered in textbooks and review articles.

Bone loss is most pronounced during aging. The loss of estrogen after menopause or after ovariectomy (in rodent models) is a well-characterized phenomenon that accounts for some of the deterioration of bone tissue during aging. Estrogen is known to stimulate osteoclast apoptosis while blocking apoptosis of osteoblasts and osteocytes. Estrogen withdrawal may also mediate its effects through the regulation of T cells. A recent review[4] highlights that T cells are involved in the deterioration of bone tissue that occurs after estrogen withdrawal. Specifically, T cell activation results in greater production of tumor necrosis factor-alpha (TNF-α) that leads to greater production of RANKL and subsequently osteoclast formation. Thus, estrogen withdrawal is recognized as a principle event in the development of osteoporosis. Additionally, increases in oxidative stress with aging contribute to the pathogenesis of bone loss. Traditionally, osteocytes were considered to be "inactive" once they became embedded in calcified bone matrix, but a recent review makes the case that a decline in osteocyte number is associated with reductions in bone strength[5] and that an increase in oxidative stress with aging may be a main mechanism by which a reduction in osteocyte number occurs.

5.2.2 Prevention of Bone Loss

Although the role of diet and exercise on symptoms, particularly BMD, has been extensively studied and reported on, the role of diet and exercise in preventing or attenuating cellular dysfunction in bone tissue—the focus of this chapter—has been less studied. It is now recognized that several mechanisms that mediate bone cell activity can be modulated by foods, food components, and physical activity. At the cellular level, foods and food components can impact estrogen-mediated pathways, the aryl hydrocarbon receptor (AHR), bone morphogenetic protein (BMP) signaling, Wnt/β-catenin signaling, peroxisome proliferator–activated receptors (PPARs), and reactive oxygen species (ROS). Physical activity mediates effects on bone cell activity through mechanical signals that are transduced and transformed into biochemical signals.

This chapter focuses on specific mechanisms by which foods and/or food components or physical activity can regulate osteoblast and/or osteoclast activity and thus may prevent dysfunction of bone cells that often accompanies the aging process. From a cellular perspective, information on how nutrition, specifically food components modulate bone cell activity is not extensive and is an expanding area of research study. Likewise, the mechanisms by which physical activity converts its mechanical signals to biochemical signals within bone cells is an active and ongoing area of study. These mechanisms are shown in Figure 5.1 that was developed to summarize, to the best of our knowledge, regulatory pathways of osteoblasts and osteoclasts that are modulated by specific food components or physical activity.

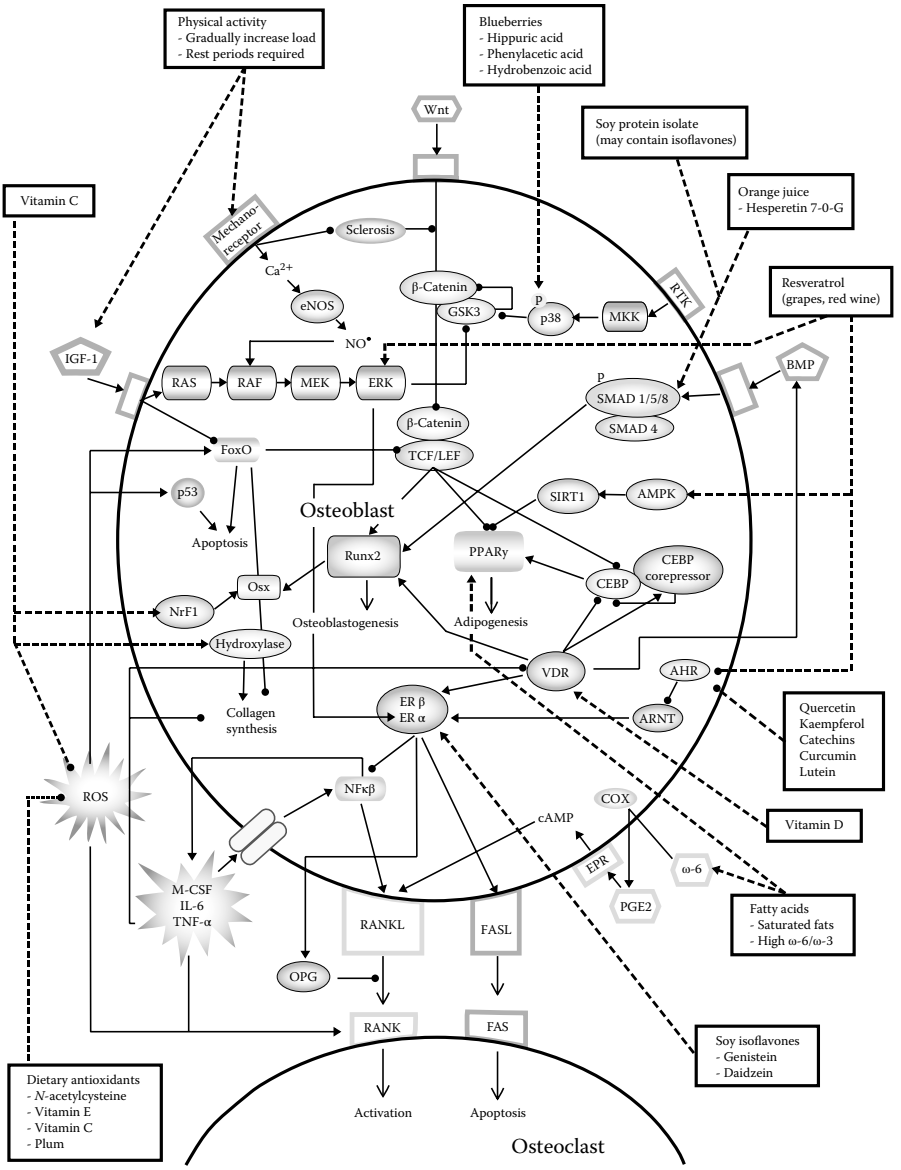

FIGURE 5.1 Impact of physical exercise and dietary modification on mechanisms of osteoporosis. Physical exercise as well as phytochemicals and nutrients from whole foods alter activities of signal transduction pathways to promote apoptosis and reduce the activation of osteoclasts as well as to promote osteoblastogenesis through reducing proinflammatory signaling, thus reducing risk for osteoporosis.

5.3 DIETARY MECHANISMS UNDERLYING PREVENTION OF OSTEOPOROSIS

5.3.1 Estrogen Receptor–Mediated Pathways: Isoflavones

There are several mechanisms by which estrogen receptor (ER) signaling affects bone metabolism. First are the genomic ER pathways. Because estrogens are hydrophobic molecules, they can readily diffuse across cell membranes and bind to the nuclear ERs.[6] The ER contains a ligand-binding domain and a DNA-binding domain. On binding its ligand, the ERs dimerize and bind to specific DNA sites known as estrogen response elements, which regulate the transcription of estrogen responsive genes. In the classical genomic ER pathway, ER binding in osteoblasts results in the recruitment of coactivators that enhance transcription of genes responsible for osteoclast apoptosis.[7–9] For example, increased expression of Fas-L on the surface of osteoblasts will result in increased activation of Fas on the surface of osteoclasts, leading to the apoptosis of osteoclasts. Additionally, increased expression of osteoprotegrin (OPG) will suppress osteoclast activation because it acts as a soluble RANKL receptor decoy.

In the nonclassical genomic ER pathway, the liganded ER can indirectly regulate gene expression by interacting with other DNA-bound transcription factors. When the ligated ER interacts with NFκβ, for example, it results in a decreased expression of macrophage colony stimulating factor (M-CSF), interleukin-6 (IL-6), and TNF-α.[10–13] Because M-CSF, IL-6, and TNF-α activate osteoclasts by upregulating RANKL on osteoblasts, their inhibition has a protective effect on bone.

Estrogens can also exert their effects through nongenomic mechanisms. Estrogen can activate rapid signaling pathways (MAPK–ERK pathway) downstream of growth factor receptors (e.g., insulin-like growth factor-1 [IGF-1] and transforming growth factor-β [TGF-β]). Activation of the mitogen-activated protein kinase (MAPK) pathway stimulates proliferation in osteoblasts and apoptosis in osteoclasts.[14] ERs located in the plasma membrane of osteoblasts and osteoclasts can induce rapid signaling pathways to also result in the stimulation of apoptosis in osteoclasts while simultaneously attenuating apoptosis in osteoblasts.[8,15]

The term "weakly estrogenic" or "weak estrogen" is often used to describe nutrients that are thought to act in a similar way to estrogens by activating these ER pathways. These include isoflavones, coumestrol, and lignans. The best characterized of these weakly estrogenic nutrients are the isoflavones, genistein, and daidzein. These isoflavones interact with both the alpha and beta ER isoforms; however, affinity for ER beta is much greater.[16,17] Daidzein has a lower affinity for ERs than genistein, but at saturable levels both have been shown to activate transcription from estrogen response elements (EREs) as effectively as 17β-estradiol. Consuming a diet rich in soy produces high physiological serum levels of free genistein and activation of ER beta.[18] Physiological concentrations of genistein suppress IL-6 production and boost OPG production in human osteoblast-derived cell lines.[19,20] Similarly, genistein and daidzein, as well as coumestrol directly suppress osteoclastogenesis induced by TNF-α, an effect that was negated by an ER antagonist.[21] Epidemiological evidence suggests that consuming whole soy foods rather than isolated isoflavones is

beneficial to bone health, particularly for fracture prevention. Clinical intervention studies regarding isoflavone supplementation often yield inconsistent findings; however, McCarty[18] suggests that this variability might be due to the fact that some isoflavones are administered as glycosides or in conjunction with soy protein. The soy or isoflavone effect on bone health, and whether it is better to study soy as a whole food to understand its role in preventing deterioration of bone tissue during aging continues to be actively studied.

5.3.2 Aryl Hydrocarbon Receptor: Resveratrol, Isoflavones

The AHR is responsible for detecting environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[22] TCDD is a toxin that can be found in cigarettes. When the AHR is activated by toxin, it recruits the aryl hydrocarbon receptor nuclear translocator (ARNT). This causes a problem because ARNT is also a coactivator of ERβ, so when AHR recruits ARNT, it can interfere with ER signaling.[23] AHR signaling inhibits the proliferation of rat calvarial osteoblast-like cells and mouse calvarial clonal preosteoblastic cells. It reduces the rate of calcium deposition and depresses mRNA expression of osteocalcin, an important protein implicated in mineralization and calcium homeostasis.[24] Several nutrients are reported to be able to inhibit the AHR and thus may improve bone health. Phytochemicals such as quercetin, kaempferol, several catechins, curcumin, and lutein are reported to act as antagonists for AHR.[25] Resveratrol, a polyphenolic compound found in the skin of red grapes and other fruits, is an AHR antagonist and prevents the damaging effects of TCDD on bone formation. TCDD alters the expression of bone specific genes; however, this effect is antagonized by resveratrol. Resveratrol has also been shown to completely antagonize the loss of mineralization induced by TCDD.[26] However, isoflavones such as daidzein cause AHR activation in vitro. This suggests that including small quantities of AHR inducers in foods may have some beneficial effects in the proliferation and differentiation of cells in animals.[27]

5.3.3 Bone Morphogenetic Protein Signaling: Soy Foods, Hesperitin

BMPs are members of the TGF-β superfamily. More than 20 BMP-related proteins have been identified and can be subdivided into several groups based on their structures and functions.[28] BMP2, BMP4, BMP6, and BMP7 act as major osteogenic inducers.[29] BMPs bind to two distinct serine/threonine kinase receptors and induce phosphorylation of Smad proteins known as BR-Smads. BR-Smads (Smad1, Smad5, Smad8) form complexes with Co-Smad (Smad4), and move into the nucleus, where they regulate transcription of target genes.[30]

BMP signaling is an extremely important component of bone development. DNA microarray analyses have revealed 184 BMP-early response genes that drive osteoblastic differentiation.[31] BMP signaling is also notably important because it activates Runx2, a master transcription factor in bone development. BMP does not directly induce the expression of Runx2, but it facilitates expression of Dlx5 in osteoblasts, and Dlx5 then induces expression of Runx2 in osteoprogenitor cells.[32] On activation of BMP signaling, Runx2 and BR-Smads physically interact with each other and

cooperatively regulate the transcription of target genes.[33] Runx2 promotes MSCs toward the osteoblastic lineage rather than the adipocyte lineage.

There are a number of nutrients that are thought to modulate bone metabolism through this BMP/Smad/Runx2 pathway. Soy-based foods, in addition to acting through ER signaling, as previously described, may impact this pathway. When OVX mice are supplemented with estrogen, there is a decline in the level of bone formation and bone resorption markers, but when OVX mice are supplemented with soy protein isolate (SPI), bone formation markers are elevated and there is a decline in bone resorption markers.[34] This suggests that SPI is largely anabolic and nonestrogenic. SPI may exert its effects by activating BMP signaling. Feeding SPI increases expression of BMP2 mRNA in bone, accompanied by increased Smad 1/5/8 phosphorylation and increased Runx2 protein.[35] Though the active components have not yet been identified, icariin, a flavonoid component of soy foods, has been shown to activate the BMP pathway and has also shown to be more potent than genistein in promoting osteoblast differentiation and mineralization.[36] Another bioactive compound that may act through this pathway is hesperitin. Hesperidin, a compound found in citrus fruits, is hydrolyzed by the gut microflora into hesperitin and conjugated into hesperetin-7-O-glucuronide (Hp7G).[37] In vitro studies have demonstrated Hp7G's ability to stimulate osteoblast differentiation as well as an increase in Smad1/5/8 phosphorylation and Runx2 expression.[37]

5.3.4 Wnt/β-catenin Signaling: Blueberry, Resveratrol

The canonical Wnt signaling pathway plays a crucial role in regulating the differentiation of osteoblasts.[38] In the absence of the extracellular Wnt signaling molecule, β-catenin, the intracellular signaling protein is held in a complex with Axin, APC, and GSK3β. GSK3β will phosphorylate β-catenin, targeting it for degradation. On activation of the Wnt receptor, β-catenin is released; it enters the nucleus and complexes with the transcription factor T-cell factor (TCF)/Lef to regulate gene expression. High levels of nuclear β-catenin/TCF drive osteogenesis, whereas lower levels drive chondrogenesis. Of great importance is the fact that an increase in Wnt/β-catenin signaling leads to higher expression of Runx2 and the subsequent commitment of MSCs to the osteoblast lineage.[39]

Recent studies have implicated this pathway in the reported beneficial effects of blueberries on the accumulation of bone mineral. Blueberry-supplemented diets have been shown to increase Wnt/β-catenin signaling and Runx2 expression.[40] This effect may be mediated by the MAP kinase p38 as it shows higher phosphorylation after consumption of blueberries. This is further supported because blueberry-induced Wnt/β-catenin is stunted by a p38 inhibitor. Once phosphorylated, p38 inhibits the activity of GSK3β, resulting in increased nuclear β-catenin/TCF. Blueberry feeding has also been shown to increase serum levels of hippuric acid, phenylacetic acid, and hydrobenzoic acid. An artificial mixture of these phenolic compounds has been shown to mimic the effects of blueberries; however, it is still unclear how these compounds activate p38. There also exists the possibility that other fruits such as dried plum might be beneficial through a similar mechanism. Another bioactive that has been shown to favorably modulate Wnt/β-catenin signaling is resveratrol. MSCs treated with

resveratrol show increased nuclear β-catenin/TCF and increased Runx2 expression.[41] There is evidence that this effect is mediated through extracellular signal–related kinase 1 and 2 (ERK1/2). Resveratrol treatment increases ERK activation; ERK in turn inhibits GSK3β via phosphorylation. The effect of resveratrol is blocked by treatment with an ERK inhibitor.

5.3.5 Peroxisome Proliferator–Activated Receptors: Fatty Acids

PPARs are a group of transcription factors that regulate energy metabolism and cell differentiation.[42] In bone, PPARγ is particularly interesting because it plays the opposite role to Runx2. PPARγ is a master transcription factor that inhibits osteogenesis and promotes adipogenesis. An important regulator of PPARγ is the CCAAT/enhancer-binding protein (CEBP) that seems to be indispensable for triggering adipogenesis.[43] PPARγ agonists that include certain antidiabetic drugs have been shown to cause bone loss and increase bone marrow fat accumulation.[42] Additionally, there is a reciprocal relationship between the expression of PPARγ and Wnt/β-catenin signaling.[44] Wnt/β-catenin signaling may suppress adipogenesis by reducing the expression of PPARγ and CEBP mRNA,[45] but the mechanism remains to be investigated at the molecular level.

This balance may help explain the association between high-fat diet and impaired bone health. Bone marrow stromal cells, when exposed to serum from high-fat-fed rats, show increased PPARγ levels and inhibition of Wnt/β-catenin signaling compared to serum from low-fat-fed rats.[44] A similar pattern is observed when the cells are exposed to an artificial mixture of nonesterified fatty acids (NEFAs) mimicking the concentration and composition of NEFA after high-fat feeding. However, it is unclear whether fatty acid metabolites affect PPARγ secondary to Wnt/β-catenin inhibition or if they can activate PPARγ directly. There has also been suggestion that polyunsaturated fatty acids (PUFAs) might regulate PPARγ because they lower adipose mass and alter bone mineral content in mice.[46] Dietary fat and PUFAs may also modulate bone metabolism through inflammatory/anti-inflammatory mechanisms; this is discussed in the Section 5.3.7 of this chapter.

PPARγ may also be modulated by resveratrol independently of Wnt/β-catenin signaling. Resveratrol activates SIRT1, a nuclear protein deacetylase. The mechanism is unclear, but it is thought to affect SIRT1 indirectly by increasing the activation of adenosine monophosphate-activated protein kinase (AMPK), which increases nicotinamide adenine dinucleotide (NAD) and in turn activates SIRT1.[47] Activation of SIRT1 by resveratrol decreases adipocyte development via inhibition of PPARγ, which in turn promotes osteoblast differentiation.[48]

5.3.6 Inflammation and Reactive Oxygen Species

Osteoporosis is largely an inflammatory disease. In fact, epidemiologic studies have shown that levels of systemic inflammation are able to predict bone loss and future fracture risk.[49] Furthermore, several inflammatory diseases are associated with bone resorption including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, celiac disease, cystic fibrosis, and chronic obstructive pulmonary

disease.[50] Along with inflammation, ROS plays a synergistic role in promoting bone loss as ROS leads to production of the proinflammatory cytokines TNFα, IL-1β, and IL-6.[51]

One of the principle mechanisms for inflammation-induced bone loss is through the RANKL–RANK interaction. Cytokines bind to homo- or heterodimeric Janus kinase (JAK) receptors resulting in the phosphorylation of signal transducer and activator of transcription (STAT) proteins. Once the STATs are phosphorylated, they are released from the JAKs and subsequently dimerize and then enter the nucleus where they regulate gene expression. The proinflammatory cytokines exert many genomic effects on the osteoblast and one of these critical effects is the increase in expression of RANKL on the surface of osteoblasts. RANKL will then interact with RANK on the surface of preosteoclasts causing them to become mature bone-resorbing osteoclasts. ROS are also able to upregulate RANKL in osteoblasts via the activation of the MAP kinase ERK that signals through STAT3.[34] Inflammatory cytokines and ROS also stimulate osteoclasts directly in two ways. First, they can upregulate RANK in osteoclasts leading to increased RANKL–RANK interaction, and second, they can actually increase the number of osteoclast precursors via stimulating the production of M-CSF.[51]

Inflammation and ROS can also affect bone metabolism independently of RANKL–RANK interaction. ROS production in MSCs has been shown to impair osteoblastogenesis and stimulate adipogenesis as a result of impaired Wnt/β-catenin/Runx2 signaling and increased PPARγ signaling.[52] This is thought to occur because the DNA damage repair gene FoxO will divert β-catenin away from TCF-mediated transcription.[53] Additionally, TNF-α has been shown to inhibit osteoblast function by inducing vitamin D resistance, blocking collagen synthesis, and stimulate osteoblast apoptosis.[51]

Intuitively, any nutrient or activity that minimizes inflammation and ROS should be protective of bone. Given that estrogen signaling, for example, is known to antagonize the effects of ROS by upregulating antioxidant systems and inhibiting cytokine production,[54] this may be a mechanism by which food components with estrogenic activity (i.e., isoflavones) contribute to the protective effects. There are also numerous nonestrogenic methods of reducing inflammation and ROS in bone. Dietary antioxidants such as *N*-acetylcysteine and vitamin E have been shown to be effective in preventing bone loss postmenopause, in aging, and in alcohol abusers. In vitro studies using bone cells have demonstrated that exposure to dried plum polyphenol extracts inhibits TNF-α induced RANKL expression and oxidative stress with coincident upregulation of Runx2 and osterix (Osx).[55,56] Similar inhibition of RANKL–RANK signaling has been reported by resveratrol.[57] However, it is unknown whether sufficient concentrations of plum polyphenol or resveratrol can be achieved through diet alone, supplemental levels may be required.

5.3.7 Prostaglandins and Fatty Acids

Prostaglandins (PGs) are lipids that act in an autocrine/paracrine manner via G-protein coupled receptors.[58] PGs are made from arachidonic acid, an n-6 PUFA, which is released from membrane phospholipids by phospholipase A2 enzymes. Arachidonic acid is then converted by cyclooxygenase (COX) to PGH2. Terminal synthases convert PGH2 into PGs of which PGE2 is known to modulate bone

metabolism. PGE2 binds its receptors EPR2R and EP4R and activates cyclic adenosine monophsophate (cAMP) dependent pathways.

Clear roles for PGE2 in bone metabolism have been difficult to define because it seems to play a role in both bone resorption and bone formation. The effects on bone resorption are primarily mediated by the upregulation of RANKL and downregulation of OPG that results in increased osteoclast activity. Furthermore, PGE2 has been shown to enhance inflammatory cytokine production (IL-1, IL-6, TNF-α) and release of parathyroid hormone (PTH) that also enhances osteoclast activation.[59] Though the principle role of PGE2 in bone was thought to be osteoclast-mediated resorption, the importance of PGE2 in bone formation is now also recognized. Local application of EP2R agonists[60] and local and systemic application of EP4R agonists[61–63] enhance bone healing in rodent models while endogenous prostaglandin production promotes osteoblast differentiation that is inhibited by COX2 deletion.[64] There are a number of factors that help define the role of PGE2. When prostaglandin production is induced by BMP-2, it enhances BMP-2 stimulation of osteoblasts and osteoclasts while PTH enhances PTH stimulation of osteoclasts.[58] Most importantly, research using rodent models suggests that while continual administration of PGE2 may lead to bone loss, intermittent administration is anabolic.[65] Overall, more research is needed to clearly define the role of PGE2 in bone metabolism. But nonetheless, this pathway may be modulated by the fatty acid composition of the diet. By decreasing the ratio of n-6 PUFA to n-3 PUFA, there is more n-3 PUFA incorporated into the cell membrane and therefore less arachidonic acid available for PGE2 synthesis. The additional n-3 PUFA can modulate COX protein expression causing a reduction in PGE2 production.[66] Diets rich in n-3 PUFA with a low n-6/n-3 ratio are also associated with lower proinflammatory cytokine levels. Overall, the effects on bone are positive with most studies showing a decrease in RANKL and/or increase in OPG from n-3 PUFA interventions.

5.3.8 Antioxidant Activity and Collagen Synthesis: Vitamin C

Vitamin C is well known as an essential nutrient for bone metabolism due to its critical role in collagen synthesis and for its antioxidant capabilities. When endogenous ascorbic acid synthesis is inhibited, mice develop osteopenia and have spontaneous fractures under conditions of increased ascorbic acid requirements and this is reversed with ascorbic acid supplementation.[67] Vitamin C is proposed to play a dual role in bone metabolism. The first is using its antioxidant capabilities to reduce osteoclast activity, and second, it is an essential cofactor in osteoblast differentiation.[67] The mechanisms have not been thoroughly elucidated, but in vitro studies have shown that treatment with ascorbic acid causes a sixfold increase in osterix, a marker of osteoblast differentiation, in bone marrow stromal cells.[68] In this situation, treatment with ascorbic acid enhanced the binding of a transcription factor (NrF1) to an antioxidant response element that regulates the production of osterix. Similarly, ascorbic acid is a cofactor for the enzyme that hydroxylates oxygen-dependent hypoxia-inducible factor.[69] Hydroxylation of this factor increase nuclear translocation that in turn increases osteogenesis by elevating vascular endothelial growth factor levels in osteoblasts.[70] In addition to modulating osteoblast and osteoclast activity, ascorbic acid is needed for normal bone matrix

production.[71] Ascorbic acid reduces the iron prosthetic group of certain hydroxylase enzymes that are essential in collagen biosynthesis and collagen cross-linking.

5.3.9 Vitamin D Receptor Pathway

Vitamin D is a steroid hormone produced in the skin on exposure to ultraviolet radiation or obtained in the diet. Vitamin D is hydroxylated, primarily in the liver, to its circulating form $25(OH)D_3$ by the enzyme 25-hydroxylase. It is hydroxylated again, primarily in the kidney, to its active form $1,25(OH)_2D_3$ by the enzyme 1-hydroxylase. When blood calcium levels are low, PTH is released and increases the activity of 1-hydroxylase to generate more active vitamin D. To exert its effects, $1,25(OH)_2D_3$ binds to the vitamin D receptor (VDR), a nuclear hormone receptor that is expressed throughout the entire body but is particularly abundant in kidney, intestine, and bone. When vitamin D binds to the VDR, it forms a complex with its heterodimeric partner: retinoic X receptor (RXR) so that it can penetrate the deep groove of DNA and bind to the vitamin D responsive elements of vitamin D-regulated genes.[72]

The classical role of vitamin is to regulate calcium homeostasis; when calcium levels are low PTH is released, which enhances the activity of 1-hydroxylase and generates more $1,25(OH)_2D_3$. This active form of vitamin D will then exert genomic effects in target tissues by binding to the VDR. In the intestine, it increases the expression of proteins needed for calcium absorption. In the kidney, it increases the expression of proteins needed for calcium reabsorption. Both these events increase blood calcium levels. In osteoblasts, however, $1,25(OH)_2D_3$ increases the expression of RANKL, the membrane-bound cytokine that activates osteoclasts. The activated osteoclast will then dissolve bone, releasing calcium into the blood. Though this last effect may seem counterproductive, the net effect is beneficial to bone because it increases overall calcium levels, which prevents chronic secretion of PTH via feedback inhibition. Also, having adequate circulating calcium causes the synthesis of calcitonin by endocrine cells in the thyroid gland. Also, calcitonin promotes the mineralization of bone.

Although vitamin D is most commonly associated with calcium homeostasis, its genomic effects extend far beyond controlling this mineral. Ligation to the VDR modulates the expression of several genes important in bone metabolism. High vitamin D will decrease transcription of 1-hydroxylase and increase transcription of 24-hydroxylase instead. This results in the formation of $24,25(OH)_2D_3$, which plays a role in mineralization rather than resorption of bone.

Vitamin D is also known to be a potent inhibitor of adipogenesis, and the mechanism is starting to be elucidated. In 3T3-L1 cells, a fibroblast-like cell, vitamin D has been shown to inhibit the expression of PPARγ.[73] This occurs because ligated VDR downregulates C/EBPβ mRNA expression and upregulates the C/EBPβ corepressor, which decreases the transcription of PPARγ. This will prevent differentiation toward the adipocyte lineage. There is also evidence that vitamin D can drive human adipose tissue–derived stromal cells to differentiate along an osteoblastic lineage at a comparable efficacy to dexamethasone, a potent glucocorticoid.[74] In these cells, vitamin D increased the expression of Runx2, Osx, BMP-2, BMP-6, and LMP-1. Although Runx2 commits MSCs to the osteoblastic lineage, Osx is another master transcription factor that acts downstream of Runx2 and is essential for bone mineralization.

BMPs will enhance the BMP/Smad/Runx2 axis driving osteoblastogenesis, and LMP-1 is thought to help mediate the effect of BMP-6 on bone.[75] Vitamin D also has the potential to enhance the expression of ERs.[76] This may enhance the bone-building effects of estrogens and estrogenic nutrients.

5.3.10 Dietary Insufficiencies

A major issue that impacts the mechanisms of cause is that of generally poor dietary habits. For instance, intakes of fruits and vegetables observed in American diets are traditionally low with only a very small minority of Americans actually eating the recommended minimum number of servings of fruits and vegetables.[77] This is most likely the reason for intakes of vitamin D, calcium, and magnesium to be below the estimated average requirement (EAR) for 70%, 38%, and 45% of the population.[78] Vitamin D and magnesium, of course, are vital for maintaining appropriate calcium status. As described in detail earlier, PTH is released in response to low serum calcium, stimulating synthesis of the enzyme 25-hydroxycholecalciferol-1-hydroxylase in the kidney. This results in increased synthesis of 1,25-dihydroxycholecalciferol (calcitriol), which activates synthesis of calcium and phosphate transporters in the intestine to enhance the absorption of calcium and phosphorus.[79–83] Magnesium is an integral component of this calcium-regulatory pathway because it is required for the release of PTH from the parathyroid gland as well as for binding of PTH to its receptor. Thus, a magnesium insufficiency is a factor in risk for osteoporosis. Lower calcitriol levels also will occur because of attenuated PTH effects, an effect that also is observed with insufficient intake of magnesium. With such a high prevalence of magnesium and vitamin D insufficiency, poor regulation of calcium absorption also would occur for a majority of the population, which when combined with insufficient calcium intake for a relatively large minority of the population leads to a population-wide risk for osteoporosis due to generally poor dietary habits.

5.4 PHYSICAL ACTIVITY MECHANISMS UNDERLYING PREVENTION OF OSTEOPOROSIS

Current bone and exercise research is aiming to identify the mechanisms by which mechanical signals are transduced and transformed into biochemical signals. It is proposed that bone cells can recognize everything from deformation, to pressure, to fluid shear, to acceleration.[84] There are also several mechanisms by which cells can recognize mechanical signals. These include stretch-activated calcium channels, which activate intracellular enzymes (e.g., phospholipase C, protein kinase C) and cause membrane depolarization with subsequent voltage-gated channel opening and further calcium entry; integrins, which are activated by deformation of their extracellular binding partners (e.g., collagen, osteopontin) by fluid shear or substrate strain; activation of G-proteins in the lipid bilayer; and cytoskeleton deformation, which provides enhanced docking and activation sites for kinases.[85] It is also worth noting that nonload-bearing exercise also increases osteoblast activity and bone strength. This has been observed when animals are exercised using high-frequency oscillatory motions.[86,87] Therefore, some of these mechanisms of signal transduction are independent of deformation.

From here, the mechanical signals converge to increase intracellular calcium, resulting in an increase in eNOS mRNA and an increase in nitric oxide (NO). NO can then potentially stimulate proliferation by binding to a regulatory site on Raf initiating a Ras–Raf–MEK–ERK cascade.[85] The role of NO is further supported by the fact that ERK activation and cell proliferation are abrogated using an NO inhibitor.[88] Ultimately, this signaling cascade will result in the modulation of gene expression, growth, and cell differentiation.

There are additional layers of complexity that play a significant role in this pathway. For example, inhibition of COX has shown that COX-1/2 induction, downstream of NO and ERK-1/2 may be a required chain in the sequence of events leading to flow-induced bone formation.[88] IGF-1 also plays a synergistic role in inducing stress-related proliferation.[89] Exercise increases osteoblast production of IGF-1, which in turn increases ERK activation. Additionally, the effects of mechanical loading are dependent on ERα.[90] Induction of ERK by calcium/NO/PGE2 results in increased phosphorylation of ERα,[91] and experimental studies using animals and bone-derived cells in vitro suggest that functional ERα is necessary for the adaptive response of bone to mechanical loading.[90] This may help explain the reduced mechanical loading response in postmenopausal women as a consequence of a decline in ERα number and/or function, resulting from estrogen deficiency.

Current research has shown that exercise-induced signals help drive osteoblast differentiation. In mice, 6 weeks of low-magnitude mechanical signals (LMMS) have been shown to increase Runx2 and decrease PPARγ expression, thereby promoting osteoblastogenesis and inhibiting adipogenesis.[92] Additionally, LMMS has been shown to actually increase the number of MSCs by 46%, thereby increasing the number of potential osteoblasts. These changes in Runx2 and PPARγ are at least in part due to increased Wnt/β-catenin signaling. Within minutes of mechanical stimulation, we see increased activation and nuclear translocation of β-catenin.[93] This is consistent with the described mechanism as induction of ERK should lead to increased phosphorylation and inhibition of GSK-3, the enzyme responsible for degrading β-catenin. Indeed, mechanical strain has been shown to suppress the activity of GSK-3.[94] Another study has also found that mechanical loading reduces the expression of Sost, which reduces the secretion of its protein product, sclerostin, in osteocytes.[95] This may have favorable effects on osteoblast differentiation because sclerostin is an antagonist of Wnt/β-catenin signaling.[96]

In addition to the actual movement and mechanical strain, duration and intensity play critical roles in determining the anabolic response of the skeleton. Bone appears to have memory of its loading history, and bone structure and strength are improved if mechanical loading is separated into short bouts.[97] In vitro studies show that continual fluid shear stress (FSS) applied to bone cells desensitizes them[98] and even a gradual increase in the magnitude of FSS does not overcome this desensitization.[99] This helps explain why in conditions such as obesity, even though there is increased load on the skeleton, bone strength is actually compromised. However, when increasing FSS is applied to bone cells with rest periods, the cells do not become sensitized.[100] These findings may explain the in vivo BMD changes in response to the gradual increase in the strength of exercise.

One last issue in regard to exercise is that osteoporosis is essentially an inflammatory disease. As a result, the anti-inflammatory effects of repeated exercise may prove to be important in reducing risk for osteoporosis. As discussed in detail in Chapter 2, 45–60 minutes of moderately stressful aerobic exercise is necessary to illicit an anti-inflammatory response through the expression of relatively large amounts of IL-6 by muscle cells. When the exercise is repeated on a chronic basis, the anti-inflammatory responses culminate in reductions in inflammatory markers. Unfortunately, although the number of clinical intervention studies on exercise and inflammation are many, as are reviews on exercise and risk for osteoporosis, the number of clinical intervention studies that address exercise, inflammatory mechanisms, and osteoporosis are very few. Interestingly, both increases in bone density and a reduction in inflammatory markers have been observed following exercise training for ~45 minutes at ~60% heart rate max and for 3–6 days per week in postmenopausal and overweight postmenopausal women.[101,102] These results are consistent with many studies that indicate that a minimum of 45–60 minutes of continuous exercise is necessary for anti-inflammatory effects to occur. They also are consistent with the concept that inflammatory mechanisms are a component of the etiology of osteoporosis and that physical exercise is an important vehicle to reduce risk for osteoporosis.

5.5 RECOMMENDATIONS

As discussed, there are many potential mechanisms by which foods and/or food components as well as physical activity modulate bone cell activity. The transfer of these cellular effects to whole bone tissue has been studied for some foods and/or food components or physical activity regimens. Identifying and more comprehensively understanding potential mechanisms of action is an important area of study to ultimately develop an overall lifestyle approach—combining healthy dietary practices and regular physical activity—to promote development and maintenance of a healthy skeleton. Based on the known mechanisms, the following recommendations are made.

5.5.1 Whole Foods and/or Food Components

For the vitamins discussed, there are dietary reference intakes that are age and gender specific but it remains a challenge to make specific recommendations regarding the quantities of whole foods or food components to consume for bone health. However, many of the foods discussed in this chapter are fruits or are components of fruits and vegetables, and both the United States and Canada have general guidelines regarding intakes of fruits or vegetables for overall health. The new "MyPlate" guidelines set by the U.S. Department of Agriculture recommends that half a plate consist of fruits and vegetables. Canada's Food Guide recommends 7–10 servings of fruits and vegetables per day for adults. By following these guidelines, risks for osteoporosis that originate from insufficient intake of various nutrients will be eliminated and go a long way to reducing the incidence of osteoporosis. Although studies investigating the relationship between intake of specific fruits and vegetables and bone health are inconclusive,[103] individuals consuming higher levels of flavonoids also have been shown to have higher BMD at femur neck and lumbar spine, common

sites of fragility fracture.[104] Based on the data on flavonoids discussed earlier, the benefits of high flavonoid consumption would be complementary to the benefits of correcting nutrient insufficiencies. Therefore, consuming a wide variety of fruits and vegetables as part of a nutritionally sufficient diet is emphasized.

There is also evidence from human studies that suggest positive effects of consuming higher intakes of n-3 relative to n-6.[105,106] In older men and women, a higher ratio of n-6 to n-3 fatty acids is associated with lower BMD at the hip.[105] Lower spine BMD was also associated with a higher ratio of n-6 to n-3 fatty acids in women not receiving hormone replacement therapy. A study in young adult males showed positive associations between n-3 fatty acids (i.e., docosahexaenoic acid, a long-chain PUFA) and BMD (total and spine) between 16 and 22 years of age. Concentrations of docosahexaenoic acid were positively associated with total BMD and BMD at the spine at 22 years of age. A positive correlation was also found between docosahexaenoic acid concentrations and the changes in BMD at the spine between 16 and 22 years of age.[106] To most effectively change the ratio of n-6 to n-3 fatty acids, there needs to be a reduction in consumption of foods rich in n-6 fatty acids and a higher consumption of foods that are rich sources of n-3 fatty acids.

Mechanistic studies investigating effects of soy isoflavones on bone tissue show favorable effects, but intervention studies in postmenopausal women, mostly those living in North America, have largely reported small or no effects of consuming high levels of soy isoflavones (either as soy protein or isoflavone supplements) to BMD. Reasons to explain this effect include the fact that lifelong exposure may modulate response to consumption of soy isoflavones, and that BMD rather than fragility fracture has been measured in these studies. Although BMD is commonly used as a predictor of fracture, it does not provide information regarding the structure of bone that is also an important predictor of fracture. Findings from the Shanghai Women's Study demonstrated that postmenopausal women with highest intakes of soy protein (18.5 g soy protein per day) have reduced risk of fracture compared to women in the lowest quintile (3.3 g soy protein per day).[107] Although women consuming the higher levels of soy protein also consumed more calories, fruits and vegetables, calcium and overall protein (both soy and nonsoy protein), the relationship between soy protein intake and bone health held after adjustment for these variables.[107]

5.5.2 Physical Activity

For recommendations regarding physical activity and bone health, the recent Canadian Physical Activity Guidelines developed by the Canadian Society for Exercise Physiology provide evidence-based guidelines aimed at preventing chronic disease, in general, through age-appropriate levels of physical activity.[108] In general, four types of exercises have been identified as important for bone health: strength training, weight-bearing aerobic, flexibility, and stability/balance exercises. A recent meta-analysis that considered the effects of exercise training on whole bone strength found that exercise enhanced bone strength at loaded sites in children but not in adults.[109] However, the authors stated the need for well-controlled studies of sufficient duration to fully determine the potential role for exercise in mediating bone strength. A meta-analysis focused on exercise for prevention and treatment of

osteoporosis specifically in postmenopausal women reported a slight improvement in BMD as well as a slight reduction in the risk of fracture.[110] Findings from both these meta-analyses identify a need for future investigations, particularly studies of high quality to more definitely establish the type and duration of exercise and age-specific exercise programs to benefit bone health.

In addition to the general findings that four types of exercise do provide risk reduction effects, the anti-inflammatory effects of the weight-bearing aerobic exercise deserves additional mention. Because this type of exercise providing anti-inflammatory effects that are separate from the benefits of the other forms of exercise, it is prudent that this form of exercise be incorporated into any activity program that is designed to produce optimal preventive effects. And the Canadian Physical Activity Guidelines appropriately address all types of exercise.

Specific guidelines for five different age groups are provided:[108] 0–4 years of age, 5–11 years of age, 12–17 years of age, 18–64 years of age, and 65 years of age and older. For infants aged less than 1 year, it is recommended that they be physically active several times a day. Examples of activities include the following: tummy time, reaching for or grasping balls or toys, playing or rolling on the floor, crawling around the home. The target is that infants and young children will progress to at least 60 minutes of energetic play per day by the age of 5 years and that these activities will help with development of motor skills. Within these guidelines are specific recommendations that "activities that strengthen muscle and bone" are performed at least 2 (over 18 years of age) or 3 days per week (5–17 years of age). Moreover, adults age 65 years or older with poor mobility are recommended to perform physical activities to enhance balance and prevent falls—for the prevention of fragility fracture. By alternating days with activities that strengthen muscle and bone with days that include moderately stressful aerobic exercise, optimal risk reduction for osteoporosis would be expected.

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6 Cancer

Jonathan Cannizzo and C. Murray Ardies

CONTENTS

6.1 INTRODUCTION

Cancer is a disease that directly or indirectly affects everyone and is the second leading cause of mortality worldwide with over 7.5 million people dying from cancer each year.[1–3] Of that 7.5 million, the American Cancer Society estimates that 600,000 deaths occur from cancer in the United States. In 2010, 1.5 million people in the United States were diagnosed with cancer, and when stratified by age, cancer is now the leading cause of death for people under the age of 85 with lung cancer being by far the biggest killer of both men and women.[4–8] Currently, approximately one out of every four deaths in the United States is due to cancer.[1,7] Cancer also carries significant economic costs that continue to grow each year with 2010 costs of ~$124 billion for cancer care being projected to increase to anywhere from ~$157 billion to ~$173 billion by 2020.[9] And these figures are only for care and treatment; they do not include other indirect costs such income and productivity losses nor do they account for the substantial emotional impact of cancer on both the patients and their families.

Unlike other chronic diseases such as hypertension, diabetes, and even heart disease, cancer is a particularly difficult disease because of the severe psychological implications. The first thing many people think of when they are diagnosed with cancer is "death sentence." And when informed, most cancer patients develop high rates of anxiety and depression, with 20%–30% at a greater risk for psychiatric morbidity and ~85% of patients exhibiting well above normal levels of stress.[10,11] This severe distress then affects activities of daily living such as appetite, sleep, and work; all of which are negatively affected by the disease.[12–14] Although psychological distress is certainly a component of many diseases, cancer is unique in the severity and universality of these effects; leading one to believe that prevention of cancer will provide important benefits well beyond the straightforward issues of biological health and health-care expenses.

Cancer of course is not a single disease but rather a collection of different, although very much related diseases (lung cancer, breast cancer [BC], ovarian cancer, prostate cancer [PC], etc.). The various forms of cancer, however, will not be discussed individually; there are excellent texts that do that such as the latest edition of *DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology* by DeVita, Lawrence, Rosenberg, DePinho, and Weinberg.[15] The different forms of cancer all share a common basis, however; one in which a variety of mutations, both inherited and acquired, lead to deregulation of cell division and ultimately result in a cancer. Because of this common basis, the general molecular and cellular mechanisms of cancer as described in *Principles and Practice* and other texts and reviews can be applied to essentially all cancers.[15–21]

In its simplest terms, developing a cancer is generally considered to occur in three stages: initiation, promotion, and progression. The initiation process of acquiring the "first" mutation most often involves reactive oxygen species (ROS) or reactive chemical species that cause some form of DNA damage in stem cells (SCs) (and sometimes in tissue progenitor cells [PCs]). (Mutations due to infidelity of DNA replication during cell division are not a topic for this chapter.) If the damage is

not repaired, then a mutation at the genetic location of the original damage will be produced in the daughter cells following cell division. This first mutation may alter a cellular function associated with controlling cell division that then accelerates the cellular mechanisms that lead to the acquisition of more mutations that then lead to the transformation of precancerous cells into cancer cells (carcinogenesis) over multiple cycles of cell division. While there is very little one can do to change an inherited initiating mutation, the various cellular mechanisms associated with acquiring new mutations, and those of subsequent promotion and progression events that follow, are in many cases amenable to prevention efforts.

From the preceding discussion, while the importance of initiating DNA damage cannot be denied, there still must be additional mutations acquired to result in a cancer. The reactive molecules that lead to the acquisition of additional DNA damage can be of either endogenous or exogenous origin. One of the most notorious exogenous sources of carcinogenic agents is tobacco smoke and it has been implicated as a causative agent for many different cancers including those of the lung, larynx, pharynx, upper digestive tract, and oral cavity (among others).[22,23] Of interest to cancer etiology and prevention, however, is that exposure to carcinogens does not necessarily result in cancer. Again, tobacco provides an interesting example in that based on lung cancer risks for smokers, about 15%–25% of lifetime smokers will be diagnosed with lung cancer; with the heaviest (>3 packs/day in some cases) and longest duration smokers incurring an incidence of lung cancer as high as 30%–40%.[24–27] While the associations are obvious that the heaviest exposures should produce the greatest risk, the "flip side" is often ignored, that is, what is it about the 75%–85% of "average" smokers who do not get lung cancer? As discussed throughout Section 6.2, there are many different mechanisms of cell function involved other than just the simple exposure to mutagens and mitogens. Some of these mechanisms include metabolic activation of oxygen and of precarcinogens into the reactive chemical species (carcinogens) that cause DNA damage, metabolic inactivation of the damaging reactive molecules, and rates/efficiency of DNA repair among others. In addition, population studies of smokers reveal that reductions in risk for lung cancer of ~40% through repeated exercise activity may occur.[28,29] Differences in diet among smokers, particularly in the consumption of higher amounts of vegetables and fruit, also account for differences in risks with higher consumption being associated with lower risks.[30,31] And as discussed in Section 6.3, it is through their effects on mechanisms of activation, inactivation, and DNA repair (among others) that diet and exercise can exert their preventive effects.

From an individual's health perspective, individual differences in the inherent mechanisms that confer risk are important, especially if one inherits a cancer-risk genotype. That these mechanisms might be modified through lifestyle-based preventive activities is even more so, and the personal and public health benefits can be profound. Interestingly, the benefits of prevention might even be applied to a serious public health problem such as smoking-induced cancer simply because a large majority of smokers seem to be unable to quit: 55%–95% fail going "unassisted cold turkey" and 75%–85% fail with pharmacological support and/or financial incentives.[32–37] The current norm in public health appears to be smoking cessation for

smokers and smoking prevention combined with diet, physical activity, and weight control for nonsmokers.[38] Perhaps a more comprehensive focus on cancer prevention that includes preventive diet and exercise behaviors for all, not just non-smokers, might prevent many more smoking-related cancers.

Understanding the molecular factors that contribute to cancer etiology and the mechanisms through which different individuals and populations are more resistant to the cancer processes is fundamental to developing effective prevention procedures that target mechanisms of cause. One of the most obvious population differences is that between women and men. Women are diagnosed far more often than men for BC even though both genders have breast tissue.[6–8,39] It is the most common malignancy in women and the second most common cause of cancer-related death in women after lung cancer.[4–8,39] Hormones clearly play an important role with estrogen having a dominant role in the cause of this cancer.[40–42] Estrogen can be metabolized into a reactive molecule capable of causing DNA damage; therefore, it can act as a mutagen (or an initiator). Estrogen also activates cell division in those cells with estrogen receptors, rendering estrogen a mitogen (or a promoter). Estrogen can therefore be considered to be a complete carcinogen because it is both a mutagen and a mitogen. And as a result of the effects of estrogen, women are expected to incur about 229,000 new cases in 2012 while the expected new cases in men is ~2,100.[7] Of course, it is not just an "estrogen thing." There are genetic risks for BC as well with mutations in the *BRCA1* and *BRCA2* genes being the most well known. The overall lifetime expectation for BC in women in the general population is about 12% in the United States (one in eight) with the expectation rising to as high as 75% with mutations in the *BRCA1* or *BRCA2* genes; genes that code for proteins involved in the repair of double-strand (DNA) breaks.[6–8,43–45] Interestingly, the risk for BC in carriers of these mutations can be significantly reduced through maintaining a healthy body weight and through physical activity, and in addition, through increased vegetable/fruit consumption and physical activity in noncarriers.[43–51] The importance of this is that even though genetic variables that confer high risk exist, they can apparently be attenuated through lifestyle modification; indicating that prevention efforts can successfully reduce risks even in the face of "bad genes."

On the male side of things, PC is the most commonly diagnosed malignancy in men.[52] It also is the second leading cause of cancer-related death in North America in men after lung cancer.[4,5,39,52,53] As with almost all cancers, the probability of getting invasive PC increases dramatically with age; ~12.5% of men >70 years of age were diagnosed in 2006–2008 compared to ~6.8% of men aged 60–69 and ~2.5% for men aged 40–59 with a lifetime risk of ~16.5% (one in six).[7] Associations between high testosterone and risk for PC also are observed although the associations are not unequivocal.[54–57] Unlike estrogen, testosterone is not known to be a mutagen, which may account for the lower consistency in the literature of a carcinogenic effect of testosterone. Testosterone and its more active metabolite dihydrotestosterone are, however, involved in stimulating proliferation and differentiation of prostate tissues (as well as apoptosis of damaged cells) to maintain normal tissue architecture, and the promoting events of PC are related to acquired mutations in the androgen receptor and in the proliferation-associated genes.[42,58–60] As genetic lesions occur within the prostate cells, they acquire a much more proliferative response to the androgens

and lose their differentiation responses, accounting for the increase in the promoting effects of higher androgen levels observed in some studies. In its most aggressive form, however, PCs are androgen independent. Interestingly, similar to BC, there is evidence that exercise can reduce risk for PC.[61–63] The exercise effects, however, appear to be more equivocal, with inconsistent positive results and apparently less of an absolute effect. Similar to those of BC, the physical activity associated with preventive effects in the epidemiological studies is of low intensity walking, yard work, housework, and other nonstrenuous exercise activities. This leads to the possibility that the benefits might not be due to the activity directly altering mechanisms of (cancer) cause but rather through ensuring insulin sensitivity due to simply not being completely sedentary; insulin resistance is known to be a risk factor for PC.[64–66]

Of these three cancers mentioned, breast (one in eight) and prostate (one in six) are by far the most common malignancies in women and men, respectively. And, while lung (including bronchus) cancer is the second-most diagnosed cancer in both genders (1 in 13 for men and 1 in 16 for women), it is by far the biggest killer in both.[4–8,39] Of particular interest is that mechanisms of inflammation have been associated with the development of each of these cancers as well as essentially, all others.[67–78] Because cell proliferation is an obligatory component of carcinogenesis, and a variety of growth factors that stimulate cell proliferation are produced during an inflammatory response (as reviewed in detail in Chapter 2), inflammatory mechanisms are relevant to any cancer. Growth factor receptors activate various components of the different mitogen-activated protein kinase (MAPK) pathways (among others) to produce their effects. These same pathways also are cross-activated by estrogen and testosterone as part of their proliferative activities. In fact, the name of the MAPK pathways underlies the fact that these signal transduction pathways were first associated with cell proliferation, growth, and differentiation and were later recognized as being central to a variety of cellular stress responses, including inflammation. With these stress-associated pathways being an integral part of the carcinogenesis (promotion) process, anti-inflammatory-based prevention procedures should therefore have a profound effect on overall cancer risk reduction.

Although gender and behavior issues create obvious differences in risk for specific cancers, in the United States, it is a particularly difficult issue. In the United States, African American women have higher mortality rates for breast, colorectal, and endometrial cancers in comparison to Caucasian women.[39] Even though Caucasian women have higher adjusted incidences of BC, minorities have higher mortality rates with this disease.[79–84] The increase in mortality rates of these diseases in women of color is most often because a diagnosis is typically made at a much later stage of cancer development. In most cases, this is due to the African American population in the United States not having similar access to health insurance and not receiving proper screenings as is much more common among Caucasian women.[85,86] Others have reported that there is no relationship between income/education level and cancer diagnosis; yet, there remains a very strong difference between races in early-stage diagnosis.[83] Even in whites who often have less intention to get screened, greater access to screenings than their African American counterparts ensures greater levels of use of early screening regardless of gender.[86] This lack of access is mostly due to lower levels of health insurance among minorities coupled with access difficulties

such as longer waits at medical facilities in underprivileged areas that are frequented more often by minorities and a lack of child care to allow time to get to screening facilities.[87,88] In addition to screening, there is some evidence that African-Americans also receive less counseling from their physicians on risk factors that play a role in cancer, factors such as diet, exercise, tobacco, and weight-gain/obesity.[89] This last, of course, brings us to the major issue for this chapter. As discussed very briefly in the preceding discussion, there are differences in behavior between individuals that appear to create large differences in risk, and participation in those risk-reduction behaviors, regardless of gender and genetics, plays a role in reducing cancer risk. And from both counseling and behavioral risk perspectives, without any knowledge of what those risky behaviors are, it is highly doubtful that meaningful changes in behavior can even be proposed or followed.

The information about preventive behaviors that is taught, whether in public schools, public service announcements, or in the physicians' offices, tends to come from professional organizations that specialize in cancer research (and treatment). There are many highly reputable scientific organizations that promote prevention, and a review of their recommendations published on their web sites reveals that there is considerable overlap in what they recommend. For example, the American Cancer Society and the American Institute for Cancer Research suggest that we should be as lean as possible without being underweight (BMI at the lower end); be physically active for 30 minutes 5 days/week; avoid sugary drinks; eat 2.5 cups of a variety of vegetables and fruits each day, choose whole grain instead of refined grain foods; eat less red meat; limit alcohol consumption to a maximum of two drinks/day for men and one drink/day for women; limit salt intake; do not use tobacco; and do not use supplements to prevent cancer.[90,91] Other organizations such as the Mayo Clinic, the Centers for Disease Control and Prevention, and the National Cancer Institute give very similar prevention tips for the layperson but also include recommendations for timely screening for precancerous lesions and to protect yourself from the sun.[91–94]

The professional publications of these groups, the latest from the American Cancer Society is used as an example here, typically follow the 2008 Physical Activity Guidelines for Americans. They also include more information than the web sites do as well as provide many examples of recommended activities ranging from walking, yoga, and mowing the lawn to running, cross-country skiing, and heavy manual labor.[95–97] The inclusion of the low-intensity activities such as walking or yoga for 3×10 minutes is a consequence of a change in exercise guidelines for public health from those based on training for improved fitness (often measured by changes in VO_2 max) to much more moderate levels of activity based on epidemiology-based research that has associated lower intensity physical activities with improved health in comparison to completely sedentary individuals.[96,97] Unfortunately, from the 1990s through to the latest 2005–2008 NHANES analyses, there has been a steady increase in the number of Americans who are inactive with 33% currently reporting no activity at all; of the remaining 76% who claim to be active ~50% do not meet the minimum guidelines for aerobic or strength activities as suggested by the Physical Activity Guidelines.[98–103] Obviously claiming to be physically active and being sufficiently active for prevention are two very different things. Thus, approximately two-thirds of the country does not participate in sufficient physical activity to have

a preventive effect. And interestingly enough, the increase in inactivity and in noncompliance with minimum aerobic and strength recommendations has occurred in a similar fashion across all ethnic and socioeconomic populations. Thus, the public health messages for cancer prevention through diet and physical activity simply are not getting to everyone (or to anyone?), regardless of who is delivering the messages or whether there is easy access to health care or not. Perhaps this is due in part to a general lack of understanding of just how important more intense physical activities and proper diet are in preventing cancer.

Because the published guidelines for prevention of cancer include many components in addition to physical activity and diet, it is very difficult to determine the role of each specific guideline in prevention efforts that are based on the guidelines in total. And interestingly enough, there are few studies available that evaluate the impact of following such prevention guidelines. However, a relatively recent (2011) follow-up of the Cancer Prevention Study-II Nutrition Cohort revealed that after 14 years of following the American Cancer Society general guidelines for prevention there were significant reductions in relative risk for all cancers for males and females to ~0.7 and 0.76.[104] In spite of a general lack of specifics, such guidelines do in fact appear to be appropriate for prevention in a general population, which then begs the question why another cancer chapter devoted to prevention through diet and exercise?

The simple answer is that since the publication of the many epidemiological studies on which the guidelines were based, there have been literally thousands of studies detailing many of the biochemical and molecular events that are a part of the etiology of cancer. In addition, many of these have detailed some of the mechanisms through which specific dietary components or exercise stress modifies mechanisms involved in the cancer process. On the basis of the mechanistic-based research, it is clear that there are different components of risk that are modified by different dietary adjustments and by different types and intensities of activity. And, based on these, more specific recommendations for diet and physical activities that target those mechanisms can be proposed.

6.1.1 Diet, Exercise, and Cancer: General Considerations

Since the landmark publication in 1981 of Sirs Richard Doll and Richard Peto[105] documenting that diet may account for ~30% of the avoidable risks for cancer, there have been many thousands of publications in the area of diet and cancer. To continue with one of the first examples, population studies indicate that there are differences in the incidence of PC between countries that have large differences in cultural-based dietary practices. For example, males in China have an incidence of PC of 0.5 per 100,000 men compared to that of the United States of 110 per 100,000.[52] There also is a significant increase in PC risk following migration of Asians (and other low-risk populations) to the United States.[106–111] In addition to the migration studies, the incidence of PC increases in Asian countries following a "westernization" of their diet and lifestyle.[112,113] Overall, diets that are high in dairy, red meat, and dietary fat and low in both variety and quantity of vegetables (a "westernized" diet) have been found to be positively correlated with risk while diets high in soy, fiber, cruciferous vegetables

(an "Asian" diet), as well as lycopene have been found to be inversely related to risk for PC.[114–126] Interestingly, similar changes in risk following the adoption of a westernized lifestyle or by emigrating to a westernized culture also are observed for BC where it traditionally has been a cancer of much lower incidence in Asian countries compared to the United States.[107,127–131] From many population studies such as these, there are consistent indications that reducing the variety and amount of vegetables in diet while increasing the amount of meat and fat contribute greatly to enhancing risk for these cancers that have a very strong endocrine component. And even in cancers that do not have a strong endocrine component, such as colorectal cancers, similar alterations in risk due to altered meat and vegetable consumption are observed in different populations and following migration to westernized cultures.[132–135]

As reported for all cancers, in many studies, an inadequate intake of various nutrients is part of the increased risks that are associated with a poor diet, while in many others, the protective effects of phenolic components have been observed.[4,29,31,105,136–150] In addition to nutritional adequacy and phytochemical content of diet, mounting evidence shows that obesity is linked to cancer risk as well.[151–154] And as is well known, the prevalence of obesity in men increased from 12.2% in 1971–1974 to 33.3% in 2005–2008 while in women the prevalence increased from 16.8% to 36.2% in the same time frame.[98–103]

While physical activity is not an absolutely essential requirement for weight maintenance, its role as a calorie burner to make it a little easier cannot be ignored. This does need to be put into perspective, however. If walking and yoga are chosen as the desired physical activities (they are recommended activities in the 2007 Physical Activity and Public Health Recommendations from the ACSM/AHA[155]) and the minimum recommended 30 min/day of activity for 5 days/week is performed, the number of additional calories expended would be less than 150 kcal each for the 5 days. Interestingly, the self-reported consumption of energy has increased by 10% for men and by 22% in women between 1970 and 2005, easily accounting for the increase in obesity in the same time frame.[98–103] These increases in consumption amount to 1701 and 2408 kcal/week for men and women, respectively. This is far more than the <750 kcal/week that would be expended following the minimum activity recommendation. Obviously, even if everyone followed the minimum recommended activity, it would still not make up for the population-wide increase in calorie intake to prevent the increase in prevalence of overweight and obesity or to prevent an increase in risk for cancer by weight gain. This does not mean of course that longer duration and higher intensity activities will not burn sufficient calories to assist with weight maintenance; they certainly will. And this is an important consideration in light of the important role that weight gain and obesity can play in promoting inflammatory signaling and risk for cancer.[151,152,156–162] The minimum activity recommendations may simply be insufficient to have any major effect on attenuating weight gain or obesity for most people.

The idea that exercise might play a direct role in prevention of cancer has been documented since the early 1920s wherein increased physical exertion of various jobs were associated with reduced cancer prevalence.[163,164] And the working hypothesis proposed by Siversten[164] (p. 376) was eerily prognostic: "That human carcinoma may be the reaction to and the result of chronic irritation of adult epithelial tissue

bathed in body fluids altered by certain metabolic products as a result of deficient muscular activity." What was not known at the time is that the weight gain associated with inactivity leads to a proinflammatory environment in abdominal adipose tissue that then leads to systemic increases in proinflammatory signaling molecules as well as insulin resistance.[165–168] Elevated insulin levels are now known to be associated with risk for a variety of cancers including those of the breast, prostate, pancreatic, and colon.[64,169–171] Increased proinflammatory signaling also is strongly associated with risk for almost all cancers and the elevated circulating proinflammatory cytokines coupled with increased insulin due to inactivity are almost certain to be among the major "metabolic products" that are responsible for the "irritation of the adult epithelial tissue" as hypothesized by Siversten. Since then, physical activity has been observed to be inversely associated with many different cancers in a variety of epidemiological studies and several mechanisms have been proposed on the basis of experiments in animal models.[28,29,46,47,51,63,172–185] The proposed mechanisms have ranged from reducing circulating levels of proliferative hormones through increased metabolism to reducing weight gain and increasing insulin sensitivity, enhancing elimination of carcinogens through increased phase II enzyme activity, and increased DNA repair.

While animal models and cell-based models provide the basis of understanding mechanisms of cancer etiology and how they might be modified, the current recommendations for risk reduction appear to be based predominantly on epidemiological associations between dietary behaviors or activity behaviors and (existing) disease. This is based in part on the relatively general nature of the existing recommendations and the fact that they have been in place for many years before the current availability of a plethora of highly specific information on the cellular basis of cancer etiology. This, however, is not a fault; it is an unavoidable limitation to the development of almost all public health policy. The need for public health policies in specific areas of health are most often recognized and recommendations formulated long before all the specific details of the etiology of risks are known. Once known, more specific recommendations may then be made.

To develop targeted prevention measures, the cellular mechanisms of cause must first be known. Then the dietary components and forms of exercise that are known to modify them can be incorporated into a series of recommendations. The ultimate recommendations must, however, have a sufficiently broad appeal and be relatively easy to implement or they will never be adopted by anyone except those most dedicated to personal health. Providing target goals for optimal prevention and a rational progression of activities designed to ultimately meet those optimal goals might be prudent. And, as with the dietary components, only those specific kinds of activity that modify causal mechanisms of cancer should become the activities recommended for optimal prevention of cancer.

6.2 ETIOLOGY OF CANCER

As mentioned in Section 6.1, cancer is really a whole family of diseases that are all related through a common etiology of acquiring a sufficient number of mutations in genes that are associated with regulating cell division to cause deregulated cell

division and metastases. Although cancer is generally considered to occur in three stages—initiation, promotion, and progression—it is actually the same basic processes that lead to various mutations that occur throughout each of these stages that cause the cancer.[15–21]

6.2.1 DNA and Chromosomal Mutations

The process of acquiring mutations often involves some sort of ROS or reactive chemical species. These reactive molecules can form adducts with DNA bases, a form of DNA damage. If the damage is not repaired, then the damaged DNA bases will be misread and mutations within the gene sequence at the location of the original damage will be produced when all the DNA is duplicated during the S phase of the cell cycle and appear in the daughter cells. Point mutations that usually arise from this process produce changes in the DNA sequence by the insertion of the wrong DNA bases. If the point mutation(s) occur(s) in the exon of a gene, then the expressed protein will likely have a single changed amino acid sequence in its primary structure, possibly affecting the ultimate three-dimensional structure and/or the physical properties of the protein.

In addition to adducts, other forms of DNA damage can occur such as DNA:DNA and DNA:protein cross-links and single- and double-strand breaks (DSBs) in the DNA. DSBs lead to extensive DNA rearrangements during the mitosis phase of the cell cycle resulting in a variety of possible chromosomal mutations in daughter cells; single-strand breaks can lead to DSBs on replication during the S phase, producing the same end result. Chromosomal mutations occur when the fragments of chromosomes fail to bind to the spindles during prometaphase and are subsequently left behind; being lost forever, along with whatever exons resided on that fragment. With multiple unrepaired DSBs on different chromosomes, components of one chromosome might rearrange with components of another to produce a translocation. Other chromosomal mutations also may occur with insertions, duplicate insertions, inversions, as well as whole chromosome duplications and deletions (aneuploidy) being seen in many cancerous cells, and they are especially common in malignancies. Where the sites of chromosomal derangements cross through exons of genes, then the protein product usually will be either nonfunctional or completely lost.

Mutations in noncoding regions of DNA also can have deleterious effects on control of cell division if they occur in the promoter region of a gene, leading to aberrant expression of the regulatory protein. Because mutations in the regulatory noncoding regions of DNA and their implications for carcinogenesis have not been as well characterized as those for specific genes, they will not be discussed here. However, with the recent publication of the various ENCODE projects, this may soon change.[186,187] The study of epigenetics is an area that is somewhat related to this. Rather than dealing with mutations, however, it is hypermethylation of CpG islands (regions of DNA that are rich in the CpG dinucleotide) that are associated with promoter regions of genes that may play a role in cancer. Hypermethylation of these CpG islands often functions to silence expression of genes, and if the gene happens to be a tumor suppressing gene, high risk for cancer is the result.[188,189] While the regulation of the methylation status of various cancer-associated genes is under intense investigation,

it is not a topic for this chapter because there is insufficient information to determine how it might be incorporated into a prevention paradigm. There is, however, some evidence that ROS-mediated damage to DNA may initiate processes that can lead to hypermethylation of CpG dinucleotides, indicating that prevention strategies to minimize ROS-mediated damage might play a role in diminishing, at least in part, risks associated with epigenetic changes.[190,191] ROS-associated damage and prevention will be discussed in detail in Section 6.2.5.1 and Section 6.3.

Any of these possible mutagenic or procarcinogenic changes to DNA structure may lead to functional properties of the expressed protein that are different from the native protein, that is, enhanced function (a constitutively active gene product or overexpression of normal function proteins resulting from chromosomal duplication events that produce multiple gene copies), attenuated function (partially inactivating mutations), or complete loss of function (inactivating mutations, chromosomal deletions, and gene silencing). And depending on the specific genes that are mutated or lost, the loss (or gain) in function may have little to no effect on overall cell function or profound effects; it just depends on which proteins have been affected and to what degree. Mutations also can be acquired during normal progression through the cell cycle simply due to the DNA replication errors that occur naturally. And if the initiating gene mutation(s) are inherited, then the initiation phase of the process has essentially been completed at the time of fertilization.

Where the mutations are in genes that are involved in controlling cell division, then rates of cell division might increase. The promotion phase typically involves the activation of more rapid rates of cellular division. Rates of cell division can be enhanced, for example, if the expressed mutant protein is a component of a signaling pathway that is involved in stimulating cell division. For example, a mutated *K-Ras* gene that produces a K-Ras protein with constitutive activity is commonly observed in colorectal cancer and less commonly in cancers of the lung and pancreas and in leukemia.[192–196] The K-Ras protein is a GTPase cell membrane protein that is activated by binding to GTP and inactivated when the GTP is hydrolyzed to GDP. A mutated K-Ras is constitutively active in these cancers and it activates the Ras-Raf-MEK1/2-extracellular signal–related kinase 1/2 (ERK1/2) (ERK-MAPK) pathway as well as the Rac1-MEK4/7-JNK1/2/3 (JNK-MAPK) pathway through activating phosphoinositide 3-kinase (PI3K), which in turn activates Rac1. These pathways are usually activated through binding of growth factors to their membrane receptors to ultimately activate a variety of transcription factors that then initiate the synthesis of many different proteins that are necessary for normal cell function, cell growth, and cell division. Some of the transcription factors activated by these pathways include NFκβ, c-Myc, Elk1, HSF-1, STAT, c-Jun, and c-Fos. These pathways also can be activated by a variety of inflammatory cytokines as well as protein kinase A and protein kinase C (PKC), indicating the importance of these pathways in cellular responses to a variety of stressors. Mutations in the *BRAF* gene also are commonly seen in a variety of cancers including colorectal, non-small cell lung cancer (NSCLC), melanomas, and adenocarcinomas of the lung.[197–200] Because the B-Raf protein is immediately downstream of K-Ras and does not activate PI3K, constitutive activation of B-Raf will tend to activate the ERK-MAPK pathway only. Mutation of the growth receptors themselves also can result in a constitutively active signal transduction pathway; for

example, mutations in epidermal growth factor (EGF) receptor have been observed in a subtype of NSCLC seen in nonsmokers as well as in colorectal cancer.[201–203] And as mentioned in Section 6.1, mutations in the androgen receptor in PCs are associated with constitutive or enhanced proliferative signaling. Mutations such as these will lead to greater proliferative signaling and result in an increase in the rate of cell division. With greater than normal numbers of cell divisions, there is then a greater likelihood of mutations occurring due to normal errors of replication. Cells with *K-Ras* or *B-Raf* mutations obviously have an increased risk for producing cancer cells because of this, but they are not yet cancer cells; only with the acquisition of additional mutations that alter other cell cycle functions can precancerous cells transform into cancer cells. And this accumulation of more mutations in subsequent generations of daughter cells defines the progression phase. Thus, promotion and progression are intimately integrated: without promotion, there will be no progression. In essence, this is why nondividing cells that have DNA damage cannot progress to become cancer cells. Cancer originates from cells that are capable of dividing, predominantly tissue SCs and possibly tissue PCs. These are the cells that are responsible for proliferating to replace tissue cells that die through normal cell aging.

Following an indeterminate number of cell divisions during the progression phase, a sufficient array of additional mutations in a variety of genes are required to ultimately give succeeding generations of proliferating cells the ability to express a cancer phenotype with certain hallmark functions. Cancer cells typically have a sustained ability to stimulate their own cell division, an inability to differentiate into normal adult tissue cells, an inability to respond to normal cellular signals that suppress cell division, an inability to respond to apoptosis signals, the ability for indefinite proliferation, an ability for invasiveness and metastasis, and an ability for stimulating angiogenesis.[17,204,205] Clearly a lot of different genes need to be mutated for all these functions to be acquired, which brings us to another concept of mutations—where the mutations are in genes that lead to the acquisition of the functions that are necessary for developing the cancer phenotype, they are called "driver" mutations. Essentially these are mutations that are directly involved in the transformation of precancerous cells into cancer cells. Mutations that occur during the multigeneration transformation process, which do not alter function of a protein or do not confer a change in function that contributes to the transformation process, are called "passenger" mutations. In a recent study of the entire genome of cultured metastatic melanoma cells from a single patient (in comparison to cultured noncancer cells from the same patient), over 33,000 somatic base substitutions were observed, with 187 occurring in coding regions.[206] With approximately 2% of the entire coding genome bearing nonsynonymous mutations in this malignant melanoma, it is clear that the vast majority of mutations are passenger mutations. And while even all the driver mutations cannot possibly be discussed here, some important examples will be mentioned because they are common to many different cancers and they provide a clear illustration of the progression phase.

Driver mutations of *Ras* and *Raf* genes already have been mentioned as mutations that enhance activation of signaling pathways that ultimately lead to cell division. Other driver mutations that are common to human cancers lead to the accelerated acquisition of mutations by altering different functions that regulate the process of

cell division. Two frequently mutated genes in human cancer are the *Rb* and *P53* genes, and in fact, just about every human cancer has mutations in one or the other (or both) of these genes.[207–212] The gene products, Rb and p53, are important regulators of the cell cycle, with Rb involved in stimulating entry into the S phase from the G1 phase (or G0) and p53 in checkpoint control. While all the details of the cell cycle and its regulation will not be discussed, a brief summary to illustrate the concepts as they relate to cancer follows.

6.2.2 Cell Cycle

The cell cycle is essentially a model for the phases through which a stem or PC will go through as it proliferates.[213–215] Mitosis and cytokinesis make up the M phase in which the processes of cell division occur. Following cytokinesis, there is a GAP1 (G1) phase during which the daughter cells grow and develop the regulatory controls necessary for their continuing function. From this phase, the cells can continue to progress to the S phase or exit the cell cycle. On exiting the cell cycle, the cells may remain quiescent (G0) or the cell may differentiate into a functional and nondividing tissue cell.[216,217] This latter is the normal function of one of the two daughter cells from some adult (tissue) SCs and of PCs; of the two daughter cells, one will typically differentiate while the other retains self-renewal functions to continue to be able to replace cells as they die.

As summarized in Figure 6.1, multipotent (in contrast to the pluripotent embryonic SCs) tissue SCs can typically be seen as long-term self-renewal cells that divide infrequently in an asymmetrical manner to renew the short-term self-renewing PCs, spending a majority of their time in G0 arrest. The PCs typically continue through the cell cycle without entering the G0 phase and divide relatively quickly (and asymmetrically) to replace tissue cells that have died, until the PCs succumb to senescence (in days or weeks), die, and need to be renewed.[216,218] When senescent cells die, most often through apoptosis, caspase 3 activates calcium-independent phospholipase A_2 (PLA_2), which cleaves arachidonic acid (AA) from membrane phospholipids leading to the subsequent release of prostaglandin E_2 (PGE_2). Along with other growth factors, PGE_2 is involved in activating G0/S or G1/S transition in tissue SCs and PCs through activation of G-coupled prostaglandin E_2 (EP) receptors that then activate Ras and PI3K, leading to activation of ERK-MAPK and JNK-MAPK pathways and subsequent production of cyclin-dependent kinases (CDKs) and the activation of mammalian target of rapamycin (mTOR) through PI3k→Akt.[219–225]

Regulation of the processes that promote retention of self-renewal properties or differentiation of daughter cells is not a topic of this chapter. In the simplest terms, however, partial withdrawal of MAPK signaling can stimulate exit from G1 while the self-renewal properties are maintained in part through Wnt, Notch, and EGF signaling from the niche cells. Differentiation can be activated by displacement of a daughter cell away from the immediate area on division and is promoted in part through signaling by transforming growth factor β (TGFβ) along with prolonged moderate MAPK activities, cell:cell contact, and other signaling events.[216,217,226–228]

Crossing the transition from G1 (or G0) to the S phase requires the coordination of a variety of cyclins and CDKs and their interaction with Rb and other proteins.

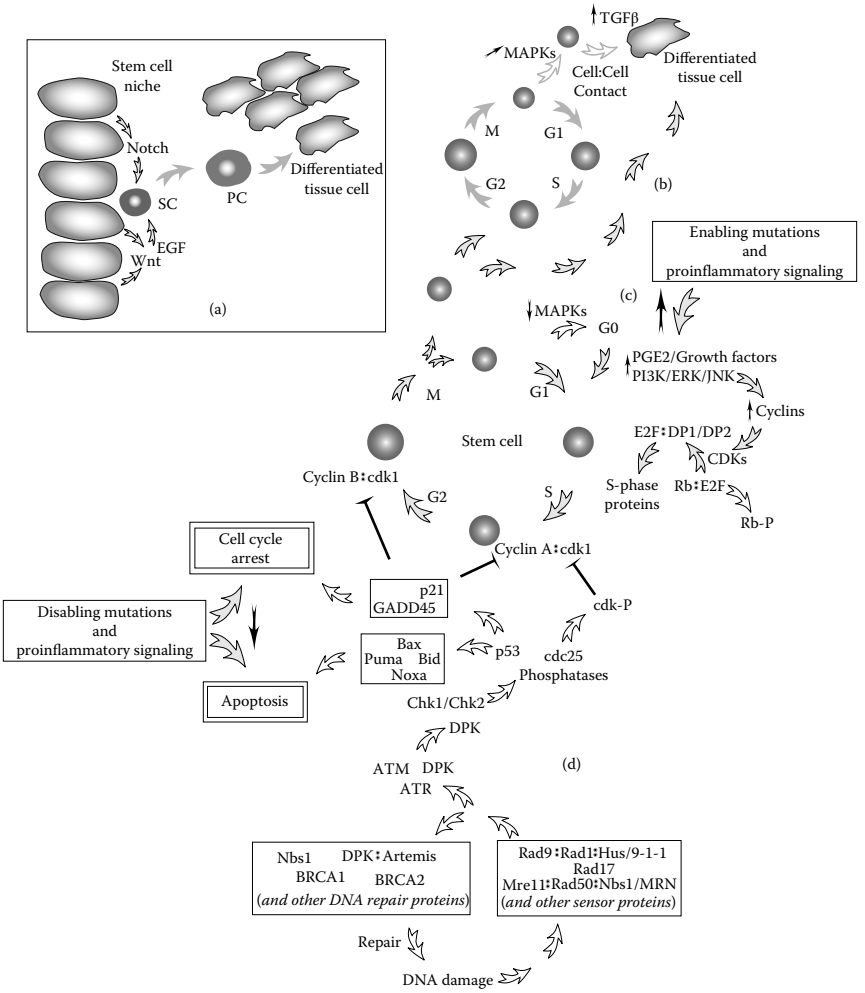

FIGURE 6.1 Summary of the molecular events that regulate cell cycle. (a) Stem cells (SC) slowly proliferate to replenish the faster proliferating progenitor cells (PC) while the PCs proliferate more quickly and differentiate into tissue cells. Wnt, Notch, and epidermal growth factor (EGF) signaling are important mediators that help maintain the stem cell phenotype. (b) In cases of damage in some tissues, SCs are activated to divide quickly to regenerate the tissue cells. (c) A decline in mitogen-activated protein kinase (MAPK) signaling is partially responsible for the SC exiting the cell cycle while prostaglandin E_2 (PGE_2) and other growth factors activate reentry into the cell cycle. (d) On encountering DNA damage, the sensor proteins activate a variety of DNA repair enzymes as well as induce synthesis of a variety of proteins that lead to activation of cdc25 phosphatases and p53 leading to cell cycle arrest and if sufficiently prolonged: apoptosis. Proinflammatory signaling and enabling mutations that confer constitutive activity in any of the proteins involved in activating entry into the cell cycle will enhance risk for cancer by enhancing rates of cell division. Proinflammatory signaling and disabling mutations that compromise function of any of the proteins involved in checkpoint control will increase risk for cancer through compromised repair of DNA damage, reduced ability to force cell cycle arrest, and attenuated apoptosis. (Details are discussed in the text.)

In cells in the G1 or G0 phase, the hypophosphorylated form of Rb forms a complex with E2F proteins and is maintained in a hypophosphorylated state, in part, through the actions of the INK4 and Cip/Kip (p21, p27, and p57) Cdk-inhibitor proteins, ensuring the cells will not progress into the S phase.[215,229–231] This transition is prevented because the Rb-E2F complexes prevent the six E2F proteins from entering the nucleus and dimerizing with DP1 and/or DP2 to form transcription factors that stimulate the expression of a variety of genes that are necessary for the S phase. With sufficient stimulation by growth factors, K-Ras is activated, which in turn activates the JNK-MAPK pathway and the ERK-MAPK pathway through PI3K, resulting in activation of the transcription factors Myc and Jun (among many others) and the subsequent expression of cyclins A, D, and E. The increased numbers of cyclins then outcompete the CDK inhibitors for binding, allowing free cyclin D to form complexes with Cdk4 and Cdk6 and cyclin E to bind to Cdk2 to activate the kinases, which then phosphorylate Rb. The Rb-E2F protein complexes then break apart, allowing the E2F proteins to dimerize with DP1/DP2 and activate transcription of a large number of S phase proteins including cyclins E and A, dihydrofolate reductase, thymidine kinase, and DNA polymerase, all of which are necessary for entry into the S phase and for DNA synthesis in the S phase.[215,229–235] The ability of E2F to induce cyclin E, which in turn regulates Cdk2 to enforce Rb phosphorylation, creates a positive feedback loop that helps contribute to the irreversibility of the G1/S transition.[236–239] Intimately involved in this process is PI3K-mediated activation of Akt, which subsequently activates mTOR to phosphorylate p70S6K, an event that is necessary for the enhanced protein synthesis associated with the proliferation activity.[224,225,240,241] Once in the S phase, the major activity of the cell is the duplication of the chromosomes followed by the G2 phase where the growing cell prepares for mitosis.

The next major transition is from G2 to the M phase and this transition also is controlled through CDK activity. Binding of Cdk1 with either cyclin A or cyclin B activates the kinase. Activation of Cdk1 with cyclin A is a relatively early event that occurs during S phase because this cyclin is induced by E2F proteins and is associated with maintaining hyperphosphorylation of Rb to ensure progression through the S phase into the G2 phase as well as entry into the M phase.[231,242] Cyclin B/cdk1 complexes that form in late G2 are an essential event for transition from the G2 phase to mitosis and activation of the kinase activity occurs when Cdk1 is dephosphorylated by Cdc25 phosphatases (Cdk125A, Cdk125B, and Cdk125C) that have been activated by Plk1, an activating event that also is performed by cyclin B/Cdk1 in a positive feedback loop.[242] This "self-activation" feedback loop ensures increasing amounts of active cyclin B/Cdc1 complexes and a definitive entry into the M phase. Once a cell progresses through the M phase, one of the two daughter cells exits the cell cycle into quiescence (G0) while the other exits to differentiate into a PC. In some cases of more extensive tissue damage, as observed for skin and intestine, SCs can take on the role of PCs and greatly increase the rate of cell cycling to replenish tissue cells, and intestinal PCs can even revert back to a SC phenotype to assist in the tissue regeneration.[227,228]

From this brief summary of a few of the relevant events, it is clear that mutations of *Rb* that lead to constitutive activity will ensure that cells consistently enter S phase from G1, independent of environmental controls. Mutations in any other genes that

lead to the production of constitutively active CDKs and E2F proteins, overproduction of cyclins, and inactivating mutations in INK4 proteins also will lead to much greater rates of cell division, and all such mutations have been observed in a wide variety of cancers.[207–212,231,243–245] The other major gene product for discussion is p53, an important component of DNA damage checkpoint controls.

Progression through either the S phase or the M phase of the cell cycle could be mutagenic or even catastrophic if there were DNA damage, compromised integrity of the DNA, or aberrant attachment of the chromosomes to spindles during the process. To avoid such events, there are checkpoint sensor proteins that continuously monitor chromosomes for the presence of DNA damage, for completed DNA replication, and for spindle integrity, and when defects are sensed, the cell cycle is shut down (arrested) until the damage can be repaired.[246–248] For the purposes of this summary, only the DNA damage checkpoints will be reviewed.

6.2.3 DNA Damage, Checkpoint Control, and Apoptosis

When DNA damage is detected by various sensor proteins and repair processes are initiated, the checkpoint functions are activated. Sensor protein complexes such as the Mre11-Rad50-Nbs1/MRN complex, which senses DNA DSBs, activate the transducer proteins ataxia telangiectasia mutated (ATM) kinase and DNA-dependent protein kinase (DPK).[247–250] ATM activation leads to the immediate attraction of a variety of proteins and enzymes that form a complex and bind to the damaged DNA, proteins including Nbs1 and BRCA1/2.[248,251,252] Nbs1 and BRCA1/2 are involved in coordinating the activity of the various proteins involved in homologous repair of the DSB, while the activated DPK can form complexes with Artemis (and others) to activate nonhomologous end-joining repair of DSBs.[249,252,253]

Rad17 and the Rad9-Rad1-Hus1/9-1-1 complex, along with a variety of other sensor proteins, detect a diverse array of DNA lesions and then recruit DNA repair complexes that perform the functions of DNA repair: base excision repair, nucleotide excision repair, mismatch excision repair, as well as activate the ATM and Rad3-related (ATR) kinase.[247–250] In addition to being involved in coordinating DNA repair mechanisms, the ATM, ATR kinases and DPKs also are important in initiating cell cycle arrest. ATM and ATR phosphorylate the checkpoint kinases Chk1 and Chk2, which then, along with DPK, phosphorylate a variety of proteins involved in cell cycle arrest including p53 and the Cdc25 phosphatases.[248,251,252,254] Phosphorylation of Cdc25A inactivates the enzyme and targets it for degradation, in turn reducing activity of the cdk's and suppressing further progression through the cell cycle. Activation of p53 leads to increased expression of p21, which blocks activated cyclin E and cyclin A/Cdk2 complexes and GADD45, which blocks activated cyclin B/cdk1 complexes, effectively shutting down progression through the cell cycle.[247,248,255] As long as the DNA damage sensor proteins detect damage, the ATM, ATR, Chk1/2 kinases, and DPK will continue to be activated and cell cycle arrest will be maintained. When the damage is repaired, the cell then reenters the cell cycle, in part due to Plk1-mediated activation of Cdc25B, which then dephosphorylates cdk1 to restore its activity.[256,257]

From the preceding discussion, a major role for p53 in this sequence of events is to ensure cell cycle arrest through inducing expression of the cyclin/cdk complex-inhibitor proteins p21 and GADD45, which then delay progression until the DNA damage is repaired. Another major function of p53 is to initiate apoptosis in the face of severe genotoxic stress.[258–263] Apoptosis is an ordered process in which cells die in response to DNA damage or hypoxia (intrinsic, mitochondria-mediated pathway) or through the activation of death receptors (extrinsic, TNF receptor 1, Fas, TRAILR1/2, and other receptor-mediated pathways), or by activation of granzymes A/B by perforins that originate from cytotoxic T cells.[264,265] The extrinsic and intrinsic pathways activate caspase 9 and then caspase 3, ultimately leading to the activation of endonucleases that then degrade chromosomal DNA and proteases that degrade nuclear and cytoskeletal proteins to completely disrupt all cellular activities. The granzymes-mediated pathways involve both caspase-dependent and caspase-independent pathways to produce the same result. The cells shrink and the chromosomes condense and fragment while extensive blebbing of the plasma occurs. They then break up into smaller apoptotic bodies that are phagocytized by adjacent cells and macrophages without initiating an inflammatory response.[264–266] For the purposes of this brief review, only the p53-mediated intrinsic pathway will be summarized.

The major proteins that activate mitochondrial-mediated apoptosis are Bax and Bak, which bind to the mitochondrial membrane and form pores that allow the release of cytochrome *c*, deoxyadenosine triphosphate, second mitochondrial-derived activator of caspase (SMAC), and other apoptogenic factors that lead to the activation of caspase 9. This caspase then cleaves caspases 3, 6, and 7 to activate them to carry out the specific molecular events of apoptosis.[267,268] Bax and Bak are normally prevented from forming Bax:Bak heterodimers (or homodimers) by being complexed with B-cell lymphoma (BCL) and myeloid-cell leukemia (MCL) proteins. Activation of p53 by checkpoint kinase activity leads to increased transcription of Bax, Bid, Puma, and Noxa. Bid, Puma, and Noxa are BH3-only proteins that can bind to BCL and MCL proteins to block their ability to form complexes with Bax and Bak, thus releasing Bax and Bak to form dimers and bind to the mitochondrial membrane and initiate the release of the apoptogenic proteins. By inducing expression of these BH3-only proteins as well as that of Bax, the ratio of noninhibited to inhibited Bax and Bak increases and apoptosis is activated.[262,267–270]

The preceding sequences of events described for p53 indicate that it is involved in activating both cell cycle arrest and apoptosis, a situation that produces somewhat of a conundrum. Where is the dividing line between sufficient DNA repair to allow reentry into the cell cycle and sufficient activation of apoptosis mechanisms that the cell self-destructs in spite of continuing successful efforts to repair DNA? This is unfortunately an area not very well understood although it may be related in part to calcium and ROS-mediated mitochondrial events.

Entry of calcium into the mitochondria activates the mitochondria permeability transition, which reduces electrochemical gradients to inhibit ATP synthesis as well as allow the release of mitochondrial calcium to the cytosol. This leads to an increase in cytosolic Ca^{++} and the activation of numerous lipases and proteases that then participate in the apoptosis program, events that have traditionally been associated with cell death through necrosis and have since been documented to occur with

apoptosis that has been stimulated by toxins.[266,271,272] In addition to these events, activated caspase 3 can cleave the plasma Ca^{++}–ATPase exchanger as well as the Na^{+}–Ca^{++} exchanger to enhance cytosolic levels of Ca^{++}, also leading to the activation of numerous lipases and proteases.[266] Thus, deregulation of Ca^{++} control is an important player in the progression of apoptotic events.

Another interesting issue with mitochondrial-associated apoptosis is that redox events also can initiate cytochrome *c* release and activate caspases to initiate apoptosis. Oxidation of cardiolipin by H_2O_2 leads to disassociation of cytochrome *c* from the inner membrane of mitochondria and its subsequent efflux.[273,274] This, coupled with the ability of ROS to activate JNK-MAPK signaling to promote apoptosis through increased expression of Bak (intrinsic pathway) and TNFα (extrinsic pathway), indicates the importance of ROS in mediating pro-apoptosis events in addition to that of p53.[275] As calcium overload in mitochondria leads to increased ROS production and subsequent cytochrome *c* release,[276] there is a progressive increase in mitochondrial-mediated pro-apoptotic signaling over time initiated by the binding of Bax and Bak to the outer membranes of mitochondria and mediated by both Ca^{++} and ROS.

Because all mitochondria are not initially affected by Bax:Bak, it is possible that it is the duration of Bax:Bak activation that impacts the progression of the apoptotic process. The longer the duration of caspase 3 activation, the greater the deregulation of cytosolic Ca^{++}; leading to increasing degrees of mitochondria impairment by Ca^{++}, increasing production of ROS, additional cytochrome *c* release, and therefore further progression into cell-wide apoptosis signaling. On the other hand, when Chk-mediated activation of p53 diminishes and Bax and Bak are again inhibited by BH3-only proteins, the cell may recover. Recovery may be, however, dependent on the number of functional mitochondria remaining, coupled with the integrity of the redox-control systems. Without sufficient ATP synthesis from the remaining mitochondria, adequate function of antioxidant enzymes, and appropriate redox control, repair from the initial damage and reversal of the ROS-mediated activation of pro-apoptosis signaling would be difficult and the cell will continue to progress through apoptosis in spite of successful DNA repair. While this may be a relatively simplistic interpretation of known events, it is meant to illustrate the complexity of the cellular events that are associated with regulation of the apoptosis recovery processes.

Thus, both apoptosis and cell cycle processes are normally well regulated and the role of p53 in these processes in the face of genotoxicity is extremely important. A nonfunctional p53 will not be able to initiate cell cycle arrest or apoptosis. Under such circumstances if cells have DNA damage and then divide, they will produce daughter cells with mutations. Mutations in the genes that code for the proteins involved in the regulation of apoptosis or checkpoint controls also will have a similar end result, greater numbers of mutations in daughter cells. Thus, mutations in these regulatory gene products would all be considered driver mutations. And as expected, mutations in the *p53* gene or in any one or more of the *ATM*, *ATR*, *Chk1/2* kinases, *DPK*, *p21*, G*ADD45*, or other of the regulatory or DNA repair genes mentioned in the preceding discussion have been observed in the vast majority of human cancers analyzed.[208,246,250–252,264,269,277–283]

From the preceding discussion, ensuring proper regulation of cell cycle, DNA repair, and apoptosis is essential to minimize risk for mutagenesis, and driver

mutations in any of the genes related to these functions produce a significantly enhanced risk for developing a cancer. Mutations in the *Rb* gene that prevent Rb from binding to E2F proteins and inactivating mutations in the *p53* gene would produce sustained proliferative signaling as well as escape from apoptosis, both hallmark functions of cancer cells.[204,205] Mutations in any of the other genes discussed in this section also would contribute to these same hallmark functions, revealing that there are many different mutations that can contribute to the same gain in function. This is essentially why so many different mutations are observed in different cancers while there really are only a half-dozen or so hallmark functions of cancer; there is no single essential mutation that produces a specific gain in hallmark function. Thus, accumulation of specific driver mutations in the promotion phase that produces a growth advantage for subsequent generations of cells is really a selection process for function and not for specific (mutated) genes. This selection for function is one reason for the heterogeneous array of mutated genes observed in cancer tumors from different individuals with a single form of cancer as well as differences in the arrays of mutations in cancers that arise in different tissues.[206,284–287] This leads to the last etiology topic before focusing on mechanisms of carcinogenesis that are amenable to prevention.

6.2.4 Cancer Stem Cells and Tumor Environment

Different cells within a given tumor also express differences in phenotype in spite of the fact that they probably originated from a single mutated SC (mSC), one of the concepts that led to the relatively recent revisions to the cancer SC hypothesis.[288,289] As mutations accumulate in subsequent generations of mSCs, the original differentiating function still may occur until a sufficient number of mutations have occurred to prevent differentiation in the daughter cells, a point when the mSCs have been transformed into cancer SCs (CSCs) that then continuously divide symmetrically. Until this happens, however, the mSCs will have produced an array of PCs with a variety of mutations (mutated PCs [mPCs]) that then produce daughter cells with slightly differing arrays of mutations and phenotypes, essentially a small array of differentiated and partially differentiated tissue cells. Some of these partially differentiated cells may retain the ability to respond to proliferating signals and subsequently divide in response to chronic stimulation. As mutations accumulate in these daughter cells, they may dedifferentiate even further to take on the proliferative characteristics of PCs. Some of these "newly dedifferentiated-progenitor" cells along with the mPCs that arose from the mSCs may even acquire the self-renewal characteristics of SCs and essentially become CSCs.[18,290] The ability of PCs in intestinal crypts to revert to a SC phenotype following damage indicates that this is certainly a possibility.[227] As these cells divide and gradually accumulate within a tumor, the tumor then acquires an array of proliferation-capable cells that can contribute to tumor mass: CSCs with unlimited self-renewal capabilities and an array of mPCs. Thus, the CSCs essentially seed the development of a heterogeneous array of larger than normal numbers of mPCs that proliferate more efficiently in response to normal proliferation signals (such as PGE_2) to drive tumor growth.

An important point for tumorigenesis and progression is that the heterogeneity of cells within a tumor is not restricted to SCs, CSCs, PCs, mPCs, and an array of dedifferentiated (and appropriately differentiated) tissue cells. Virtually all (solid) tumors contain a variety of inflammatory associated stromal cells that contribute to the tumorigenesis process, cells that include macrophages, lymphocytes, natural killer (NK) cells, mast cells, endothelial cells, and fibroblasts.[78,291,292] While the actual order of events that leads to infiltration of the tissue with the individual types of stromal cells is unknown, the possibility exists that it starts as mSCs, and mPCs initially begin to accumulate and the distance from the new precancerous cells to the existing capillaries increases, causing a corresponding decrease in oxygen availability in these cells. Hypoxia induces activation of the transcription factor hypoxia-inducible factor 1 (HIF-1), which subsequently activates transcription of a variety of proteins including glycolytic enzymes, pyruvate dehydrogenase kinase (PDK), glucose transporters 1 and 3 (GLUT1 and GLUT3), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), TGFα, as well as the monocyte chemoattractant protein MCP-1.[293–297] The increase in glycolytic enzymes will confer a metabolic advantage under the hypoxic conditions experienced by some tumor cells. In addition, the expression of PDK suppresses oxidative metabolism of pyruvate by inactivating pyruvate dehydrogenase. This will, through a series of moderate product-inhibition effects on the enzymes within the glycolytic pathway, enhance cellular levels of glycolytic intermediates, including glucose 6-phosphate, which is the primary substrate that enters the pentose-phosphate pathway. This metabolic pathway is necessary for nucleotide synthesis; thus, increased PDK activity enhances an essential synthesis function in cells that are dividing quickly.

The increase in MCP-1 contributes to the attraction and activation of monocytes, which, following a cascade of proinflammatory signaling events, leads to the gradual accumulation of other inflammatory cells, including platelets. Activated platelets release a large variety of factors including IL-1β, TNFα, platelet-derived growth factor (PDGF), TGFβ, EGF, basic fibroblast growth factor (bFGF), and the chemokines RANTES, platelet factor 4, and IL-8, adding to the VEGF and IGF released in response to HIF-1.[298–300] While platelets are traditionally associated with blood clot formation, they also are intimately involved in tissue remodeling. The PDGF, TGFβ, as well as the various chemokines initiate chemotaxis of tissue macrophages and fibroblasts into the area that adds to the heterogeneity of the precancerous tissue. The PDGF also has the effect of stimulating mitogenesis of fibroblasts while TGFβ stimulates the macrophages to release (among other factors) fibroblast growth factor (bFGF) and PDGF, both of which are essential for angiogenesis in tissues.[301–303] Thus, through the infiltration and activation of inflammatory-associated cells, not only is a continuous supply of growth factors available for stimulating proliferative activity in any cells that can respond to them (SCs, mSCs, CSCs, PCs, and mPCs), but also an activation of the processes that lead to angiogenesis, which helps to sustain growth of the developing tumor, another hallmark function.[78,204,205,291,292,304]

Continued inflammatory signaling by the infiltrating inflammatory cells can activate expression and release of CD40 from platelets, which then activates the release of a variety of matrix metalloproteinases and serine and cysteine proteinases from endothelial cells, macrophages, mast cells, and others. These proteases then degrade

collagen, VE-cadherin, fibronectin, and other extracellular matrix adhesion molecules to contribute to the ability of a growing tumor to invade adjacent tissues, again, another hallmark function of cancer.[304–306]

Thus, the infiltrating stromal cells provide essential contributions to the tumorigenesis process without actually being involved in a classic inflammatory response such as one that occurs with infection or tissue damage, revealing why "inflammation" has consistently been associated with risk for cancer. This does not mean that inflammatory conditions from infection or damage cannot contribute to this process because they certainly can. Any damage or infection within the precancerous tissue will activate a localized inflammatory response that will then attract more inflammatory cells to the local region and exacerbate the already protumorigenic effects of the initial infiltrating stromal cells. Inflammatory signaling molecules from damage/infection sites elsewhere in the body or from proliferating adipose tissue also can enter the circulation and these too can exacerbate the protumorigenesis environment of a developing tumor.

6.2.4.1 Contribution of Obesity and Insulin Resistance to the Tumor Environment

Obesity, especially visceral obesity, also is associated with a "proinflammatory state" in which proinflammatory cytokines that enter the circulation originate from the adipose tissue itself.[157,160,307–310] The inflammation-associated cytokines include TNFα, IL-6, IL-1β, and MCP-1. Once they enter the circulation, they can then enhance proinflammatory signaling in precancerous and cancerous tumors, a major factor in the known link between obesity and an increased risk for many cancers.[311,312] While other mechanisms associated with infiltrating inflammatory cells that contribute to tumorigenesis will not be reviewed here, it is important to note that because these inflammation-associated signaling processes are essential to the cancer process, anti-inflammatory measures should therefore reduce risk for tumorigenesis.

Closely related to the increase in risk due to obesity is an increase in risk for cancer with insulin resistance. Metabolic syndrome, which includes insulin resistance as a required symptom of the syndrome, and obesity are very tightly associated, very likely through the inactivity that often leads to obesity.[158,313,314] The proinflammatory cytokines (especially TNFα) produced by adipocytes, coupled with the prooxidant effects of inactivity, lead to insulin resistance in peripheral tissues and a compensatory increase in insulin levels. Insulin enhances activation of the ERK-MAPK pathway (through PI3K) and increases production of IGF-1 by liver, both of which activate (through PI3K) the ERK-MAPK, JNK-MAPK, and p38-MAPK pathways as well as mTOR signaling (PI3K-PKB-Akt-mTOR-P70S6k) to produce growth and proliferative responses. It is no wonder that insulin resistance is associated with risk for a variety of cancers![64,165,315–322] Add to these signaling effects the role of glucose as a major substrate for both ATP synthesis and DNA synthesis, and the increase in serum levels of glucose and insulin that occur with insulin resistance can only be an advantage to proliferating CSCs, mSCs, and mPCs in a slightly hypoxic tumor environment. And this advantage is certain to be enhanced through the commonly-observed increase in expression of glucose transporters and of insulin receptors in many cancer cells.[321,323–328]

In addition to the insulin-mediated enhanced risk for cancer, the increased levels of glucose in serum comprise an independent source of risk. The aldehyde group of open-chain glucose can form a Schiff base with available amino groups to initiate the formation of advanced glycation end-products (AGE).[329–331] These products cause cellular and DNA damage through enhancing rates of lipid peroxidation and they also activate proinflammatory signaling pathways through interacting with AGE receptors (RAGE).[330,332–336] Thus, obesity can contribute to risk for cancer through a cascade of events initiated by adipose-mediated inflammatory signaling that leads to insulin resistance and elevated insulin, IGF-1, and glucose. The elevated insulin and IGF-1 levels enhance activity of the proliferative and growth signaling pathways while the increased glucose enhances substrate supply for the growth. In addition, the elevated levels of glucose can enhance cellular and DNA damage (DNA damage is discussed in Section 6.2.5) and initiate additional proinflammatory signaling as well, truly risk-enhancing for the tumor environment.

From this brief review of cancer etiology, it is clear that accumulating mutations in SCs and PCs are the driving force behind tumorigenesis, while signaling processes from infiltrating somatic cells (and circulating cytokines) are essential accessories to the process. Once formed, there is little that can be done to repair mutations. For some mutations, however, there are now drug therapies that utilize a variety of inhibitor molecules to attenuate the effects of specific gain-in-function mutations, with many more "in the pipeline." From the perspective of prevention, these therapies are "after-the-fact" attempts to manage the deleterious effect of the mutations to prevent further development of an existing tumor or the development of a secondary tumor. For prevention to be most effective, however, preventing the mutations in the first place is the best strategy. And from this standpoint, reducing the DNA damage as well as increasing repair of the inevitable remaining damage is a logical focus.

6.2.5 DNA Damage

6.2.5.1 Reactive Oxygen and Nitrogen Species

DNA damage is caused by a large variety of reactive molecules that are produced during metabolism of both endogenous and exogenous molecules. ROS are one class of reactive molecules produced that can either attack DNA directly or they can initiate oxidative damage to other biological molecules, the products of which then cause the DNA damage.[105,277,337–339] While a comprehensive discussion of radical chemistry is beyond the scope of this chapter, examples of some of the more biologically relevant reactions will be discussed.

The major endogenous sources of radical species in biological systems include the generation of superoxide anion radical ($O_2^{\cdot-}$) by mitochondria during aerobic metabolism, by the NADPH-oxidase activity of activated G-coupled proteins, by phagolysosomes of activated neutrophils and macrophages, by cytochrome P450 monooxygenases (CYP), as well as nitric oxide (NO·) produced by nitric oxide synthase in vascular endothelial cells and activated neutrophils and macrophages.[340–348] Neither $O_2^{\cdot-}$ nor NO· is particularly toxic in aqueous solutions; however, the superoxide anions can spontaneously dismutate to produce hydrogen peroxide: $O_2^{\cdot-} + O_2^{\cdot-} + 2H_2O \rightarrow H_2O_2 + O_2 + 2OH^-$. This dismutation is greatly accelerated by

the various superoxide dismutase (SOD) enzymes, thus ensuring that the predominant soluble oxidant in the cells will be H_2O_2. The three isozymes of SOD include the cytosolic copper–zinc form of SOD in the cytosol, nucleus, and mitochondrial membrane (Cu/ZnSOD—SOD1), the extracellular copper–zinc form (ECSOD—SOD3), and the manganese form (MnSOD—SOD2) in the mitochondrial matrix. H_2O_2 also can be produced directly by xanthine oxidase–mediated oxidation of hypoxanthine and xanthine during uric acid synthesis. While the nonradical H_2O_2 is a relatively weak oxidant, it can easily be reduced by superoxide or by multivalent metal ions such as Fe^{2+} or Cu^{+} to produce hydroxyl radicals that are possibly the most aggressive oxidants known: $O_2^{\cdot-}(\text{or } Fe^{2+}) + H_2O_2 \rightarrow \cdot OH + HO^- + O_2(\text{or } Fe^{3+})$. Because superoxide can reduce Fe^{3+} and Cu^{2+} so much faster than it can reduce hydrogen peroxide, in all likelihood the more relevant in vivo reaction involves superoxide as a reducing agent for metal ions with the reduced metal then reacting with the hydrogen peroxide. The production of hydroxyl radicals through these reactions is of major importance to DNA damage and mutagenesis. Hydroxyl radicals have both strong oxidant and electrophilic characteristics, which make them particularly damaging to DNA (and anything else for that matter).[349–351] It is most likely that H_2O_2 produced elsewhere in the cell diffuses into the nucleus where it then reacts with a metal ion to produce ·OH, which then reacts with susceptible atoms of the closest DNA molecule in the immediate vicinity.

A major endogenous reactive nitrogen species (RNS) is peroxynitrite ($ONOO^-$), a strong oxidant that is produced when nitric oxide (NO·) reacts with $O_2^{\cdot-}$. The $ONOO^-$ can react with CO_2 to produce carbonate radicals ($CO_3^{\cdot-}$) and nitrogen dioxide radicals ($NO_2\cdot$), both of which are oxidants. The RNS have traditionally been thought of as relevant to infection or damage-related inflammatory reactions because of the production of NO· by various inflammatory cells. With the recognition that infiltrating stromal cells are intimately involved in tumorigenesis, these NO·-derived reactive species are now known to contribute to the tumorigenesis process through causing DNA damage without an actual infection or damage-associated inflammatory response being present. This source of DNA damage is in addition to that produced by hydroxyl radicals that are derived from normal metabolic and signaling processes. Thus, the major endogenous oxidants produced include ·OH, $ONOO^-$, $NO_2\cdot$, and $CO_3^{\cdot-}$. While all possible reactions of these molecules with each susceptible component of the different DNA molecules cannot be described here, several of the predominant reactions will be used to illustrate the general principles of how DNA damage ultimately leads to risk for cancer.

One mechanism through which hydroxyl radicals can cause damage to DNA molecules is through abstracting a hydrogen atom from C-H bonds of the deoxyribose component of DNA; predominantly from the C5 position due to its greater exposure to the external solvent when in the double-stranded configuration.[349,352,353] Abstracting the H leaves behind a carbon-centered allyl radical that converts to a peroxyl radical through addition of molecular oxygen and then an oxyl radical that ultimately leads to strand breaks at this position. As described in Section 6.2.1, unrepaired single-strand breaks can lead to DSBs during replication and these DSBs can then lead to a variety of chromosomal mutations in daughter cells. DSBs also can be caused by ionizing radiation such as that emitted by uranium, plutonium, and radon.

Hydroxyl radicals also can abstract a hydrogen atom from the C5 methyl group of thymine leaving an allyl radical, which then leads to the production of 5-hydroxymethyluracil and other end-products following the addition of oxygen. Addition reactions of ·OH with DNA bases can lead to a wide array of DNA damage products. ·OH adds to the various double bonds of the DNA bases, predominantly at the C4, C5, and C8 of purines and at the C5 and C6 of pyrimidines to produce a variety of OH adduct radicals.[348,349,352–356] These radical products can then be oxidized, or reduced, to produce an array of stable adducts including 8-oxo-7,8-dihydrodeoxyguanosine (8-oxo-dG) and 8-oxo-7,8-dihydrodeoxyadenosine (8-oxo-dA), thymine glycol, and 5-methyldeoxycytidine (5-methyl-dC) among many others. When these lesions are not repaired and the cell progresses through the S phase, replication errors are made and mutations will occur. For example, in a variety of *Escherichia coli* and cell culture model systems, 8-oxo-dA and 8-oxo-dG lesions lead to A → C transversions and G → A transitions/G → T transversions in daughter cells, respectively. Thymine glycol will lead to T → C transitions and 5-methyl-dC leads to C → T transitions. If these mutations occur in the coding region of a gene that is responsible for cell cycle control or DNA repair, then risk for cancer can be greatly enhanced.

Hydroxyl radicals also can initiate peroxidation reactions in the unsaturated fatty acids of phospholipids that result in the production of a wide variety of reactive products; the major reactive products include malondialdehyde, acrolein, crotonaldehyde, and 4-hydroxynonenal.[354,355,357–361] Malondialdehyde forms exocyclic adducts by reacting with the exocyclic amino groups of C2, C4, and C6 of guanosine, cytosine, and adenosine, respectively, potentially leading to G → T transversions and C → T and A → G transitions. Acrolein, 4-hydroxynonenal, and crotonaldehyde also react with the exocyclic amino groups of DNA to form a variety of propano-adducts, while reactions of these with peroxide can produce the much more reactive epoxide derivatives, which react with exocyclic amino groups as well to form a variety of etheno adducts. These adducts are associated with producing a variety of mutations including G → T and G → C transversions, C → A transversions and C → T transitions, and A → T and A → C transversions, with etheno-dG adducts being approximately 100 times more common than those of etheno-dA and etheno-dC adducts. Most interestingly, all of these adducts are increased under conditions of oxidative stress.

In addition to the preceding, DNA lesions resulting from oxidation by $ONOO^-$, $NO_2\cdot$, and $CO_3^{\cdot-}$ also occur. Of these three major products of $NO + O_2^{\cdot-}$ reactions, peroxynitrite is not only relatively stable but it also can readily diffuse across cellular membranes, rendering it the major NO·-related oxidant capable of oxidizing DNA.[353–355,362] $ONOO^-$ (and its protonated form peroxynitrous acid) preferentially oxidizes guanosine to 8-nitroguanosine and 8-oxo-dG. 8-Nitroguanosine spontaneously depurinates leaving an apurinic site leading to G → T transversions. The 8-oxo-dG (also produced by ·OH) is in turn far more reactive with $ONOO^-$ than is the unmodified dG.[353,363] Subsequent oxidation of 8-oxo-dG by $ONOO^-$ produces oxazolone, spiroiminodihydantoin, and guanidineohydantoin. Thus, under the "normal" transforming conditions of a precancerous tumor, the mutagenic 8-oxo-dG (G → T transversions) will be produced by both ·OH and $ONOO^-$, and with a second oxidation event by $ONOO^-$ oxazolone (G → T transversions),

spiroiminodihydantoin (G → T and G → C transversions) and guanidineohydantoin (G → C transversions) will result. Peroxynitrite also reacts with the exocyclic amines of dG, dA, and dC to produce reactive diazo and nitroso adducts that hydrolyze to produce xanthine, hypoxanthine, and uracil. These DNA lesions can lead to both G → C transitions and G → T transversions. ROS and RNS oxidation products are therefore responsible for producing G → C and G →T transversions as well as a variety of other mutations.

6.2.5.2 Xenibiotics

Exposures to exogenous compounds (xenobiotics) that are directly reactive, or that can be activated to reactive compounds, also are major contributors to DNA damage and subsequent risk for mutagenesis and cancer. Tobacco smoke, whether through first- or second-hand exposure, is an obvious source of exogenous compounds with carcinogenic potential. In addition to tobacco smoke, humans are exposed to a wide variety of carcinogenic xenobiotics in the air, water, and food. A major source of exogenous carcinogens is combustion products arising from a variety of common activities such as smoking tobacco (and other things), smoking and grilling meats and other foods, burning oil, gas, coal, and other biomass derivatives in mobile (automobiles) and stationary (steel mills, power stations, refineries, waste incineration, etc.) devices, and from residential wood and vegetation burning. These contain hundreds of different carcinogenic compounds including a wide variety of polycyclic aromatic hydrocarbons (PAHs), nitrosamines, ketones, aldehydes, quinones, metals, and free radicals, among others.[364–373] In addition to breathing air that contains such compounds, these same emissions are deposited in the water and soils and they then enter the food chain such that it is difficult to eat or drink anything that does not contain at least trace amounts of these compounds. Add to this the vast array of naturally occurring toxic (and potentially carcinogenic) chemicals made by the plants themselves, and the number of different reactive compounds in the environment that could contribute to risk for cancer is far too extensive for all of them to be discussed here. Instead, a few general principles of how they contribute to the processes of DNA damage will be highlighted.

Exogenous sources of carcinogens tend to produce different effects in different tissues, effects that tend to be greater in tissues of first exposure.[374–378] Those tissues of first exposure will get the greatest concentration of potentially harmful molecules, and if they are inherently reactive (ROS, RNS, aldehydes, ketones, epoxides, etc.), these tissues will suffer the greatest damaging effects with tissues further "downstream" suffering much less damage simply because of the relatively short half-life of the reactive molecules. With airborne carcinogens the order of exposure will most likely be oral/nasal → pharyngeal → trachea → lung → circulation whereas with water-soluble carcinogens it would more likely be oral → pharyngeal → esophagus → GI tract → liver → circulation. Many exogenous carcinogens require activation to a reactive form before they can cause DNA damage. And, the majority of the activation reactions are catalyzed by various forms of cytochrome P450 enzymes (CYP).[377–379]

Tobacco products probably comprise the single greatest voluntary source of exogenous carcinogens for humans. Tobacco products have been estimated to be used by 32.2% of men and 18.5% of women for a total prevalence of ~25% of the

U.S. population (with similar figures in other high-income countries) while in low-income countries as much as ~50% of men and ~8% of women smoke for a total of over 1.1 billion smokers worldwide.[380,381] Tobacco smoke contains a wide variety of carcinogens including an array of PAHs, nitrosamines, aldehydes, metals, nitrogen oxides, acrolein, and many others, compounds that are found in almost all combustion-associated air pollution.[367,370,382] Because of this, tobacco smoke provides a highly relevant model for risk for cancer from most exogenous sources. And of all of the exogenous compounds found in combustion products, the PAHs and nitrosamines produce the greatest mutagenic potential, with benzo[a]pyrene (BaP) and the tobacco-specific nitrosamine—4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) being among the best characterized of these.

BaP and NNK are both known to produce mutations as well as to cause lung cancer in a variety of animal models.[370,382] For these compounds to exert their mutagenic effects, they must first be activated by CYP. CYP enzymes comprise a gene superfamily of over 2000 heme-containing monooxygenase enzymes in more than 200 related gene families that (collectively) are present in all eukaryotes (and many prokaryotes as well) and are responsible for metabolizing a wide array of compounds.[379,383–386] In brief, CYP enzymes comprise the majority of phase I enzyme activities that are part of a general two-step progression in the metabolic transformation of lipid soluble xenobiotics into water-soluble compounds for easy excretion in the urine. Other phase I enzymes include the flavin-containing monooxygenases, peroxidases, dehydrogenases, and esterases among many others. The basic purpose of phase I enzymes is to add a functional group such as a hydroxyl or an oxide to the molecule so that phase II enzymes can then transfer a more water-soluble component to it. Phase II enzymes include several families of enzymes such as the UDP-glucuronosyl transferases (GT), glutathione-S-transferases (GST), and the acetyltransferases (among several others). Binding of a substrate to the heme iron of CYP triggers the reduction of the iron by an electron from CYP reductase, the ferrous iron then binds molecular oxygen. Following a second reduction to form an iron-peroxo complex and a near immediate protonation to the iron-hydroperoxo form, the activated oxygen then reacts with the substrate; one oxygen atom is inserted into the substrate while the other leaves as water. In some cases, the leaving product is relatively nonreactive and subsequent conjugation reactions allow for easy excretion. In others, the products that are released are highly reactive electrophiles that readily form adducts with a variety of other molecules, including DNA. In addition to the production of reactive products from substrates, CYP reactions also can become uncoupled. Although relatively stable, the originally formed iron-oxygen complex can rearrange to produce a superoxide that dismutates within the enzyme and then disassociates to generate free H_2O_2. The superoxide also can disassociate freely from the enzyme and subsequently react with the H_2O_2 to produce ·OH; thus, uncoupled CYP enzymes can be a significant source of damaging ROS in addition to the production of electrophilic substrates. Of the 18 mammalian gene families, the *CYP1*, *CYP2*, *CYP3*, and *CYP4* families are most active in the activation of xenobiotics, and in addition, members from each of these families have been documented to produce significant quantities of ROS as well.[377,378,386,387]

As mentioned earlier in this section, PAHs and nitrosamines are important contributors to environmental-associated DNA damage with BaP and NNK being well-studied models for each. The olefins of BaP can be oxidized by CYP1A1, CYP1A2, CYP1B1, and by CYP3A4 to produce an array of BaP-epoxides with BaP-7,8-diol-9,10-epoxide (from CYP1A1) being the major product of BaP metabolism because of the relative high content of this isozyme in lung.[370,379,382,385] The various diol-epoxides produced from BaP are electrophilic compounds that are highly reactive with the exocyclic amines of DNA. Because guanine is the base that is most susceptible to attack, a preponderance of G → T transversions are produced following treatment with BaP. In some cases, oxidation of the olefins can result in the formation of an aldehyde group instead of an epoxide, a reactive group that also reacts with the same exocyclic amines.

Activation of the tobacco-specific nitrosamine NNK also is performed by a variety of CYPs. α-Methyl hydroxylation of NNK by CYP1A1, CYP1A2, CYP2A1, CYP2B1, and CYP2E1 (among others) produces 4-(3-pyridyl)-4-oxo-butane-1-diazohydroxide (plus formaldehyde) while α-methylene hydroxylation by CYP1A2, CYP3A4, and CYP2A6 produces a keto-aldehyde with the concomitant release of methyl-diazohydroxide.[370,382,385,388] Of these products it is the diazo group that confers the reactivity resulting in pyridyloxobutyl and methyl DNA adducts, which can lead to G → A transitions and to a lesser extent to G → T transversions. CYP2A6 also can hydroxylate nicotine at the 2' position, which results in opening of the pyrrolidine ring, making it susceptible to nitrosylation with the subsequent formation of NNK, an endogenous reaction that adds to the NNK that is already present in tobacco smoke.

Food-borne xenobiotics comprise another potential source of environmental mutagens and the aflatoxins provide a highly relevant example. Similar to BaP, aflatoxins are (five-member) multiring structures that can be activated to diol-epoxides by CYP3A4 (a major hepatic CYP), which then can bind to susceptible DNA molecules. The resulting aflatoxin-N^7-G adducts produced lead to G → T transversions.[377,389–393] Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which commonly infect grains and nuts such as corn, sorghum, wheat, rice, peanuts, and various tree nuts. The fungi proliferate extensively under warm and moist storage conditions to produce what may be the most important food-borne exogenous carcinogen. While exposures to aflatoxin in wealthier countries are nearly universal, the amounts tend to be relatively low and are not considered a major risk, the more modern technology available for harvesting, handling, and storage of foods limits growth of the contaminating fungi. Consumption of aflatoxin-contaminated foods is a major factor for hepatic cancer throughout parts of China and Africa where many people harvest and store their own local produce in less than adequate conditions. The risks for hepatic cancer due to aflatoxin exposures are especially great in regions where hepatitis infections are prevalent. In populations with both hepatitis infections and high aflatoxin exposures, the incidence of hepatocellular cancer is as much as 60 times greater in comparison to either condition alone, indicating the extensive synergy between chronic infections and chronic exposures to these xenobiotic carcinogens. In such populations, hepatic cancer is often the primary cause

of cancer-related death and a major cause of death overall. Although aflatoxin is not considered a major exogenous carcinogen in the United States or Europe, it is a major one for many in the world and illustrates the potential for higher-level exposures to food-borne carcinogens to be a major factor in cancer, especially in the face of coexisting chronic inflammation.

Processed meats and red meats also can comprise a source of potentially mutagenic compounds, although they are only moderately associated with increased risk for cancers, including colorectal, gastric, and esophageal cancers.[132,134,394–397] The heme content of red meat, the presence of nitrate and nitrite in processed meats that lead to the formation of N-nitroso (N-N=O) compounds *in vivo*, and the formation of heterocyclic amines and PAHs during high-temperature cooking (barbecuing and searing) appear to be associated with risk in these studies. Heme iron is known to contribute to the generation of ROS with subsequent increases in lipid peroxidation. And the increased formation of malondialdehyde and subsequent DNA damage can contribute to carcinogenesis. The ability of PAHs to cause DNA damage following activation by CYP has been described earlier in this section, and interestingly, total daily PAH exposures from food are similar to those from smoking a pack of cigarettes; in epidemiology studies, they even relate more closely to levels of DNA adducts in blood than smoking does.[398] This last of course would make sense because everyone eats but only ~25% smoke and also may explain why PAH-related DNA adducts in lungs of smokers are only about 1.5–2 times higher than in nonsmokers. Along the same lines, overall exposure to nitrates from vegetable consumption is actually much higher than that from preserved meats, leading one to believe that even though CYP2E1 activation of nitrosamines can lead to the methylation of guanine and G → A transitions, the contribution of this reaction from dietary meat in and of itself is not a major source of risk for cancer.[398,399] This is not to say that each of these reactions do not contribute to risk because they do, but it does illustrate that there are few situations where a single source of exposure to exogenous mutagens can provide the majority source of risk for any cancer. The last meat-based mutagens to mention are the heterocyclic amines that are produced from surface proteins and amino acids that are pyrolyzed during grilling and searing. N-hydroxylation of heterocyclic amines by CYP1A2 followed by esterification reactions, catalyzed by a variety of phase II transferases, lead to release of an aryl nitrenium ion that then can bind to guanine, producing G → T transversions.[398,400]

While all of the above-mentioned mutagenic reactions occur in humans, the moderate associations between meat consumption and cancer risk indicate that rather than being a major source of risk in and of itself, high levels of processed and grilled meat consumption may more likely exacerbate the presence of other significant risks such as persistent ROS stress from chronic inflammation or high-level exposures to tobacco smoke.

CYP-mediated reactions that result in the activation of carcinogens are an important component of risk for cancer from xenobiotics. Activities of the individual CYP enzymes, however, are not constant and they also tend to be very different for different individuals.[382,401–403] Chronic exposure to different xenobiotics (and exercise) can differentially alter expression of the CYP enzymes. Drinking alcoholic beverages, for example, can enhance CYP2E1 in many tissues and enhance the activation of NNK

and other nitrosamines, while chronic exposure to PAHs and to heterocyclic amines induces activity of CYP1A1.[398,404–407] Such increases in CYP activity are associated with an increased risk for many cancers and may be related to an increase in both carcinogen activation and ROS production by these CYP isozymes. Inactivation of the electrophilic products by phase II enzymes before they can cause damage also is an important component of risk. And similar to phase I enzymes, phase II enzymes vary widely among individuals, and the activities can be modified by exposure to xenobiotics as well as by exercise.[173,402,408–411] Because phase I and phase II enzymes are regulated through different mechanisms, it is possible that an increase in the former could be accompanied by an even larger increase in the latter, making it an awkward assumption that an increase in phase I activities necessarily means an increase in cancer risk. And to make things even more complicated, enzymes such as CYP1A1 that are involved in activating PAHs to electrophilic epoxides also are involved in hydroxylation of the epoxides to inactivate them. Because of these metabolic complexities, absolute risks due to exposure to different carcinogens in the air, water, and food are difficult to assess in the general population, and although not a topic for this chapter, the concept deserves a brief discussion.

Extensive efforts have been made over the past three decades to incorporate measures of exposure along with a variety of biological markers into risk assessment for cancer, a concept pioneered by Perera and Weinstein with the development of the field of molecular epidemiology.[412,413] This field is essentially the study of individualized cancer risk in populations through integrating measures of exposure to different carcinogens with the measured individual differences in carcinogen metabolism (activation/inactivation), along with the observed array of DNA damage and mutations.[207,378,401,412,414–416] Results of such studies have been instrumental in tying exposures to specific sources of carcinogens to risk for specific types of cancer, as long as there are known source-specific mutagens. For example, NNK is unique to tobacco smoke and the presence of pyridyloxobutyl DNA adducts at specific codons, and G → A transitions at the same locations in lung tumors, will tie the cancer to tobacco use. In contrast, using PAH-associated DNA damage or mutations as markers for smoking risk would not work because the PAHs found in tobacco smoke also are found in our food, air, and water.

These kinds of studies are not without their own difficulties in interpretation. For example, without the presence of a unique mutagen in a particular environmental source, it is often difficult to differentiate DNA damage due to exogenous sources from that arising from endogenous sources. One major reason for this is that the ultimate forms of DNA damage caused by various reactive compounds arising from air and water pollution are often indistinguishable from those caused through endogenous mechanisms. In instances where it is possible to differentiate the two, such as in those experiments that used labeled versions of the common air pollutants formaldehyde, vinyl chloride, and ethylene oxide, the endogenous DNA damage is more than a thousand-fold greater, and even with the administration of near toxic (to humans) doses of methanol (a common industrial solvent and fuel additive) to rats, hydroxylmethyl DNA adducts from methanol are still lower than this same adduct arising from endogenous sources.[417,418] DNA damage from exogenous sources of pollutants in general also must be evaluated against a background of as many as 50,000 DNA

adducts/cell that arise from all endogenous sources. When such data is taken into consideration, although risks for cancer are clearly enhanced by exogenous sources, total exogenous risks have been estimated to be from 1% to as high as 19% of the total cancer risk.[364,415,419–422] Of these values, the greater values are obtained when carcinogenic compounds in food that result from various storage (e.g., contamination from aflatoxin-producing molds) and cooking conditions (smoking and grilling) are included as environmental sources. This is not to say that environmental air and water pollution is not a source of risk and that efforts to protect the environment from pollution are not essential to minimizing risks to our future health, but it is still (relative to endogenous sources) a minority source of the total average risk for the general population.

An issue that is related to air and water pollution is that of exposures to exogenous carcinogens in the workplace and (geographically) localized exposures due to industrial accidents. Of these, the first is a major source of risk for what actually may be a relatively small segment of the population (~152,000 cancer deaths annually as currently estimated by the WHO out of the ~7.5 million annual cancer deaths worldwide).[3,7,423] The second is certain to be even lower, but it is very difficult to estimate on a global scale simply because of the limited number of accidents and the extended time scale of the resulting carcinogenic effects. In addition, short of changing jobs or moving before an accidental release, there is little an individual can do about exposures such as these.

An area of exogenous risk that has received a lot of attention lately is that of "endocrine disruptors." Endocrine disruptor compounds (EDCs) exert their carcinogenic effect predominantly through mechanisms that ultimately lead to enhanced entry into the cell cycle; they predominantly act as promoters that in some cases (e.g., FD&C Red #3) also may cause DNA damage.[424–430] EDCs were originally thought of as xenoestrogens or environmental estrogens for their ability to bind to estrogen receptors as revealed through the study of dichlorodiphenyltrichloroethane (DDT) and diethylstilbestrol. They are now recognized as having endocrine and metabolic effects through interactions with estrogen and androgen receptors, various retinoic X receptors, and the aryl hydrocarbon receptor (AhR). Common EDCs include pesticides such as the organochlorine pesticides (OCPs), which include DDT (banned in 1972 in the United States), heptachlor epoxide, and oxychlordane; the dioxins that originate from paper-bleaching agents and incinerating PVC products including polychlorinated dibenzodioxins (PCDDs) and polychlorinated biphenyls (PCBs); the plasticizers bisphenyl-A (BPA) and phthalates; and the flame retardant polybrominated diphenyl ether (PBDEs). Of these compounds, the OCPs, BPA, phthalates, and PBDEs all interact with estrogen receptors (ERs) and androgen receptors (ARs) to exert their disruptive developmental effects while the PCDDs and PCBs bind to the AhR to produce their effects.

Risks for BC will be discussed to illustrate that much of the enhanced risk for cancer following exposure to many of these compounds appears to be influenced by the timing of the exposures. In utero exposures to many of these compounds are known from animal studies to enhance risk for BC by increasing proliferation of the ductal tissues during organogenesis and early growth (and adolescence) and delaying differentiation of the terminal buds to lobules to enhance the duration of

their sensitivity to proliferative signaling, therefore increasing risk for cancer. While these risk-enhancing effects can be mediated by the estrogenic activity of a variety of EDCs, the various dioxins also may play a role in these developmental effects. Dioxins bind to the AhR that then dimerizes with the ARNT protein to bind to the xenobiotic response element to induce expression of a variety of proteins (including CYP1A1). Because of its proximity to the estrogen response element, there is considerable cross talk between AhR- and ER-mediated effects with 2,3,7,8-tetrachlorodibenzodioxin (TCDD) being capable of eliciting estrogenic effects.[431,432] In addition, ERs act as co-activators of AhR-mediated responses, indicating that it is possible for dioxins and xenoestrogens to act in an additive or possibly even synergistic fashion, leading to enhanced risks during susceptible periods of growth.

Induction of CYP1A1 (msp1 polymorphism) by dioxins is associated with hydroxylation of estrogen to a metabolite that retains its biological activity and also causes DNA damage.[433–435] These effects would be consistent with the elevated risk for BC observed with occupational exposures to both classes of EDCs.[426] Environmental exposures during adolescence also might increase risk for BC through the potential additive effects of these EDCs with the ovarian estrogens, and possibly compounded by the effect of the many other growth factors that are present during adolescence and that lead to hyperphosphorylation of ERs to greatly enhance their promoting activities. Risks for PC also appear to be affected by EDCs when exposures occur during fetal development and adolescence through similar mechanisms, a logical result because prostate SCs and PCs express the full repertoire of ERs as well as ARs and AhRs.[436,437]

As with other environmental risks, there may be little that can be done about exposures to EDCs from air and water pollution, however, because many of the exposures to these compounds come from what might be termed voluntary exposures, some risks can be minimized. Simply avoiding using any pesticides if at all possible, minimizing the use of plastic storage containers for foods and beverages and avoiding purchasing canned foods or those already in plastic will go a long way to reduce exposures to these compounds during critical times. While an awful lot of commercial interests might not like it, repetitious public health announcements expressing such sentiments might actually help to reduce behaviors that enhance risk for BC and PC in the same way a variety of antismoking campaigns have modestly reduced smoking behaviors in the United States and other countries.[438–440]

From the preceding discussion, it is clear that there are many endogenous and exogenous sources of compounds that lead to DNA damage and to enhanced rates of cell division that in combination lead to elevated risks for cancer. There also are a wide variety of possible DNA alterations in addition to those already mentioned. As one can imagine, many of the activated lipid-peroxidation species mentioned in the preceding discussion (such as malondialdehyde and acrolein) also may react with each other, in addition to DNA, to form more complex adducts including a variety of DNA cross-links, each with their own mutagenic potential.[348,354,360,441] The activated compounds that react with exocyclic amines of DNA also can react with exposed amino groups of any nearby protein, leading to inactivation of these proteins, as well creating a variety of inactivating protein:protein and protein:DNA cross-links. While the details of these forms of DNA and protein damage are beyond the scope

of this chapter, the potential carcinogenic effect is not. The DNA:protein cross-links will obviously have their own direct mutagenic potential because they are a form of DNA damage. In instances where the protein damage produces a loss of function of any of the enzymes that are associated with the regulation of signal transduction pathways, or cell cycle control, or those involved in phase II activities, or in protein or DNA synthesis and repair, then risks for mutations also may be enhanced.

The repair or replacement of damaged proteins and damaged DNA in proliferating cells ensures that risks for mutagenesis are minimized. When the processes of repair and synthesis are compromised and the damage persists, then the probability of producing mutations increases. Not coincidently, it is the same ROS, RNS, and other electrophilic oxidant species that cause damage to proteins and to DNA that also can alter activities of the various signal-transduction pathways to initiate (directly and indirectly) proinflammatory and proliferative signaling. Some of these same oxidants also are involved in enhancing synthesis and repair functions (through altering activity of the signal transduction pathways). Therefore, anything that can interfere with the tight integration of oxidant/antioxidant status with the regulation and integrity of signal transduction pathways also can contribute to risk for cancer. This leads us to our last major topic of cancer etiology.

6.2.6 Nutritional Insufficiencies

Nutritional insufficiencies comprise a risk factor for cancer that is common in the United States. For instance, intakes for vitamins C, D, and E are well below the EAR for 25%, 70%, and 60% of all Americans, respectively. Intakes also are below the EAR for folate and zinc (>10%); iron, copper, and selenium (~5%–6%); and for calcium (38%) and magnesium (>45%), and each of these insufficient intakes are associated with increased DNA damage, mutagenesis, and risk for cancer.[105,136,139,145,442–446] The implications of these insufficiencies are very important for cancer risk in the United States, especially when one considers the endemic nature of them; the majority of Americans consume insufficient amounts of more than one nutrient. As described in his triage theory of insufficiency-based risk, Dr. Ames hypothesizes that short-term survival of an organism takes precedence over long-term survival in the face of micronutrient insufficiencies as an evolutionary trait, ensuring that the immediate nutrient requirements for current metabolic and cell function requirements (presumably, e.g., activities such as ATP generation, protein and membrane synthesis, and production of membrane gradients) are satisfied at the expense of longer-term functions such as DNA synthesis and repair.[445,446] This theory is both attractive and practical in part because it puts into a biological and evolutionary perspective one of the realities of basic chemistry, that is, diffusion. As specific nutrients are being modified or conjugated to other macromolecules while being used, then the local concentration of the original form must decline. If the rate of use is high, then the local concentration will decline faster than in other locations of slower use, causing the nutrient to diffuse from the areas of higher concentration to those of the fastest use. This will of course compromise other functions at some other locations, *unless* optimal amounts of the nutrient are available. With such a high penetration of insufficient nutrient intake in the U.S. population, optimal nutrient availability for most is

highly doubtful. And as a result, many cell functions will be compromised to some extent. From the preceding discussion, it is seen that a majority of Americans consume insufficient amounts of vitamin C, D, and E, with obvious implications for both ROS-mediated damage and inflammatory and proliferative signaling.

The antioxidant function of vitamins C and E is well known with vitamin C acting as a soluble antioxidant that can quench a variety of soluble ROS including superoxide anions and hydroxyl radicals while vitamin E acts as a lipid-soluble antioxidant to arrest lipid peroxidation reactions.[447–451] Vitamin C also reduces vitamin E radicals back to the functional form while glutathione (GSH) reduces (directly or through glutaredoxin activity) the ascorbate radical or the dehydroascorbate forms back to ascorbate. GSH reductase (GSR) then uses NADPH as an electron source to reduce the glutathione disulfide (GSSG) back to GSH + GSH, while thioredoxin reductase (TrxR) can reduce oxidized ascorbate to the ascorbate form using NADPH as the electron source. The importance of these reactions is twofold, with the first relating to ROS-mediated damage. A reduction in antioxidant function due to an insufficient intake of vitamin C can lead to a reduction in antioxidant function of vitamin E, resulting in increased lipid peroxidation and DNA damage from ROS, malondialdehyde, and other peroxidation products. The second is the effect this may have on activity of various signal transduction pathways.

ROS (and other oxidizing agents) can modify activity of signal transduction pathways by oxidizing redox sensor proteins such as thioredoxin (Trx) and peroxiredoxin (Prx). Trx is a component of a complex redox-regulation system that includes Trx, TrxR, GSH, GSR, GSH peroxidase (GPX), and Prx.[450,452] These sensor proteins function as antioxidant enzymes through their peroxidase activities and also are important regulators of activity of the signal transduction pathways.[168,450,453–458] For example, Trx forms inhibitory heterodimers with apoptosis signal-regulating kinase 1 (ASK1) and disassociation can occur when Trx is oxidized, leading to activation of the p38 and JNK-MAPK pathways. The transcription factors Jun/Fos (AP-1), heat shock factor 1, and p53 also can be indirectly activated by Trx through Trx-mediated oxidation of redox-factor 1 (Ref-1).[454,458–462] Because GSH is an essential component of this redox system, a nutritional insufficiency that contributes to a nonoptimal antioxidant status can enhance use of GSH as an antioxidant while compromising its use as a cofactor in the redox system. This could then compromise redox-based control of the signal transduction pathways, inappropriately enhancing their activation. Selenium insufficiency (5%–6% of the population) also contributes to this dysregulation because the selenoprotein TrxR is an important component of the Trx/Prx redox-regulation system, explaining in part the common associations between insufficiencies of selenium and increased risk for cancer.

In addition to the antioxidant compounds mentioned, a variety of antioxidant enzymes also contribute to redox-based control of signal transduction pathways. The antioxidant enzymes SOD, catalase (CAT), and GPX (also a selenoenzyme) contribute to redox regulation in the cell through the inactivation of ROS, a function that also is commonly known to reduce ROS-induced damage to DNA, proteins, and phospholipids.[168,344,345,450,453–460] Reducing ROS will attenuate ROS-mediated oxidation of Trx and therefore help to ensure "appropriate" signal transduction activities. Because of the heterogeneous distribution of these antioxidant enzymes,

the redox-sensor enzymes, and the various sources of ROS with differing rates of ROS production within cells, there also will be differing spatial distributions of ROS oxidants within the cell as well, producing an activation pattern of the various signal transduction pathways that is specific to the particular spatial distribution of ROS.[458,459,463] As the redox environment within a cell changes due to changes in metabolic requirements, activities of the different signal transduction pathways also change and the resulting patterns of activation of various transcription factors are altered as well. It is through these temporal and spatial alterations in redox potential that alterations in protein synthesis are made to modify cell functions to better handle the various stressors. Other factors that can alter the internal redox state of a cell include responses to various growth factors and other signaling molecules, production of ROS from either endogenous or exogenous sources, or even exposures to exogenous toxins and xenobiotics. "Flooding" cells with damaging ROS or toxins could interfere with the delicate regulatory balance between activity of the antioxidant (and redox) enzymes and the activity of the signal transduction pathways, resulting in overactivating or underactivating the pathways. On the other hand, excessive amounts of soluble antioxidants could disrupt the redox environment to inappropriately alter regulation as well. Both situations could obviously alter overall cell function, and the direct activation of ERK-MAPK and p38-MAPK pathways by cigarette smoke extracts resulting in proinflammatory signaling (independent of damage or mutagenic effects) is indicative of the former.[464,465] The latter concept is illustrated by the prevention of the mitochondrial biogenesis and enhanced insulin sensitivity that is normally stimulated by exercise through supplementation with vitamin C (1 g) and vitamin E (400 IU).[466] Maintaining tight control of the signaling processes within the cell therefore depends on the localized concentration of the various antioxidant and redox enzymes and their cofactors. Because iron, copper, zinc, and selenium are components of one or more of all of the antioxidant and redox enzymes, the prevailing insufficient intake (5%–10% of the population) of these minerals is certain to be a factor in preventing optimal control of the various signal transduction pathways and thereby enhance risk for cancer.

In addition to the direct effects of ROS on the various signaling pathways, they also can affect them indirectly. PGE_2 is an important activator of proliferative signaling in tissue SCs and PCs and $cPLA_2$ can be activated by ROS, leading to enhanced production of PGE_2.[467–471] This is one more avenue for enhanced proliferative signaling to occur as a result of enhanced ROS stress. It also is one that would be enhanced in the event of nonoptimal antioxidant status. Thus, from all of the preceding discussion, nutritional insufficiencies in antioxidant-associated nutrients and cofactors exacerbate the risks associated with exposures to exogenous and endogenous ROS, mutagens, and mitogens.

The last issue to discuss in regard to nutrient insufficiencies is that of vitamin D, calcium, and magnesium. These represent the dietary (intake) insufficiencies with the greatest prevalence for both vitamins and minerals, 70%, 38%, and >45%, respectively. The importance of vitamin D and magnesium for maintaining calcium status is well known. When levels of calcium in the serum decline, parathyroid hormone (PTH) is released, stimulating synthesis of the enzyme 25-hydroxycholecalciferol-1-hydroxylase in the kidney and ultimately enhancing

intestinal calcium and phosphorus absorption.[472–476] Because magnesium is required for both the release of PTH from the parathyroid gland and binding of PTH to its receptor, a magnesium insufficiency can have detrimental effects on both calcium regulation and absorption and is known to be a major factor in risk for osteoporosis. Lower calcitriol levels also will occur because of attenuated PTH effects, an effect that also is observed with insufficient intake of magnesium.

Inadequate vitamin D is commonly known to be associated somewhat with increased risk for cancer; the associations have not consistently been observed in all studies.[477–480] The risk-reduction effects of vitamin D have been proposed to be mediated in part through an increased expression of p21 and p27 that leads to cell cycle arrest and by enhancing differentiation through modulating JNK-MAPK activities, effects that have been observed in cancer cells in culture. Calcitriol also inhibits the production of proinflammatory prostaglandins and cytokines by suppressing COX2 expression and NFκβ signaling in both cancer and noncancer cells. These latter effects would be important in prevention and illustrate how nutritional deficits (vitamin D) might enhance risk factors (inflammation) that appear to be far removed from the commonly known function of the nutrient (promoting calcium absorption). In the one double-blind, placebo-controlled clinical trial for prevention in postmenopausal women that used calcium and vitamin D supplements (individually or in combination), relative risks of less than 0.4 were observed for all cancers in each of the supplemented groups in comparison to nonsupplemented.[479] Although these results support the associations between low vitamin D and enhanced risk for cancer, the study was of relatively short duration (4 years) and may not necessarily apply to the "life-time-to-date" associations often tested for in many epidemiology studies. These results also support the concept that not knowing the nutritional status of subjects in epidemiological studies can limit the interpretation of the results; calcium, vitamin D, and magnesium insufficiencies (and any others for that matter) may be a serious confounder for interpreting risks in such studies because they exist in a large majority of subjects.

This brings us to the various avenues that are amenable to prevention. Minimizing exposure as much as possible (or practical) to potentially carcinogenic compounds is clearly a very important behavior. While moving or changing jobs are not often convenient strategies to avoid exogenous mutagens, reducing the voluntary exposures to them certainly is. The use of tobacco products and self-exposure to second-hand smoke are voluntary behaviors that should be avoided or at the very least minimized. Preparing and eating smoked meats also can be limited and food storage conditions can be altered to minimize contamination. Risks that arise from endogenous mechanisms also can be attenuated, although they are certainly less amendable to complete prevention. The major endogenous factors that are best associated with prevention include the metabolic production and inactivation of ROS and RNS, activation and inactivation of electrophiles from xenobiotics, and the repair of the damage to DNA and proteins that is caused by the various ROS, RNS, oxidants, and electrophiles. In addition to these, the various signaling pathways that are associated with cell cycle regulation also are important targets, with the primary prevention effort being directed at correcting nutrient deficiencies and insufficiencies. If this primary objective can be met through optimal dietary habits, then many of the other prevention

targets that relate to signaling processes, such as those resulting in enhanced phase II activities, will be addressed at the same time.

6.3 ETIOLOGY OF PREVENTION

As described in Section 6.1.1, there is considerable evidence from both epidemiologic and mechanistic-based researches that a variety of factors associated with eating a proper diet and exercising regularly may significantly reduce risk for many cancers. Perhaps, this should be restated from a slightly different perspective: it is the variety of nutritional insufficiencies, coupled with an inadequate consumption of plant-based foods, and a lack of sufficient physical exercise that profoundly increase the risks for cancer. The difference in phrasing is philosophical; cancer should not be considered "normal" but rather it should be considered an unfortunate consequence of our poor lifestyle choices. In the context of our cellular and molecular-based approach to disease etiology and prevention, exposures to various carcinogens can lead to a variety of damaging events that result in cellular-stress responses and dysfunctions. And, continuing exposures to these damaging agents can lead to the development of a cancer. Unfortunately, many in the United States already have subclinical cellular dysfunctions due to an inadequate consumption of different essential nutrients, a situation that leads to greater signaling through cellular stress-response pathways than would otherwise occur, with subsequent enhanced proinflammatory and proliferative effects. When these nutritional insufficiencies are combined with prolonged exposures to multiple carcinogens from a variety of sources, the result is a perfect recipe for enhancing risk for cancer. They also are the starting point for prevention.

6.3.1 Nutrition and Diet

Logic dictates that eliminating nutritional insufficiencies should reduce risks for cancer in part by ensuring optimal antioxidant status and redox regulation of signal transduction pathways. Proper nutrition also should help through maintaining adequate availability of nutrients for the purposes of synthesis and repair, and it should be a relatively simple thing to do. After all, all one has to do is eat a proper diet, such as that described in the MyPlate (www.choosemyplate.gov) recommendations. The difficulty with this concept, as explained in Chapter 8, is that people eat for many reasons and the habits of eating that are learned and ingrained over a lifetime are extremely difficult to break. The simplest way to deal with part of this problem is to use dietary supplements, and many attempts to reduce risk for cancer through the use of dietary supplements have been made, albeit with varying degrees of success.

In many of the prevention trials, supplements that contain antioxidant-related compounds such as β-carotene, vitamins E and C, and selenium have been tried.[481–489] The use of antioxidant supplements has often been based on the negative associations observed between cancer incidence and fruit and vegetable intake in epidemiological studies and on the assumption that the protective effect was apparently due mainly to the antioxidant and β-carotene content of these foods. Although decreased

incidences of gastric cancer have been observed in China with vitamin E, selenium, plus β-carotene supplements; supplementation with vitamin C and molybdenum, retinol and zinc, vitamin E alone, vitamin C alone, or with multivitamins, in both China and the United States, have had little to no positive effect. Similar inconsistent results are seen with PC as well as with many other cancers, and increased risks for cancer, while certainly unintended, have been observed with some supplements. In the cases of PC (SELECT), supplementation with vitamin E, selenium, or both significantly enhanced risk for cancer. In both the ATBC and CARET studies, supplementation with β-carotene significantly enhanced risk for lung cancers while supplements with antioxidant vitamins had little to no effect. In several other recent longer-term (>10 years) clinical trials, the use of multivitamin/multimineral supplements (or multi-antioxidant supplements) produced only very small nonsignificant preventive effects, or no preventive effects at all, while the evidence for their use in epidemiological surveys is also weak to nonexistent.[487,490–496]

With a focus on mechanisms of prevention for this section, a comprehensive review of these types of studies is not the point. These few studies do, however, illustrate several important issues. In the smoking–lung cancer studies, the enhanced risk was associated with heavy alcohol consumption. Although the enhanced carcinogenic effect of β-carotene in conjunction with heavy drinking in smokers was one that could not be predicted on the basis of the epidemiological evidence at that time, there was earlier evidence from animal and human studies that chronic ethanol consumption may significantly alter β-carotene and retinoic acid metabolism to enhance vitamin A toxicity.[497,498] Perhaps, if such information were more widely known, it might have been incorporated into concepts of risk and the potential for risks when using supplements in special populations. One more issue of unintended consequences is that of antioxidants interfering with normal cell function. While antioxidant supplements have often been thought to be beneficial as a prophylactic to reduce ROS-mediated damage, increases in mitochondrial function and in insulin sensitivity that are normally caused by exercise and that are partially responsible for the preventive effects of exercise can be prevented by supplementation with vitamin C (1 g) and vitamin E (400 IU) in amounts that are often used in supplements.[466] Whether this phenomenon contributes to the lack of beneficial effect of using supplements in many studies is unknown; however, with insulin resistance being a source of risk for carcinogenesis, it should not be overlooked.

In relation to the special populations' issue, the preventive effect of the antioxidant compounds on gastric cancer appeared to occur predominantly in populations with a relatively high incidence of chronic undernutrition and malnutrition. In hindsight, one would expect that providing nutrient supplements to a predominantly malnourished population to correct nutrient deficiencies might have a much greater impact on prevention than on people with marginally insufficient diets and no overt deficiencies. And this may be especially true when the population with the much poorer diet also is exposed to greater amounts of carcinogens, aflatoxins in the case of the referenced studies on gastric cancer conducted in China and the United States. Although nutrient insufficiencies can enhance risk for cancer by enhancing mechanisms of cause, nutrient-based supplements may only be a major contributing factor for prevention in the face of chronic undernutrition or outright malnutrition.

Another issue may be that nutrient supplements simply cannot overcome the detrimental risks associated with moderate exposures to carcinogens when they are coupled with behaviors that enhance risks, behaviors such as consuming too much alcohol, smoking (or using) tobacco products, eating too many calories, eating too few fruits and vegetables, and not participating in any physical activity. The first few of these behaviors have been addressed earlier and reducing risks for a variety of cancers includes altering behaviors to maintain weight, to avoid using tobacco products or exposures to tobacco smoke, and to minimize the use of alcohol. These preventive activities have one thing in common; they all reduce exposures to endogenous and exogenous sources of carcinogens and proliferative signaling but do very little to *enhance* protection. The latter two, however, deserve a detailed discussion because increasing fruit and vegetable consumption (and participating in appropriate physical activity) can enhance a variety of mechanisms of "protection" to reduce risk for cancer.

Because of the known inverse association between consumption of plant-based foods and cancer, it stands to reason that increasing consumption of a variety of fruits and vegetables confers risk-reduction benefits. And from the preceding, it apparently is not because of any one specific nutrient or compound with antioxidant properties. Rather, it is far more likely that it is an optimal nutrient status gained from consuming such a diet combined with the consumption of an array of bioactive non-nutrient compounds called phytochemicals that protects against cancer.[148,410,499–506]

The major forms of phytochemicals include the various phenolics (flavonoids, phenolic acids, stilbenes, coumarins, and tannins), carotenoids (α-carotene, β-carotene, lutein, lycopene, zeaxanthin, and astaxanthin), and organosulfur compounds (isothiocyanates, indoles, and allelic sulfur compounds), many of which are weak to moderate antioxidants.[410] The highest concentrations of phytochemicals are found in herbs, spices, and dried fruits (~1%–5% by weight) with somewhat lower amounts in fresh fruits, nuts, grains, vegetables, and oils (0.05%–1% by weight).[507] Common foods and their phenolic contents are listed in Chapter 2, Section 2.5.2.2 and won't be listed here. It is important to note that many of the foods that are high in phytochemical content also are consumed much more frequently in a traditional Mediterranean diet than in the American diet and it is consumption of these foods that is associated with the reduced incidence of cancer (and other chronic diseases) in the Mediterranean cultures in comparison to the United States.[49,140,150,508–510] The mechanisms of prevention that are enhanced by phytochemicals include reducing proinflammatory signaling, attenuating the production of mutagenic electrophiles, enhancing the elimination of mutagenic compounds, enhancing antioxidant control through increasing expression of antioxidant and redox enzymes, and increasing DNA and protein repair.

6.3.2 Inflammatory Signaling

As described in Section 6.2.4, inflammatory signaling by infiltrating stromal cells is an important factor in the development of tumors, stromal cells that include macrophages, lymphocytes, NK cells, mast cells, endothelial cells, and fibroblasts. Each of these cells can produce an array of proinflammatory prostaglandins and cytokines (for details please see Chapter 2) in response to the activation of pattern recognition

receptors (PRRs) by pathogen-associated molecular patterns and damage-associated molecular patterns, and through paracrine and autocrine activation by the same prostaglandins and cytokines.[511–517] The receptor-mediated activation process for production of the proinflammatory cytokines involves activation of the NFκβ, p38-MAPK, and JNK-MAPK pathways, which in turn activate the various transcription factors that then induce expression of IL-6, pro-IL-1β, TNFα, and IFN-β.[512,514,518,519] And because both elevated calcium and ROS can activate these same signal transduction pathways, any metabolic stress and cellular damage also will lead to the activation of proinflammatory signaling as well.[520–523]

One means to attenuate inflammatory signaling is to attenuate activation of the NFκβ, p38-MAPK, and JNK-MAPK pathways; this will in turn reduce the expression of the proinflammatory cytokines. And in a variety of animal studies as well as cell culture studies, a variety of flavonoids (quercetin, apigenin, epigallocatechin gallate (EGCG), theaflavin, genistein, anthocyanins, and kaempferol), phenolic acids (ellagic acid, capsaicin, and curcumin), and the stilbenoid resveratrol significantly inhibit activity of one or more of these pathways.[147,508,524–534] And where tested, in many of these same studies, a significantly reduced expression of IL-6, IL-1β, TNFα, IFN, ICAM, and MCP-1 have been observed as well, indicating that consumption of these phytochemicals can reduce proinflammatory signaling.

The mechanisms through which they reduce proinflammatory signaling are diverse. Sulforaphane can inhibit PRR signaling by directly attenuating TLR4 activation while curcumin inhibits both TLR4 and NOD1/2. Resveratrol, EGCG, quercetin, and luteolin inhibit TBK1 (MyD88-independent pathway) and resveratrol also inhibits TRIFF (MyD88 activation pathway), all of which attenuate the NFκβ, p38-MAPK, and JNK-MAPK signal transduction pathways.[528] As described in detail in Chapter 2, PRR-mediated activation of these pathways is essential for both damage- and infection-mediated inflammatory responses. JNK-MAPK and p38-MAPK activate a variety of transcription factors (including cJun, ATF2, IRF3, IRF5, IRF7, JunB, JunD, and Iκβζ) that dimerize in various combinations and, along with NFκβ (p50/p65 dimer), induce expression of the proinflammatory cytokines. The ability of curcumin, resveratrol, sulforaphane, EGCG, and luteolin to attenuate PRR activation and subsequent proinflammatory signaling therefore is an important factor in reducing inflammation-associated risks for cancer.

In addition to blocking PRR activation, NFκβ signaling also can be attenuated through inhibiting IKK activity. IKK phosphorylates Iκβ, which is normally complexed to NFκβ in the cytosol. On phosphorylation, NFκβ is released and it then can enter the nucleus and bind to its promoter. A variety of theaflavins (including EGCG) directly inhibit IKK while quercetin, curcumin, kaempferol, apigenin, morin, anthocyanins, procyanidins, and lycopene suppress either nuclear translocation of NFκβ or binding of NFκβ to DNA, attenuating expression of the proinflammatory cytokines.[524,525,532,534]

Activity of the MAPK pathways also is attenuated by a variety of phenolics including apigenin, luteolin, quercetin, resveratrol, EGCG, and kaempferol. These suppress ERK1/2-MAPK, JNK1/2-MAPK, and p38-MAPK pathways while cyanidins can attenuate ERK activation and curcumin can inhibit PKC, a calcium-activated enzyme that activates various components of the MAPK pathways.[524,525,532,534]

These effects also lead to a suppression of proinflammatory signaling. Different cell types, however, respond differently to phenolics; for example, luteolin attenuates all MAPK pathways in respiratory epithelial cells but not the JNK-MAPK in macrophages.[524,525,532,534–536] EGCG also is known to have different effects on different lineages of the same cell type with inhibition of IKK occurring in some keratinocytes and blocking of p38 activation in others (HaCa cells).

Because both ROS and increased cytosolic calcium also can activate these same MAPK pathways to produce a metabolic stress–mediated proinflammatory response, inhibiting these effects is an important preventive measure. The inhibition of PKC by curcumin has already been noted, an effect that also occurs with quercetin and luteolin and contributes to the dampening of the various MAPK pathways.[537] As discussed in Chapter 2 (section 2.5.2.2), the antioxidant effects of individual phytochemicals tend to be relatively weak, and even at the highest recorded levels, consumption of any individual compound from specific foods rarely approaches within 10%–20% of that necessary for achieving effective antioxidant effects. However, when mixtures of foods (with very different phytochemical contents) are consumed, then antioxidant effects are observed at a total phytochemical dose that is up to five times less than the dose necessary when a single food source of phytochemicals is eaten.[410,463,538] With half-lives in the 1–2-hour range for most phytochemicals, the antioxidant effects are transient at best, but may still have a dampening effect on ROS-mediated activation. From a wide array of studies, it is clear that different components of the MAPK and NFκβ signaling pathways are attenuated by different phytochemicals, as are the different activating mechanisms (PRRs, ROS, and PKC), and in some respects in a cell-specific manner. Because of this, to have an overall preventive effect through suppressing cytokine-mediated inflammatory signaling, an array of phytochemicals would need to be consumed.

In addition to the cytokines, the eicosanoids PGE_2, LTB_4, and TXA_2 also have potent proinflammatory effects and they are usually the first ones produced in response to any type of damage or stress. These molecules are produced in almost all cells as a direct response to the calcium-, ROS-, or MAPK-mediated activation of PLA_2 along with the coordinated activities of cyclooxygenases, lipoxygenases, and the various eicosanoid synthases.[467–471,520,539,540] These eicosanoids are responsible for initiating proinflammatory responses; responses that include the chemoattraction of macrophages, neutrophils, and other inflammatory cells into the area as well as their activation. These effects are in addition to activating the proliferation of any SCs or PCs by PGE_2 in the immediate area. Once activated by eicosanoids, the inflammatory cells produce the inflammatory cytokines that then enhance the attraction and activation of additional inflammatory cells and platelets. Thus, an eicosanoid-induced enhancement of inflammatory responses within a tumor environment can be an important contributor to the carcinogenesis process.

Inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes would reduce synthesis of eicosanoids, and the phytochemicals EGCG, quercetin, and kaempferol have been observed to directly inhibit either COX or LOX activity to attenuate production of PGE_2, LTB_4, and TXA_2, as do tyrosol, hydroxytyrosol, apigenin, luteolin, and oleuropein from extra virgin olive oil.[525,541,542] And, similar to their effects on MAPK and NFκβ signaling, single phenolics rarely attenuate activity of both

enzymes in all cells, necessitating the consumption of a variety of phytochemicals to obtain an optimal preventive effect. Interestingly, the anti-inflammatory effects of extra virgin olive oil are observed in clinical trials with a daily consumption of as little as 25 g/day (less than 12% of the average daily caloric requirements for adult males), an easily attainable amount if extra-virgin olive oil (EVOO) is used for most culinary needs. These anti-inflammatory effects also are observed in conjunction with the Mediterranean diets where the average consumption of a wide array of phenolics (including those from EVOO) is greater than 1 g/day (~1.3 g/day for females and ~1.5 g/day for men), but not observed with the typical U.S. diet that has an intake of phenolics that averages less than 450 mg/day.[49,140,150,508–510,543,544] Perhaps, the three times greater intake of phenolics common to the traditional Mediterranean diet (in comparison to the U.S. diet) represents some form of a threshold effect, maybe it takes more than 1 g/day of a mixture of phenolics that are obtained from whole foods to produce a reduction in synthesis of proinflammatory eicosanoids. On the other hand, it is possible that consuming an insufficient amount of the essential fatty acid α-linoleic acid (ALA) in combination with a low consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (from marine sources) contributes to the relatively proinflammatory effect of the "typical" U.S. diet.

Both EPA and DHA, commonly found in cold-water fish oils, are known to have anti-inflammatory properties. EPA is synthesized from the essential fatty acid ALA, with conversion rates of approximately 1%–8% in men and 8%–21% in women; DHA is subsequently synthesized from EPA with less than 1% of dietary ALA being converted to DHA for both.[545,546] Less well known are the anti-inflammatory effects of AA, which is synthesized from the essential fatty acid linoleic acid. As reviewed in detail in Chapter 2, lipoxins A_4 and B_4 are synthesized from AA, resolvins D1–4 and protectin D1 are synthesized from DHA, and resolvin E1 is synthesized from EPA. These anti-inflammatory eicosanoids are produced through the coordinated activities of COX2 and 5-, 12-, and 15-LOX and are responsible for initiating and maintaining the resolution phase of inflammation. Lipoxins produce their pro-resolution effects through activating G-protein-coupled receptors on neutrophils, macrophages, endothelial cells, and monocytes that then result in a suppression of p38-MAPK, p42/44-MAPK (ERK 1/2), PI3K activities and inhibiting the activation of NFκβ, producing potent anti-inflammatory effects. Resolvins also have been observed to inhibit activation of NFκβ and both resolvins and protectins greatly enhance the phagocytosis of neutrophils by macrophages without the concomitant production of ROS (thus reducing inflammation-associated ROS-mediated damage) and to inhibit the migration of neutrophils into tissues. In addition to these direct anti-inflammatory effects, lipoxins enhance the release of IL-10 from macrophages, which also suppresses synthesis of the proinflammatory cytokines through activating expression of the suppressor of cytokine synthesis (SOCS) proteins. While the resolution effects of these eicosanoids are essential for shutting down inflammation responses following pathogen and sterile damage–associated stress, their role in diminishing the pro-carcinogenic effects of inflammatory signaling in a tumor environment is largely unknown. The possibility exists, however, that with ~30% of the U.S. population not meeting the AI for ALA, an insufficient availability of endogenously formed EPA and DHA might contribute to an enhanced risk for cancer. This last concept is a

possibility already proposed for CHD based on a significant decline in risk for CHD in Eastern European populations that change from using predominantly low-ALA oils to high-ALA oils for cooking.[547]

EPA and DHA also may exert some of their resolution effects through direct activation of receptors in addition to being precursors for lipoxins and resolvins. For example, both EPA and DHA are peroxisome proliferator-activated receptor gamma (PPARγ) agonists, and activation of this receptor is known to inhibit p38-MAPK, ERK-MAPK, and the NFκβ pathways, resulting in decreased inflammatory signaling in a variety of inflammatory cells.[548–552] This direct anti-inflammatory effect is important because it indicates that increasing consumption of exogenous EPA and DHA not only could correct an insufficiency produced through inadequate consumption of ALA, but it also may provide a pharmacological effect. What we mean by this is that with an adequate consumption of ALA, existing synthesis activities will produce sufficient amounts of EPA and DHA to ensure normal proresolving functions and therefore reduce risks for disease that were produced from an inadequate consumption of ALA. Synthesis of EPA, DHA, and the various lipoxins, resolvins, and protectins is, however, highly regulated and it is doubtful that simply increasing EPA and DHA as substrates would significantly enhance rates of synthesis of resolvins and protectins beyond that which would exist in cases of an adequate intake of ALA. However, due to their role as direct acting PPARγ agonists, these PPARγ-mediated anti-inflammatory effects would be in addition to their normal resolution effects mediated through the regulated synthesis of resolvins and protections. If this is the case, then increasing EPA/DHA supply beyond that obtained from adequate ALA consumption could produce enhanced anti-inflammatory effects. From an array of studies, there appear to be consistent protective effects of consuming EPA and DHA in the 250–500 mg/day range without consistently observing (systemic) anti-inflammatory effects while at higher intakes in the 2–3 g/day range a reduction in systemic markers of inflammation such as C-reactive protein or IL-6 are consistently observed.[553–559] In addition, PPARγ agonists are known to inhibit platelet aggregation, and increased blood-clotting times are observed only at the higher doses of EPA and DHA.[560–563] These general observations would be consistent with such a concept; consuming lower amounts of EPA/DHA essentially corrects an inadequate consumption of ALA while consuming higher amounts produces an additional pharmacological effect through direct activation of PPARγ receptors.

From the preceding discussion, it is clear that consuming mixtures of different phytochemicals that are obtained from a wide variety of fruits, vegetables, and EVOO as well as EPA and DHA from fish oils can reduce proinflammatory signaling by attenuating the synthesis of both proinflammatory cytokines and eicosanoids. These effects are mediated through dampening the activation of the p38-MAPK, ERK-MAPK, and NFκβ pathways, inhibiting both COX and LOX activities and activating PPARγ receptors. The fact that only partial inhibition of these pathways is achieved from these dietary components is very important. Complete blocking of these pathways would prevent any inflammatory responses from happening and that obviously could ultimately result in severe damage and death. By dampening the inflammatory responses, the essential inflammatory responses are preserved while at the same time the risks for excessive or chronic responses are reduced. These

anti-inflammatory effects also appear to occur with a total consumption of phenolics that is well above 1 g/day, although an exact threshold for this effect is unknown. Even without the knowledge of an exact threshold level, it is clear that consuming effective amounts of phytochemicals within the context of a predominantly vegetarian diet is not only practical but also common to some traditional cultures. In addition to their effects on inflammatory signaling, mixtures of phytochemicals also can have transient antioxidant effects that might contribute to reducing ROS-associated risks for mutations. And as with the dampening effects on inflammatory signaling, the transient nature of the antioxidant effects also may be a desirable characteristic. As described in Section 6.3.1, antioxidant supplements (even in typical OTC doses) that appear to produce a prolonged excessive antioxidant effect can have detrimental effects, presumably through interfering with the normal regulation of redox control and resulting in detrimental effects on activities of the various signal transduction pathways. For optimal cellular antioxidant function, it may be more desirable to enhance activities of the various antioxidant and redox enzymes to maintain appropriate regulatory control while at the same time increasing the ability to deal with higher levels of ROS and other oxidants.

6.3.3 Antioxidant and Redox Control

Enhancing antioxidant enzyme activity should provide two benefits in regard to reducing risk for cancer. The first is through reducing the amount of ROS-mediated damage within cells. As described in Section 6.2.5, a variety of RNS and ROS (whether from intracellular or extracellular sources) can cause damage to DNA, cellular phospholipids, and proteins increasing risk for the production of an array of mutations in subsequent generations of proliferating cells. In addition to the mutagenic potential of ROS and the products of ROS reactions, they also can modify activity of various signal transduction pathways. Chronic activation of these pathways, especially the various MAPKs, by excessive levels of ROS can lead to enhanced proliferative responses. This occurs directly in proliferating cells and indirectly through activating proinflammatory signaling, which then enhances proliferative responses. Activated inflammatory cells also produce ROS and RNS themselves, which in turn add to the potential for damage and detrimental signaling. The integration of the effects of ROS/RNS-mediated damage and stress responses in proliferating cells, with the same effects resulting from proinflammatory signaling, would be especially important in the tumor environment. And because of the known detrimental effects of supplementing otherwise healthy (and presumably nutritionally replete) athletes with antioxidants,[466,564–567] enhancing expression of antioxidant enzymes and redox-control enzymes may be the more prudent approach to reducing ROS-mediated risks for cancer. Increasing expression of antioxidant enzymes would enhance the ability to reduce damage caused by excessive entry or production of ROS/RNS that may occur as a result of inflammation or transient exposures to metabolic or chemical stress. By enhancing expression of redox-control enzymes, overactivation of signal transduction pathways by oxidants also may be minimized.

As discussed earlier in Section 6.2.6, the redox sensor proteins Trx and Prx are integral components of the redox sensing system, which are important regulators of

activity of various enzymes throughout the cell, including those of signal transduction pathways. As described, the inhibitory complex formed between Trx and ASK1 disassociates on oxidation of Trx, leading to the activation of various MAPK kinases (MAPKKs) by ASK1 and ultimately to that of p38 and JNK.[450,452,458,459,568] The consequences of JNK/p38 activation on cell proliferation and inflammatory signaling activities have already been discussed. By increasing the activity of the various antioxidant enzymes and antioxidant compounds in a cell, excessive or inappropriate activation of proliferative or inflammatory signaling might be minimized.

Trx also mediates the activation of Ref-1 through its reduction in the nucleus, which also may be important in terms of risk for tumorigenesis. Ref-1 was first recognized as being the mammalian AP-endonuclease (base excision repair of apurinic and apyrimidinic sites from ROS damage) and subsequently as a direct activator of the Jus/Fos heterodimer AP-1, HIF-1, and p53 and as an enhancer for p21 expression.[461,462,569] These latter two activities of Ref-1 are indicative of one possible mechanism for reducing risk for tumorigenesis from ROS and ROS-mediated DNA damage. As discussed in Section 6.2.3, activation of ATM, DPK, and ATR by the DNA-damage sensor proteins initiates a series of events that lead to cell cycle arrest and the activation of p53 and DNA repair. Through the reduction of Ref-1 by Trx, these same events will occur, increased DNA repair by Ref-1 and increased cell cycle arrest through enhanced p53 activation and p21 expression. This leads to the concept that increasing content of reduced Trx also may increase the amount of reduced Ref-1 and thereby reduce risk for tumorigenesis by increasing DNA repair and increasing the time available in the cell cycle for repair. Sensitivity to apoptosis also may be enhanced through the increased activation of p53, another mechanism through which risk for tumorigenesis may be reduced. While these functions may have possible preventive effects, greatly enhanced Trx content has been observed in several cancer cell lines and many cancers, and high levels of Trx are associated with high risk for metastatic progression of cancers.[462] This association is likely due in part to the activation of HIF-1 by Ref-1. HIF-1 is a transcription factor that initiates expression of a variety of proteins necessary for maintaining cellular metabolism in a hypoxic environment (PDK, GLUT1/3), for angiogenesis (VEGF, IGF, and TGF), and for enhanced protein synthesis and growth (AP-1). The possibility exists, however, that these effects that comprise some of the hallmark functions of a tumor result from an array of driver mutations that lead to in part a greatly increased expression of Trx. This would not necessarily preclude the preventive role that an enhanced expression of Trx might play. When Trx expression is induced, along with that of an array of other protective proteins that function as an integrated system of redox sensors and antioxidants, the total effect should be protective, as opposed to the risk-enhancing effects of a greatly increased Trx content alone. If indeed this is the case, it indicates the potential difficulty in making global conclusions of risk on the basis of the functions of a single protein in transformed cells.

Trx functions within a complex system of cellular redox enzymes that includes Trx, TrxR, GSH, GSH, GPX, and Prx with the antioxidant enzymes SOD and CAT also being important in regulating the overall cellular redox state.[450,452,462,570–573] GPX utilizes two GSH as a source of electrons to reduce H_2O_2 to water while GSR reduces

the resulting GSSG back to two GSH using NADPH as a source of electrons. The antioxidant activities of both Trx and Prx work in a similar manner with the SH groups of these peroxidases providing the electrons to reduce the H_2O_2 to water. To regain their reduced state, TrxR reduces Trx using electrons from NADPH while (reduced) Trx can directly reduce Prx. Similar to Trx, Prx also forms dimers with a variety of proteins to form inhibitory complexes, including c-Myc, macrophage migration inhibitory factor (inhibits migration of macrophages away from areas of inflammation, a necessary component of the resolution phase of inflammation), and p66 in mitochondria. The involvement of c-Myc in enhanced Cdk synthesis for entering cell cycle was mentioned in Section 6.2.2, and p66 is involved in promoting mitochondria-mediated apoptosis following its phosphorylation by JNK. Prx also can bind with ASK1 to attenuate activation of p38/JNK-MAPK in a manner similar to Trx. While Prx is involved in many other signaling pathways, it is evident that at least in the case of p66, Prx can attenuate signaling that leads to apoptosis. An overabundance of Trx that might maintain Prx in a reduced state may ensure a sustained antiapoptotic effect that might lead to increased risk for cancer. An overabundance of Prx might do the same, and as discussed in the most recent reviews,[427,535,538] an overexpression of either one alone significantly enhances a variety of functions that increase risk for cancer, including inhibited mitochondria-mediated apoptosis. The various functions that lead to enhanced risk will not be discussed here because the point of this is to relate that the uncoordinated increase in expression of either Trx or Prx can lead to dysregulation of cellular functions that then increase risk for cancer, and an increased expression of both (individually) has been observed in many cancers. On the other hand, a coordinated increase in both might ensure that a balanced regulatory function of these redox sensors can be maintained, while at the same time an increase in antioxidant protection would be realized. And, such a coordinated induction is possible because Trx and Prx as well as mitochondrial SOD (Sod2), CAT, GSR, glutamate cysteine ligase (rate limiting for GSH synthesis), GST, heme oxygenase-1 (HO-1), UDP-GT, and NADPH:quinone oxidoreductase-1 (NQO1) are all induced through binding of the Nrf2:Mah heterodimer transcription factor to the antioxidant response element (ARE) (aka: electrophilic response element [ERE]) of their promoter regions.[411,531,574,575]

The Nrf2:Keap1 complex is a cytosolic sensor protein in the cytosol that responds to a wide array of oxidants and electrophilic compounds. Phosphorylation of Nrf2 by protein kinases, oxidation of SH groups by H_2O_2 or other oxidants, and covalent modification of SH groups through a variety of mechanisms can disassociate the Nrf2:Keap1 complex and lead to binding of Nrf2 to the ARE transactivation binding sites.[531,576–578] The ability for H_2O_2 to disassociate the complex is a major factor in how a transient increase in ROS can lead to enhanced antioxidant control through the induction of the various antioxidant enzymes and redox sensor proteins. And a wide array of phytochemicals can contribute to all three mechanisms of Nrf2 activation, revealing an important component of dietary-based prevention.

Expression of GSH, GSR, HO-1, Sod2, CAT, GCL, Trx, Prx, and UDPGT are all induced by a variety of phytochemicals including quercetin, resveratrol, diallyl sulfide, s-allyl cysteine, lycopene, EGCG, curcumin, and sulforaphane.[408,433,579–590] Although the step-by-step mechanisms through which each of the phytochemicals

work to activate Nrf2:ARE binding have not been completely worked out, from several recent reviews there is good evidence for several possibilities.[411,530,576,578,591] The hydroxyl groups on the catechol polyphenols (including flavonoids and phenolic acids such as EGCG, quercetin, catechin, cyanidin, and caffeic acid) are susceptible to autoxidation, which leads to the formation of a quinone. The quinone can then react with and bind to susceptible SH groups of the Keap1 to disassociate the complex. Other phytochemicals that have a Michael acceptor center such the α,β-unsaturated ketone of curcumin and the caffeic acid phenethyl ester, or an isothiocyanate group such as phenethyl isothiocyanate, benzyl isothiocyanate, and sulforaphane also are known to form adducts with Keap1 because of the electrophilic nature of these reactive centers. The formation of quinones through autoxidation of the catechol polyphenols also can lead to the formation of H_2O_2 because they participate in a redox resonance reaction where the quinone cycles between being reduced and then oxidized again, producing H_2O_2 at each redox cycle. The hydrogen peroxide generated by this process could directly oxidize the SH groups to force the disassociation. Another possibility would involve the variety of protein kinases that are known to phosphorylate Nrf2, including p38-MAPK, JNK-MAPK, ERK-MAPK, and PKC. Oxidation of the redox-sensor Trx by H_2O_2 leads to the disassociation of the Trx:ASK1 complex and ASK1 can then phosphorylate MEKKs, which subsequently activate p38- and JNK-MAPKs. Generation of H_2O_2 also is associated with activating a variety of forms of PKC through phosphorylation of tyrosine, again, indicating the possibility that redox cycling of catechol polyphenols can contribute to phosphorylation of Nrf2. PKC also can contribute to this process by phosphorylating Raf, which subsequently activates MEK1/2 and then ERK-MAPK.

From a variety of studies reviewed,[392,543] an array of phytochemicals activate Nrf2 through the different mechanisms: resveratrol (grapes and other fruits) through Nrf2 phosphorylation; capsaicin (hot peppers) through Nrf2 phosphorylation following PI3K/AKT activation; diallyl sulfide (garlic) through Nrf2 phosphorylation by p38-MAPK and ERK-MAPK; EGCG (green tea) through Nrf2 phosphorylation by AKT, ERK-MAPK, and p38-MAPK, as well as direct oxidation by H_2O_2, and through covalent modification of Keap1; curcumin (turmeric) through phosphorylation by PKC, p38-MAPK, and subsequent to activation of PI3K; isothiocyanates (cruciferous vegetables) through phosphorylation by ERK-MAPK, p38-MAPK, AKT, and by covalent modification Keap1; and caffeic acid (coffee, tea, and wine) through covalent modification of Keap1. From these examples, Nrf2 is commonly activated through phosphorylation by a variety of redox-sensitive kinases and covalent modification of Keap1, presumably through a quinone intermediate, also occurs relatively frequently. These mechanisms reveal that rather than antioxidant properties, it is much more likely that it is the prooxidant and electrophilic properties of phytochemicals that confer their protective effect. Because Nrf2 activation leads to an increased expression of all of the major classes of antioxidant and redox enzymes, diets that include an array of these compounds should enhance both redox control of signal transduction pathways as well as increase protection from (temporary) exposures to large amounts of ROS and RNS as might occur during an active infection or exposures to exogenous-derived reactive compounds. These protective functions may, in part, explain the strong associations between diets that contain relative high amounts of

various phytochemicals and a reduced risk for breast, prostate, and colorectal cancers described in Section 6.1.1 as well as for lung, renal, and GI cancers.[501,504,592–594]

In Section 6.3.2, the ability of various phytochemicals to suppress proinflammatory signaling through attenuating the various MAPK pathways was described. These effects are, however, seemingly directly contradictory to the enhanced activities of various MAPKs by the same phytochemicals for Nrf2 activation. Rather than being a conundrum, these opposing results are expected due to the timing of the events and the specific effects that are measured. The activation processes reflect the rates of the chemical reactions necessary for the events to occur. These would include the autoxidation of the hydroxyls and subsequent generation of H_2O_2 (followed by oxidation of susceptible –SH groups) or the various Michael reactions that also can occur, all of which would be relatively short-term phenomena considering the half-life of the phytochemicals. From the data on flavonoids, the bioavailability of these compounds is in the 2%–20% range, which results in biological concentrations in the 250 nM to 30 μM range.[463,538,595,596] With a half-life of from 1 to 2 hours, the biological effects of these phytochemicals following ingestion are sure to be transient; possibly a moderate effect that declines quickly over a couple hours (or less). As a guess, considering typical eating patterns and the amounts eaten, the activation effects of phytochemicals might last for an hour or so, possibly once or twice a day (well, on days they are actually consumed). Thus, the activation time of the various MAPK pathways and Nrf2 would be relatively transient. The half-lives of the proteins expressed are much longer; MnSOD has a half-life of approximately 5 hours while that of CAT, GPX, and Trx are in the 24–48-hour range.[597–600] Ultimately, it is the transient activation of intracellular signaling that produces a longer-term attenuation of the signaling processes through increased expression of the antioxidant and redox enzymes. The net effect is to dampen the average or chronic signaling processes that can lead to an enhanced risk for tumorigenesis while still allowing for enhanced signaling due to short-term cellular stresses. In these situations, regular consumption of these phytochemicals would dampen the overall total daily signaling activity and result in a protective effect.

6.3.3.1 Cell Cycle

Because cell progression into and through the cell cycle is dependent on activity of the cellular signaling pathways as well as the redox state of the cell, a phytochemical-induced increase in redox control coupled with dampened activity of the signaling pathways would then alter the progression of stem and PCs through the cell cycle. From a number of reviews on the subject, a variety of cell signaling functions in cancer cells have been observed to be altered by a wide variety of phytochemicals, changes that can affect different aspects of the progression phase of carcinogenesis.[500,578,601–606] For example, benyl isothionate (BITC), phenethyl isothionate (PEITC), curcumin, quercetin, resveratrol, theaflavins, genistein, sulforaphane, apigenin, and EGCG (among others) have all been documented to enhance rates of apoptosis in cancer cells. Mechanisms of this effect have been associated, in part, with increased caspase activation (isothiocyanates, sulforaphane, and apigenin), decreases in cyclin D1 and cdk (curcumin and resveratrol), and inhibition of NFκβ (BITC, resveratrol, genistein, theaflavins, EGCG, and curcumin). Inhibiting

NFκβ is an important mechanism not only because it attenuates apoptosis (in part through inducing Bcl proteins) but also because it is a component of the activation pathways for proinflammatory signaling.

Cell cycle arrest and suppression of growth also have been observed with many phytochemicals, including BITC, luteolin, resveratrol, apigenin, genistein, EGCG, curcumin and quercetin.[601–605,607] These effects have been observed to be associated with suppression of ERK-MAPK and JNK-MAPK activities, reduction in activation of Ras, Myc, Jun, and/or Fos (curcumin, EGCG, quercetin, genistein, and resveratrol), and reductions in expression of p38, Cdc2, and Fos while increased expression of p21 also has been observed. And each of these growth suppression and enhanced apoptosis effects from cell culture studies has been associated with phytochemical-mediated reductions in chemical-induced tumorigenesis in a variety of animal studies.

From the standpoint of redox control, nutrient-based dietary supplements have had little effect other than correcting various nutritional deficiencies while supplements that contain individual phytochemicals have their own limitations due to issues of toxicity. In addition, the majority of the mechanistic studies from the preceding discussion are based on treating transformed cells (or animals with chemical-induced or transplanted tumors) with individual phytochemicals in amounts that are unlikely to be obtained from a diet. This raises some issues as to how well these studies apply to diet-based prevention in humans, before they have transformed cells (i.e., cancer) and because human diets actually contain a wide array of different phytochemicals (in differing and lower amounts), each with their own array of (often overlapping) effects. The value of these studies is not that they reveal how any single phytochemical can prevent cancer through dietary modification or supplementation but rather how a variety of phytochemicals through an array of relatively subtle effects on many different mechanisms of cellular function may integrate into an overall protective effect. And considering the integrated risks of proinflammatory signaling and prooxidant stresses within the tumor environment, the suppression of inflammatory signaling as well as the enhanced antioxidant capacity to optimize control of the cellular redox state could have profound overall risk-reduction effects.

6.3.3.2 DNA Damage and Repair

As described, the enhanced antioxidant capacity through induction of SOD, CAT, GPX, Trx, and Prx that is mediated through Nrf2 activation will reduce the levels of ROS in the cell and enhance redox control of signal transduction pathways. The ability of ROS and RNS to cause mutations directly through DNA damage and indirectly through lipid peroxidation–induced DNA damage also will be reduced. Reductions in DNA damage in peripheral lymphocytes in humans and various tissues in animals have been observed where diets have been modified by increasing consumption of fruit juice, fruit and/or vegetables, and whole berry extracts.[608–612] In many of these studies, whether the reductions were due to antioxidant effects of the dietary compounds or induction of the various antioxidant enzymes is difficult to tell. Based on the ability of various phenolics to activate Nrf2 responses through a variety of mechanisms, it is, however, reasonable to infer that a Nrf2-mediated induction of antioxidant and redox-control enzymes is at least partially responsible for the

reduction in oxidative DNA damage. Enhanced DNA repair also could explain some of the observed reduction in oxidative DNA damage.

As described in Section 6.3.3, one of the effects of phytochemicals is the transient production of H_2O_2 through quinone/semiquinone cycling. This may lead to a transient oxidation of Trx and the subsequent activation of Ref-1. Because this endonuclease is a functional component of base excision repair (BER), its transient (and moderate) activation by Trx oxidation may be an important factor in enhancing DNA repair. This increase, coupled with a Ref-1-mediated activation of p53 and expression of p21, would help explain the enhanced apoptosis and cell cycle arrest observed for a variety of phytochemicals as well. Selenium has been observed to enhance BER in human fibroblasts in association with enhanced REF-1 activation while β-cryptoxanthin (a carotenoid) as well as ascorbate plus α-tocopherol appear to enhance DNA repair and reduce markers of DNA damage in HeLa cells and Ref-1 heterozygous mice, respectively.[613–615] And interestingly, silibinin (from milk thistle) enhances p53 stabilization as well as upregulates GADD45 following UVB radiation of mouse epidermal cells. Because GADD45 is a downstream target of p53 (as well as being implicated in DNA repair and cell cycle arrest), each of these results is consistent with a role for regulation of Ref-1 activity by Trx in enhancing DNA repair.

In studies where regulatory mechanisms of DNA repair have not been determined, there are relatively consistent results that various phytochemicals including curcumin, indole-3-carbinol, resveratrol, ellagic acid, red raspberry extract, and quercetin can enhance rates of DNA repair.[609,616–618] In human dietary studies, flavonoid-rich diets (multiple fruits and vegetables) or addition of kiwi to the diet significantly enhanced DNA repair with enhanced expression of DNA repair enzymes being observed in some, although not in all.[619–621] In some instances, the enhanced DNA repair was related to nutritional deficits being corrected rather than to a direct effect of the phytochemicals per se, again indicating the difficulty of interpreting human studies where nutritional insufficiencies are common. In one particular study using human cells exposed to 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (a heterocyclic amine found in grilled meats), a reduction in DNA adducts was observed following cotreatment with sulforaphane and quercetin; however, this effect was associated with a sulforaphane-mediated increase in UDP-GT and GST enzymes and a quercetin-mediated reduction in CYP1A2 activity.[622] The results of this last study indicate other mechanisms through which dietary phytochemicals can modify risk for carcinogenesis, inhibition of phase I activity that leads to the production of DNA-damaging electrophiles and induction of phase II activities that lead to the inactivation of them.

6.3.3.3 Phase I and Phase II Enzymes

As discussed in Section 6.2.5.2, the activation of various PAHs, nitrosamines, heterocyclic amines, and other xenobiotics to electrophilic intermediates by phase I enzymes such as CYP1A1, CYP1A2, CYP2A1, CYP2B1, and CYP2E1 are responsible for the actual production of the carcinogens from these parent compounds. And it is the phase II enzymes GST, UDP-GT, and NQO1 that are responsible in part for conjugating hydrophilic compounds to the electrophiles in order that they can be inactivated and easily excreted. Phase II enzymes also can be induced by binding of

Nrf2 to the ARE/ERE response element of their genes, an event activated by a wide array of phytochemicals.

In a variety of studies, a consistent induction of GST, UDP-GT, and/or NQO1 has been observed following consumption of curcumin, benzyl isothiocyanate, coumarin, indole-3-carbinol, sulforaphane, or infusion of cruciferous extracts (isothiocyanate mixtures).[622–627] Similar results are observed in cell cultures following treatment with a wide variety of phytochemicals including EGCG, sulforaphane, quercetin, catechin, indole-3-carbinol, phenethyl isothiocyanate, organosulfur compounds from garlic, and coumarins.[628–632] In addition, chemically induced lung, liver, mammary, esophageal, and colon cancers also are significantly reduced with EGCG, N-acetylcysteine, quercetin, genistein, multiple types of berries, curcumin, resveratrol, tea polyphenols, and indole-3-carbinol in a variety of animal models.[143,626,633–640] The reduction in risk in many these studies is associated with increased inactivation of the reactive carcinogens. While certainly not an exhaustive list, phytochemicals are capable of inducing phase II enzyme activity and reducing incidence and multiplicity of tumors that are caused by specific carcinogens. The application of each individual model to the general population, however, is a little less direct because relatively high doses of individual carcinogens that are unlikely to be seen in humans are used, as is the amount of specific phytochemical. The basic message, though, is that different phytochemicals can reduce risk for a variety of chemical-induced cancers through inducing expression of these phase II enzymes as observed in a variety of animal models and because humans are exposed to a wide array of possible carcinogens (at much lower doses) consuming a variety of phytochemicals should produce a general protective effect against all (most?).

In addition to reducing risk by inactivating active carcinogens, inhibiting cytochrome P450 activities also may contribute to risk reduction in some cases. In a wide variety of studies, activation of many different procarcinogens to carcinogens by CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2E1, and CYP3A4 activities (as well those of other activating CYPs) is inhibited to differing degrees by quercetin, EGCG, curcumin, phenethyl isothiocyanate, benzyl isothiocyanate, and sulforaphane.[601,641–646] On the other hand, some phytochemicals also have been observed to enhance expression of several CYP enzymes. Indole-3-carbinol, quercetin, flavone, tangeretin, and phenethyl isothiocyanate, for example, induce expression of CYP1A1 and CYP1A2 in a variety of tissues including the intestines, lung, and liver while St. John's Wort is a very effective inducer of CYP3A4 and CYP2B6.[601,647–650] Some phytochemicals also can induce activity of certain CYPs while inhibiting others. Diallyl disulfide from garlic, for example, can increase CYP2B1 activities while inhibiting CYP2E1, and the anthraquinone emodin can induce CYP1A1 while at the same time inhibiting its activity. Because the absolute activities of individual CYP enzymes are difficult to associate with specific risks for individual cancers and because of the different effects that various phytochemicals can have on CYP activities, it is even more difficult to determine how risks for cancer may be modified through such a mechanism. Because of the consistent induction of phase II enzymes by a variety of phytochemicals and the consistent association with a reduction in risk for cancer by such changes, it is far more likely that the risk reduction benefits of a high-phytochemical diet are conferred through this mechanism rather than through

inhibiting phase I enzymes. This brings us to the last dietary component that consistently reduces risk for cancer through enhancing antioxidant and redox-control enzymes and the phase II enzymes—caloric restriction.

6.3.4 Calorie Restriction as Prevention

Of all the dietary modifications, calorie restriction (CR) while maintaining appropriate nutrient intake appears to be the most consistent means to reduce risk for cancer.[651–654] In animal models, calories are typically restricted to approximately 60%–80% of what an ad libitum-fed animal might consume with mechanisms of risk reduction including reduced insulin levels, increased insulin sensitivity, reduced ROS/RNS production, enhanced antioxidant and redox control enzyme activities, and reductions in circulating proinflammatory cytokines.[652,655,656] In addition to these, circulating IGF-1 is reduced while that of adiponectin is increased. These last two would be expected to occur because of the reduced calorie intake and the resulting reduction in adiposity. In response to increases in serum glucose following eating (and overeating), the insulin that is released not only activates glucose transport into cells through translocation of GLUT4 transporters to the cell membrane but it also stimulates synthesis of IGF-1 by the liver; resulting in increased circulating IGF-1. Because of the reduction in calories with CR, glycemic loads are reduced, along with both insulin and IGF-1. Adiponectin is inversely associated with adiposity and as logic would dictate with continuing CR, adiposity is reduced, and circulating adiponectin increases concomitantly.

The risk-reduction benefits of reduced IGF-1 and increased adiponectin are somewhat interrelated because of their common effects on mTOR. mTOR phosphorylates p70S6K and it is activated by Akt to promote both cell growth and proliferation. The PI3K/Akt pathway is activated by insulin and IGF-1 as well as by other growth factors and by increased nutrient supply. The subsequent proliferative and growth effects of activated mTOR are factors in obesity-related risks for cancer as well as being commonly seen in tumors.[224,657] Under conditions of CR, the PI3K-mediated activation of Akt is suppressed, resulting in decreased mTOR activity. In addition, the lower-energy environment in cells produced by CR increases AMP levels in the cell, enhancing AMP-dependent protein kinase (AMPK) activity, which directly inhibits mTOR.[658] The increased levels of adiponectin that also occur with CR not only enhance AMPK activity to mediate a decrease in mTOR activity but they also enhance expression of PGC-1α.[659] Activation of PGC-1α (described in detail in Chapter 4) is associated with increased insulin sensitivity by acting as a coactivator of GLUT4 synthesis as well as possibly being involved in enhancing expression of insulin receptor substrate-1 (IRS1), providing an additional suppressing effect on circulating insulin by enhancing insulin sensitivity, which in turn lowers IGF-1 even further. Thus, the growth and proliferative suppressing effects of CR appear to be mediated in part through a coordinated increase in AMPK activity that inhibits mTOR as well as a decline in insulin and IGF-1-mediated activation of the PI3K/Akt pathway, further reducing mTOR activity.

As mentioned earlier in this section, a reduced production of ROS and RNS with enhanced redox control also occurs with CR. These responses are consistent with

those mediated by Nrf2 binding to the ARE/ERE response element and indeed CR is associated with activation of this important regulator.[656,660,661] As one might expect with activation of Nrf2, activities of phase II enzymes also are induced by CR and are considered to be a major reason for decreased DNA damage and tumorigenesis in carcinogen-exposed, CR animals.[651,660,662–665]

CR thus appears to be an effective treatment for reducing risk for cancer on the basis of cellular mechanisms that are altered in a variety of animal CR models. Data on risk reduction by CR in humans is in extremely short supply although there are a few studies documenting reduced risk in populations with traditionally low (or forced) caloric intake, and in CR trials, similar alterations in insulin, IGF-1, adiponectin, and in metabolic indices occur. Unfortunately, such severe reductions in calorie intake are very difficult for the majority of humans. And while reductions in IGF-1, enhanced insulin sensitivity, and enhanced phase II activities occur in humans under conditions of CR, the altered IGF-1 levels appear to be associated in part with reduced protein intake. Elevated IGF-1 levels are observed with protein intakes that exceed the 0.8 g/kg recommendation while diets that restrict protein intake to this level from customary higher intakes, even without CR, are associated with lowered IGF-1 levels.[666–668] With a median intake of ~91 g/day for adults between 19 and 50 years, 1.4 and 1.2 g/kg/day for American males and females (19–50 years) respectively,[669] protein intake in sedentary people might be considered an independent risk for cancer through elevating IGF-1 and a factor to be considered for non-CR recommendations for prevention.

6.3.5 Exercise

Physical activity has been recognized as an important lifestyle issue in risk for cancer for many years and it is a key component of risk-reduction recommendations of many organizations.[95,97,155,670] Physical activity is widely documented in epidemiology studies to be inversely associated with risk for all cancers. In cases where individual cancers are studied, however, the inverse association is relatively modest or inconsistent for some (e.g., prostate, lung, and ovarian cancers) while there is a consistent and strongly protective association for colon, breast, and endometrial cancers.[46,671] From the standpoint of how exercise affects cellular functions, however, one might expect a more consistent as well as a stronger inverse association. Perhaps, some of the limitations inherent in the epidemiology research are in part responsible for this. Diet and physical activity are notoriously difficult to accurately assess in such studies and an accurate assessment of possible confounding nutritional, body composition, and environmental variables are essentially unfeasible.[182,672] Also, with an approximate 33% prevalence of complete inactivity coupled with an additional ~32% prevalence of physical activity that does not meet the minimal requirements for preventive effects, we would conjecture that there simply may be too few subjects who actually exercise at enough different levels of intensity to observe a statistically significant inverse association in all but the largest epidemiological samples. In addition, a pervasive lack of a nutritionally adequate diet for a large majority of Americans may be a serious confounding factor that prevents some of the positive effects of exercise to be observed. This last issue, coupled with the long latency

period for the development of cancer, also would make it very difficult to assess the ability of physical activity to reduce risk for cancer in all but the longest duration clinical trials as well. As discussed in Chapter 1, it is precisely these limitations that form the basis for developing a focus on using various biomarkers of risk in the context of properly designed clinical trials in this area of research.[182]

When it comes to specific mechanisms of cancer etiology that are affected by exercise, the array of cellular functions that are modified are very similar to those described for dietary components. While not typically determined in cancer-associated studies, these mechanisms have been extensively studied in a wide variety of exercise-specific experiments and are highly relevant to cancer.

6.3.5.1 Exercise and Insulin Sensitivity

As discussed in Section 6.2.4.1, elevated insulin enhances risk for cancer through enhancing growth and proliferative signaling within the tumor environment through direct activation of PI3K activities and indirectly through insulin-mediated increases in hepatic expression of IGF-1. Increased glucose levels lead to enhanced cellular damage through the formation of Amadori products and to DNA damage from enhanced lipid peroxidation as well as increased proinflammatory signaling through activation of RAGE. Enhancing insulin sensitivity would therefore reduce risk for cancer through two principal mechanisms: reducing circulating levels of insulin and reducing circulating levels glucose. The ability of physical activities to enhance insulin sensitivity and reduce circulating levels of both insulin and glucose has been discussed in detail in Chapters 3 and 4 and will be summarized here.

A major component of the exercise-insulin issue is that inactivity can cause insulin resistance in skeletal muscle, a component of risk for approximately 33% of Americans who report that they are completely inactive. An enhanced local production of ROS due to inactivity mediates an induction of TNFα synthesis that in turn enhances lysine phosphorylation of IRS1. This enhances its degradation as well as impairs its ability to complex with p85, thus reducing insulin sensitivity.[165–167,673–678] Fortunately, insulin sensitivity can return to normal relatively quickly with exercise.

The ability of both endurance training and resistance training to enhance insulin sensitivity has been well documented, as has a transient increase (~24 hours) in insulin sensitivity following acute exercise.[679–688] Interestingly, insulin sensitivity is enhanced to a greater degree at higher intensity activities when the exercise is performed 3 days/week, with even greater insulin sensitivity observed when weight training is combined with endurance exercise, although little to no association with increasing intensity is observed with daily exercise. Repeated exercise training is known to enhance GLUT4 and IRS synthesis.[689–694] These increases are likely initiated in part through phosphorylation or deacetylation of PGC-1α and phosphorylation of estrogen receptors following an exercise-mediated activation of MAPKs, AMPK, and SIRT1. Increased expression of both GLUT4 and IRS has the effect of increasing the activation of glucose transport at a given level of insulin. Acute exercise also is known to increase insulin sensitivity for as long as 17 hours following the activity, with this effect also mediated in part by AMPK.[695–698] The Rab-GTPase:AS160 is normally inhibited following phosphorylation by Akt (insulin → IR/IRS → PI3K → PI3,4,5-TP:PDK1 → Akt) to allow Rab-GTP-mediated

translocation of GLUT4 proteins to the cell membrane. This Rab-GTPase as well as TBC1D1 also can be phosphorylated by AMPK, leading to insulin-independent glucose transport. In addition to AMPK-mediated effects, muscle contraction also appears to activate Akt. Thus, through the transient inhibition of Rab-GTPases and the increased expression of GLUT4 and IRS proteins, repeated exercise can enhance insulin sensitivity. With daily exercise, one would expect the prolonged acute (and transient) increase in insulin sensitivity through AS160/TBC1D1 phosphorylation (the "last bout effect") to be additive to the increase due to an increased expression of GLUT4 and IRS (the "repeated bout effect") to maximize sensitivity while with alternate-day activity the more transient last bout effect would have dissipated by the second day to reduce the effective daily average level of insulin sensitivity. This average, however, appears to be enhanced by more strenuous alternate-day activities as observed in some studies. Whether a slightly greater average insulin sensitivity that may occur as a result of more strenuous alternate-day activities results in significantly lower risk for cancer compared to less strenuous alternate-day activities is unknown. Based on the kinetics of the formation of Amadori products and their contribution to damage and proinflammatory signaling, however (as discussed in Chapter 4), it is doubtful there may be a significant difference.

Because skeletal muscle is the largest organ that is responsive to insulin and is an important contributor to glucose homeostasis through its removal, alterations in skeletal muscle sensitivity to insulin produce significant changes in risk for cancer. Importantly, most kinds of physical activity enhance insulin sensitivity in skeletal muscle and also are associated with a reduction in risk for cancer, with cellular mechanisms that are directly associated with tumorigenesis being reduced through enhanced insulin sensitivity. Thus, to contribute to a reduction in risk for cancer through maintaining "optimal" insulin sensitivity, moderately stressful exercise activities of either strength training or endurance training should be performed at a minimum of every other day.

6.3.5.2 Exercise, Redox Control, and Phase II Enzymes

Exercise training is commonly known to improve athletic performance in part by enhancing metabolic function of skeletal muscle through increased biogenesis of mitochondria. And it is the transient increase in production of the cellular oxidant H_2O_2 due to the high metabolic demands of the exercise that is responsible in part for many of the metabolic adaptations to exercise.[168,564,699–703] These adaptations not only include mitochondrial biogenesis, but in several of the more recent studies, enhanced activities or expression of one or more of the redox control enzymes (GSR, GPX, Trx, and Prx) and/or of the phase II enzymes (UDP-GT, GST, and NQO1) have been observed in a variety of tissues.[173,704–710] The ability of acute endurance-type exercise to activate Nrf2:ARE binding also has been observed and this would be expected because Nrf2 is a transcription factor that is known to be associated directly with mitochondrial biogenesis, as is PGC-1α, MEF2, and Tfam (among others).[710–719] The importance of Nrf2 binding to ARE/ERE promoter sequences is that Nrf2 is a major transcription factor responsible for initiating transcription of the redox control enzymes as well as phase II enzymes as discussed in Section 6.3.3. Because it also is necessary for mitochondrial biogenesis, it is apparent that physical activities that

lead to mitochondrial biogenesis in skeletal muscle also can lead to enhanced redox control and phase II enzyme activities.

A brief summary of the activation of mitochondrial biogenesis follows.[694,710–720] To sustain muscle contractions, the metabolic production of ATP is greatly enhanced, with both the muscle contractions themselves and the enhanced activity of the various metabolic enzymes activated by increased cytosolic calcium (as well as a variety of allosteric regulators). The enhanced metabolic production of H_2O_2, which occurs concomitant with the enhanced ATP synthesis, then activates the various MAPKs, Nrf2, and NFκβ. Increased cytosolic calcium also activates PKC to further activate the MAPK pathways as well as calcineurin A (CnA) and Ca-calmodulin-dependent protein kinase (CaMK). The CnA and CaMK in turn activate the transcription factors cyclic AMP response element-binding protein (CREB) and myocyte enhancer factor-2 (MEF2) while p38-MAPK activity mediates activation of the transcription factor ATF2 as well as MEF2 and CREB. MEF2, CREB, and ATF2 then enhance expression of PGC-1α. PGC-1α then coactivates Nrf2, Nrf1, NFAT, and other factors to initiate transcription of the mitochondrial genes that are necessary in part for mitochondrial biogenesis. Posttranslational modification of PGC-1α such as phosphorylation by AMPK or deacetylation by SIRT1 also activates PGC-1α to enhance its coactivation activity and increased AMPK activity also activates SIRT1. Thus, there are essentially two "pathways" to activate mitochondrial biogenesis through exercise, enhanced MAPK, CaMK, and CnA activities through ROS and Ca^{++}-mediated mechanisms (among others) and additionally through direct activation of PGC-1α through AMPK and SIRT1. And one would expect maximal activation of both of these pathways with maximal exercise, a concept that has been tested to its extreme relatively recently where in a variety of studies extremely low-volume intervals of maximal or near-maximal intensity exercise (e.g., 5×30 seconds of maximum effort on a cycle-ergometer with 4 minutes rest between each interval performed 3 days/week) produces significant elevations in aerobic capacity, induces mitochondrial biogenesis, and enhances insulin sensitivity.[693,721–727]

The reason for this digression into mitochondrial biogenesis is to illustrate the signaling processes that mediate mitochondrial biogenesis also can enhance insulin sensitivity and activity of redox control enzymes and phase II enzymes. These responses are initiated through activating the MAPK signaling pathways, CaMK, and CnA as well as AMPK and SIRT1 activities and coordinated by PGC-1α and Nrf2, each of which can be activated directly or indirectly through physical activity–mediated release of Ca^{++} and/or enhanced production of H_2O_2 and/or elevated AMP. While the activation of PGC-1α appears to be required for both enhanced mitochondrial biogenesis and insulin sensitivity, Nrf2 activation does not appear to be essential for enhancing insulin sensitivity nor is it considered a requirement for muscle hypertrophy. In addition, muscle hypertrophy responses and mitochondrial biogenesis responses tend to be mutually exclusive.[660,728,729] One reason for this last item is that enhanced AMPK and SIRT1 activities that typically occur with longer-term endurance exercise (both) inhibit mTOR, an essential mediator of muscle hypertrophy. While this does not necessarily mean that resistance exercises cannot activate Nrf2 sufficiently to enhance expression of the redox control and phase II enzymes, no research was found to document any changes in redox-control

or phase II enzymes following resistance training. Thus, based on currently available data, it is the longer-duration moderate to high stress and shorter-duration maximal stress endurance-type exercise that appears to enhance mitochondrial function, and in some cases VO_{2max}, which likely mediates an enhanced expression of redox control and phase II enzymes and not resistance exercise alone.

Much of the molecular-based information on mitochondrial biogenesis and insulin sensitivity has been performed on muscle cells of one type or another, as has much of the research on redox control enzymes and exercise. Because sarcomas of skeletal muscle are very rare in comparison to cancers of the lung, breast, colon, liver, and prostate (and others), cancer risk reduction through enhanced Nrf2 signaling in nonmuscle tissue is an important consideration. In the relatively few studies that have investigated the induction of either the various redox-control or phase II enzyme activities, some inconsistencies in results have been observed.[173,704–710,730] In studies that used 6–8 weeks of endurance training in rats, GPX and GSR activities have been increased in heart and skeletal muscle but not in liver. In a longer-term study with beagle dogs and a similar duration study using swimming rats, enhanced hepatic GST activities have been observed with a substantial increase in the Ya_1 subunit. This is relevant because the Ya_1 subunit is associated with activation of the ARE response element to which Nrf2 binds. Eight weeks of running exercise training in rats also has been observed to enhance Trx activity in the brain and a similar duration of running exercise enhances UDP-GT activity in the lung. In these studies, the duration of the exercise periods ranged from 40 minutes/day for the beagles and from 60 minutes to 120 minutes for the rat studies at what would be termed moderately stressful intensity. Why some studies saw no change is very difficult to say and may reflect differences in strain and possibly age of the animals used. Ultimately, there simply are not enough studies performed to make any specific definitive conclusions other than to recognize that in the majority of available studies where enhanced activities of Nrf2-dependent enzymes are observed in nonmuscle tissues, it is following longer-duration moderate-intensity exercise training, a form of exercise that also has relevance for DNA repair.

6.3.5.3 DNA Repair

As discussed in Section 6.3.3.2, oxidation of Ref-1 by Trx in the nucleus enhances DNA repair by Ref-1 and increases cell cycle arrest through a Ref-1-mediated enhanced p53 activation and p21 expression. An enhanced content of Trx mediated by activation of Nrf2 would be expected to enhance DNA repair and reduce risk for tumorigenesis through this mechanism and in addition through enhanced p53/p21. As might be expected, in those few studies that investigated exercise and DNA repair, reductions in DNA damage as well as enhanced activity of DNA repair has been observed.[731–736] These effects appear to be related in part to the enhanced ROS production during acute exercise periods and occur in muscle, brain, liver, and circulating leukocytes. The DNA repair enzyme oxoguanine DNA glycosylase (OGG1) is mainly responsible for the repair of oxidative damage to guanine and is the specific enzyme identified as being induced by exercise. Interestingly, the gene for this enzyme has Nrf2-binding sites in its promoter region.[737–739] With Nrf2 activation associated with mitochondrial biogenesis as well as being necessary for induction of

OGG1, it is no surprise that the type of repeated exercise used in the referenced studies was moderately stressful endurance exercise (running or swimming) for 60–90 minutes and is suggestive that longer-term exercise might be necessary to enhance DNA repair. The implication of a duration effect (60–90 minutes) is very interesting. The IL-6 promoter contains an ARE consensus sequence that is activated by Nrf2, and IL-6 also has been implicated as a required signaling molecule for repair of hepatic mitochondrial DNA through induction of OGG1 and the prevention of ROS-induced DNA fragmentation in lung, preventive effects that do not happen in IL-6 knockout mice.[740–742] Thus, the possibility exists that IL-6 is a coactivator along with Nrf2 for augmenting the enhanced DNA repair following longer-term exercise. Thus, the form and duration of exercise that increases IL-6 production may be optimal for enhancing DNA repair while mitochondrial biogenesis, antioxidant and redox control enzyme, and phase II enzyme induction can occur with shorter duration exercise.

6.3.5.4 IL-6-Mediated Anti-Inflammatory Effects of Exercise

The ability of physical activity to reduce inflammatory signaling has been discussed in detail in Chapters 2 and 4, so it will be presented in summary here. With approximately 1 hour or more of continuous moderate-intensity exercise, serum levels of IL-6 can be elevated as much as 100-fold before diminishing to resting levels over a period of from 3 to 5 hours.[743–750] This increase in IL-6 originates from the active skeletal muscle cells during nonexhaustive whole-body exercise and has been observed with activity as low as ~6 METS; the IL-6 response does not occur with small-muscle group exercise. The nonexhaustive nature of the exercise is associated with no increase in TNFα, a response that does occur with exhaustive and/or damaging exercise. As a result of the large increase in IL-6, expression of IL-10, sIL-1ra (soluble IL-1 receptor antagonist), sIL-1r (soluble IL-1 receptor), and sTNFr (soluble TNF receptor) is induced.

The sIL-1r and sTNFr bind to circulating IL-1α, IL-1β, and TNFα while the sIL-1ra inhibits IL-1α and IL-1β from binding to their cellular receptors. The IL-10 activates the Jak-STAT pathway to activate synthesis of the SOCS proteins, which then inhibits the synthesis of IL-1α, IL-1β, IL-6, IL-8, and TNFα.[751–753] IL-10 also suppresses both MAPK and NFκβ signaling.[751–754] Thus, as a result of the large initial production of IL-6, there is a profound anti-inflammatory effect of the longer-term moderate intensity endurance-type exercise. As a result of repeated exercise of this type, one would expect a reduction in serum markers of inflammation such as C-reactive protein (CRP).

From a variety of clinical trials testing the association between repeated exercise and CRP, those trials that utilized moderate to strenuous exercise of a duration from 45 minutes to 1 hour tended to demonstrate a reduction in CRP while in those with exercise durations of 30 minutes there were no changes in CRP observed.[755–764] These results are consistent with an anti-inflammatory effect mediated by IL-6 induction from the longer duration exercise periods. They also reveal a possible reason for the longer duration activities to be associated with enhanced DNA repair.

In summary, exercise can produce a variety of effects that can reduce mechanisms that contribute to cause for a wide variety of cancers. Daily or alternate daily exercise

training of almost any form and resistance exercise training as well as most daily low to moderate physical activities (gardening, dancing, light-effort bicycle riding, golf, household chores, etc.) are associated with an increase in insulin sensitivity, which lowers circulating insulin and glucose and therefore reduces their associated promotional effects. Moderate intensity (30 or more minutes continuous; five times a week), vigorous (20 minutes or more; three times week), and high or maximum intensity (~0.5–10 minutes duration repeated approximately three to six times, three times a week) exercises that enhance mitochondrial biogenesis are associated with transiently activating MAPKs, NFκβ, and Nrf2 to enhance expression of antioxidant enzymes, redox control enzymes, and phase II enzymes and to increase DNA repair activity. While enhancing insulin sensitivity occurs with less intensive and shorter duration activities to reduce risk for cancer, the enhanced redox control, phase II, and DNA repair benefits appear to accrue only with the more intense and longer duration activities that also enhance mitochondrial biogenesis. It is only with the longer duration (~1 hour or longer) of moderate intensity activities that the significant anti-inflammatory effects and possibly the enhanced DNA repair activities mediated in part by IL-6 will occur.

6.4 SUMMARY AND RECOMMENDATIONS

Cancer is a major public health issue that causes approximately one in fourth deaths in the United States each year and costs over $125 billion each year just for care and treatment. While there are many different cancers that arise from different tissues, the general processes of carcinogenesis are remarkably similar for each.

6.4.1 Cancer Etiology

Essentially, cancer arises from tissue SCs and possibly PCs that divide and accumulate mutations in genes that code for any of a variety of proteins that are directly involved in regulating different aspects of the cell cycle or that are involved in signaling processes that in turn lead to entry into the cell cycle. The initiation stage is the process that leads to the acquisition of the first driver mutation that enhances risk for cancer. This mutation could occur when a SC divides before the complete repair of existing DNA damage or it could be inherited. Most chemical modifications to DNA bases (DNA damage) lead to the production of point mutations during the S phase of the cell cycle. If single-strand breaks are present, they can lead to the production of DSBs during the DNA-replication process and DSBs that persist into the M phase ultimately lead to a variety of chromosomal mutations. These point or chromosomal mutations are then present in the daughter cells, and if they are again stimulated to divide in the face of additional unrepaired DNA damage, then additional mutations also will be acquired in the subsequent generation of cells. While the vast majority of mutations acquired during the promotion phase are passenger mutations, the relatively few driver mutations that enhance rates of cell division, that interfere with DNA repair, or that impede checkpoint control ultimately promote the development of a tumor and its subsequent progression to malignancy over many cell division cycles.

Because regulating entry into the S phase of the cell cycle, preserving the integrity of checkpoint control, and maintaining efficient DNA repair are so important to the fidelity of DNA duplication and cell division, mutations in proteins that are involved in one or more of these processes are very common to all human cancers. Mutations in the genes that code for proteins of the signal-transduction pathways that subsequently lead to growth and cell division such as *BRAF* and *KRAS*, or genes that are directly involved in G0 → S or G1 → S transition such as *RB*, are very common in human cancers. Enabling mutations in these will enhance rates of cell division. The *TP53* gene also is very commonly mutated in almost all human cancers and appears to be an important mutation that leads to malignancy. This is most likely because P53 is vital to maintaining checkpoint control. When sensor proteins detect DNA damage, this protein is activated to enhance cell cycle arrest and prevent progression through the cell cycle until the DNA damage is repaired. P53 also induces expression of a variety of proteins that block BCL and MXL proteins from inhibiting Bax and Bak, two regulatory proteins that stimulate the release of cytochrome *c* and other apoptotic proteins from mitochondria. On release of the apoptotic proteins, caspases are activated to initiate the process of apoptosis, ultimately ending in cell death (unless the DNA is repaired in a timely manner to stop the process and continue safely through the cell cycle).

If the dividing cells acquire disabling mutations in any of the genes that code for proteins that are involved in checkpoint control or DNA repair, then the cells will likely progress through the cell cycle to produce additional mutations in subsequent generations. As the dividing mutated cells accumulate additional mutations in a variety of genes over an indeterminate number of generations, they ultimately acquire the functional characteristics of cancer cells, a phenotype with specific hallmark functions: a sustained ability to stimulate their own cell division, an inability to differentiate into normal adult tissue cells, an inability to respond to normal cellular signals that suppress cell division, an inability to respond to apoptosis signals, the ability for indefinite proliferation, an ability for invasiveness and metastasis, and an ability for stimulating angiogenesis.

Once the carcinogenesis process is initiated and SCs and PCs proliferate, a small array of mSC and PCs will accumulate within the tumor environment and develop slightly hypoxic conditions as they accumulate some distance from the existing capillaries. The slight hypoxic environment activates HIF-1 and subsequently a variety of proteins that confer a growth advantage for cells in this environment are induced, along with chemoattractant proteins that enhance the infiltration of the local region with activated macrophages, lymphocytes, NK cells, mast cells, endothelial cells, platelets, and fibroblasts. Thus, the developing tumor environment is really a heterogeneous mixture of mSCs, mPCs, and tumor-associated cells that include a large number of inflammatory cells and platelets. The activated inflammatory cells are intimately involved in the promotion and progression phase. Activated inflammatory cells release a variety of growth and proliferative signaling molecules including PGE_2, IL-1β, TNFα, PDGF, TGFβ, EGF, bFGF, RANTES, platelet factor 4, IL-8, and IL-6 (among others). The wide array of inflammatory signaling molecules released from the activated proinflammatory cells initiate cellular responses in the mSCs and mPCs that are tightly integrated with the already impaired cell cycle control,

inadequate DNA repair, and deficient apoptosis signaling processes. This integration of the extracellular initiated and intracellular initiated/impaired signaling processes leads to the development of tumors with immortal, self-stimulating, and invasive CSCs that can independently stimulate their own angiogenesis to sustain continued tumorigenesis essentially anywhere in the body.

Of the two fundamental processes that are essential for carcinogenesis, the first is enhanced proliferative signaling that accelerates cell division of SCs and PCs. One source of proliferative signaling is derived from driver mutations within the SCs and PCs. Another is from the array of growth and proliferative signaling molecules released from activated inflammatory cells. A third is PGE_2 that is released from apoptotic cells that have died through normal senescence; very often senescent PCs. A fourth would be PGE_2 that is released by any cell following almost any kind of cell stress; thus, there are plenty of additional sources of proliferative signaling available. While short-term production of the prostaglandin due to transient cell stresses very rarely leads to a proliferative response, longer-term signaling from chronic damage–associated stresses will. Chronic inflammation is especially important to this process because it is not only a source of a steady supply of many different growth and proliferation signaling prostaglandins, cytokines, and growth factors but it also greatly enhances production of a variety of ROS and RNS. This is, of course, the second fundamental process that is necessary for carcinogenesis: a source of reactive molecules to cause DNA damage.

The major endogenous sources of radical species include mitochondrial respiration, NADPH-oxidase activity of activated G-coupled proteins and by phagolysosomes of activated neutrophils and macrophages, cytochrome P450 monooxygenases, and nitric oxide synthase in vascular endothelial cells and activated neutrophils and macrophages. The major radical molecules produced from these processes are $O_2^{\cdot-}$ and NO·, which then participate in a variety of reactions to produce H_2O_2, $ONOO^-$, $CO_3^{\cdot-}$, $NO_2\cdot$, and ·OH. Of these, the H_2O_2 does not actually cause DNA damage, but as the major cellular oxidant, it can enhance activity of the various signal transduction pathways to contribute to growth and proliferative signaling. Of the DNA-damaging species, the most aggressive oxidant is the hydroxyl radical. In addition to causing DNA damage, the ·OH also causes lipid peroxidation to produce a variety of DNA-damaging products that include malondialdehyde, acrolein, crotonaldehyde, and 4-hydroxynonenal. Because H_2O_2, NO·, and $ONOO^-$ readily diffuse across cellular membranes, their production by activated inflammatory cells in the tumor environment contribute to the carcinogenesis process by providing a steady supply of additional ROS and RNS that then leads to DNA damage and the ultimate production of a variety of transitions and transversions.

Exogenous compounds arising from the environment also can play an important role in carcinogenesis by adding to the DNA damage from endogenous sources, compounds such as PAHs, nitrosamines, aldehydes, metals, nitrogen oxides, acrolein, and many others that are common to combustion products. These carcinogens are present in tobacco as well as being common air and water pollutants. Tobacco, of course, is probably the most "effective" source of carcinogens due to environmental exposures because of the very high concentrations of carcinogens achieved during normal and repeated use of tobacco products. Although relatively

very rare, environmental exposures due to industrial accidents also may produce high-concentration exposures, albeit far less frequently in comparison to smoking. Other environmental carcinogens include the aflatoxins, the N-nitroso compounds formed from nitrates and nitrites in processed meats, and the heterocyclic amines and PAHs formed from barbecuing and searing meats.

Many of the "environmental" compounds are not in and of themselves reactive. They require metabolic activation to become active DNA-damaging species, usually by the *CYP1*, *CYP2*, *CYP3*, and *CYP4* families of the phase I enzymes. Once activated to an electrophilic species, they then react with susceptible components of the different DNA bases to produce DNA damage that can lead to the production of an array of transitions and transversions. These reactions are a normal component of a series of reactions that are designed to modify the original compounds to make them more easily eliminated in the urine. The production of reactive intermediates by CYP is sometimes necessary for a second CYP reaction that often involves the hydroxylation or demethylation of the electrophile to make it more water soluble. Thus, some CYP reactions generate electrophiles while others can eliminate them. Additional reactions by phase II enzymes also are involved in this process. UDP-GT, GST, and the acetyltransferases all are involved in conjugating relatively large water-soluble components to the electrophiles, essentially inactivating them. Phase II enzymes are therefore important in reducing the potential for DNA damage by the electrophiles that arise from xenobiotics following phase I activation.

Overall, DNA damage from both exogenous and endogenous sources are very important in producing risk for cancer. Although DNA damage from exogenous sources may comprise up to 20% of the total damage, individual behaviors such as smoking and frequent consumption of BBQ meats can significantly enhance risk for cancer by greatly increasing exposures to exogenous carcinogens. Reducing significant exposures to environmental carcinogens by not smoking is a relatively straightforward affair; do not smoke or use tobacco products (and so much easier said than done), while avoiding breathing contaminated air, and drinking contaminated food and water can be more problematic because of the endemic nature of air and water pollution. The behavioral risks associated with diet and physical activity may be more amenable to change.

The etiology of cancer is complex when individual cancers are considered, however; there are mechanisms common to all with a majority of the risk arising from endogenous mechanisms that enhance proliferative signaling and DNA damage. These endogenous mechanisms can be exacerbated by a variety of exogenous factors that relate to environmental exposures to carcinogenic xenobiotics that can enhance DNA damage and/or proliferative signaling. All of these risks can be enhanced to a remarkable degree through poor diet and through inadequate (or nonexistent) physical activity.

6.4.2 Diet-Based Prevention

Diet-based reduction in risk for cancer is based on two general concepts. The first is that poor dietary habits can significantly enhance existing risks. Perhaps, the most common detrimental dietary behavior today is the overconsumption of calories,

a situation that applies to many of the over 70% of the population that is either overweight or obese. The cellular dysfunction that ensues in fat cells that are subject to chronic lipid overload leads to the production of a variety of adipokines and proinflammatory molecules including adiponectin, leptin, and visfatin as well as TNFα, IL-6, IL-1β, and MCP-1. These signaling molecules are necessary to activate the infiltration of the growing adipose tissue with the proinflammatory cells and fibroblasts that are necessary for adopogenesis. These signaling molecules also enter the circulation and can enhance growth and proliferative signaling in the tumor environment as well as to produce additional risks.

The additional risks from weight gain are associated with the enhanced circulating levels of TNFα that are implicated in developing insulin resistance in skeletal muscles. The insulin resistance leads to higher levels of circulating insulin and glucose, both of which provide growth advantages to the proliferating cells within the tumor environment. The chronic increase in insulin due to insulin resistance and the intermittent elevations in insulin due to overeating at mealtime enhance production of IGF-1 by the liver to provide an additional source of growth and proliferative signaling. The increased glucose levels provide essential substrates for the developing tumor cells as well as contributing to an enhanced production of AGEs, which then activate proinflammatory signaling through binding to the RAGEs of macrophages that are within the tumor environment. Thus, overeating behaviors are a significant source of risk for the development of cancers.

Eating an inadequate amount of nutrients also is a very common source of risk for cancers. Unfortunately, a majority of Americans consume a diet that is insufficient in one or more of the essential nutrients. While outright deficiencies are very rare, the very low consumption of fruits and vegetables that is endemic to American culture is associated with an insufficient intake of vitamins C, D, and E as well as folate, zinc, selenium, calcium, and magnesium. Each of these insufficiencies is associated with an increased risk for various cancers, most likely through compromised antioxidant status that leads to greater DNA damage and to inadequate control of various signaling pathways to enhance proliferative and proinflammatory signaling.

The second concept relates to the possibility that specific dietary components may reduce risk to a greater degree than correcting inadequate nutrient consumption alone will. A variety of foods contain beneficial phytochemicals that modify cellular functions such that proinflammatory and proliferative responses to common cellular stresses might be attenuated. In addition, the inactivation of reactive carcinogens and DNA repair also may be enhanced by these phytochemicals.

Production of the proinflammatory eicosanoids and cytokines in response to cellular stress and to infection and infection-related damage is mediated by activation of the MAPK signal transduction pathways, NFκβ, PLA2, COX, and LOX. Regulation of these activities depends in part on the activation of PRRs by components of infectious agents or cellular debris. The phytochemicals curcumin, resveratrol, sulforaphane, EGCG, and luteolin are known to attenuate PRR activation and subsequent proinflammatory signaling. Others, including quercetin, curcumin, EGCG, kaempferol, apigenin, morin, anthocyanins, procyanidins, and lycopene, directly suppress NFκβ signaling to reduce expression of proinflammatory cytokines. In addition, MAPK pathways also can be directly inhibited by these compounds; this ultimately

moderates inflammatory responses to infectious agents and reduces the likelihood of excessive "peripheral damage" due to exaggerated inflammatory responses. A variety of compounds from EVOO such as tyrosol, hydroxytyrosol, apigenin, luteolin, and oleuropein as well as EGCG, quercetin, and kaempferol also directly inhibit COX and/or LOX to reduce the production of proinflammatory eicosanoids. In addition to moderating inflammatory responses, all of these inhibitory effects also would reduce the activation of proinflammatory signaling by noninfectious cellular stress.

Other compounds with anti-inflammatory properties are EPA and DHA. American diets tend to be low in α-linolenic acid, which is the precursor essential fatty acid for EPA and DHA synthesis. These fatty acids are essential for production of the resolvins and protectins that contribute to the resolution phase of inflammation. They also can directly activate PPARγ to inhibit MAPK pathways and platelet aggregation, both of which attenuate proinflammatory signaling.

The activities of the various MAPK pathways also are relevant to regulation of the cell cycle. Cell cycle arrest and suppression of growth by BITC, luteolin, resveratrol, apigenin, genistein, EGCG, curcumin, and quercetin in association with suppression of ERK-MAPK and Jnk-MAPK activities and a reduction in activation of Ras, Myc, Jun, and/or Fos have been observed. In addition, rates of apoptosis are enhanced by these phytochemicals and all these alterations are associated with reductions in tumorigenesis.

MAPK activities also are dependent in part on the redox state of the cell that in turn is dependent on the degree of metabolic stress, ROS/RNS production, and the relative activities of the antioxidant and redox-control enzymes. Trx, TrxR, GSH, GSR, GPX, and Prx along with the antioxidant enzymes SOD and CAT are essential to regulating the overall cellular redox state. Expressions of these enzymes are all induced through binding of the Nrf2:Mah heterodimer transcription factor to the ARE. A variety of phytochemicals including quercetin, resveratrol, diallyl sulfide, s-allyl cysteine, lycopene, EGCG, curcumin, catechin, cyanidin, caffeic acid, and sulforaphane have been observed to enhance Nrf2 binding as well as expression of the various antioxidant redox-control enzymes. Nrf2 activation is associated with covalent binding of some phytochemicals to susceptible SH groups of Keap1, while others generate H2O2, which then disassociates Keap1 from Nrf2. The activation of Nrf2 also may occur through the transient H2O2-mediated activation of p38-MAPK, JNK-MAPK, ERK-MAPK, and PKC, which are known to phosphorylate Nrf2. Increased expression of these antioxidant and redox-control enzymes optimize control of the redox state of cells to reduce the likelihood of exaggerated proinflammatory or proliferative responses to a variety of cellular stresses.

Other enzymes that are regulated by Nrf2 include glutamate cysteine ligase, GST, HO-1, UDP-GT, and NQO1. Induction of these phase II enzymes by administration of EGCG, N-acetylcysteine, quercetin, genistein, multiple types of berries, curcumin, resveratrol, tea polyphenols, and indole-3-carbinol leads to an enhanced inactivation of carcinogens, a reduction in DNA damage, and attenuation of lung, liver, mammary, esophageal, and colon cancers in a variety of studies. The reduction in DNA damage observed in some of the studies reveals an additional possibility for risk reduction—DNA repair. While inconsistent in the few human studies, induction in DNA repair activities following treatment with curcumin, indole-3-carbinol,

resveratrol, ellagic acid, red raspberry extract, and quercetin have been observed consistently in animal studies. In some studies, this increase has been related to the activation of Ref-1, a component of the BER as well as an activator of p53.

Though not classically considered a risk for cancer, consumption of protein in amounts greater than the recommended 0.8 g/kg/day are associated with an increase in IGF-1 levels. Increased synthesis of IGF-1 also occurs due to the chronic "insulin overload" that results from calorie excess and from insulin resistance and is a source of risk due to the proliferative effects of these hormones. Interestingly, a reduction in IGF-1 with calorie restricted diets is one reason for the effectiveness of this dietary strategy. The association with protein intake is that reductions in protein intake to AI levels lead to reductions in IGF-1 that are similar to those seen with CR. Other effects of CR include enhanced insulin sensitivity mediated by activation of PGC-1α and AMPK by adiponectin as well as an induced expression of antioxidant and redox-control enzymes and of phase II enzymes, effects mediated by activation of Nrf2. As expected from all of these changes, CR is associated with reduced DNA damage and attenuated tumorigenesis. The reason to single out the protein intake earlier is to suggest that while CR is an extremely difficult lifestyle to follow for most people, reducing protein intake to achieve lowered IGF-1 levels might be more a more palatable dietary strategy to reduce risk.

6.4.3 Dietary Recommendations

Reducing diet-based risks for the development of cancers entails two basic guidelines. The first is to consume a diet that matches caloric intake with caloric expenditures and meets the RDA/AI guidelines for lipids, protein, carbohydrates, and each of the nutrients. The second is to include in a variety of foods that contain beneficial phytochemicals as integral components of the diet. The various dietary insufficiencies that are common to the "average" American diet are relatively easy to correct and the USDA has published dietary guidelines many times over the past decades that describe how to do just that. The latest *My Plate* guidelines are an excellent starting point. To maximize the risk-reduction benefits of a nutritious diet through the consumption of the various phytochemicals, the additional servings of nuts/seeds and beans/peas that are outlined in Chapter 2 also are recommended.

As is obvious from the fact that the majority of the population consumes too many calories with too few nutrients, the constant barrage of information about proper diet, food groups, "healthy foods," and serving sizes in health classes in schools, "health clubs," and in the media simply is not having much of a positive effect. Perhaps, correcting poor dietary habits is not such as easy thing to do after all; the current approach of giving good advice without providing detailed guidance on how to change behavior certainly has not worked very well. Because altering risk for cancer entails altering a lifetime of poor dietary habits, a behavioral approach to learning a new diet rather than a knowledge-only approach may be more successful. Most details of the dietary recommendations have already been described in other chapters, so they will not be reproduced here. For a general proposal on how to integrate the dietary recommendations within a behavioral approach to lifestyle change, the reader is referred to Chapter 9 where all of the dietary recommendations have been incorporated into a recommendation for behavioral modification (Figure 6.2).

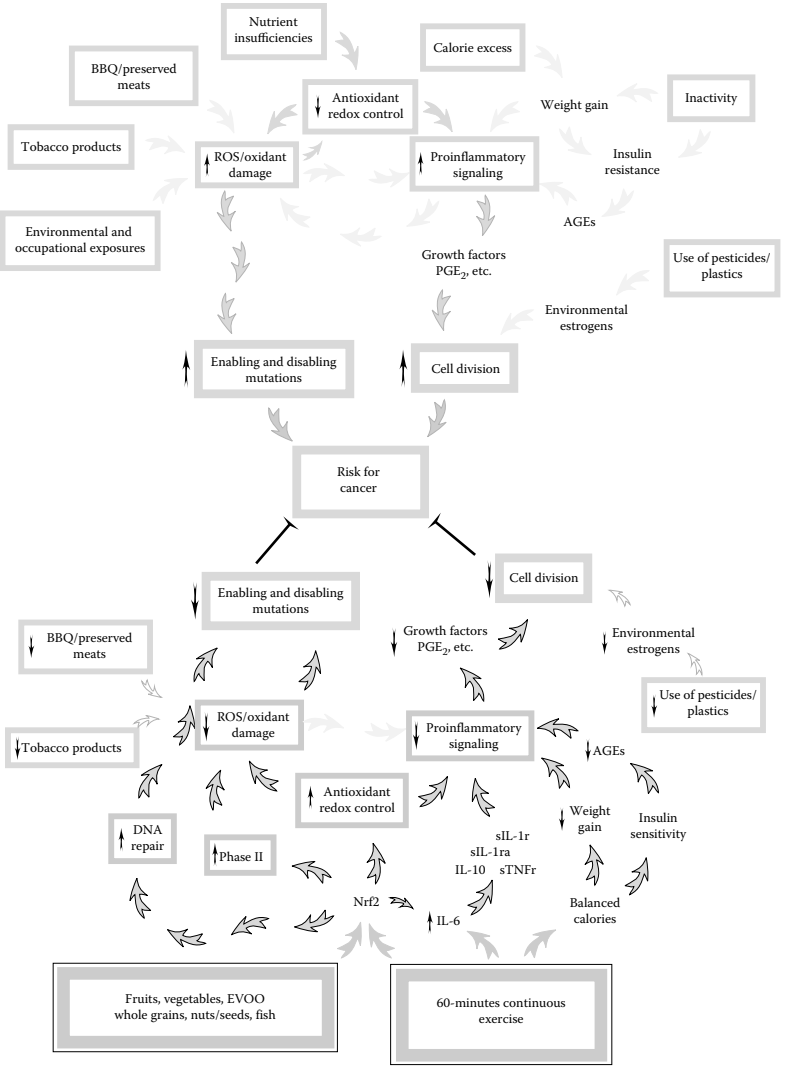

FIGURE 6.2 Summary of effects of diet and exercise on risks for cancer. Nutrient insufficiencies, calorie overload, and inactivity lead to compromised antioxidant and redox status, enhanced proinflammatory signaling, and elevated reactive oxygen species (ROS)/oxidant-mediated damage. These lead to increase cell proliferation and the ultimate production of a variety of enabling and disabling mutations that drive the cancer processes and may account for as much as 80% or more of the total risk. These effects are enhanced through voluntary and involuntary exposures to a variety of procarcinogenic compounds from the environment. Consumption of a calorie-balanced, phytochemical-rich diet along with 60 continuous minutes of daily exercise will optimize redox and antioxidant control, DNA repair, and phase II activities and attenuate proinflammatory signaling to minimize both cell proliferation and mutagenesis. Altering behavior to reduce the voluntary use of higher-risk products such as tobacco, BBQ meats, pesticides, and plastic products also will reduce risk for a large minority of the risks. (Details are discussed in the text.)

6.4.4 Exercise

While an active lifestyle has been known to be inversely associated with risk for cancer for many decades, the recognition that inactivity is a specific risk is a relatively new phenomenon. The role of inactivity in increasing cancer risks is, of course, intimately related to that of obesity. Physical activity certainly can be an effective calorie burner to avoid weight gain if performed frequently and for a sufficiently long time. Inactivity also is closely associated with the development of insulin resistance and therefore all of its inherent proinflammatory and proliferative risks. The inactivity-induced insulin resistance is independent of that arising from weight gain and therefore applies to the normal-weight component of the population that is inactive. An important issue is that almost any kind of physical activity that is performed for 10 minutes or more several times each day is associated with maintaining insulin sensitivity. Whether resistance training, "aerobic" type training, or just being involved in activities such as gardening and other yard work or social dancing, regular and daily physical activities avoid inactivity-induced insulin resistance. The more intense physical activities appear to increase insulin sensitivity in part through increased expression of GLUT4 and IRS, most likely mediated through repeated activation of PGC-1α by an exercise-mediated activation of MAPKs, AMPK, and SIRT1. Acute exercise also can enhance AMPK activity to phosphorylate AS160 and TBC1D1 to mediate a prolonged activation of existing GLUT4 transporters.

In addition to being highly beneficial in maintaining insulin sensitivity and in promoting weight maintenance, other benefits of exercise also can be very important. The enhanced mitochondrial biogenesis and the improved "aerobic capacity" that accompanies some forms of exercise are mediated in part through PGC-1α, MEF2, Tfam, and Nrf2. The type of physical activity that improves "aerobic capacity" is typically of moderate intensity and longer duration (30–60 minutes) although repeated bouts (three to five) of very short-term (60 seconds) maximum intensity exercise also are known to induce mitochondrial biogenesis through the same molecular pathways. Most notable is that Nrf2 is involved in mediating mitochondrial biogenesis and that the forms of exercise that increase aerobic capacity also enhance activity of a variety of important protective enzymes in nonmuscle tissue. These enzymes include the antioxidant and redox control enzymes SOD, GPX, Trx, and GSR as well as the phase II enzymes GST and UDP-GT to enhance redox control and to reduce ROS- and non-ROS-mediated DNA damage.

Rates of DNA repair also can occur with the more stressful, longer duration exercise. Enhanced activity of the DNA repair enzyme OGG1 has been observed following moderately stressful endurance exercise such as swimming or running for 60–90 minutes. This duration is the same time range for induction of IL-6 synthesis by muscle cells and both OGG and IL-6 have Nrf2-binding sites in their promoter. With IL-6 being implicated as a possible coinducer of DNA repair along with its known anti-inflammatory effects, it is evident that longer duration aerobic exercise may produce the greatest cancer-preventive effects.

The anti-inflammatory effects of exercise are mediated by IL-6 through increased expression of IL-10, soluble IL-1 receptor antagonist, soluble IL-1 receptor, and soluble TNF receptor. Binding of any circulating IL-1α, IL-1β, and TNFα to sIL-r and

sTNFr will reduce the effect of these circulating proinflammatory cytokines while sIL-1ra is an antagonist for IL-1 binding to its receptor, thus reducing the proinflammatory effects of these cytokines. The IL-10 activates synthesis of the SOCS, which then inhibits the synthesis of IL-1α, IL-1β, IL-6, IL-8, and TNFα to further reduce proinflammatory signaling. In clinical trials, the longer duration aerobic exercise is associated with reductions in CRP and is consistent with the concept that longer durations of Nrf2 activation are necessary for an anti-inflammatory effect to be realized.

6.4.5 Exercise Recommendations

Based on the molecular mechanisms of risk for carcinogenesis and for altering those mechanisms, there are several levels of risk reduction that can be obtained through exercise. Daily or alternate daily "aerobic" and resistance exercise training or daily low to moderate intensity physical activities such as gardening, dancing, light-effort bicycle riding, and golf increase insulin sensitivity.

To obtain the greater protective benefits of enhanced expression of antioxidant enzymes, redox control enzymes, phase II enzymes moderate intensity aerobic exercise that enhances mitochondrial biogenesis (30 or more minutes continuous; five times a week), vigorous (20 minutes or more; three times a week) and high or maximum intensity (~0.5–10 minutes duration repeated three to five times; three times a week) is necessary.

To maximize the cancer-preventive effects through additional anti-inflammatory and enhanced DNA repair, the longer duration (1 hour or longer) moderate intensity activities that enhance expression of IL-6 by myocytes need to be performed on a daily basis, which thus becomes the recommendation for exercise. These longer duration activities also are important in helping to avoid weight gain. If the long duration activities cannot be performed everyday due to time limitations, then it may be possible that similar reductions in risk may be attained through daily exercise using an alternate-day schedule: alternating the longer duration activities with shorter duration high intensity activities.

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7 Neurodegenerative Disease

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CONTENTS

7.1 INTRODUCTION

7.1.1 Aging and Neurodegenerative Diseases: The Current Perspective

The past decade saw the oldest baby boomers step into their 60s, many of whom are entering their retirement today, and will continue to do so at a precipitous rate until 2030. It is estimated that the number of people aged 65 years and above will soar to about 72.1 million, which is more than twice their number in 2000 (*Aging Statistics-Administration on Aging, Department of Health and Human Services*). This meteoric demographic shift conflated with the increased life expectancy estimated for the coming years has overarching implications for health care and the economy as a whole as it encroaches into already weakened pension and social security systems.[1,2] This pivots the global health-care priorities more toward management of age-related chronic illnesses such as cardiovascular diseases, osteoporosis, and neurodegenerative diseases (NDs).[3,4] Many of these illnesses pose a challenge to assessing their true nature and severity as they are often underrepresented in mortality statistics and there are not enough reliable epidemiological studies that focus on the suffering and disability such diseases cause.[5] Neuropsychiatric disorders cover the top 5 of the 10 leading causes of disability worldwide and account for about 37% of years lived with disability—a metric of equivalent years of healthy life lost through time spent in states of less than full health.[1,6] Moreover, the societal, economic, as well as emotional costs of managing such conditions are not restricted to the individual and often involve loss of the caregiver's productivity and psychological well-being. In the face of such staggering facts juxtaposed with dwindling resources to cope with them, it is imperative that we regroup our efforts toward better prevention or management of neurological and psychiatric disorders.

NDs form a significant component of neurological disorders and are inextricable from the current global concern of age-related chronic illnesses because their incidence and the attendant cognitive and motor deficits are known to increase exponentially with age.[7] NDs are conventionally characterized by a progressive loss of neurons in distinct anatomical subunits of the nervous system, thus leading to different clinical manifestations.[8] The longstanding hallmark of these disorders has been the presence of signature molecular lesions or plaques upon pathological examination of the diseased brains.[9] However, this classification has been debunked by recent progress in the neuropathological diagnosis of these conditions, which have revealed that these lesions are not singularly associated with a particular disease; rather, there is considerable overlap within the molecular as well as clinical features of this group of diseases.[9,10] This naturally confounds the diagnosis—clinicians often have to rely on highly subjective family history profiling to supplement genetic testing—which is complicated

by the lack of specific biomarkers for the diseases. The status quo of ND management lacks in two prominent areas: (1) a unifying dogma pinning the molecular events common to the progression of these diseases, which would tremendously reduce efforts aimed at targeting each disorder separately and (2) impetus for preventive strategies by identifying modifiable lifestyle factors that could delay or alter the progression of the disease. These two areas form the crux of this chapter. We shall begin with an introduction of some of the most common diseases in this category, followed by a detailed assessment of their molecular etiology and mechanisms amenable to preventive manipulation. We shall then discuss the potential of diet and exercise as plausible means to prevent/attenuate neuronal degeneration and delineate the putative mechanisms for their proposed effects. Lastly, we will look into some recommended regimens for diet and physical activity aimed to slow down disease progression and thus eventually delay the onset of morbidity organic to the disease.

There are close to 20 major NDs,[8,9] but our discussion will be limited to the 4 most commonly encountered, and which we believe would cover the salient aspects of this group of illnesses: Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS).

7.1.1.1 Alzheimer's Disease

More than a hundred years have passed since Alois Alzheimer published his seminal report on senile plaques (SPs) causing cognitive dysfunction in a middle-aged woman;[11] AD is now the most common form of dementia in the United States, accounting for almost 60%–80% of dementia cases.[12] It is estimated that approximately 5.4 million Americans have AD, 96% of whom are aged 65 years or more. This number is expected to nearly triple and to account for more than $1.1 trillion in health-care costs by 2050.[12] Although AD has not been a major contributor to the burden of mortality when compared to other illnesses such as heart disease, cancer, and stroke, it rose from the eighth leading cause of death in 1999 to the sixth in 2010, surpassing diabetes mellitus.[13] This is aggravated by the profound socioeconomic burden on immediate family members and/or caregivers during the disease course and after the patient's death.[14–16]

The neuropathogenesis of AD is linked to two types of lesions or protein aggregates—SPs and neurofibrillary tangles (NFTs)—although their presence has not been unequivocally tied to the disease itself.[17] NFTs are intraneuronal protein clusters of hyperphosphorylated form of the microtubule-associated protein tau (MAPT), whereas SPs are extracellular aggregates of beta-amyloid (Aβ)—a cleavage product of amyloid precursor protein (APP). It is posited that the neurodegenerative changes at the molecular level kick-start 5–10 years before a clinical diagnosis is made based on the cognitive deficits and other neuropsychiatric changes impairing normal life functions.[17,18] Typically, AD manifests itself in the form of amnesic type of memory impairment, language, and visuospatial deficits, whereas the motor and sensory abnormalities and seizures do not surface until the late stages of the disease.[18] AD is initially thought to progress from a pre-dementia stage featuring mild cognitive impairment beneath the level of clinical diagnosis to the clinical onset of mild dementia during which declarative memory changes and depression become prominent. The next stage of moderate dementia affects other domains of cognition

and also the noncognitive behavioral aspects, which put tremendous strain on the caregiver. This then develops into a full-blown impairment of cognitive function or the final stage of dementia, wherein patients are unable to articulate their needs or carry out even the simplest of motor tasks and are entirely dependent on their caregivers. The life expectancy of an AD patient is reduced by one-third upon clinical diagnosis, and the average duration of survival is between 5 and 8 years,[19] but it is hoped that future interventions targeting the crucial time-window of subclinical dementia would delay onset or even prevent the development of the disease.

7.1.1.2 Parkinson's Disease

PD—or "Shaking Palsy" as James Parkinson first described it in 1817[20]—is the second most common ND after AD, affecting more than 1% of 55-year-old individuals and more than 3% of those over age 75 years.[21] It is primarily a motor disorder resulting in the progressive loss of functionality in the upper extremities, although cognitive dysfunction and other psychiatric problems are fairly common.[22] With the incidence of PD expected to double within the next 20 years, it is predicted to overburden the caregiving system considering the level of disability it causes.[23] The annual economic burden of PD in the United States is estimated to be $10.8 billion—a combination of direct and indirect costs—of which 58% is related to direct medical costs.[23] The indirect costs, including loss of productivity for patients and caregivers due to early retirement or termination, have societal and psychological costs associated with it and often take a greater toll than represented.

The classic clinical features of PD include tremors, hypokinesia (decreased movement), bradykinesia (slow movement), and rigidity. Psychiatric abnormalities include depression and visual hallucinations. Dementia is seen in at least 20% of the cases.[21] Pathologically, the brains of PD patients are found to be deficient in dopaminergic neurons in the substantia nigra region, with intracytoplasmic inclusions called Lewy bodies in the remaining nigral neurons.[24] With the discovery of new subtypes of the disease that do not display Lewy body pathology, a combination of neuropathological and genetic studies is needed to make an informed decision. Since there are no definitive diagnostic tests for PD, a high premium is placed on the clinical manifestations of the disease, especially the motor symptoms. Bradykinesia is an easily recognizable and the principal diagnostic criterion; it involves difficulty in programming and executing actions involving movement and also features slower reaction times. Patients have difficulty with daily activities like eating or buttoning a shirt. It is posited that a combination of muscle weakness, rigidity, tremors, inaccurate movement, and the slowing of thought contribute to bradykinesia.[25] It is also known to correlate best with the extent of dopamine deficiency in the substantia nigra region. "Freezing" or motor block, in which patients are unable to initiate voluntary movements and appear "stuck" to the ground, is another classical symptom of PD. Apart from this, patients also present with postural deformities ("dropped head" or "bent spine"), decreased stride length while walking, speech disorders (failure to find words), and drooling. There is also significant cognitive retardation, dementia, occurrence of sleep disorders, and a general lack of initiative to carry out daily activities.[26,27]

Despite the promise offered by levodopa and other such symptomatic medications, the prognosis of PD patients is bleak, involving considerable motor disability within 5–10 years following the onset of the disease.[27] Lack of potent drug therapy and specific biomarkers for early detection of the disease underscore the urgent need to develop better diagnostic techniques as well as investigate the role of any preventive measures that could reduce disability in susceptible individuals.

7.1.1.3 Huntington's Disease

George Huntington's landmark description of inherited chorea—an involuntary movement disorder—spawned a cascade of scientific research on the disease that later came to bear his name.[28] We now know that it is an incurable autosomal dominant ND caused due to an aberration in the *HTT* gene coding for the protein huntingtin.[29] The mutated gene contains an expanded CAG trinucleotide repeat sequence. While the mean onset of disease is 40 years, it could decrease in people with high CAG repeat sizes.[30] HD is found in about 4–10 people per 100,000 in western countries, although the number could be as low as 0.38 or as high as 15 in some population groups, possibly due to their geographical isolation.[31,32] As with any disease causing severe disability, HD has far-reaching ramifications for the caregiver and the physician, who respond differently from the patient considering that they are influenced by different aspects of the disease,[33] thus stressing the need for developing a holistic approach toward management of HD.

HD neuropathology depicts selective atrophy in the neostriatal region comprising the caudate and putamen, although with disease progression, there is widespread brain atrophy.[31] The medium spiny neurons in the striatum are most vulnerable to degeneration resulting in an increased undesirable motor stimulation of the body,[34] which translates to the clinical manifestations of the disease. The diagnosis of HD is not as confounding as that for other NDs owing to its high genetic predictability. Upon positive genetic diagnosis, the classical features essential for a clinical diagnosis could be grouped under extrapyramidal movement disorder, cognitive dysfunction, and psychiatric or behavioral disturbance.[31] The breakdown of motor function is due to a combination of voluntary and involuntary movement disorders. The involuntary movements initially proceed through Chorea—a condition whose etymology is linked to the Greek word for "dance"—characterized by rapid, irregular, jerky movements of the trunk, face, and limbs. As the disease progresses, the chorea usually becomes less pronounced and is replaced by bradykinesia and rigidity, and eventually, akinesia, which are functionally more debilitating.[35] The deficits in voluntary movements start off as gait abnormalities and slowed performance in executing functions such as handwriting and equipment usage and lead to a progressive decline of fine motor skills, speech, and mobility to a point where the patient loses volitional control over limb movements and becomes nonambulatory and completely dependent on the caregiver for daily activities.[22,31] Cognitive deficits in HD are often apparent before a clinical diagnosis is made; they include bradyphrenia (slowness of thought), defective recall, and personality changes. Patients suffer from "subcortical dementia," which affects the ability to learn and recall new skills as well as the visuospatial memory. In advanced stages, dementia leads to a global decline of all cognitive faculties.[31] As far as the psychiatric manifestations of the disease is concerned, depression offers the

most cause for concern, considering that it is prevalent in about 40% of the patients, and that they are at a higher risk to commit suicide.[31,36]

What makes HD the most tractable of ND in addition to its genetic predictability is the bounty of reliable biomarker and clinical flags available for the subclinical or premanifest stage of the disease—the stage most amenable to preventive interventions in progressive disorders.[32] This could probably serve as a perfect model system for designing early strategies for diseases with more equivocal diagnostic criteria such as AD and PD.

7.1.1.4 Amyotrophic Lateral Sclerosis

The term "ALS" was first coined by Jean-Martin Charcot in 1874, who is not only credited for expertly outlining the link between the clinical features of ALS to the autopsy findings but also for pioneering the clinicopathological approach in the medical community.[37] ALS—also popularly known as Lou Gehrig's disease—is an adult onset, rapidly progressive, and the most common form of motor neuron disease resulting in the degeneration of both the upper and lower motor neurons. It is characterized by muscular atrophy, weakness, and fasciculation resulting in paralysis, and eventually, death within 3–5 years of onset of symptoms.[38] Not only does ALS entail the morbidity and the heavy socioeconomic costs common to most neurodegenerative conditions, but also one of the worst mortality rates.[39–41] It is estimated to be present in 4–6 individuals per 100,000, with the prevalence peaking in the 60–75 age group, although some pockets in the Western Pacific have much higher prevalence.[42] The most common ALS cases are sporadic, that is, lack a genetic component (95%), whereas the rest have a family history (familial).[43] A significant proportion of the familial cases result from mutations in the gene coding for the copper-zinc superoxide dismutase enzyme (SOD1).[44] There is no specific etiology for the disease, although some environmental factors such as exposure to heavy metals, epidemiological features, and trauma are implicated in addition to genetic predisposing factors.[38,45,46]

The clinical presentation of the disease is a combination of upper and lower motor neuron symptoms. It may originate most commonly as an asymmetric focal weakness of the extremities (difficulty gripping and stumbling) or have bulbar origins (dysphagia or dysarthria),[47] or less commonly, have respiratory muscle onset.[48] Regardless of onset, the disease eventually spreads to, and wastes away other muscles of the body. Another cardinal feature of ALS is the hyperreflexia of the atrophied muscles, with the lack of any sensory disturbance.[47] The classical upper motor neuron symptoms include poor dexterity and gait, difficulty in swallowing, stiffness, and imbalance. Lower motor neuron symptoms are a result of muscle weakness, atrophy, fasciculations, and cramps[38] and include difficulty in performing fine motor tasks such as buttoning and writing, leg weakness leading to problems in climbing, arising, as well as balance. Weakness of the tongue and pharyngeal muscles could lead to coughing, choking, and aspiration of food, which could be potentially fatal. Although essentially a motor neuron disease, a small number of ALS patients develop frontotemporal dementia, and about 30%–50% show frontotemporal executive function deficits.[47] These may become apparent before or after the onset of motor dysfunction and are usually subtler than the symptoms of full-blown dementia. Early decline in interpersonal conduct, mental rigidity, distractibility, and impairment in verbal fluency and attention are some of the characteristics observed in such patients.[49] The

clinical course of the disease is nearly linear with the degeneration inexorably progressing throughout the body and death usually occurring due to respiratory failure.[47]

The knowledge gleaned from the prevalent, clinical manifestations of the most common NDs along with the monumental societal and economic costs associated with their symptomatic management clearly highlight one thing: the need for cost-effective strategies to counter the etiological mechanisms preceding the clinical or morbid states of the disease, thus greatly reducing the disability burden inherent to them. In this regard, the potential of diet and exercise as disease-modifying lifestyle factors has generated a lot of optimism in the neurological community, due to their reported benefits as well as their relatively seamless incorporation into our daily lives.

7.1.2 Neurodegenerative Diseases, Diet, and Exercise: What Is the Connection?

There is no doubt of the tremendous influence our environment and lifestyle have on our overall state of well-being; they are known to influence the progression of many chronic diseases, including those affecting the nervous system.[50] However, in line with our ever-increasing obsession with short-term gains, we have found means to circumvent our nutritional and exercise needs, to our own detriment. Very often, the negative consequences of this do not manifest until mid-to-late life, but when they do, there is little scope for reparative measures. Thus, to have a fighting chance against such chronic illnesses, we need to start early.

In this regard, it is important to understand the dynamic relationship between the brain and the environment. Our lifestyle has a dramatic influence on brain plasticity and neuronal function, which in turn determine the clinical course of NDs, when corrected for genetic factors.[51] The etiology of most NDs being multifactorial, diet and exercise as potential preventive modalities offer several advantages: (1) they have a broad scope of action, (2) they are noninvasive and safe, (3) they have strong translational potential,[52] and (4) they are relatively cheap and can be easily incorporated in our daily life. Moreover, there is evidence to suggest a synergistic role between diet and exercise in brain function.[53,54] These implications have spawned a number of epidemiological studies investigating their role in neurodegenerative conditions, and the results have been encouraging, if not conclusive.

Studies correlating a high-calorie diet with increased risk for AD indirectly point to the salutary role of dietary restriction (DR) in preventing the disease.[55,56] In particular, a diet rich in saturated fat and cholesterol is associated with an increased risk for dementia, and AD in particular,[55,57,58] whereas fish consumption and high intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) is thought to reduce the risk in most cases.[59–63] Also, there is overwhelming evidence linking adherence to a Mediterranean-type diet (vegetables, legumes, cereals, fish, and MUFA) to reduced risk of AD, cognitive decline, and overall, reduced AD-related mortality.[64–67] However, it remains to be established whether or not these dietary components work synergistically, thus warranting their use as a whole diet, as opposed to the individual components. [68] In addition to diet, exercise is another modifiable factor known to retard disease progression, modulate pathogenic mechanisms, and reduce cognitive decline associated with AD and pre-dementia syndromes.[69–72] Interestingly, exercise is independently associated

with reduced AD risk, even when considered together with diet, thus suggesting that a combination of diet and exercise could provide additive benefits to patients.[73]

In PD, although there is no conclusive basis for the role of overall fat intake in pathogenesis,[74] consumption of unsaturated fatty acids may proffer a reduced risk.[75] Similar to AD, a Mediterranean-type diet plus reduced consumption of saturated fat and moderate intake of alcohol may protect against PD.[76,77] The preconceived dismal role of exercise in rehabilitation of PD patients has been recently debunked, thanks to the abundant bench and translational research in the area.[78,79] Exercise is now associated with improvement in the motor symptoms such as postural stability and tremor,[80,81] nonmotor symptoms including depression,[82] and overall functional outcome.[83,84] Also, the studies utilizing resistive and high-intensity exercise paradigms in early and late stages of the disease underscore the importance of forced rather than self-selected physical activity, to attenuate disease progression.[85–87]

There is not a lot of epidemiological data supporting the role of dietary modification in delaying progression of HD. However, a diet low in tryptophan—an amino acid whose abnormal metabolism is implicated in the pathogenesis—is argued to attenuate the progression of disease or delay onset of symptoms in high-risk candidates.[88,89] Also, animal studies suggest that DR could have a neuroprotective effect in HD by boosting the body's defense against protein aggregation and apoptosis.[90] The link between exercise and HD progression is yet to be clinically established, but there are some positive results from animal studies.[91–93]

Dietary and exercise interventions in ALS have shown moderate effects in the clinical level and warrant further investigation.[94] Dietary vitamin E supplementation has by far been the most promising intervention at the clinical level.[95,96] Contrary to conventional knowledge, a low-calorie diet or DR does not offer any benefits in the pathogenesis or functional outcome of the disease.[97–99] This discrepancy is attributed to the malnourished and hypermetabolic state of ALS patients, which would only worsen with caloric restriction.[100] Thus, it is postulated that ALS patients could show increased survival and improved quality of life by consuming a high-energy or a ketogenic diet.[101,102] As far as exercise and ALS are concerned, there has been a conspicuous lack of consensus due to studies associating vigorous physical activity to increased ALS risk.[103–105] However, evidence supporting the use of moderate physical activity and resistance training in improving muscle strength and function and enhancing the quality of life in ALS patients justifies further consideration.[106–108]

A comprehensive analysis of the clinical as well as the preclinical data from the various nutritional and physical activity–based studies strongly point to their plausibility as effective preventive and/or adjuvant therapy. They promise to relieve the overwhelming burden on drug therapies by offering means to delay the need for their intervention. However, there are many caveats associated with such epidemiological studies. For example, many of these studies were case-control and not randomized control trials, which introduces a huge correlation-causation bias. Also, there was a disproportionate focus on functional outcomes of diet and exercise modalities rather than the biochemical/molecular changes they initiate; this could have rejected some of the nascent, promising interventions that did not have overt functional effects yet. Some of the other confounding issues are that regarding statistical power, poor patient compliance, and dosage standardization.[94] This calls for not only better designed randomized

control trials but also a comprehensive understanding of the molecular mechanisms in play. Some questions that need to be addressed at this stage, and which would appraise the potential of diet and exercise in the etiological process of NDs, are as follows: What mechanisms do diet and exercise modulate, relevant to ND prevention? Where do these mechanisms feature in the global molecular etiology of the disease?

To be able to better answer these questions, a detailed assessment of the molecular etiology of NDs is needed, which forms the basis of Section 7.2.

7.2 MOLECULAR ETIOLOGY

7.2.1 Genetics

The multifactorial nature of NDs is evident from the presence of both familial and sporadic forms of diseases. Although the familial forms of diseases represent a minority of the cases (5%–10%), their genetic causes have provided many insights into the pathogenic mechanisms associated with the sporadic forms as well. Although they lack a Mendelian component, sporadic forms have many susceptibility genes associated with them, which—in conjunction with various environmental factors and the aging of the body—are known to increase the likelihood of the disease.[109]

AD: Familial forms of AD are caused by fully penetrant mutations in three genes: *Amyloid precursor protein* (*APP*), *Presenilin 1* (*PSEN1*), and *Presenilin 2* (*PSEN2*). The APP, as the name suggests, is the precursor of Aβ, a 4-kDa peptide and principal component of SPs found in AD patients. APP is proteolytically processed by a group of enzymes called α, β, and γ secretases to obtain two major Aβ fragments, $A\beta_{40}$ and $A\beta_{42}$, consisting of 40 and 42 residues, respectively. $A\beta_{42}$ is the pernicious one of the two and is the main culprit implicated in AD pathogenesis.[110] The presenilins form a component of the γ secretase complex and along with the other secretases determine the balance between amyloidogenic and nonamyloidogenic fragment content of Aβ. The mutations in the three key genes involved in synthesis and processing of APP is thought to tip the balance toward production $A\beta_{42}$ and cause the familial form of the disease.[111]

One of the well-established genetic susceptibility factors for AD is the *apolipoprotein E* (*APOE*) gene.[112,113] The *APOE-ε4* allele is linked to a three-fold increased risk of AD, although its impact on the disease risk decreases with age. It is associated with increased plasma concentrations of cholesterol and thought to enhance Aβ deposition and formation of SPs; however, it is neither sufficient nor necessary to cause the disease.[113] The other susceptibility genes whose polymorphs carry an increased risk for the sporadic forms of AD include the β-secretases, *BACE1* and *BACE2*, and other components of γ-secretase activity, *Nicastrin* and *PEN2*,[114–116] thus establishing errant Aβ biosynthesis as the common theme linking both the familial and the sporadic forms of the disease.

PD: As with AD, the etiology of the most commonly encountered sporadic form of PD seems to be a complex interplay of susceptibility genes, environmental exposure, and aging. The key causative genes for the rare, familial form of the disease are α-*synuclein*, *parkin*, *ubiquitin carboxyl-terminal hydrolase L1* (*UCH-L1*), *PTEN-induced putative kinase 1* (*PINK1*), and *leucine-rich repeat kinase 2* (*LRRK2*). The protein α-synuclein is thought to function as a molecular chaperone and be involved in vesicular trafficking.[117,118] Mutations in the gene (*PARK1* and *PARK4*) are known

to increase the tendency of α-synuclein to form aggregates and eventually form Lewy bodies.[119,120] Mutations in the *parkin* gene (*PARK2*) are known to cause early-onset PD devoid of the classical Lewy body formation.[121] Mutated parkin, a ubiquitin E3 ligase, is thought to interfere with the normal functioning of the ubiquitin-proteasome system essential for the degradation of misfolded proteins.[122] The implication of a mutation in the *UCH-L1* gene in PD cemented the role of the ubiquitin-proteasomal dysfunction in the pathogenesis.[123] The mutation in *UCH-L1* impairs its hydrolase activity, thus affecting ubiquitin hydrolysis and eventual death of dopaminergic neurons.[124] The *PINK-1* gene encodes a mitochondrial protein involved in stress response pathways, mutations in which could hinder the ability of neurons to cope with stress.[125] The other major causative gene is the *LRR2* gene, whose physiological role is not precisely established, but is known to cause an autosomal dominant form of the disease.[126]

Many of the susceptibility factors of PD include variants of the genes implicated in the familial forms. The Rep1 polymorphism in the promoter region of the α-*synuclein* gene,[127] a variant in the *LRRK2* gene,[128] and mutations in the gene encoding the *MAPT*[129] have been associated with increased PD risk. The other susceptibility factors include genes coding for proteins involved in dopamine transport and metabolism and the detoxification of xenobiotics. A plethora of studies have been carried out in this regard, yet conclusive results of an increased risk of PD have been associated only with polymorphisms in the genes encoding for proteins glutathione S-transferase P1[130] and cytochrome P450 2D6.[131]

HD: HD is inherited in an autosomal dominant manner and is caused by expanded CAG repeats in the exon 1 of the *HTT/huntingtin* gene. The number of CAG repeats—which code for a track of glutamine residues in the protein—is the major contributor of variability in terms of age of onset and severity of symptoms as it determines the ability of the mutated huntingtin to form aggregates.[132,133] Wild-type huntingtin is thought to be involved in embryonic development, vesicle transport, postsynaptic signaling, transcriptional regulation, and cell survival pathways.[134] Some of the other genetic variants altering the age of onset of the disease are those in the NR2A and the NR2B subunits of the N-methyl-D-aspartate receptor (NMDAR).[135] Also, the polyglutamine expression in HD has been shown to impair the expression of a number of genes encoding synaptic and intraneuronal signaling proteins, suggesting that synaptic dysregulation could be a key pathogenic mechanism.[136]

ALS: ALS is mainly a sporadic disorder with about 5%–10% prevalence of familial cases. The key causative genes for familial ALS are *SOD1*, *alsin* (*ALS2*), *senataxin*, and *VAMP-associated protein B* (*VAPB*). Superoxide dismutases are a class of free-radical scavenging enzymes, with SOD1 specifically involved in converting the highly reactive superoxide anion, a by-product of oxidative phosphorylation (OXPHOS) in the mitochondria, to the relatively benign hydrogen peroxide. SOD1 mutations account for about 20% of the familial cases, and although most mutations reduce enzyme activity, others do not affect catalytic function.[137] However, neither age of onset nor the course of the disease seem to correlate with SOD1 activity,[138,139] suggesting that the effects of mutant SOD1 are a result of a "gain-of-toxicity" rather than "loss-of-function." Mutations in the *ALS2* gene coding for alsin are predominantly loss-of-function and are known to render neurons vulnerable to oxidative stress and disrupt vesicle trafficking.[140,141] The function of alsin in unknown,

but it was found to suppress mutant SOD1-mediated neurotoxicity in motor neuron cell lines.[142] Mutations in the *senataxin* gene encoding for a DNA and RNA helicase involved in the defense against oxidative DNA damage[143] were associated with juvenile autosomal dominant form of ALS.[144] Defects in the *VAPB* gene are linked to adult-onset autosomal dominant and atypical ALS and cause decreased anchoring of lipid-bound proteins and disruption of vesicular transport.[137,145]

Studies aimed at identifying candidate susceptibility genes for ALS have so far met with conflicting results. However, information gathered from the genes causing the familial forms of the disease have highlighted the importance of polymorphisms in DNA repair genes, angiogenesis factors, and genes coding for neurofilaments in ALS etiology.[109]

7.2.2 Environment

The impact of the environment on the etiology of NDs cannot be underestimated. In fact, many of the susceptibility factors discussed in Section 7.2.1 have emerged from gene–environment interactions accumulating over a period of time, thereby necessitating studies to identify such factors relevant to ND etiology. The results from such studies have not always been conclusive due to biases and confounding factors inherent in such case-control groups. Nevertheless, few have clearly emerged as potential risk factors for NDs (Table 7.1).[109,136,146–148]

Metal exposure is probably one the most thoroughly studied environmental factors in ND pathogenesis and has led to a better understanding of the intracellular processes that could trigger the disease. Higher levels of metals have been detected in regions of the brain specific to the insult caused by the disease. For example, higher copper and zinc levels were found in the SPs and NFTs in AD brains, and PD midbrains have higher iron levels. High levels of these metals are linked to oxidative stress conditions in the brain, as they are capable of generating free radicals and setting off the oxidative stress cascade.[149]

Exposure to metals in the perinatal period is deleterious to neuronal development. Low levels of lead exposure during the developmental stage is thought to have a protracted negative effect on APP processing and Aβ biosynthesis in later stages of life.[146] It is also known to induce oxidative DNA damage and reduce DNA methyltransferase activity. The ability of lead to have late onset effects on the activities of enzymes and on DNA oxidation revealed an interesting facet to the gene–environment interaction—epigenetics. Epigenetics is an umbrella term for all the mechanisms involved in gene expression changes and their subsequent inheritance, by means not involving DNA sequence changes.[150] It principally involves processes such as DNA methylation, histone deacetylation, and RNA editing, which occur after DNA synthesis. DNA methylation patterns are established *in utero* and are susceptible to environmental modification until postnatal development, thus establishing a gene imprint that lasts for a lifetime.[151] Early life exposure to lead is thought to enhance or repress the functions of various genes by influencing DNA methylation. In AD, hypomethylation of the APP promoter and the subsequent increase in APP and Aβ production could contribute to the oxidative damage. DNA methylation is also linked to DNA repair and oxidative DNA damage, thus potentially modifying

TABLE 7.1
Some of the Important Environmental Factors Associated with ND Etiology

Disease	Environmental Factor	Comment
AD	Metals (iron, copper, zinc, lead)	Increased risk; inconclusive results
	Pesticides	Increased risk
	Solvents	Increased risk; inconclusive results
	Brain injuries	Increased risk
	Infection and inflammation	Increased risk
	Caloric restriction	Protection
	Antioxidants	Protection
	Mediterranean diet, fruits, vegetables	Protection
	Fish, MUFA, PUFA	Protection
PD	Metals (iron, copper, manganese, lead)	Increased risk; conflicting results
	Pesticides, herbicides	Increased risk
	Head injuries	Increased risk
	Tobacco	Protection
	Caffeine from coffee and tea	Protection
	Mediterranean diet, fruits, vegetables	Protection
	Fish	Protection
HD	Metals (iron, copper, manganese)	Increased risk; conflicting results
	Environmental enrichment (cognitive and physical activities)	Protection
ALS	Metals (lead)	Increased risk
	Pesticides, insecticides	Increased risk
	High-intensity sports	Increased risk
	Head injuries	Increased risk

Source: Modified from Migliore, L. and Coppedè, F., *Mutat. Res.*, 674, 73–84, 2009. With permission.

stress-response pathways. Moreover, such changes in gene expression patterns are not limited to metal exposure, but also other environmental components such as diet and chemicals. Epigenetic changes can thus translate early environmental exposure to far-reaching changes in cellular mechanisms, which could eventually be useful in understanding our susceptibility to age-related diseases. Although its role in cancer is well established, its contribution to NDs is still in its infancy.

The role of "designer drugs" and other agricultural chemicals is best established in PD, wherein the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) led to the establishment of MPTP-based animal models of the disease. MPTP and related pesticides such as rotenone, paraquat, and maneb possess oxidative stress-generating properties,[152] which cause mitochondrial dysfunction, a classical feature of many NDs. Some of the other interesting environmental components implicated in NDs are air pollution and diet. Air pollution is associated with neuroinflammation and deposition of $A\beta_{42}$ and α-synuclein, beginning at childhood.[153] There is evidence that nano-sized particulate matter could gain entry into the brain and cause NDs.[154]

The influence of diet in NDs has been previously described and will continue to feature in the following discussion.

7.2.3 Aging

Aging is an inevitable physiological process that slows down our sensory, motor, and cognitive machinery with time. While the probability of developing NDs increases remarkably with age, it is not irreversibly linked to neurodegeneration and death. What separates people who age and people who are struck with a ND while they age is the rate of aging and the ability of the body to compensate for age-associated deficits.[155]

Aging is broadly characterized by increased oxidative stress,[156] impaired energy metabolism,[157] accumulation of dysfunctional proteins,[158] and DNA damage.[159] Disease-specific genes, susceptibility factors, and the environment trigger molecular events similar to aging and exacerbate its effects to a toxic level in specific neuronal populations.[155] Aging is associated with increased levels of oxidized proteins, DNA, and lipids in the brain. Age-related protein modifications such as carbonylation, nitration, and lipid peroxidation markers such as 4-hyrodynonenal (4-HNE) and malondialdehyde are markedly elevated in NDs.[160–162] Protein accumulation is a prominent feature of aging and is thought to be a combination of increased protein damage and the breakdown of the proteasomal and autophagic systems handling such proteins.[163] However, accumulation of aggregate components such as Aβ, α-synuclein, and SOD1 in the diseased brains is greater than that seen in normally aging brains.[164,165] Aging also alters neurotransmitter pathways and the production of neurotrophic factors like brain-derived neurotrophic factor (BDNF)—changes that are enhanced in diseased brains.[166,167]

Taken together, the etiological model of NDs is built on the premise that aging exposes the chinks in the neuronal armor, and a combination of genetic and environmental factors decide whether the weakened neurons succumb to the disease or not. Aging is a perpetual component in almost all cellular processes responsible for neurodegeneration and shall be reintroduced wherever relevant in the following sections (Sections 7.2.4–7.2.7) delineating the molecular mechanisms of causation.

7.2.4 Oxidative Stress

Oxygen is essential for most cellular processes in our body, yet the by-products of its metabolism result in the formation of reactive intermediates, which could lead to oxidative stress, if left unchecked. These reactive oxygen species (ROS) serve important physiological functions such as signal transduction, gene transcription, and response to noxious stimuli, at low concentrations.[168] The deleterious effects of ROS become apparent at higher concentrations, which under normal circumstances are nullified by the robust antioxidant defense consisting of enzymes and free-radical scavengers, thus maintaining homeostasis. When the oxidant-antioxidant balance goes awry, oxidative stress and its attendant toxic events ensue.

Oxidative stress is the hub of all processes leading to neurodegenerative damage. Whether it triggers the cascade of events that eventually lead to the death of neurons

is debatable, but it is intimately involved in the major events leading to the formation of the protein aggregates—the pathological hallmark of NDs—as well as the consequent damage exacted by them resulting in neuronal demise. The brain is particularly vulnerable to the noxious effects of oxidative stress due to many potentiating factors. It is one of the highest consumers of oxygen and glucose on a per weight basis, making it more susceptible to ROS overload.[169] Neuronal membranes are rich in unsaturated fatty acids, making them easy candidates for free-radical-mediated lipid peroxidation, causing membrane damage and the disruption of the activity of membrane-bound proteins.[170] It also shows lower basal antioxidant capacity, which is aggravated by the reduced antioxidant surveillance and regenerative capacity of the aged brain.[171]

The mitochondrion serves as the main source of intracellular ROS through the OXPHOS reaction occurring in the electron transport chain, wherein molecular oxygen is reduced to generate the highly reactive superoxide anion ($O_2^{\bullet -}$). This reaction is essential as it activates molecular oxygen, whose reaction with physiological molecules is otherwise energetically unfavorable.[171] Once generated, the superoxide ion is capable of triggering the production of more ROS such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hydroxyl radical ($HO^{\bullet}$), and hydroperoxyl radical ($HOO^{\bullet}$). Of particular interest is the Fenton chemistry that involves the iron catalyzed reaction of hydrogen peroxide with superoxide ion forming the highly reactive $HO^{\bullet}$.[172,173]

$$Fe^{3+} + {}^{\bullet}O_2^- \rightarrow Fe^{2+} + O_2 (\text{Step I})$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH^- + OH (\text{Step II})$$

Overall,

$${}^{\bullet}O_2^- + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2$$

These transition-metal catalyzed reactions are key to the disease pathogenesis because the brains in most NDs show elevated levels of Cu^{2+}, Zn^{2+}, and Fe^{3+}, thus potentiating the reaction and the formation of ROS.[147] Aβ aggregation and α-synuclein-mediated damage in AD[174] and PD,[175] respectively, have been shown to involve interaction with transition metals and the concurrent formation of hydroxyl radicals. ROS is also generated by the peroxisomes that mainly produce H_2O_2, and NADPH oxidase (NOX), which releases H_2O_2 and $^{\bullet}O_2^-$. Both aging and AD show an increase in NOX activity.[176] Another mitochondrial ROS source is the enzyme monoamine oxidase that catalyzes the oxidation of biogenic amines such as dopamine, serotonin, and norepinephrine and is implicated in PD pathology.[177]

Along with ROS, reactive nitrogen species (RNS) generated as by-products of nitric oxide ($NO^{\bullet}$), also contribute to oxidative stress. Nitric oxide, a free radical in itself, is an important signaling molecule functioning almost like a neurotransmitter in the

CNS.[178] It plays a role in immune response and vasodilation of smooth muscles, thus regulating blood flow.[176] During inflammatory conditions prevalent in the ND brain, NO is released by activated microglia and endothelial cells by the action of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase, respectively. NO then reacts with superoxide to form peroxynitrite ($ONOO^-$)—a potent RNS, which could decompose to form $HO^{\bullet}$.[169] iNOS-mediated NO release and $ONOO^-$ generation is thought to be one of mechanisms of mutant-SOD1 toxicity in ALS[179] as well as the reduction in levels of aconitase—an enzyme of the tricarboxylic acid cycle sensitive to inhibition by ROS—in the striatum of HD patients.[134]

Our body has devised various adaptive responses to combat the oxidative onslaught of ROS and RNS. The antioxidant system comprises enzymes such as the superoxide dismutases (Mn-SOD and Cu/Zn-SOD) that convert $^{\bullet}O_2^-$ to H_2O_2 and O_2 and catalase and glutathione peroxidases (GSHPx) that convert H_2O_2 to water. Also, there are nonenzymatic components that act as direct antioxidants by scavenging/chelating ROS or by inducing antioxidant enzymes that include thioredoxin, glutathione (GSH), vitamins E and C, uric acid, and ubiquinone.[176,177] Evidence of reduction in antioxidant enzyme activity and scavengers is noted in both AD[180] and PD.[181] GSH depletion is one of the markers of nigral degeneration, and its levels parallel the severity of PD.[179] In addition to antioxidants, there are protein chaperones responsible for tagging oxidatively modified proteins for their eventual disposal, such as heat shock protein 70 (HSP70), glucose-regulated protein 78 (GRP78), and HSP27, whose activities are also compromised in NDs.[182] One of the crucial targets of ROS is proteins, particularly at their cysteine residues.[183] In addition to oxidation by transition metals, proteins are also vulnerable to covalent modifications by lipid peroxidation products such as 4-HNE, acrolein, and malondialdehyde. The formation of these adducts sets into motion a host of other processes that result from either the loss of protein function[184] or the toxicity of the adduct itself.[185] Acrolein and 4-HNE are known to disrupt sugar and glutamate uptake, by acting on membrane Na^+/K^+- and Ca^{2+}-ATPases and glutamate and glucose transporters, which in turn cause severe energy depletion and Ca^{2+} influx, leading to synaptic degeneration and neuronal death.[171,186] Ca^{2+} ions are signaling gateways responsible for cell–cell communication, synaptic plasticity, and neuronal integrity.[187] Its cytosolic levels are tightly regulated, and any process—or in this case ROS—causing Ca^{2+} overload leads to a suicidal cascade fueled by further influx of mitochondria-derived ROS and RNS.[177]

There is considerable ROS-mediated DNA damage in NDs that hampers DNA repair mechanisms leading to mutations in the sequences coding for essential proteins.[188] Mutant SOD1 toxicity in ALS is thought to involve oxidative DNA damage due to reduced capacity of DNA repair enzymes.[189] The CAG trinucleotide expansion seen in HD is also found to be due to erroneous DNA repair induced by damaged DNA.[190] While the primary hypothesis of oxidative stress–triggered neurodegeneration rests on the breakdown of antioxidant defense in the face of ROS overload, there are many other processes that result from and further contribute to the ROS insult (Figure 7.1). There is continuous crosstalk between oxidative stress and other cellular mechanisms (discussed in Sections 7.2.5–7.2.7), forming a self-amplifying loop of events that cause synaptic damage and neuronal death.

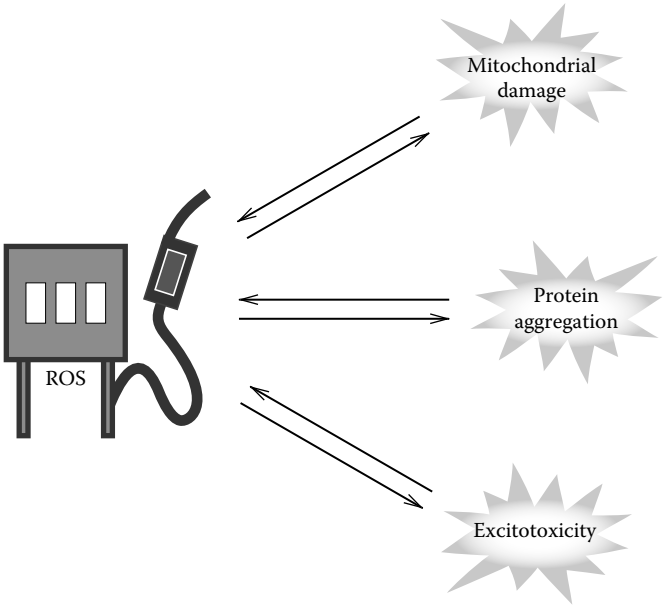

FIGURE 7.1 Oxidative stress fuels many other toxic insults in ND etiology. Oxidative stress causes and results from mitochondrial damage, protein aggregation, and excitotoxicity thereby forming a self-amplifying loop.

7.2.5 Mitochondrial Dysfunction

Mitochondria are responsible for ATP production that fuels most cellular processes. Being an energy-intensive organ, the brain depends on the mitochondria for most of its energy supply by a process called oxidative phosphorylation. Mitochondria are not only the major sites of ROS generation but are also involved in the adaptive response against them by activating antioxidant enzymes.[191] They control apoptotic pathways, buffer cytosolic Ca^{2+}, and maintain respiratory efficiency by their ability to undergo fission/fusion.[192] Mitochondrial axonal transport is extremely important for maintaining neuronal polarity, and thus their signaling ability.[193] It is evident that the unimpeded functioning of mitochondria is vital to neuronal integrity and survival. Mitochondrial dysfunction in NDs occurs due to a combination of several factors, the most important triggers of which are disruption in its ability to undergo fission/fusion and changes in its DNA. As discussed in Section 7.2.4, oxidative stress is found to play a role both upstream and downstream of mitochondrial deregulation.

Human mitochondrial DNA (mtDNA) is circular, double-stranded and exists in multiple copies within the cell.[194] As this gives rise to the possibility of coexistence of wild-type and mutant forms, the deleterious effects of mutations do not have any functional significance until they exceed a threshold, which is lower in neurons due to their sole reliance on OXPHOS for energy. This along with higher ROS exposure,

lack of histone protection, and limited DNA repair machinery make mtDNA highly vulnerable to oxidative damage leading to mutations.[195] These mutations could gradually impair the electron transport chain, effectively reducing ATP production and increasing ROS generation, which in turn act as a positive feedback spawning further mutations and oxidative damage.[195] Decline of mitochondrial function due to ROS and mtDNA mutations is prevalent in aged brains and is exacerbated in NDs due to several disease-specific susceptibility factors.[196]

mtDNA mutations are believed to cause the increased oxidative damage seen in AD patients,[197] although it is not a direct causative agent of the disease.[198] mtDNA single-nucleotide polymorphisms and haplogroups seem to influence AD risk;[199] mtDNA haplogroups have been linked to APOE-4 in some cases.[200] ROS-mediated impairment of mtDNA base excision repair could also contribute to mitochondrial damage.[201] Certain haplogroups have also been associated with the modification of mitochondrial complex I activity and increased oxidative stress characteristic of PD.[202] mtDNA mutations are elevated in the oxidative stress–vulnerable nigral neurons of PD patients, which indicates that they could be partially responsible for the widespread mitochondrial damage seen in PD—an implication strengthened by both clinical and preclinical data.[203,204] Although the causality of mtDNA mutations in PD has been challenged,[205,206] its interplay with other causative factors of PD like familial gene mutations[207] suggests that its role cannot be disregarded.

In addition to fidelity of mtDNA, mitochondrial dynamics are also crucial to maintaining cellular homeostasis. Mitochondria undergo fission (splitting) and fusion (combining) that facilitate exchange of essential components and the dilution of toxic ones, thus establishing a healthy equilibrium. Fusion is carried out by the GTPases mitofusin 1 & 2 (Mfn 1 & 2) and optic atrophy protein 1 (OAP1), and fission, by dynamin-like protein 1 (DLP1), and the outer mitochondrial membrane protein Fis1.[195] The balance of fission and fusion is contingent upon changes in metabolic status, oxidative stress, and ion homeostasis and could have serious impact on mitochondrial functions involving calcium buffering, apoptosis, and ROS generation.[208] Dysfunctional mitochondrial dynamics in AD is evident from the changes in the number and morphology of mitochondria in the hippocampal neurons in addition to lower levels of fusion proteins (Mfn 1 & 2 and OAP1) and higher levels of Fis1, suggesting an equilibrium shift toward increased mitochondrial fragmentation.[209,210] APP and subsequent Aβ overproduction have been shown to impair mitochondrial dynamics in very early stages of the disease, even before the appearance of plaques.[211] Nitrosylation of DLP1 by Aβ-mediated NO release is thought to be one of the mechanisms for the observed effects, thus providing a cellular mechanism tying oxidative stress and mitochondrial dysfunction in Aβ-toxicity.[212] Recently, it was found that the Aβ-mediated mitochondrial damage was aggravated by a truncated form of the protein tau—the aberrantly phosphorylated form of which is a major component of NFTs in AD.[213] Caspase-mediated truncation of tau enhances aggregate formation,[214] thus suggesting that mitochondrial deregulation probably mediates tau toxicity. In turn, mitochondrial damage may also promote tangle formation by enhancing tau phosphorylation.[215]

Mitochondrial dysfunction is probably one of the most pronounced pathogenic features of PD. This is no surprise considering that the causative genes for the familial form of the disease as well as the susceptibility factors including α-synuclein, parkin, PINK-1, and LRR2 are associated with the mitochondria.[195] Both environmental and genetic factors implicated in the disease etiology seem to perturb mitochondrial homeostasis by inhibiting the activity of complex I. MPTP or rotenone-mediated inhibition of its activity causes pronounced oxidative stress, which further drives mitochondrial activity and ATP production down.[216] Lower ATP levels reduce the energy-intensive vesicular loading of dopamine, causing a spike in the cytoplasmic dopamine levels and an increase in oxidative stress due to its enhanced oxidation.[179] The by-products of dopamine oxidation—semiquinones and quinones—are capable of inhibiting complex I activity by interacting with GSH, thus establishing a bidirectional relationship between dopamine oxidation and complex I inhibition.[179]

The proteins implicated in the familial form of the disease are generally associated with the mitochondrial membrane and influence the activity of complex I. α-synuclein associated with the inner mitochondrial membrane reduces complex I activity, triggering apoptosis,[217] whereas parkin and PINK1 have prosurvival roles probably by working in conjunction to inhibit the fatal mitochondrial cytochrome c release.[191] Cytochrome c release is also one of the events triggered by mutant SOD1 aggregates in ALS, in addition to blocking protein importation and ROS production.[191] Reduced levels of the antioxidant Mn-SOD and reduced function of the electron transport chain are the other features in ALS contributing to mitochondrial damage. The attendant energy deficit affects mitochondrial membrane potential, causing Ca^{2+} influx and membrane permeability eventually causing cell death.[218] This mechanism is also common to huntingtin-mediated toxicity in HD.[219] Impaired complex-II activity and disruption of fusion–fission complexes are the other mechanisms of mitochondrial dysfunction in HD.[191] In summary, the mitochondrial dysfunction-oxidative stress cycle mediates both the downstream effects of pathological components of NDs and the prodromal effects of the genetic and environmental factors causing them.

7.2.6 Protein Aggregation

Protein aggregates are considered to be of seminal importance in ND etiology as they provide the first visual pathological indicator for the disease. Protein inclusions/amyloid plaques have been the stand-alone theme tying diverse NDs together (Table 7.2), until further deliberative studies unraveled other mechanisms associated with the disease. An intriguing feature of these aggregates in addition to their characteristic β-pleated structure is that they are composed of otherwise ubiquitous proteins found throughout the body, but form plaques in only in disease-specific regions in the CNS.[220] It is thought that the genetic mutations in the proteins implicated in the familial forms of the disease accelerate this process of aggregation, whereas environmental and susceptibility factors might mirror the same at a lower rate, in the sporadic forms. For example, mutations in the gene encoding α-synuclein (*SNCA*) increases its propensity to form aggregates (seen in Lewy bodies) in the familial form, whereas environmental factors such as rotenone or MPTP are known

TABLE 7.2
Protein Aggregates in Some of the Common NDs

Disease	Protein Component of Aggregates	Cellular Location
AD	Aβ	Extracellular
	Hyperphosphorylated tau	Cytoplasmic
PD	α-synuclein	Cytoplasmic
HD	Huntingtin (with polyglutamine tracks)	Nuclear
ALS	SOD1 (only in the familial form)	Intracellular

to promote α-synuclein-containing inclusions in the sporadic form.[221] Similarly, the preponderance of the amyloidogenic $A\beta_{42}$ fragment caused by mutations in genes encoding APP and presenilins is also promoted by the APOE-4 haplotype (*APOE-4*), a known susceptibility factor for sporadic AD. Although the underlying mechanism is not clear, a combination of reduced Aβ clearance and cholesterol modulating effect of APOE-4 is thought to contribute to Aβ deposition.[220] In some cases, the aggregates show mixed toxicity—toxicity of the aggregate itself (toxic gain of function) as well as toxicity due to loss of function of the aggregated protein. An excellent example of this is the MAPT, which is involved in axonal transport.[220] Overall, the protein aggregates are thought to exert their toxicity by a complex mix of overlapping mechanisms including mitochondrial dysfunction, altered gene expression, oxidative stress, sequestration of other proteins, disruption of axonal transport, excitotoxicity (discussed in Section 7.2.7), and synaptic dysfunction.[220]

Although the "amyloid hypothesis" proposing that protein aggregation triggers the cascade of events culminating in neurodegeneration has been the gold standard of the ND paradigm,[222] an increasing body of evidence challenges this notion.[17,223,224] One of the reasons for this conflict is the lack of correlation of number and the size of plaques with the severity of the disease in AD postmortem tissue;[225] the other is the fact that SPs are seen in the cortical tissue of many cognitively normal 70-year-olds.[226] The intensive research spurred on by these revelations now suggest that the fibrillar aggregates are an epiphenomenon of the disease and that a common trigger sets off both the neurodegeneration and protein aggregation.[17] There is evidence for the presence of soluble oligomeric species of Aβ,[227] globular or ringlike protofibrils of α-synuclein,[228] and protofibrillar intermediates of huntingtin,[229] all of which are toxic in their respective disease conditions.[224] It is speculated that the preaggregate protofibrils might be the actual malignant entity, possibly even more toxic than the fibrillar aggregates. Hence, it is pragmatic to inhibit the formation of these neurotoxic protofibrillar intermediates to prevent neuronal death. This requires a detailed assessment of the processes in play that influence the formation of these moieties.[224]

Since the prefibrillar theory has not been unequivocally proven, there is no knowledge of the concrete steps responsible for their formation, or which of the several intermediate species is most toxic. However, we do have knowledge of the possible factors that could seed the process, or in other words, present avenues for its prevention. Oxidative damage, again, is one of the culprits initiating the misfolding of proteins and thought to be a primary event in the generation

of protofibrils.[224] Other covalent modifications such as nitration,[230] SUMOylation (small ubiquitin-like modifier),[231] and phosphorylation[232] are also involved in the aggregation process. Under normal conditions, these misfolded proteins are handled by various housekeeping proteolytic machinery (proteasomal and autophagic) that serve to either refold them or tag them for degradation, thereby circumventing their toxic effects. Cellular stress also stimulates the assembly of a bevy of stress response proteins called molecular chaperones, which aid in combating the stress. This bastion of stress-handling pathways is broken down in NDs and contributes to the formation and/or reduced clearance of the toxic protofibrils and amyloid aggregates.

Proteins misfolded due to genetic mutations, environmental factors, or oxidative stress induce the synthesis of HSPs that chaperone them and modulate their conformation, movement across membranes, or tag them for degradation.[233] Some of the most common HSPs include HSP70, HSP27, ubiquitin, and HSP32/heme oxygenase. Overexpression of HSPs seems to reverse the neuronal loss caused by the proteins implicated in various NDs, thus suggesting that misfolding is one of the causes of neurodegeneration.[220] Induction of HSP70 reversed the neurotoxicity seen in α-synuclein and polyglutamine-toxicity models in *Drosophila*.[234] The induction of heme oxygenase-1 is being pursued as a promising therapeutic strategy in combating AD neurodegeneration.[233] Ubiquitin is another chaperone and the smallest of the HSPs involved in targeting proteins to the proteasome—the degradation hub of short-lived proteins.[235] The ubiquitin-proteasome system (UPS) plays a crucial role in regulating protein turnover and controls functions as diverse as neuronal functioning, DNA repair, synaptic plasticity, and cell signaling.[176] When proteins get misfolded, they are tagged by a chain of ubiquitin molecules to the 26S proteasome—a multiprotein complex with proteolytic activity. The ubiquitination reaction is catalyzed by a series of activating (E1), conjugating (E2), and ligating enzymes (E3) and reversed by deubiquitinating enzymes (DUBs).[124] The dysfunction of any of the components of the UPS could conceivably result in the accumulation of damaged proteins and eventually, cell death.

Both oxidative stress and aging are known to impair the UPS by increased oxidative modifications of the proteasomal proteins as well as the ubiquitinating enzymes involved in the system.[236,237] Hence, it is no surprise that UPS dysfunction is also a prominent phenomenon in ND etiology, wherein both aging and oxidative stress have been exhaustively implicated. Aggregates positive for ubiquitin have been found in almost all ND pathologies,[238–241] suggesting either that the UPS defense system was probably engaged, yet overpowered by the excess production of the toxic proteins or its efficiency was reduced or some combination of both. Decreased proteasomal function has also been found throughout the ND spectrum,[242–244] thus lending further credibility to this hypothesis. The fact that some of the familial forms of PD are caused by mutations in the genes encoding parkin—an E3 ligase, and UHCL1—a DUB, underscores the importance of this system in PD etiology. Proteasomal dysfunction promotes accumulation of aggregate-prone α-synuclein, which in turn is known to depress UPS function further, thus setting up a vicious circle.[124,242] Thus, disease-specific genetic factors or global oxidative stress could first activate and then impair the UPS, which could eventually cause toxic protein buildup in addition to

other ramifications such as additional oxidative stress, mitochondrial damage, and neuronal death.[67,176]

In addition to the UPS-mediated protein degradation, other proteolytic systems such as lysosome-mediated autophagy,[245] matrix metalloproteinases,[246] and calpains[247] are also implicated in NDs. In all cases, maintaining the tenuous balance between synthesis of essential proteins and the degradation of damaged ones seems to be of utmost importance in preventing the neuronal damage caused by these protein aggregates.

7.2.7 Excitotoxicity

Glutamate is the principal excitatory neurotransmitter in the nervous system that plays a role in synaptic plasticity, learning, memory acquisition, and other cognitive functions.[248] It is involved in fast transmission of neuronal signals by binding to and activating excitatory amino acid receptors/ligand-gated ion channels, which cause receptor depolarization and neuronal excitation. While deficits in this excitatory transmission are known to cause several neuropsychiatric conditions,[249] excessive or prolonged stimulation by glutamate could cause a condition called excitotoxicity, which eventually causes neuronal death. Excitotoxicity has been implicated in the etiology of several acute as well as chronic NDs.[250]

Glutamate receptors can be divided into two categories: ionotrophic and metabotrophic, wherein the ionotrophic receptors are subdivided into NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate. AMPA receptors can be directly activated by glutamate, mediating rapid neurotransmission, whereas NMDARs require prior depolarization by AMPA/kainate receptors for activation.[250] NMDAR activation causes release of Mg^{2+} and an influx of Ca^{2+}, which activate intracellular signaling pathways modulating surface presence of AMPA receptors on the postsynaptic membrane, thus forming a regulatory loop for synaptic plasticity.[251] Usually, glutamate neurotransmission is temporary and it is rapidly reabsorbed by high-affinity pumps located on nerve terminals and astrocytes.[249] Excitotoxicity presents a condition where the reuptake ability of these pumps is compromised due to excess extracellular glutamate, causing prolonged depolarization spiraling into a sequence of events involving disruption of calcium balance, nitric oxide synthesis, generation of ROS, and activation of cell death pathways.[252] Elevated intracellular levels of calcium is the secondary trigger of excitotoxic damage and probably the most profound as it marks the beginning of an irreversible sequence of events culminating in cell death.[250] High intracellular levels of calcium could activate nucleases causing DNA fragmentation, calcium-dependent proteases like calpain causing cytoskeletal breakdown, and lipases causing membrane disruption, all of which are detrimental to cell survival.[253] Hence, the ability to buffer calcium may be the determinant of which cells survive the excitotoxic insult.[254]

Mitochondria are both regulators of calcium and the chief downstream targets of its overload. Mitochondria can help reduce cytosolic calcium by an energy-dependent process, but could also dump toxic levels of Ca^{2+} through the Na^{+}/Ca^{2+} exchanger, when metabolically impaired or physically damaged.[187] Impaired energetics in the mitochondria and the concurrent reduction in ATP levels hamper energy-driven processes within the cell, which is no longer able to maintain its membrane potential.

The already depolarized cell potentials reduce the threshold of glutamate required to cause excitotoxicity, further adding to the damage. The resultant surge in intracellular calcium increases ROS formation, which aggravates the mitochondrial dysfunction that initiated this cycle in the first place.[250] Thus, agents that cause mitochondrial dysfunction by interfering with the electron transport chain or other metabolic processes could theoretically render neurons more vulnerable to even mild excitotoxic insults. This type of slow excitotoxicity is seen in both HD and PD, both shown to have profound impairment in mitochondrial energy metabolism.[255] It is postulated that the loss of nigral cells in PD could have an excitotoxic component,[256] as the damage induced by MPTP—a known mitochondrial toxin—involves glutamate release, and is reversed upon administration of NMDAR antagonists.[257] Also, parkin is known to negatively regulate the number of glutamatergic synapses; mutated parkin implicated in PD causes an increase in the number of synapses, thereby increasing vulnerability to excitotoxic damage.[258] In HD, there is marked decrease in mitochondrial complex II activity, which causes long-term potentiation of the NMDA-mediated synaptic excitation in the striatum.[259] Also, mutant huntingtin aggregates directly disrupt mitochondria, reducing membrane potential and the threshold of calcium required to open the mitochondrial permeability transition pore, whose prolonged opening causes the release of apoptotic factors.[187]

Oxidative stress is also closely tied to excitotoxicity and is often a downstream effect of increased intracellular Ca^{2+}. Free radicals are produced by the action of calcium-dependent enzymes such as phospholipase A_2 (PLA_2), nitric oxide synthase, and xanthine oxidase as well as by dysfunctional mitochondria that result from such high Ca^{2+} levels.[250] In addition to the deleterious effects of free radicals on membrane lipids, essential proteins, and DNA (discussed in Section 7.2.4), they could also cause inhibition of glutamate transporters that reuptake glutamate from the synapses, causing prolonged stimulation of postsynaptic receptors and increased calcium influx, triggering yet another feed-forward cycle resulting in cell death.[189] Inhibition of glutamate transporter (EAAT2) by oxidative modification is one of the mechanisms by which mutant SOD1 causes motor neuron death.[260] In fact, this uptake deficit was found specifically in ALS motor neurons, and not in other NDs, suggesting that excitotoxicity could play a significant role in ALS pathogenesis.[261] Experiments investigating the influence of excitotoxicity on AD etiology revealed that β-amyloid sensitizes neurons to excitotoxic cell death by deregulating calcium homeostasis in cultured neurons.[262] Also, the oxidative stress induced by Aβ oligomers involves an NMDAR mechanism,[263] thus highlighting the potential of excitotoxicity to amplify the neurodegenerative process seen in AD. Thus, although excitotoxicity is generally thought to play a secondary role in the ND etiology, its mechanisms dovetail with the cardinal features seen in most NDs: oxidative stress and mitochondrial dysfunction, causing neuronal death.

7.2.8 ND Etiology: At the Crossroads of Prevention and Treatment

The etiological spectrum of NDs forms an intermeshing network of mechanisms such as oxidative stress, mitochondrial dysfunction, accumulation of protein aggregates, and excitotoxicity (Figure 7.2). In addition to these, inflammatory

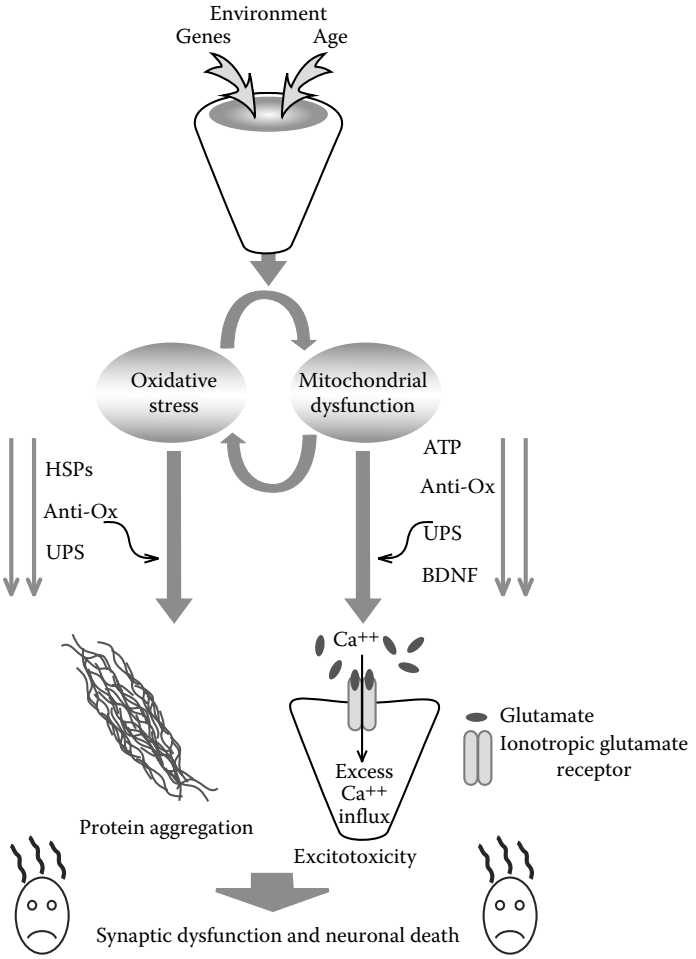

FIGURE 7.2 The molecular etiology of neurodegenerative diseases in a capsule. Genetic and environmental factors combined with accelerated aging are thought to cause NDs. Crosstalk between oxidative stress pathways and mitochondrial dysfunction seems to drive the process. Impaired heat-shock proteins (HSPs), antioxidants (anti-ox), and proteasomal (UPS) degradation of misfolded proteins cause them to aggregate and form plaques, disrupting normal cellular function. Also, reduced ATP production by damaged mitochondria coupled with reduced levels of protective and trophic factors (anti-ox, brain derived neurotrophic factor, [BDNF]) causes excessive membrane depolarization and excess activation by glutamate. This causes a heavy influx of calcium leading to excitotoxicity that eventually leads to synaptic dysfunction, release of apoptotic factors, and neuronal death.

responses have also been noted in the later stages of pathogenesis, especially in AD.[264] Genetic and environmental factors aggravate the effects of the aging nervous system by accelerating the molecular events that cause neuronal dysfunction. Save for purely Mendelian NDs, it is seen that, often what start off as seemingly insidious triggers accrue over a period of many years, gradually weakening the

defense and stress response mechanisms of the body causing neuronal dysfunction and death, thus accounting for the characteristic progressive nature of deficits seen in most NDs. Neuronal death is thought to mainly occur by apoptosis, with oxidative stress, glutamate overactivation, genetic mutations, and decreased levels of neurotrophic factors serving as death signals. The apoptosis is mediated by opening of mitochondrial pores and release of proapoptotic cytochrome c, which triggers the caspase cascade culminating in cell phagocytosis and nuclear fragmentation.[265]

One of the prime goals of preventive therapy in NDs is increasing the healthy interim period before debilitation sets in. This involves identifying mechanisms that deteriorate with age—those that could be fortified to afford the neurons increased ability to fight degeneration. Focus on the early events of ND etiology has delineated key mechanisms that could be preventively averted: oxidative stress, mitochondrial impairment, and impaired stress response. Attenuation of these deficits could involve boosting the adaptive response of neurons by increased synthesis of neurotrophic factors, HSPs, and other prosurvival factors as well as by improving energy metabolism.[155] Diet and exercise are thought to bolster this adaptive response by various mechanisms, thus considerably reducing risk of ND. An understanding of the mechanistic underpinnings of this salutary role is essential for designing better drugs and alternative therapies aimed at preventing the disease.

7.3 PREVENTIVE MECHANISMS MEDIATED BY DIET

Two representative diet paradigms are considered here, as they cover most of the neuroprotective effects of diet vis-à-vis ND prevention.

7.3.1 Dietary Restriction: Less Is Good for the Brain

We are constantly barraged with facts about how overeating is the root cause of all maladies, creating an almost mass paranoia about everything we eat. The situation may actually not be as dire as it seems, but the principle behind the health benefits of low calorie intake is unshakeable. DR—a 10%–40% reduction of calories with maintenance of macronutrient intake—is widely reported to increase longevity and "healthspan" in laboratory animals.[266] Recently, it showed similar beneficial effects on the metabolic health and overall function in primates, although its effects on longevity are ambiguous.[267] Nevertheless, the extrapolation of the beneficial effects of DR to living species beyond the lab spells good news for its potential in humans.

In addition to its effects on survival and overall well-being, DR also slows down age-related changes in the nervous system. It reduces age-inflicted oxidative damage as well as cognitive and motor deficits in mice.[268,269] Since accelerated aging is one of the chief risk factors for neurodegeneration, DR mediated resistance to age-related deficits could potentially delay onset or progression of NDs. Encouragingly so, DR has been found to reverse damage to hippocampal neurons and increase resistance to lipid peroxidation and excitotoxicity in animals models of AD.[270] It also reduced dopaminergic and striatal neuronal loss in animal models of PD[271] and HD,[272] respectively and improved motor function in both. This along with

strong epidemiological evidence supporting the benefits of reduced calorie intake in reducing ND risk[55,75] warrant further investigation.

7.3.1.1 DR Mechanisms: Neurotrophic Factors

Neurotrophic factors, along with neurotransmitters and hormones, belong to the global class of signaling proteins responsible for neuronal survival and synaptic plasticity. The most notable of them—BDNF—is induced by DR. BDNF plays a crucial role in synaptogenesis, nerve outgrowth, and ultimately neuronal survival.[273] Its reduction has also been implicated in the age-related changes leading to memory decline and synaptic dysfunction.[274] BDNF has been known to promote resistance to oxidative damage, metabolic deficits, and excitotoxicity inherent in ND pathogenesis.[273] Its neuroprotective effects stem from its ability to induce the expression of genes encoding for proteins that combat stress: antioxidant enzymes[275] (Cu/Zn-SOD, Mn-SOD, GSHPx, and catalase) and antiapoptotic proteins[276] (Bcl-2 family members). It also stabilizes calcium homeostasis by interacting with glutamate receptors, thereby promoting synaptic stability, as well as by inducing the expression of genes coding for calcium-dependent proteins and the glutamate receptor subunits.[277] These prosurvival actions of BDNF are mediated by two sets of transducers within the cell: the kinases (mitogen-activated protein kinase, protein kinase C, tyrosine kinase [TrkB], Ca^{2+}/calmodulin-dependent kinase), which are the primary transducers and transcription factors (nuclear factor kappa-B and cAMP response element-binding protein [CREB]), which eventually modulate the expression of genes coding for antioxidant enzymes, HSPs, antiapoptotic proteins, and more neurotrophic factors.[182]

The ability of neural progenitor cells to differentiate into neurons and glial cells is crucial to replace cells damaged due to aging or disease. DR is also known to promote this process, known as neurogenesis. BDNF is thought to play a role in enhancing the survival of such newly divided cells, thus linking DR and BDNF to maintenance or perhaps enhancement of cognitive function during aging and diseased conditions.[273]

7.3.1.2 DR Mechanisms: Heat-Shock Response and Protein Chaperones

Heat-shock proteins, as their name suggests, form the first line of defense against high temperatures usually associated with inflammation or infection. Their roles as protein chaperones and their involvement in ND etiology has been previously discussed (Section 7.2.6). DR increases the levels of HSP70 and GRP78 in cortical, hippocampal, and striatal neurons.[273] These molecular chaperones can protect neurons against oxidative and excitotoxic injury[278] and participate in protein remodeling and disposal pathways.[279] It is presumed that DR activates the synthesis of these chaperones through the BDNF-mediated induction of transcription factors as mentioned earlier (Section 7.3.1.1).

DR was also found to offer increased resistance to metabolic and oxidative distress in the synapses, as indicated by maintenance of intact glutamate and glucose transport as well as mitochondrial function.[280] It is also thought that the protective proteins induced by DR concentrate in the synaptic compartments.[277] These findings are important because synapses are pivotal to signal transduction, yet extremely vulnerable to damage due to repetitive calcium influx and oxyradical production.[273]

In a way, they are the "Achilles' heel" of neuronal circuitry with their dysfunction being regarded as a primary marker of aging and neurodegeneration, even before neuronal death. DR thus augments the ability of synapses to cope with the stress prevalent in NDs.

7.3.1.3 DR Mechanisms: Epigenetics

Aging is generally associated with globally decreased, yet locally increased DNA methylation, leading to the silencing of many genes essential for survival. DR is known to reverse these DNA methylation changes and increase genomic stability.[50] DR also plays a role in histone modification, which could cause either gene activation or repression, depending on the type of modification. Histone deacetylation is associated with transcriptional repression as it leads to a more condensed chromatin structure. The enzymes that catalyze the deacetylation of histones are called histone deacetylases (HDACs); their actions are critical to understand how DR affects neuronal survival at the transcriptional level.

HDAC activity is increased during DR, suggesting that global deacetylation may be protective against age-related stress.[281] Of particular importance among the HDACs is the Class III NAD^+-dependent enzyme sirtuin-1 (SIRT1), which is a prime mediator of the health-promoting effects of DR.[282,283] SIRT1 is highly sensitive to the metabolic status of the cell and improves stress resistance of the cell by deacetylating and thus negatively regulating the activities of transcription factors and regulatory proteins.[50] For example, it deacetylates and represses the O subclass of forkhead family of transcription factors (FOXO), thereby inhibiting FOXO-mediated apoptosis.[284] Similarly, its effect on the DNA repair protein Ku70 also prevents stress-induced cell death by sequestering proapoptotic proteins away from the mitochondria.[282] DR-mediated SIRT1 induction could promote cell survival and retard degeneration in many NDs.[285]

7.3.1.4 DR Mimetics: A Shortcut?

Reducing calorie intake is a draconian regimen and likely to have poor adherence by most of the population. Not surprisingly, this has sparked intense research toward finding more convenient alternatives that provide the benefits of DR by circumventing the arduous path of calorie-cutback. DR mimetics such as resveratrol (RES)[50] and 2-deoxy-D-glucose[277] have demonstrated DR-like effects in various pathological conditions. RES, a polyphenol found in grapes, red wine, and berries, has potent neuroprotective effects in many models of NDs.[285] RES-mediated activation of SIRT1, and the consequent suppression of FOXO and p53-mediated apoptosis, is thought to be one of its chief neuroprotective mechanisms.[286] RES also has potent antioxidant effects, although its low bioavailability makes a direct radical scavenging effect less likely.[50] Majority of its antioxidant effects probably stem from its ability to induce antioxidant enzymes and maintain mitochondrial function.[287] In addition, RES also mirrors DR-mediated HSP induction and improved protein handling through a mechanism not related to its antioxidant effects, thus highlighting the versatility of its actions.[288]

DR has met with positive results in all ND models except ALS so far.[99] This could perhaps mean that the negative effects of reduced energy intake nullify/outweigh the prosurvival effects of DR in already undernourished ALS patients or that DR

does not equally benefit all populations of neurons.[266] In fact, ALS patients seem to benefit from a high-energy ketogenic diet.[102] A ketogenic diet might also delay the weight loss seen in the later stages of HD.[289] Thus, DR has overarching effects on all levels of neuronal circuitry including synapses (glutamate and calcium homeostasis), postsynaptic membranes (BDNF and glutamate), intracellular machinery (kinases, transcription factors, and HSPs), the genome (epigenetic modifications), and finally at the level of neuronal differentiation itself (neurogenesis). These mechanisms pose a stronghold against the cellular stress rampant in most NDs.

7.3.2 Mediterranean Diet: *What* You Eat Is as Important as How Much

The Mediterranean diet (MeDi) is heralded as the model for good eating, due to not only its health-promoting effects but also its high palatability.[290] It is characterized by a higher proportion of plant-derived foods (fruits, vegetables, nuts, legumes, and cereals), fish, olive oil, low to moderate intake of wine, and a low intake of red meat and poultry.[291] High adherence to MeDi has been associated with reduced risks of mild cognitive impairment, AD, and PD.[77,292] Although the MeDi combines nutrients that have individually been associated with reduced ND risks, its study as a diet is more pragmatic as people consume food as a whole rather than a mix of disparate nutrients.[292] To date, no synergistic relationship among the MeDi components has been established; however, they seem to impinge on similar molecular pathways en route to their beneficial effects.

Vegetables, especially the green leafy and cruciferous ones (broccoli, cabbage, brussels sprouts, and kale) have been associated with a slower decline of cognitive function with age.[293,294] The MeDi owes most of its neuroprotective properties to a chemical class of compounds called flavonoids found in many fruits, vegetables, cereals, wine, and tea. They are a subclass of naturally occurring polyphenolic compounds historically known for their potent antioxidant effects. However, just like RES, their actions are not restricted to their free-radical scavenging activity; rather, they modulate diverse signaling pathways influencing neuronal survival, memory, and cognition.[295] For a detailed description of the types of flavonoids and the foods that contain them, see Chapter 2, Section 2.5.2.2.

The polyphenols found in fruits and vegetables are mainly of the anthocyanin type and abundant in bilberries, black raspberries, and chokeberries (concentration ranging from 600 to 1500 mg/100 g of fresh weight).[296] Polyphenols mediate some of their antioxidant effects by inhibiting the activity of the calcium-dependent enzyme PLA_2, which is involved in lipid peroxidation and mitochondrial deregulation.[297] They are also excellent metal chelators (e.g., green tea catechins), which are particularly beneficial in AD pathogenesis, wherein transition metals promote Aβ deposition, and also in other neurodegenerative conditions, where metals could lead to free-radical production.[298] Also, some grape-derived polyphenols may potentially inhibit Aβ oligomerization, which is one of the primary goals of AD prevention.[299]

Another fascinating aspect of the repertoire of beneficial effects of flavonoids is their ability to trigger cascades that oversee all aspects of neuronal function including synaptic plasticity, memory, and neurogenesis.[292] It is postulated that they

do so by either increasing the expression of BDNF, thereby initiating the cascade of events similar to those activated by DR, or by directly activating the CREB pathway.[300] Flavonoids presumably bind to the TrkB receptor, which activates CREB-mediated transcription of genes essential for synaptic remodeling.[301] This also activates the PI3 kinase/Akt pathway, which inhibits proapoptotic proteins and promotes neurogenesis.[292] These actions of flavonoids are mildly reminiscent of the prosurvival mechanisms activated by RES, which incidentally is a nonflavonoid polyphenol and another major component of the MeDi found in red wine, grapes, and berries. Since there is considerable overlap between the rescue mechanisms mediated by polyphenols and DR, such polyphenols could probably make excellent candidates as DR mimetics.

Unsaturated fatty acids (UFA) form the next significant component of the MeDi and are found mainly in olive oil, nuts, seeds (MUFA), and fish (PUFA). Olive oil is also rich in antioxidant principles and tocopherol and polyphenols, which probably contribute to the beneficial effects of MUFA. Fish (especially fatty fish) are rich in n-3 PUFA (e.g., docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]), which are thought to have neuroprotective effects in NDs.[302] DHA, the representative n-3 PUFA, is associated with general memory and learning[303] and with reduced Aβ & tau accumulation[304] and reduced apoptotic activity in AD models.[305] Of particular importance in the effectiveness of PUFA is the balance between n-3 and n-6 (linoleic acid is the most common n-6 PUFA), as n-6 PUFA is suspected to have atherogenic effects. Hence a high n-3/n-6 ratio (found in fish oil) provides a better risk/benefit profile in terms of neuronal heath.[68] The neuroprotective effects of UFA mainly originate in the membrane and the processes associated with it. They help maintain the fluidity and the integrity of the membrane, thereby controlling synaptic function and neuronal transmission. They may also modify the activities of several membrane-bound proteins (PLA_2, protein kinase C, and acetyltransferase), neurotransmitter receptors, and ion channels.[292] They could thus conceivably work in conjunction with other factors like BDNF and flavonoids that activate processes beginning at the membrane level.

MeDi is also an excellent source of vitamins that have diverse neurophysiological functions, and whose augmentation could have several beneficial effects. Vitamin E found in nuts, seeds, and green leafy vegetables and is a potent antioxidant with possible effects on gene expression. The verdict from clinical studies of vitamin E as a preventive agent in NDs (AD, PD, and ALS) although not conclusive is promising.[306] Vitamin D_3 found in fish (salmon, mackerel, and blue fish) is associated with enhancement of cognitive functions, mediated by its antioxidant, anti-inflammatory, and neutrophin-inducing effects.[292] Folic acid or folate found in green vegetables, citrus fruits, and whole grains is an essential cofactor in enzymatic processes involved in cell differentiation, proliferation, and survival.[273] Folic acid deficiency is associated with reduced S-adenosylmethionine and increased homocysteine levels; the former is responsible for DNA methylation and could modulate epigenetic pathways, whereas the latter is known to cause oxidative DNA damage and impaired DNA repair.[147]

The MeDi diet thus offers a potpourri of nutrients in a palatable format that individually or through composite mechanisms promotes neuronal defenses and

survival. In addition to the two paradigms discussed in the preceding discussion, a lot of dietary supplements consisting of neuroprotective herbs and antioxidants are proposed to prevent the progression of NDs. They include herbs such as *Panax ginseng*, *Ginkgo biloba*, *Withania somnifera*, *Curcuma longa* and antioxidants such as N-acetylcysteine, Coenzyme Q_{10}, creatine, GSH, and lycopene.[273,307]

7.4 PREVENTIVE MECHANISMS MEDIATED BY EXERCISE

Physical inactivity is positively correlated with the risk of many age-related chronic illnesses including NDs. Studies in humans and rodents have unequivocally established the beneficial effects of regular exercise on cognitive functions.[308–310] It can improve nuanced tasks such as planning, decision making, as well as reverse cognitive dysfunction in both aged and diseased brains.[310,311] Regular aerobic exercise combined with muscle strengthening is expected to act as a disease-delaying strategy by promoting neuronal activity and brain plasticity.

7.4.1 Exercise Mechanisms: BDNF is the Key Player

One of the notable molecular consequences of exercise is the increase in the levels of BDNF—the neurotrophic factor also responsible for most of the prosurvival effects of DR. Exercise promotes the expression of BDNF in the hippocampus, thus providing a molecular base for its actions on learning and memory.[312] Interestingly, inhibiting BDNF also abrogates the exercise-mediated beneficial effects on synaptic plasticity, suggesting that BDNF is essential for exercise-mediated neuroprotection.[313] BDNF activates downstream pathways similar to those discussed under DR mechanisms involving kinases such as mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase (CAMK), which converge on CREB-mediated gene transcription of essential proteins and maintenance of Ca^{2+} homeostasis and glutamate activity, which eventually lead to efficacious signal transduction at the synapses and neuronal survival. Exercise also promotes hippocampal neurogenesis with BDNF suggested to support survival of the newly formed cells.[314] There is a growing body of evidence to show that BDNF acts as a "metabotrophin," a portmanteau coined to suggest that its effects derive from its crosstalk with processes involved in both energy metabolism and synaptic function.[315]

7.4.2 Exercise Mechanisms: Energy Metabolism–Synaptic Plasticity Interface

Energy balance has a marked influence on neuronal and cognitive plasticity.[316] As we now know, defective energy metabolism has multifaceted effects on cellular mechanisms that affect neuronal transmission such as Ca^{2+} homeostasis, glutamate activity, and stress response, which are crucial components of ND etiology. Any entity that perturbs the energy status of the cell (like physical activity) could thus theoretically affect neuronal function. It is postulated that exercise exerts its protective effects on neuronal function by a combined action on processes involved in both energy metabolism and synaptic plasticity.[51] One of the reasons for this assumption

is that its actions primarily stem from BDNF upregulation, which as previously discussed modulates both these systems. Further evidence that exercise upregulates proteins, which are both components of metabolic and synaptic pathways and are also implicated in cognitive function, support the hypothesis that it functions at energy metabolism–cognition interface.[317] Voluntary exercise decreases oxidative stress and improves energy homeostasis in the mitochondria, possibly by increasing the levels of mitochondrial uncoupling protein (UCP2)[293]—a member of the family of mitochondrial inner membrane proteins that allow protons to leak across membranes, thereby reducing OXPHOS and the production of superoxides.[182] They can thus protect neurons against oxidative damage and maintain mitochondrial function under conditions of duress. The oxidative stress-alleviating effects of exercise also contribute to the actions of BDNF and CREB—which in turn activate BDNF expression. Moreover, negative manipulation of mitochondrial metabolism also inhibits the exercise-mediated elevation of BDNF expression and its downstream events, thus confirming the role of exercise in modulating these interdependent systems.

In addition to neural cues, visceral signals of the energy status of the cell are also picked up by the CNS to modulate neuronal functions.[51] Insulin-like growth factor-1 (IGF-1) plays a role in regulating lipid metabolism and insulin action.[102] The IGF-1 receptor is expressed in the hippocampus and known to be involved in synaptic and cognitive function.[318] Exercise stimulates the uptake of IGF-1 into the brain, wherein it modulates pathways downstream to BDNF activation and improves synaptic plasticity.[51,319]

7.4.3 Exercise Mechanisms: Epigenetics

Exercise can control the expression of BDNF by modifying the epigenome, thus providing another example of how the environment can produce long-lasting changes that eventually affect our neuronal circuitry.[52] Exercise promotes chromatin remodeling of the *Bdnf* gene by modulating histone deacetylation and methylation, which in turn cause stable elevations in gene expression, thus providing for a means to regulate synaptic function at the genomic level. Along with enhancing cognitive function, both exercise and BDNF have been linked to reduction of depression—an interesting prospect considering that depression is a prevalent comorbidity in most NDs.[320,321] These epigenetic modifications provide a channel to transfer the salutary effects of exercise across generations. With an ever-growing trove of encouraging results emerging from studies on diet and exercise, it is only natural to investigate whether they could synergize to produce better results. A combination of exercise and DHA supplementation enhances the BDNF-mediated synaptic regulation as well as learning ability.[54] Flavonoids and exercise also synergize to promote cell survival and neuronal plasticity.[322] Additionally, exercise has also been found to reverse the noxious effects of unhealthy diets.[323] Thus, in addition to interaction among themselves, components of a diet also interact with other lifestyle interventions like exercise to amplify the health-promoting influence of one another. This presents an interesting modality that when subjected to standardization and well-designed trials could open up new avenues for the preventive care of NDs.

7.5 SUMMARY AND RECOMMENDATIONS

7.5.1 Summary: Etiology and the Preventive Role of Diet and Exercise

An intricate combination of age, environmental factors, and genetic factors decides whether our nervous system degenerates or not. Environmental factors such as heavy metal exposure or poor diet could potentiate the effects of aging by contributing to the oxidative stress, metabolic dysfunction, and weakened stress response mechanisms. Epigenetic modifications triggered by environmental influences beginning early in life could also have long-term repercussions in terms of ND risk. Finally, disease-specific genes and susceptibility factors are what determine the degeneration of specific anatomical regions within the nervous system, the latter playing a dominant role in the sporadic forms of the disease. Oxidative stress seems to be the constant factor in the complex etiological process. It generates ROS that detrimentally modify proteins, lipids, and DNA. Oxidative modification of proteins by ROS as well as lipid peroxidation products causes them to lose their activity or enhances their tendency to aggregate. This is further enhanced by an age-related decline of protein chaperones and the UPS—two systems responsible for the processing of damaged proteins. Aggregated proteins or amyloid plaques add to the oxidative insult and impair signal transduction, setting the cells up for a programmed death cascade. Oxidative stress also damages mitochondria resulting in metabolic deregulation, Ca^{2+} dysfunction, and further oxidative stress. This self-propagating cycle causes the mitochondrial release of proapoptotic factors and subsequent cell death. Energetically impaired mitochondria also render neurons vulnerable to excessive activation by glutamate culminating in excitotoxicity—a condition characterized by synaptic dysfunction, excess calcium influx, further mitochondrial deterioration, and apoptotic cell death. It is therefore clear that the molecular cascade of events surrounding neuronal degeneration is far from linear, and more often than not, involves cross-communication among various processes. However, there are some key features that stand out, which when targeted offer considerable promise for preventive intervention in NDs. They are oxidative stress, energy dysfunction, and stress response.

The environment has a tremendous influence on our brain functions. Channeling this impact to our benefit is a challenge; modifying lifestyle parameters like what we eat and whether we run may play a significant role in achieving that. Proper diet and regular exercise have favorable effects on the nervous system. They positively influence learning, memory, and cognition as well as motor functions. Eating a low-calorie diet rich in fruits, vegetables, nuts, fish, and olive oil with moderate consumption of wine seems to protect from neurodegeneration. Regular aerobic exercises and muscle strengthening activities also stimulate processes in the body that could ward-off neuronal damage. Both diet and exercise are able to exert their effects by bolstering the expression of neurotrophic factors and stress response proteins, which maintain energy homeostasis and safeguard neuronal function (Figure 7.3). They seem to augment antioxidant and protein chaperone response and initiate favorable epigenetic changes, thus acting at an incipient stage of the disease course.[155] They also aid in the genesis of neuronal cells and help maintain synaptic plasticity. It is speculated that restricting calories and subjecting the body to physical activity provide a kind of preconditioning effect by delivering small bouts of stress, so that the

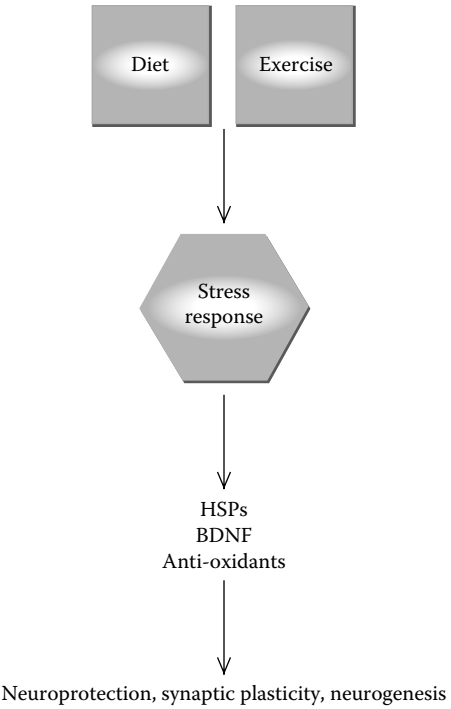

FIGURE 7.3 Diet and exercise stimulate adaptive stress response to provide resistance against neurodegenerative diseases. Diet and exercise stimulate the release of brain-derived neurotrophic factor (BDNF), heat-shock proteins (HSPs), and antioxidants that have multifaceted actions boosting the body's defense mechanisms against stress, thereby promoting synaptic and neuronal plasticity as well as neuronal differentiation.

body could boost its adaptive response for a future, more severe insult. This effect is called hormesis[182] and probably explains the actions of many dietary constituents whose effective body concentrations are too low to have a direct consequence.

Diet and exercise could potentially intervene early, which is ideal for the almost traceless progression of these diseases. ND diagnosis is usually based on the manifestation of clinical symptoms, which is often a point of no return as far as the disease course is concerned. Diet and exercise have multimodal neuroprotective effects, which are desirable in preventing or delaying the progression of diseases with such complex etiology. Thus, maintaining a low-calorie diet with sufficient "brain-healthy" foods and thorough exercise regimen (see recommendations in Section 7.5.2) may well be the elixir to keeping these malicious, yet fascinating diseases at bay.

7.5.2 Dietary Recommendations

Oxidative stress being a global factor in the molecular etiology of NDs as well as inflammatory diseases, the recommendations suggested in Chapter 2 on Inflammation (Section 2.7.3) mostly apply to ND prevention. The crux of the dietary

protocol rests on reduction of calories as well as consumption of brain-healthy foods. It relies heavily on plant-derived foods and fish to meet the body's need for energy and macronutrients. It is inspired from the elements of the MeDi, which consists of foods rich in polyphenols, especially flavonoids. Consumption of one serving of low-fat dairy per day is recommended (approximately 120 kcals), to maintain calcium intake. The caloric content of the total recommendations including one serving of dairy is approximately 1500–1700 kcals, with fat constituting a relatively lower share of the total caloric intake. An exception to this caloric restriction is for ALS patients; they seem to benefit from a high-calorie, high-fat diet, which is low in carbohydrates[101,102] (fat:carbohydrate ratio—approximately 4:1). Nevertheless, to maintain a healthy eating pattern, it is recommended they follow a similar diet plan but with modifications (ALS modifications are listed alongside in parentheses).

Finally, since disease prevention is a long-term goal, it is impractical to expect an austere adherence to the recommended diet. Indulging in a treat once in a while is okay, as long as there is not too much digression from the optimum calorie intake and adequate intake of healthy foods.

Fruits—Minimum of 2.5 cups each day with at least three different selections from each category each week:

A: elderberries, blueberries, pomegranate, blackberries, raspberries, Saskatoon berries, blackcurrant, raisins, figs, or prunes
B: plums, oranges, grapefruit, lemons, cantaloupe, cherries, cranberries, red/black grapes, or apples
C: kiwi, bananas, pineapples, mangos, pears, star fruit, guava, strawberries, peaches, or any other fruit

Vegetables—Minimum of three one-cup servings each day with at least three different one-cup selections from each category each week:

A: chili peppers, broccoli, water cress, garden cress, spinach, arugula, and Brussels sprouts
B: cauliflower, tomato, lettuce, green beans, sweet peppers, and red cabbage
C: onion, garlic, celery, green cabbage, asparagus, and Swiss chard
D: squash, zucchini, pumpkin, sweet potatoes, carrots, and potatoes

Nuts and Seeds—Minimum of one one-quarter cup serving each day with a minimum of three different selections from each category each week:

A: chestnuts, pecans, walnuts, and pistachios
B: hazelnut, almonds, Brazil nuts, cashews, macadamia nuts, or peanuts

Beans and Peas—Minimum of one one-cup serving each day with a minimum of two different one-cup selections from each category each week:

A: black beans, Adzuki beans, kidney beans, peas/pea pods, and lima beans
B: lentils, pinto beans, chili beans, chick peas, and navy beans

Whole Grains and Cereals—Minimum five 1-oz servings of whole grains each day with a minimum of one selection from each category each week: (—one to two servings per day in the case of ALS patients)

A: buckwheat, wheat, and bulgur
B: oats, corn, and barley
C: wild rice and rice

Oils—Used for cooking, salads, and dipping; approximately 1 tbsp/day (2 tbsp in case of ALS patients)

A: Extra virgin olive oil

Meats—Minimum of two servings of high EPA/DHA fish each week (four servings in case of ALS patients)

Beverages—In addition to water, tea, and coffee, consumption of red wine (one 4–5 oz glass for women and two for men per day) is recommended for its high phenolic content.

7.5.3 Exercise Recommendations

In addition to the requirements for aerobic exercises recommended in Chapter 2, additional muscle strengthening, flexibility, and balance training exercises as suggested by the American College of Sports Medicine/American Heart Association nexus are also recommended. The muscle strengthening exercises consist of 8–10 exercises (10–15 repetitions for each exercise) on two or more nonconsecutive days per week, involving the major muscle groups. Weight-bearing calisthenics and other resistance training exercises could be incorporated to maximize muscle strength. Flexibility exercises (8–10 exercises), once again focusing on major muscle groups (3–4 repetitions and holding each repetition for 10–30 seconds), for a minimum of 2–3/week, as well as balance training exercises for 3 days/week are also recommended.[324]

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8 Hunger and Satiety Signaling

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CONTENTS

8.1 INTRODUCTION

Food intake is a complex behavior that is nonetheless quite familiar since most of us engage in it several times a day. The complexity derives from the flexibility inherent in most of its parameters and the integrated activity of numerous and diverse influences. This short chapter reviews what is known of the factors that influence food intake in humans and other general omnivores, that is, animals (mammals in this instance) that consume, and are able to derive usable energy from, almost any type of food or nutrient.

8.1.1 Why We Eat

Eating is the behavior that takes nutrients into the body from the environment. Nutrients include both micronutrients (minerals and vitamins) and energy-containing macronutrients (carbohydrates, lipids, and proteins). It is axiomatic that under normal circumstances eating is the primary source of energy for the body and that if an individual is going to be weight stable energy intake must equal the energy expended during exercise and metabolism over an extended period of time. Taking in more energy than is expended necessarily leads to weight gain and vice versa.

The other important factor in the calculus of eating and energy balance is that unlike the case for other commodities such as water, consumed but not yet used energy can be stored in the body, mainly in adipose depots (fat tissue). Eating less energy than is expended, at least in the short term, results in more of this previously stored energy being used, and when this occurs chronically body weight is lost primarily from body fat. Taking in more energy than is expended builds up the adipose tissue reservoir, and this is presumably what is happening in the contemporary *epidemic of obesity.*

8.2 NEUROBIOLOGICAL CONTROLS OVER FOOD INTAKE

Eating is a behavior and as such is controlled and coordinated by the brain; an excellent review of this control is found in the work by Langhans and Geary.[1] Historically, it was thought that there are two critical brain *centers* that control the intake of food. The lateral hypothalamic area (LHA) was called the eating center because when it was electrically or pharmacologically stimulated in experiments, animals started eating. Chronic stimulation of the LHA resulted in animals becoming obese, that is, having excess stored fat. Experimental destruction of the LHA resulted in animals that ate very little or not at all and that often died without food being infused directly into their stomachs. In contrast, the ventromedial hypothalamic nucleus (VMN) was called the satiety center because when it was stimulated animals stopped eating and when it was destroyed animals overate and became obese. Both the LHA and the VMN receive sensory input related to energy needs and reserves, and the two were considered to work in a reciprocally coordinated manner to determine eating.

It is now recognized that this model is overly simplistic and that diverse circuits interconnecting numerous brain areas are involved.[2–5] Relying on receptors located throughout the body as well as within the brain itself, numerous brain circuits are made continuously aware of relevant information regarding energy status throughout the body. This includes knowing about immediately available energy (e.g., blood glucose), energy being consumed or that was recently consumed and awaiting entry into the blood from the intestines, energy stored in adipose tissue, energy stored in liver and other tissues, and so on. This information is integrated with knowledge of current energetic needs or anticipated needs, environmental factors such as where and when food might be available, the social situation (predators, offspring, conspecifics), memory of past experiences, hedonic factors, and many other factors as well (see the reviews by Seeley and Woods[6] and Levin et al.[7]).

Based on these myriad inputs, the brain is able to make informed decisions about when to eat, what to eat, how much to eat, and so on and to integrate food seeking and consumption with other behavioral and metabolic priorities. Another fundamental mandate of the brain is to ensure adequate circulating energy (i.e., blood glucose and fatty acids) for immediate tissue needs.[6–8] Eating is consequently well coordinated with the control of plasma glucose, fatty acids, and other nutrients. Under most circumstances, the levels of energy-rich fuels in the blood are relatively constant, with extraction from the blood by active tissues matched well to secretion into the blood by liver and fat cells, and this is true at rest as well as during exercise.

Meals are the major exception to this rule as they are times of elevated plasma glucose and other nutrients. An important mandate of the brain is to restrain meal size so as to circumvent excessively large increments of plasma nutrients while nonetheless allowing adequate nutrients to be consumed.[9–11] This is accomplished in large part by knowing when meals are likely to occur and making appropriate anticipatory responses that keep plasma fuels as low as possible during and soon after eating. This requires coordinating ongoing information about when food is likely to be available, calories being consumed (via satiation signals), the levels of fuels already in the plasma (via direct sensing by specialized cells in the brain and elsewhere), and the amount of energy present in various storage depots (via adiposity signals). In sum, a key function of the brain is to coordinate diverse processes that allow optimal circulating and stored levels of energy-rich nutrients.

8.3 HOMEOSTATIC VERSUS NONHOMEOSTATIC INFLUENCES OVER FOOD INTAKE

Eating is often considered a homeostatic behavior, that is, a reflexive response to insufficient energy, either in the blood or in its ongoing usage by the brain. Glucose supply to the brain is a key variable since unlike most other tissues, nerve cells obligatorily derive energy mainly from glucose and, because very little energy can be stored within the brain, it requires a steady stream of adequate glucose input via the circulation. This need for continuous glucose was the basis of the popular glucostatic theory of eating,[12–14] and it posited that when immediately available glucose is low the brain responds by initiating eating. The intended consequence of this low glucose–induced eating was an increase of glucose entering the blood from the intestines, and this in turn was thought to provide a signal to the brain to stop the ongoing meal. Although simple and appealing, the theory fell into disfavor because levels of glucose within the normal physiological range have little or no impact on eating behavior.[15,16] It is true that if the blood glucose level or glucose utilization in the brain is lowered to extremely low levels eating is initiated, but this is considered an emergency response necessary to prevent coma or death.[17] Thus, although eating in this situation of extreme immediate glucose need can certainly be considered homeostatic, it does not reflect normal eating.

Eating can perhaps better be considered a homeostatic behavior with respect to adiposity.[18] The amount of fat in the body is generally thought to be regulated. When individuals lose weight due to dieting or insufficient available food, they tend to regain the weight to precisely their former level given sufficient time with adequate food availability. Likewise, when otherwise weight-stable individuals voluntarily overeat and gain weight in experiments, they tend to lose the excess weight and return to basal levels over time once the period of overeating is over.[19] All of these data are consistent with the concept of a strict regulation of body weight (body fat) by the brain, and with the act of eating being recruited to assist in the regulation. An important point is that most of the energy available from any individual meal is not available for a considerable interval after the meal is ended, such that the time constant for considering the level of adiposity as a homeostatically regulated behavior is much longer than for other instances of homeostasis. In this light, eating can

be considered a behavior that is used in the homeostatic regulation of body weight, but only minor adjustments are possible to be made at any one time or meal.[11,18] In fact, considerable evidence suggests that in humans matching food intake to energy expenditure often takes days or weeks to be accurate.[20,21] In contrast to food intake being a homeostatic slave to the regulation of body weight, there is compelling evidence that most instances of food intake are influenced to a large extent by nonhomeostatic factors.[10]

8.3.1 Meals

Meals are the units of food intake, and an important point is that the factors that determine when meals will start are different from those that determine when meals will end. As indicated in Section 8.3, single factors such as glucose availability or stomach contractions were historically thought to determine both meal onset and meal offset, but these single-factor theories have not withstood the test of time. With respect to when meals are taken, the best evidence is that meal initiation is based on habit, convenience, social situation, opportunities, time of day, and so on. Such a flexible system that is not tied to metabolic need allows optimal coordination of meals with other activities.[10]

In contrast to meal initiation, the amount of food that is eaten once a meal has begun is more directly linked with metabolism and overall energy homeostasis. During a meal, ingested food is continuously analyzed for its nutrient composition, and this begins even before it enters the mouth. The sight and smell of many foods initiate the secretion of hormones and gastrointestinal (GI) enzymes that will facilitate the processing of what is about to be eaten.[22] For individuals who habitually eat at the same time each day, these same secretions are initiated as that time approaches. These secretions are collectively called cephalic responses since they are initiated by the brain around mealtime but prior to any ingested food actually being absorbed into the blood. Cephalic responses readily become associated via a classical conditioning process with stimuli that reliably predict the onset of meals, such as time of day or certain environments.[9,22] This is a useful adaptation in that it enables the GI system to better prepare to digest and absorb nutrients efficiently while preventing prandial excursions of blood glucose from getting excessively high. If an individual is prevented from making premeal anticipatory cephalic responses, and nonetheless eats the same-sized meal as he or she otherwise would, blood glucose rises into the diabetic range during and soon after the meal.[9]

8.3.2 Signals That Contribute to Meal Termination (Satiation Signals)

Satiation is the phenomenon of *fullness*, and in experiments its occurrence heralds the end of a meal and hence determines meal size. Satiety, on the other hand, refers to factors that prolong the start of a second meal after a first one has ended in freely feeding individuals. Satiety is generally considered to result from a signal that is generated in the processing of a first meal and that has a long half-life; as its levels wane below some threshold, the urge to eat is initiated. However, although the concept of satiety may apply to freely feeding individuals in an environment devoid of temporal

and other possible food cues, other, nonhomeostatic, factors, as discussed earlier in Section 8.3, are more likely to determine when meals begin. Although little is known about satiety, considerable information is now available about satiation.

The GI system begins at the mouth and ends at the anus. Each segment along the way is characterized by numerous specialized cell types. These include muscles, secretory cells, receptor cells, and many more. Taste receptors are clustered in groups (taste buds) on the tongue as well as being scattered over much of the oropharyngeal region, and they have recently been identified on intestinal cells as well, implying that specific tastants in meals are being detected throughout the GI tract and the progress of individual nutrients can thus be tracked and appropriate hormones and enzymes secreted at the right time, customizing the digestive process to what is being eaten.[23,24]

In the mid-twentieth century, a paradigm shift occurred that moved theorizing about the controls of food intake from the simplistic single-factor theories such as glucose utilization to a neuropeptide-centric model of energy balance control. In 1973, it was discovered that the duodenal peptide cholecystokinin (CCK) decreases food intake when administered to animals.[25,26] CCK is produced predominantly in I-cells in the initial part of the small intestine after the stomach, the duodenum, and it is released prandially in response to specific nutrient signals in consumed foods such as fatty acids and amino acids.[27] Prior to this breakthrough work on satiation, CCK had been characterized for its role in other digestive processes, including gastric emptying, stimulation of the gall bladder and bile flow and pancreatic secretions.[28–30] The hypophagia produced by CCK administration was considered to be part of a negative feedback loop, such that when partially digested nutrients are sensed in the duodenum CCK is released to aid digestion and reduce further food intake. When the flow of newly consumed nutrients into the duodenum stops, CCK secretion is no longer stimulated.

When CCK is released from I-cells in the wall of the duodenum, some of it enters the blood to produce hormonal effects in other organs. In addition, some of the secreted CCK molecules act locally on CCK receptors (CCK-1R) located on sensory endings of fibers of vagal afferent nerves that pass to the hindbrain where they interact with other signals to produce satiation. If CCK activity during a meal is prevented by administering antagonists to CCK-1R, animals and humans eat larger than normal meals.[31,32] This implies that our own endogenous CCK normally limits meal size and is an important component of satiation response.

Although there has been considerable interest in utilizing CCK-1R agonists to reduce food intake in overweight or obese humans, experiments to date have not been efficacious.[33] This is due in part to the fact that whereas the administration of CCK at the onset of each meal always reduces the amount eaten in that meal, individuals compensate by eating more often, that is, they increase meal number, leading to no net reduction in daily energy intake.[34] More recently, several longer acting formulations of CCK agonists have been developed and may prove therapeutically beneficial for weight loss.[33] Despite the disappointment of CCK as a weight loss agent, the demonstration that a GI peptide secreted during meals can impact food intake changed the way the neurobiological controls of food intake have been examined over the past 40 years.

Numerous other peptides secreted by the GI tract during meals have since been reported to act as satiation signals.[27] The list is long and growing, and it includes peptide YY (PYY), pancreatic polypeptide, glucagon-like peptide-1 (GLP-1), glucagon, amylin, somatostatin, uroguanylin, and others. It is important to note that each of these gut peptides is secreted in response to specific properties of the food being digested, and each has unique functions in causing appropriate enzymes or other molecules to be secreted into the intestinal lumen or else to speed up or slow down the passage of the partially digested food (or chyme) along the GI tract. What these peptides share in common is an ability to limit meal size when they are administered just prior to the onset of a meal. One gut peptide, ghrelin, is synthesized primarily in the stomach and has a functionally opposite action on food intake. When administered to humans or animals, ghrelin initiates meals and causes overeating.[35,36] If the time that a meal will occur is well known, individuals secrete most of these peptides cephalically, beginning an hour or so before the meal begins[9,22,37–40] (see Figure 8.1), presumably to jump-start the digestive system and consequently allow more efficient digestion without the risk of hyperglycemia.

To summarize, food intake (i.e., meals) tends to be initiated at times that are convenient or habitual. At every step of the way along the GI tract, the food is analyzed by sensory cells. Depending on the nutrient content of the food, a customized cocktail of peptides is secreted to facilitate digestion and nutrient absorption. These same peptides generate hormonal and neural signals that are relayed to the brain, enabling coordination among the various organs involved in digestion. The signals also combine to create the sensation/perception of fullness and satiation.

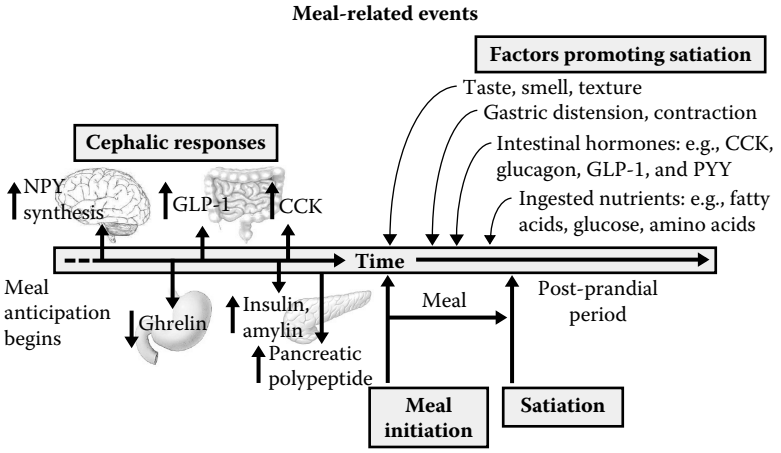

FIGURE 8.1 Different parameters and signals in anticipation of, during, and following a meal: cephalically induced hormone and neuropeptide release occurs to prepare the body for an influx of nutrients and to prevent potentially damaging hyperglycemia. During a meal, many factors may act as satiation signals. When the meal is completed, many of the same peptides help complete the digestion process.

8.3.3 Signals Related to Adiposity

Food intake is also influenced by the amount of fat stored in the body, or adiposity. Several hormones are secreted into the blood in proportion to adiposity, and they circulate to the brain and interact with receptors in many brain areas, especially in the hypothalamus. These hormones are called adiposity signals because they provide continuous feedback to the brain about how much fat is in storage, and this information can then be integrated with other information related to metabolism and energy balance. Insulin, a hormone secreted by the endocrine pancreas that is best known for its blood glucose–lowering action, was the first identified adiposity signal.[4,41] Insulin is secreted in direct proportion to adiposity, and it acts in the hypothalamus to cause a net catabolic action, that is, it reduces food intake and lowers body weight. Leptin, a hormone secreted by fat cells, is also secreted in direct proportion to body fat and, like insulin, is transported from the blood into the hypothalamus and other brain areas where it acts on leptin receptors on neurons influencing energy homeostasis. Also like insulin, when leptin is administered into the brain animals eat less food and lose weight. There are many reviews of these phenomena.[4,42–44] The point is that an experimental or therapeutic increase of either the insulin signal or the leptin signal in the brain conveys the message that the body is fatter than it actually is, and the brain responds by reducing food intake. Conversely, when either the insulin signal or the leptin signal is reduced within the brain, overeating and body weight gain occur. A number of other compounds have been proposed to be functional adiposity signals, including amylin,[45] which is cosecreted with insulin from the pancreas, and several adipose tissue–derived cytokines including interleukin-6 and tumor necrosis factor-α. However, most research on adiposity signals has been focused on leptin and insulin.

Adiposity signals are transported across the blood–brain barrier from the blood into the brain by a receptor-mediated, saturable pathway in brain capillary endothelial cells.[46,47] Within the brain, adiposity signals act on their respective receptors located in several areas, especially within the arcuate nucleus (ARC) of the hypothalamus, a region that receives and integrates diverse information pertinent to energy balance; that is, in addition to having receptors for insulin and leptin, ARC neurons are sensitive to individual nutrients (such as glucose and some fatty acids and amino acids), to satiation signals, and to inputs from numerous brain areas involved with hedonics, learning, biological rhythms, and others.[48,49] The ARC contains two major types of neurons that integrate all of these inputs and project to other hypothalamic nuclei as well as to other brain areas (Figure 8.2). The neurons located predominantly in the ventromedial portion of the ARC synthesize and secrete two neuropeptides, agouti-related protein (AgRP) and neuropeptide Y (NPY).[4,43,50] These neurons primarily project to the hypothalamic paraventricular nuclei (PVNs) and the lateral hypothalamus (LH). Electrical or pharmacological stimulation of NPY/AgRP neurons produces an anabolic response that includes a rapid initiation of food intake and reduced energy expenditure. Animals consume excessive food (i.e., are hyperphagic) and become obese when AgRP or NPY is administered chronically. The adiposity signals insulin and leptin have an inhibitory action on these neurons, reducing food intake and causing weight loss when administered chronically.

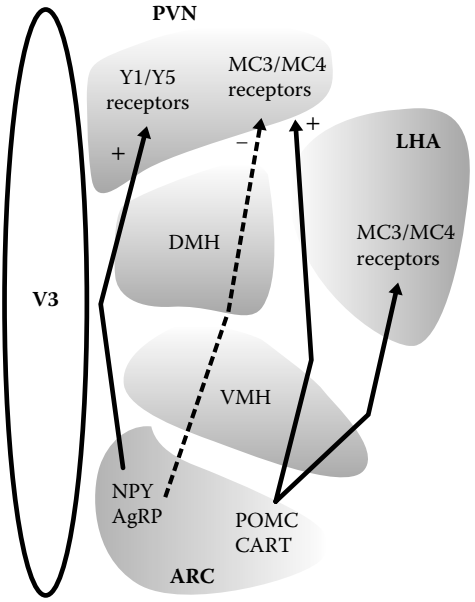

FIGURE 8.2 Neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC) project to the paraventricular nucleus of the hypothalamus (PVN): NPY acts on Y1/Y5 receptors in PVN to stimulate food intake; simultaneously, AgRP acts to inhibit MC3/MC4 receptor signaling to further increase food intake. Proopiomelanocortin (POMC) neurons project to MC3/MC4 receptors in the lateral hypothalamic area (LHA) and the PVN, releasing α-melanocyte-stimulating hormone to inhibit food intake. The depicted unilateral view occurs on both sides of the 3rd ventricle (V3). DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus.

The lateral region of the ARC (see Figure 8.2) contains neurons that synthesize two different neuropeptides, proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). POMC is a large prohormone that is the precursor molecule for several active neuropeptides, the exact neuropeptides generated in any cell depending on the cleavage enzymes present. In the ARC, POMC is cleaved into the active neuropeptide α-melanocyte-stimulating hormone (αMSH), which is secreted from the axon terminals of the ARC POMC neurons and acts on melanocortin receptors (MC3 and MC4). The axons of POMC/CART neurons project to the PVN and LH, where they elicit their catabolic effects by inhibiting food intake and increasing energy expenditure.[5,43] Insulin and leptin act on POMC/CART neurons to release αMSH, and intact melanocortin receptor signaling is required for their hypophagic effects. Several excellent reviews on this ARC integrative system that influences energy homeostasis are available.[5,51,52]

8.3.4 Interactions of Satiation and Obesity Signals

Given all of these different kinds of controllers over energy balance and influences on food intake, it is important to consider how they interact. As discussed earlier in Section 8.3.1, eating can best be considered in terms of meals, with daily food intake

being based on both meal frequency and meal size. Many types of experiments have revealed how these meal parameters can interact. When constraints are put on meal size such that only small amounts can be consumed at one time, animals eat more meals per day and tend to keep their body weights relatively constant.[34] Analogously, when the number of times a day an individual can initiate a meal is limited, meal size increases and weight remains stable.[53] This flexibility of meal patterning in the defense of body weight implicates a strong influence of adiposity signals over meals and total food intake.

As discussed in detail earlier in Section 8.3.2, satiation signals such as CCK are generated during meals in response to the quality and quantity of nutrients being consumed. As the various satiation signals accumulate, they eventually cause the meal to stop.[10] The sensitivity of the brain to satiation signals is based in part on adiposity signals, and their levels in turn are a function of how fat or thin the individual is. If an otherwise weight-stable individual goes on a diet (or does not have sufficient food to maintain weight), the levels of insulin and leptin that are secreted decrease in proportion to the loss of body fat. The lower insulin and leptin signal in turn reduces sensitivity to satiation signals. As a result, more food is eaten during a meal before satiation occurs, that is, larger meals are eaten, and this will continue until the weight that was lost is regained. Conversely, when animals or people are experimentally overfed and gain weight, the insulin and leptin signals are increased and there is increased sensitivity to satiation signals. Smaller meals are eaten and weight is lost. Relevant experiments can also be done in other ways. For example, when a normal-weight animal is administered a very small amount of insulin[54] or leptin[55] into the brain, the ability of CCK to reduce meal size is greatly enhanced. This influence over meal size by satiation signals is one reason why weight tends to remain stable in individuals over long periods and also a reason why body weight often rebounds when one's enthusiasm for a diet regimen wanes.

8.4 SUMMARY AND RECOMMENDATIONS

8.4.1 Summary

Numerous questions have been considered with respect to the consumption of food, including the timing, content, and size of individual meals. Although food intake is often considered a homeostatic behavior, in actuality it is influenced by a combination of both homeostatic and nonhomeostatic factors; thus, evidence does not support the concept that food intake is a reflexive, homeostatic-type response to an acute shortage of energy. Alterations of food intake over long intervals are consistent with a homeostatic control of body fat, with adiposity signals such as insulin and leptin influencing food intake based on energy levels stored in adipocytes. Short-term feedback is provided during meals that is based on nutrient content and is signaled to the brain by GI peptides including CCK, PYY, and GLP-1. These are considered satiation signals because they are released in response to the ingestion of food and act to reduce meal size.

The times at which we eat are largely determined by habit, convenience, or opportunity rather than a depletion of stored energy. A coordinated brain-initiated cephalic secretion of numerous hormones occurs before anticipated meals and primes the

digestive system to better prepare for the upcoming caloric load. These cephalic responses are easily entrained to routine feeding times. Superimposed on these metabolic factors are diverse interacting nonhomeostatic factors, including stress, learning, palatability, social influences, and many others.

As discussed earlier in Section 8.3.4, satiation and adiposity signals interact to determine meal size, providing compelling evidence of a homeostatic influence over body weight/adiposity. At the same time, such control makes eating seem automatic or robotic, which, of course, it is not. The reason is that homeostatic influences should be considered as providing a background tone, with other factors intervening and/or overriding much of the time. We are all familiar with feeling full or satiated after a particularly large meal but nonetheless eating more when an appealing dessert is presented. Hedonics, experience with certain foods, opportunity, social situation, habits, stress, and emotions, as well as diverse other factors, can all impact the amount eaten in any given meal.[56] There is also evidence that the ability of satiation signals to influence meal size can be changed by learning and experience.[10] The plethora of hedonically pleasing and calorically rich foods that is constantly available in many societies is often considered a major causal factor in the general increase of body weight known as the obesity epidemic. The important point is that despite the tightly integrated homeostatic factors that control food intake and body weight, these controls are easily trumped by nonhomeostatic influences. Chronic intervention by nonhomeostatic factors can lead to increased body weight and associated detrimental health outcomes, including diabetes and cardiovascular disease.

8.4.2 Recommendation

Most people are unaware of how powerfully nonhomeostatic cues can affect eating behavior. Based on the habitual nature of eating, it therefore might be tempting to recommend that dietary interventions should be approached using behavioral therapy. The primary goal for such therapy would be to learn, be conscious of, and permanently change dietary behavior to a pattern consistent with a healthier lifestyle. However, such dietary changes are generally only effective in the short term, with most weight being regained after a number of months or years.[57,58] Approved pharmacological treatments, including appetite suppressants and lipid absorption inhibitors, are generally effective in producing long-term weight loss so long as treatments are continued. However, current formulations generally produce only modest weight loss. Surgical interventions comprise an attractive alternative for some obese individuals in that besides causing sustained weight loss over many years they also improve glucose homeostasis.[59,60] Successful bariatric surgery often leads to substantial reductions in food intake and changes in meal patterning and food preferences, including lowered dietary fat intake following surgery.[61,62] Given the ineffectiveness of alternative treatments, bariatric surgeries provide a viable treatment option for some obese individuals.

ACKNOWLEDGMENT

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9 Summary and Recommendations

C. Murray Ardies

CONTENTS

9.1 OVERVIEW

Probably the most important concept that has emerged from each of these chapters is the pervasive role that inflammation-associated signaling plays in the etiology of diabetes, osteoporosis, atherosclerosis, cancer, and neurodegenerative diseases (NDs), chronic diseases that are responsible for the vast majority of the many hundreds of billions of dollars spent as annual direct and indirect costs for health care in the United States. The second is that the prevailing inadequate dietary and activity habits of Americans compromise cell functions and enhance proinflammatory signaling to such a degree that they are major contributors to the etiology of these chronic diseases. The detrimental effects of nutritionally insufficient diets, consuming too many calories, and participating in too little physical activity and the impacts of poor diet and activity habits on the progression of chronic diseases are profound. In essence, it is through our lifestyle of mindless ease and plenty that so many of us are in a constant, low-level proinflammatory state. The term *mindless* simply refers to the idea that most people are completely unaware of how detrimental their lifestyle really is to their own health, and even if they are aware they rarely change their behaviors. As a result, a large majority in the United States is highly susceptible to cellular and tissue dysfunctions from any cellular stressors regardless of their source and therefore at high risk for chronic disease.

On a positive note, a tremendous amount of information has been reviewed here to illustrate that many of these risks, risks that we have come to accept as normal, do not really have to exist. With appropriate changes in food choices to

ensure a well-balanced and nutritionally adequate diet that also includes the consumption of a wide array of protective phytochemicals, and with the addition of vigorous physical activities to our daily lives, many adverse cellular stresses can be handled with ease and the proinflammatory environment dampened, along with reducing the risks for many chronic diseases. A proper diet will ensure that optimal amounts of nutrients are available so that normal cell functions are not compromised by nutrient inadequacies. Increasing consumption of protective phytochemicals and participating in vigorous activities produce transient changes in the cellular redox microenvironment, which then modifies the activities of various intracellular signaling pathways. These changes result in the enhanced expression of many different proteins that enhance cellular redox control, protect cells from oxidative damage, attenuate proinflammatory signaling, and enhance repair of damage.

On the basis of these excellent reviews of very complex biological processes, the major effects of diet and exercise on the etiology of chronic disease are summarized next and specific recommendations are made for altering lifestyles to optimize prevention.

9.1.1 Diet and Chronic Disease

Because the vast majority of Americans consume a diet that is insufficient in one or more nutrients, the ability of cells to synthesize the wide array of essential proteins and enzymes that maintain metabolic and redox control, maintain damage-free DNA and RNA, repair and/or replace damaged proteins and cellular phospholipids, and perform any of the other cellular functions that are necessary for cell survival is certain to be less than optimal. Essentially, immediate-need metabolic activities that contribute to very-short-term cell survival (such as adenosine triphosphate [ATP] synthesis) take precedence over other cellular functions that are not required with such immediacy (such as DNA repair). These immediate-need functions, if they are met by depleting (or significantly diminishing) local nutrient *supplies*, can end up short-changing longer term functions for cell survival that also depend on an optimal supply of the same nutrients.

Cellular survival is dependent, in a large part, on the appropriate regulation of the cellular redox state. This is because the activities of the various signaling pathways that regulate the activation of transcription factors ultimately depend on the local redox microenvironment at different locations throughout the cell. Because a majority of Americans eat less than the estimated average requirements (EAR) for vitamin C (25%), vitamin E (60%), selenium (5%), iron (5%), zinc (8%), and copper (6%), nutrients that either function as antioxidants or are components of antioxidant and redox-control enzymes, antioxidant function and redox control are certain to be less than optimal for most. Dysregulation of antioxidant and redox control leads to a cascade of effects that can result in an enhanced synthesis of a variety of signaling molecules that lead to proinflammatory effects. Although small alterations in the microenvironment at the cellular level due to dietary insufficiencies are rarely if ever fatal to the cell (and they certainly are not noticeable to the individual), they will lead to enhanced inflammatory risks. Production of reactive oxygen species (ROSs) is a common by-product of normal metabolism, and with reduced antioxidant and redox control a more oxidizing microenvironment within the cell is the result. This tends

to elevate ROS-mediated activation of eicosanoid and cytokine synthesis to produce a mildly proinflammatory environment. These proinflammatory signaling responses that are responsible for enhancing the risk for chronic diseases are, however, not often those of a *classic* inflammation response that is often described as following infections or cuts and scrapes. Rather, they are characteristic of a cellular stress response, one that produces many of the same end results. In general, the production of proinflammatory signaling molecules results in the activation of resident inflammatory cells (macrophages, dendritic cells, and mast cells, among others) and the infiltration of the local tissue by circulating inflammatory cells (neutrophils, monocytes, and platelets, among others). This results in additional proinflammatory signaling by the activated inflammatory cells as well as enhanced damage to cellular proteins, DNA, and membrane lipids through various ROS/reactive nitrogen species (RNS)-mediated mechanisms because of an increased production of ROS/RNS by the activated cells. In addition to inflammation-associated damage, alterations in cell functions due to responses to the various inflammation-associated cytokines occur. While the common nutrient insufficiencies are important in exacerbating proinflammatory signaling by compromising both antioxidant function and redox control, overconsumption of calories introduces another form of stress with proinflammatory consequences.

As described in Chapter 4, the metabolic stress of constant fatty acid overload in adipocytes leads to endoplasmic reticulum stress and dysfunction. This stress leads to increased JNK activity as well as the release of excess free fatty acids (FFAs), various eicosanoids, and cytokines, including tumor necrosis factor-α (TNF-α) from the adipocytes. The TNF-α and FFAs that enter the circulation are associated with mitochondrial dysfunction and insulin resistance in both adipocytes and skeletal muscle. The eicosanoids, along with TNF-α, and other cytokines attract circulating monocytes into the adipose tissue as well as act in a paracrine fashion to activate local tissue macrophages and preadipocytes (progenitor cells for adipose tissue) to initiate adipocyte hyperplasia. The accumulation of new cells then leads to hypoxia-mediated activation of a variety of cell activities, including synthesis of growth factors by vascular endothelial cells, macrophages, and fibroblasts to stimulate angiogenesis to support the new tissue cells. Angiogenesis is briefly discussed in Chapter 2 as a component of wound healing and is considered an integral part of damage-associated inflammatory responses that ultimately result in the resolution of the inflammation responses when the damage is repaired. In the case of adipose tissue, it is the same signaling molecules that result in the hypertrophy/hyperplasia → angiogenesis responses, except in this case it is ER stress due to fatty acid overload that initiates the process, not overt cellular damage in the traditional sense. There is also no resolution as long as the calorie overload continues. The process of angiogenesis also happens to be a very important response to hypoxia as part of the etiology of tumorigenesis, as described in more detail for cancer by Cannizzo and Ardies in Chapter 6. Thus, while angiogenesis is an essential component of wound healing following damage-induced inflammation, the same signaling molecules arising from the same types of cells are also components of the tissue hypertrophy/hyperplasia → angiogenesis responses that occur with developing obesity and with tumorigenesis. The same array of eicosanoids and cytokines produced

by stressed adipocytes and by activated macrophages (and other inflammatory cells) are instrumental in stimulating the proliferation of both progenitor cells and stem cells in any tissues and therefore an important component of cancer etiology as well as tissue repair. Metabolic stress of chronic calorie overload thus leads to increased proinflammatory signaling that can have far-reaching effects including aiding in tumorigenesis when circulating cytokines from growing adipose tissues add to the proinflammatory environment of a growing tumor.

The ingestion of compounds from environmental sources provides a common source of cellular stress that also enhances risk for cancer. From constantly breathing airborne pollutants to smoking and using tobacco products; consuming toxins, mutagens, and mitogens from drinking water, food, and food storage containers; and ingesting too much ethanol, exposures to exogenous transition metals, polycyclic aromatic hydrocarbons, various aldehydes, and many other carcinogenic compounds are varied but ubiquitous. Unfortunately, the cytochrome P450–mediated processes that are part of the elimination processes lead to the production of ROSs and in many cases a wide variety of reactive intermediates. In response to the various oxidants, proinflammatory signaling responses are initiated, often adding to the insufficiency-induced proinflammatory environment. The enhanced DNA damage coupled with increased proliferative signaling provides the perfect environment for enhancing risks for tumorigenesis.

As described in detail in Chapter 3, circulating proinflammatory molecules and elevated FFAs that occur as a result of chronic calorie overload are instrumental in the development of type 2 diabetes (T2D). Bourey, Najjar, and others explain that insulin resistance and visceral obesity represent chronic subacute inflammatory states, with TNF-α playing an important role in causing the insulin resistance. In addition, the role of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1)-mediated insulin clearance by the liver should not be overlooked. Impaired insulin clearance leads to increased insulin levels and subsequently the downregulation of peripheral insulin receptors to produce insulin resistance. In the liver, these effects result in enhanced de novo synthesis of lipids, leading to both fatty liver and an increased distribution of fat to adipose tissue to compound the already elevated adipose-mediated proinflammatory signaling. Impaired expression of CEACAM1 concomitant with nonalcoholic fatty liver disease often precedes insulin resistance in the obese, leading one to believe that impaired clearance of insulin by the liver is a primary event in developing T2D along with that of proinflammatory signaling. Inactivity simply compounds the problem by producing a chronic low-level proinflammatory environment in skeletal muscles, which is also a major factor in developing insulin resistance.

In a similar manner, osteoporosis also is now considered to be largely an inflammatory disease, as described by Dodington and Ward in Chapter 5. One reason for the inflammatory nature of osteoporosis etiology is the monocyte lineage of osteoclasts, the bone cells responsible for the degradation of bone. As differentiated monocytes, osteoclasts have many of the same receptors as macrophages and are thus able to produce proinflammatory cytokines and both osteoclasts and osteoblasts are sensitive to proinflammatory signaling as well as cell–cell interactions. In response to the production of proinflammatory cytokines, enhanced expression of

receptor activator of nuclear factor κ-β ligand (RANKL) occurs in osteoblasts, which increases RANKL-mediated activation of receptor activator of nuclear factor κ-β (RANK) on preosteoclasts to induce maturation of the bone-resorbing osteoclasts. Proinflammatory cytokines also increase the expression of RANK, leading to enhanced activation of existing osteoclasts. On activation, osteoclasts greatly increase their bone resorption activities and, in addition, produce macrophage colony-stimulating factor (M-CSF), which subsequently increases the proliferation of the preosteoclasts, further enhancing bone resorption. It is also important to recognize that a majority of Americans consume less than the EAR of vitamin D (60%), calcium (38%), and magnesium (45%), which are major contributing factors to poor mineralization of bone in the first place. Although it is difficult to identify the particular cellular stresses that initiate proinflammatory signaling within bone cells, it is certainly possible that the normal metabolic production of ROSs contributes to the activation process because of the poor antioxidant and redox control due to inadequate nutrient consumption. Any intrinsic production of proinflammatory molecules would certainly be enhanced by the entry of proinflammatory cytokines from the circulation, further increasing resorption. The enhanced bone resorption would be coupled with the impaired regulation of calcium homeostasis and insufficient calcium and vitamin D intake, which impairs bone formation; this is a perfect recipe for osteoporosis.

The nutritional insufficiencies and caloric excesses also exacerbate the development of NDs, as described by Raghavan and Shah in Chapter 7. Similar to the situation in bone, oxidative stress and proinflammatory signaling also appear to be major contributing factors in these diseases. Age-associated declines in mitochondrial function (potentially exacerbated by excess FFAs and TNF-α from weight gain and adipogenesis) and in the ubiquitin–proteasome system, coupled with increased oxidative stress and subsequent oxidative damage, lead to the gradual accumulation of misfolded proteins and protein aggregates over decades. Environmental exposures to transition metals that initiate ROS production also are important in mechanisms of cause, again illustrating the importance of maintaining optimal antioxidant and redox control. Coupled with these processes is an enhanced glutamate release leading to a calcium-associated excitotoxicity. Because mitochondria are important regulators of intracellular calcium homeostasis, the increasing degree of mitochondrial dysfunction that occurs with aging is also implicated as a major factor in ND.

The source of cellular stress that is the proximate cause of atherosclerosis is the intermittent low sheer forces and sometimes turbulent blood flow at curved and bifurcate sections of coronary (and other) arteries. The mechanical stress produced by this type of flow initiates proinflammatory signaling to produce a hypertrophic and proliferative response in the blood vessels at these sites. It is essentially an angiogenesis response to strengthen the vascular walls to compensate for inadequate flow; it also happens to result in the activation and accumulation of macrophages in the arterial wall. Here, the macrophages perform their normal scavenger function of phagocytizing oxidized membrane lipids, advanced glycation end products (AGEs), oxidized cholesterol, and oxidized lipoproteins when they bind to the macrophages' pattern recognition receptors. This enhances proinflammatory signaling further and ultimately results in the production of fatty streaks, plaque, and potentially vascular occlusion

and/or thrombi. This process is exacerbated by poor antioxidant function that leads to more oxidation events, poor redox control that enhances proinflammatory signaling, insulin resistance that leads to an enhanced production of proinflammatory AGEs, increased blood pressure that increases mechanical stresses, and increased levels of oxidizable low-density lipoprotein and high-density lipoprotein (HDL). The details of these processes as discussed in Chapter 4 clearly indicate that contrary to popular interpretations of earlier epidemiology research atherosclerosis is essentially a non-resolving inflammatory disease initiated by mechanical stresses and is not a disease caused by cholesterol. In fact, cholesterol appears to be a relatively minor player in the etiology of atherosclerosis in comparison with the pervasive proinflammatory effects of inadequate diets, calorie excess, and inactivity. As described in some of Robert's earlier work, HDL also can be proatherogenic, further illustrating the hazards of making conclusions of cause and of prevention on the basis of epidemiological correlations.

A major role of dietary insufficiencies and calorie excess in risk for chronic diseases is clear. While calorie excess is certainly a major concern in the United States, poor food choices are also exceedingly important. Not only are poor choices responsible for the prevailing dietary insufficiencies but they also result in a very low consumption of phytochemicals. Because approximately 90% of Americans eat less than the recommended amounts of fruits and vegetables, the major sources for phytochemicals, they rarely consume enough to have a consistently beneficial effect. The wide array of phytochemicals in fruits and vegetables provide very important effects that greatly enhance cell survival. The enhanced protective effects include attenuating proinflammatory signaling, enhancing antioxidant function, reducing ROS and oxidant damage, increasing redox control, increasing phase II enzyme activities, enhancing DNA repair, and increasing the sensitivity of proliferating cells to checkpoint control and to apoptosis.

The various phytochemicals appear to modify cell function through modifying redox states at the microenvironment level within cells. Catechol polyphenols autoxidize to form quinones, which then cycle between reduction and oxidation states to produce hydrogen peroxide (H_2O_2). This then alters the oxidation states of Trx, Prx, and other redox-control enzymes, leading to the transient activation of mitogen-activated protein kinase (MAPK) signal transduction by Trx following the disassociation of oxidized Trx from apoptosis signal-regulating kinase 1. The transcription factors AP-1, Hsf1, and P53 are also activated by Ref1 following its oxidation by Trx. Phosphorylation of Nrf2 by MAPKs and oxidation of Nrf2 by H_2O_2 leads to its disassociation from Keap1, and it subsequently binds to the antioxidant response element (ARE) to induce the expression of Trx, Prx, mitochondrial superoxide dismutase (Sod2), catalase (CAT), glutathione reductase (GSR), glutamate cysteine ligase, glutathione-*S*-transferase (GST), glutathione peroxidase (GPX), heme-oxygenase-1, UDP-glucuronosyl transferase (UDP-GT), and NADPH:quinone oxidoreductase-1 (NQO1). Following autoxidation, some polyphenols bind covalently to Keap1 to produce the same effects. By inducing Trx, Prx, GST, GSR, Sod2, GSH, GPX, and CAT, intracellular antioxidant and redox control is optimized to reduce oxidant-mediated damage and attenuate proinflammatory signaling, thus producing a global reduction in risks for chronic diseases.

Among the effects of a reduction in ROSs and proinflammatory cytokines is a reduction in RANKL–RANK signaling to reduce osteoclast activation and proliferation. In addition, the phytochemical-associated transient increases in p38 and extracellular signal-related kinase (ERK)–MAPK activities are associated with the activation of Wnt/β-catenin signaling and an enhanced expression of Runx2 in bone, leading to the differentiation of mesenchymal stem cells to the osteoblast lineage and increasing osteogenesis. Both of these lead to a reduced risk for osteoporosis.

Reduced ROS damage and expression of proinflammatory prostaglandins and cytokines in turn attenuates both nuclear DNA damage and proliferative signaling to reduce risk for cancer. The activation of Ref1 and P53 also increases the sensitivity of proliferating cells to checkpoint controls and enhances DNA repair, whereas the induction of UDP-GT, NQO1, and GSTs enhances the elimination of carcinogens to further reduce risk for tumorigenesis. The reductions in cellular ROSs and in proinflammatory signaling in combination with the induced Sod2 will help sustain mitochondrial function as well as insulin sensitivity, reducing the risk for both diabetes and NDs. In the central nervous system (CNS), polyphenols also appear to induce the expression of brain-derived neurotrophic factor (BDNF) by activating the cyclic adenosine monophosphate (AMP) response element binding (CREB) protein, possibly through the transient activation of ERK–MAPK. These latter effects enhance neural survival and synaptogenesis and thus contribute to the reduction in risk for NDs.

Thus, transient activation of Trx, MAPKs, Ref-1, and Nrf2 (among many others), mostly through oxidant-mediated effects, appears to be a primary event in the induction of the protective benefits of phytochemicals. These effects are in stark contrast to the detrimental effects of a constant increase in the cellular oxidation state that occurs with a chronic low-level increase in the cellular production of oxidants. The detrimental effects are essentially the same whether the increased oxidation state is derived from endogenous ROS/RNS (inflammatory and nutrient insufficiency) origins or exogenous (toxins and carcinogens) origins.

9.1.2 Dietary Recommendations

With a large majority of Americans consuming a poor diet, fairly substantial changes will need to be made in order for most to obtain an optimal diet, one that is both nutritionally adequate and preventive. The *MyPlate* recommendations provide a very good starting point for ensuring nutrient intake. The minimum recommendations given here are intended for adolescents through 50 years and provide approximately 1900 cal each day on average. For the purposes of obtaining optimal prevention effects, however, with the exception of three servings of dairy, the *MyPlate* recommendations have been modified in order to increase the polyphenol content of the diet to levels observed in traditional Mediterranean diets (Table 9.1). This is accomplished by recommending minimum numbers of servings each week from specific groupings of fruits and vegetables and by separating out nuts/seeds and beans/peas (also good sources of protein) as two separate food categories with their own minimums. In essence, these changes add 0.5 cups of fruit/day and 1.25 cups of vegetables/day to the minimum intake in comparison with the *MyPlate* minimum recommendations for these two categories. In addition, phytochemical-rich extra

TABLE 9.1
Recommended Daily Food Servings

Food Group	Minimum Daily Servings (~1900 kcal)	Standard Serving Size	Approximate Kilocalories/ Serving	Additional Servings Each +400 kcal
Fruits	5	0.5 c.	71	1
Vegetables	6	0.5 c.	38	1
Cold-water fish	1 (2X week)	4 oz.	230	
Beans/legumes	2	0.5 c.	110	1
Nuts/seeds	1	0.25 c.	240	
Dairy (skim/low fat)	3	1 c. milk 2 oz. cheese	86	
Breads/cereals	5	1 oz./1 slice	78	
Extra virgin olive oil	2	1 tbsp.	100	
[a]Red wine	1	4 oz.	85	

Note: *Fruits:* minimum of 2.5 cups each day with at least three different selections from each category each week:

A: elderberries, blueberries, pomegranates, blackberries, raspberries, saskatoon berries, blackcurrants
B: plums, oranges, grapefruit, lemons, cantaloupe, cherries, cranberries, red/black grapes, or apples.
C: kiwi, bananas, pineapples, mango, pears, star fruit, guava, strawberries, peaches, or any other fruit.

Vegetables: minimum of 3 cups each day with at least three different selections from each category each week:

A: chili peppers, broccoli, water cress, garden cress, spinach, arugula, Brussels sprouts
B: cauliflower, tomato, Romaine lettuce, green beans, sweet peppers, red cabbage
C: onion, garlic, celery, green cabbage, asparagus, Swiss chard
D: squash, zucchini, pumpkin, sweet potatoes, carrots, potato

Nuts and seeds: minimum of one 0.25-cup serving each day with a minimum of two different selections from each category each week:

A: chestnuts, pecans, walnuts, pistachios
B: hazelnut, almonds, Brazil nuts, cashews, Macadamia nuts, or peanuts

Beans and legumes: minimum of one 1-cup serving each day with a minimum of two different 1-cup selections from each category each week:

A: black beans, adzuki beans, kidney beans, peas/pea pods, lima beans
B: lentils, pinto beans, chili beans, chick peas, navy beans

Whole grains and cereals: minimum five 1 oz. servings of whole grains each day with a minimum of one selection from each category each week:

A: buckwheat, wheat, bulgur
B: oats, corn, barley
C: wild rice, rice

[a] Highly recommended. You should not use alcoholic beverages if you are pregnant or if you have been advised by your physician not to drink.

virgin olive oil is the only recommended oil to use for cooking, salads, and *spreads* (dipping). The number of servings in the meat category is a total of two servings of cold-water fish each week. This is to ensure sufficient consumption of EPA/DHA as well as to slightly reduce the overall protein intake. The consumption of other meats is not prohibited but rather not recommended as a daily or even a weekly habit. The five serving minimum for the grains/cereals category is restricted to servings for whole grains, helping to maximize phytochemical as well as vitamin and mineral intake. When the minimum numbers of servings are adhered to, the diet will on average meet the recommended dietary allowances or adequate intakes for all nutrients as well as providing somewhere between 1730 and 1960 kcal. The range of calorie values is because of the differences in caloric content of different foods within any one category as well as the presence or absence of the recommended servings of fish. The daily average of the minimum recommendations over a week is approximately 1900 kcal. In addition, by following these recommendations a minimum daily consumption of phenolics of approximately 1.3 g will result, well within the low-end range of a traditional Mediterranean diet and much higher than the average of less than 0.5 g/day reported for the "American" diet. Protein intake for this recommendation approximates to an average of 80 g/day (~70–~95 g/day depending on whether fish is eaten or not), somewhat lower than the average intake of ~91 g/day for all adults 19–50 years of age, as reported in Chapter 6. The protein intake of these recommendations is equivalent to the 5 to 6 oz. of protein equivalents for adults recommended by the *MyPlate* guidelines and consistent with the recommendation for an intake of approximately 80 g/day by Bourey, Najjar, and others and by Raghavan and Shaw. The last recommendation is that the bulk of the calories should be eaten within 2 hours of finishing daily exercise. As discussed in Chapter 3, there is very little evidence to support stressing the importance of breakfast or of multiple small meals. On the other hand, it would be very important to take advantage of an exercise-induced increase in insulin sensitivity, especially for prediabetics and diabetics. Finally, although red wine is the only recommended alcoholic beverage, the *one serving minimum* is not included in the calorie/nutrient totals because it is not recommended for anyone who, for a variety of reasons, should not drink alcohol.

Randomly selected servings from each of the recommended lists that conformed to the recommended minimums in Table 9.1 were entered into daily food logs (*NutriBase 10 Professional Edition*; CyberSoft, Inc., 3851 E. Thunderhill Place, Phoenix Arizona, 85044) and analyzed for macronutrient, nutrient, and caloric contents in order to calculate the averages for calorie, protein, and nutrient intakes of the aforementioned minimum recommendations. The approximate calorie content for servings in each food group in Table 9.1 was obtained from the 2005 Dietary Guidelines for Americans (Department of Health and Human Services [DHHS]/U.S. Department of Agriculture [USDA]), in which calorie contents of mixed-food servings were reported. This will account for the discrepancy between the values obtained from the nutrient analysis by *NutriBase* for the actual caloric content of daily food menus (minimum servings = ~1900 kcal average, ~1730 kcal without salmon, and ~1960 kcal with salmon) and those obtained (~1700 kcal without salmon and 1830 kcal with salmon) using the USDA values for mixed-food servings. Because there are many foods available that are not listed in the recommended servings categories, the

DHHS/USDA values may be more appropriate to use in estimating calorie contents of food servings for calories beyond the ~1900 minimum. It is important to note that as with any calorie analysis there will be differences in values obtained for the same foods simply because of natural variations in macronutrient and water content; therefore, any of the values used here can only be considered to be a reasonable approximation. The minimum phenolic content of the recommendations was based on the total content of a day's minimum recommended servings where the foods were specifically selected from the lists of foods to have the lowest tabled polyphenol content (from Chapter 2, Table 2.1). Therefore, it is assumed that randomly selecting foods from the required categories each day, in combination with any that are in various seasonings, will actually result in a daily polyphenol intake that averages a little more than 1.3 g, similar to a traditional Mediterranean diet. With the average sedentary adult female and male requiring approximately 2000 and 2400 cal each day, respectively, more calories than the 1900 daily average recommended minimum will be required for most people. Certainly, more will be required if they follow the recommended activity recommendations (discussed in Section 9.1.5) that will consume 500 cal or more; this leaves room for food options. For additional calories beyond the minimum recommendations, approximately half are recommended to come from fruit and/or vegetable and/or bean/legume sources, whereas the remainder should be considered *optional*. There is also nothing wrong with the occasional junk food snack, a chocolate confection or candy cane, indulging in your favorite barbecue ribs, a bowl of butterscotch ripple ice cream, or even overindulging at a party once in a while; there are plenty of optional calories embedded into the recommendations to do so. It just should not be done every day (or even every week).

9.1.3 Behavioral Issues

From eating too many calories for mostly the wrong reasons to poor food choices, the dietary behaviors of most Americans are in need of a complete overhaul. A major issue that needs to be dealt with is that the eating behaviors that are responsible for our current high prevalence of overweight and inadequate nutrient intake are behaviors that have been ingrained in us for a lifetime. For the most part we have learned them unquestioningly from our parents, and we continue them unthinkingly as adults. As detailed in Chapter 8 by Begg and Woods, these habitual behaviors not only include the timings and sizes of meals but also the tastes, textures, colors, and aesthetics of the specific foods that are most often chosen (in many cases, the same as those that were originally supplied by our parents) to be eaten, with each of these factors influencing different homeostatic and nonhomeostatic aspects of consumption through largely unconscious means. Because most people seem to be completely unaware that many different environmental cues affect eating behavior, it is apparent that eating is a relatively mindless behavior, that is, most details of the behavior itself and the details surrounding the behavior are simply not paid any attention. Because of the largely mindless aspect of eating behavior and the large array of nonhomeostatic influential factors, hunger and satiety are the least of the many factors that are important in the initiation and cessation of eating. It is also clear from their discussions that the habit of eating at certain times not only conditions the CNS to

prime the gastrointestinal (GI) tract for eating at those times but also primes us to be hungry at those same times, even if we had just completed a meal an hour earlier at a nonhabitual time. Further, when eating at nonhabitual times (celebratory dinners, special brunches, out-for-coffee, parties, while on vacation, etc.) our ingrained hunger/satiety mechanisms do not come into play so readily, making it much easier to overeat: I am sure we have all been to parties where we just eat and eat and eat (and maybe even drink a little as well) and never quite feel satiated.

Although a chapter on hunger and satiety might seem out of place in a book on chronic diseases, it actually provides important information that should be considered along with dietary recommendations. It is tempting to think that because of the strong habitual nature of eating integrating some components of cognitive behavioral therapy into the process might be helpful in changing eating behavior, or at least be more helpful than the simplistic "just eat less" or "just eat more fruits and vegetables" give-advice approach. To quote Begg and Woods, "The primary goal for such therapy would be to learn, be conscious of, and permanently change dietary behavior to a pattern consistent with a healthier lifestyle" (Chapter 8, Section 8.4.2). The learning and conscious part of their advice would certainly fit the cognitive component of a cognitive therapy approach. Old behaviors built up over the lifetime of the individual have to be *unlearned*, while completely new permanent ones need to be learned; it is a task that is not to be taken lightly. One only need look to the data on weight loss to get an insight into the difficulties of the problem.

As they noted, most people who successfully lose weight in weight-loss programs ultimately regain the weight within months or years. In the subjects' defense, however, this may have something to do with the actual goal of weight loss itself. Once weight-loss goals are accomplished, the weight-loss diet can be abandoned. The unfortunate consequence of what is clearly a rational thought process is that the old eating habits are easily regained and weight gain ultimately ensues. With a current and very public focus on obesity as a (or more probably the) major health issue today, weight loss becomes the default focus for prevention, as though losing weight is the major relevant factor in regaining optimal health. If it is believed by the general public to be the major health problem then old habits will not be changed, and risks for chronic diseases will remain.

The major focus of the recommendations here is that a permanent change in eating behavior must be made; it must be made in context with learning to be aware of how much food and which specific foods need to be eaten to attain optimal health, which includes appropriate weight control. The ultimate goal is to create new eating behaviors that consciously optimize nutrient and phytochemical intake along with appropriate conscious caloric control to regulate body weight at a healthy level. The implication of this is that learning the new (and *permanent*) diet is one priority and another priority is learning caloric control. At the risk of stepping on the toes of various professionals (I am certainly not trained as a behavioral therapist), the following is a suggestion for one possible approach to learning the new (and permanent) lifestyle diet.

These learning objectives might be met by obtaining cups, containers, and bowls that conform to standardized 0.25, 0.5, and 1.0 c. serving sizes and to volumes that equate to an approximate 4 oz. serving size for fish (about half of a salmon steak).

From the foods and servings in Table 9.1, selections of foods from each recommended category that conform to the minimum recommendations plus sufficient additional servings to meet average caloric needs can be made with one caveat: the standard-size containers *must* be used to measure out the appropriate size and number of servings for the *entire* day at once. For the first 2 weeks of this procedure, using the average 2000 and 2400 kcal for women and men, respectively, is recommended. To meet the 2000 and 2400 kcal target, calories beyond the minimum 1900 kcal servings should come exclusively from fruits, vegetables, beans/peas, and/or whole grains and the tabled caloric content of the servings should be used as a guide to determine the appropriate number of additional servings. Visualizing an entire day's worth of food (and the inherent calories) is one possible way of learning to get a feel for the different volumes of foods and how they can be variously apportioned throughout the day. (Each day's number of servings and approximate caloric content also should be recorded for later reference as well.) The servings can then be placed on the usual dinnerware and consumed at customary times to minimize homeostatic disturbances. Although this does not necessarily conform to others' recommendations that include using smaller sized dinnerware than the current and customary large sizes, the intent of using serving-size portions is to learn and be conscious of serving sizes, regardless of their ultimate dinnerware environment. Although seemingly trivial, the goal of this initial effort is to continually be conscious of what the different standardized servings for the new diet look like as they are put in different combinations on different serving ware each meal and to be conscious of how much is being eaten. Using the common rule of thumb for behavior modification, it may take at least a month of using the serving-size containers to start to break the old habits of filling the plate and to learn to recognize and use appropriate serving sizes. At the same time, the physical presence (and use) of the serving-size containers might serve as a constant reminder of the learning tasks each day. Although this particular procedure in learning new diets has not been tested rigorously, it is a logical extension of the concept that unconscious interactions between intrinsic and extrinsic factors are an integral part of the preexisting eating behaviors; therefore, we need to focus on and learn to visualize and be conscious of the new foods and their portions as part of learning the new eating behavior.

As new dietary behaviors are being acquired, the second major goal of learning weight control in the context of the new diet can start. Sometime during the first week or two of practicing rigid control of food servings, measuring and recording body weight every day (at the same time and under the same conditions as much as practical) could start. Attempts at changing intake should not be made at this time because the purpose is to monitor weight at the specific food intake being used. Only after at least 2 weeks of recording body weight should attempts be made to relate weight to daily food intake. The reason for this is that a weekly average for body weight should be used as the *reference* body weight and not the weight on any single day. This is to minimize the effects of daily variations in body weight due to the presence or absence of food in the GI tract and fluctuations in hydration state. Once weekly averages are available, adjustments to food intake can be made to see the effect this may have on body weight. For example, one daily serving of grains and one of meat could be removed each day for 2 weeks to see if the reduction of calories

results in a loss or stabilization of body weight. Alternatively, all alcoholic beverages could be removed to the same end. On the other hand, servings of specific foods or beverages could be added and the effects observed. The purpose of such interventions is not necessarily to lose or gain weight but rather to learn to be conscious of the effects that adjusting food intake may have on body weight. Learning these adjustments may also be important in developing an intrinsic sense for what would be a realistic weight-loss goal based on experience with the new dietary behavior. After 4–6 weeks of this initial learning phase, one should have a reasonably good record of food intake and average body weight each week to be able to see what it might take to maintain or lose body weight. After this learning phase has been completed, weight-loss or weight management goals can be discussed and set. The purpose of a paradigm such as this is to suggest that the learning of a new and mindful eating behavior is the first priority of an overall dietary program and that any weight management issues are dealt with only after starting to learn the new dietary behaviors.

Part of this learning phase includes learning to consume unaccustomed quantities of what may be many new foods. Again, this is not a trivial learning experience. Eating the unaccustomed foods in unaccustomed amounts might be in some cases a somewhat unsettling experience. The new foods simply will not conform to the habitual tastes and aesthetics of the usual foods and portions and therefore will not meet the expected nonhomeostatic stimuli, possibly disrupting the satiety and pleasurable feelings normally associated with eating. Further, because of the innumerable pleasant memories of the tastes of various foods and the satiety that accompanies eating them, memories that must have been formed over hundreds and perhaps thousands of repetitions of the same eating behaviors, the old habits might be badly missed as well. Issues relating to shopping for unaccustomed foods (how do you even know they are ripe or ready?) or preparing them also may lead to frustrations. Because of such issues, engaging the services of a professional dietitian, nutritionist, or personal trainer might help to facilitate the process. At first, daily or weekly contacts with such a professional, especially one familiar with using a behavioral-type approach, for discussing and resolving such issues, or for other counseling needs, might be very helpful. Less intensive *update* sessions later on in the program also might be highly useful to help enhance adherence to the new lifestyle. Similar professional assistance is advisable for the exercise component of the recommendations as well.

9.1.4 Exercise and Chronic Disease

The lack of physical activity for the majority of Americans is a serious issue with respect to risks for chronic diseases. One rather visible consequence of a sedentary lifestyle is a tendency to gain weight simply because of not expending sufficient calories. Sedentary recreational pursuits (computer games, television, social networking, reading, watching movies, etc.) and sedentary occupations make a deadly combination. When one considers the difficulty in changing eating habits, enjoying an increasingly sedentary lifestyle as we age from early adolescence through middle age without decreasing caloric consumption to compensate is certainly understandable. Unfortunately, the end result for a large majority of Americans is a constant gradual increase in adiposity and body weight and ever-worsening fitness, making

it even harder to consider physical activities for recreation (and health). And, the increasing adiposity greatly enhances for chronic diseases through the proinflammatory mechanisms of adipose hyperplasia and angiogenesis.

Another major consequence of inactivity is the development of insulin resistance in skeletal muscle. Inactivity induces a continuous small increase in ROS production by skeletal muscle cells that then produces a mild chronic proinflammatory environment, ultimately leading to TNF-α-mediated inactivation of insulin receptor substrate proteins and impaired insulin function in skeletal muscle. As a result of insulin resistance, levels of glucose in the blood are elevated and the formation of Amadori products is increased. These adducts are eventually modified to form various AGEs, which then activate AGE receptors on macrophages, smooth muscle cells, and vascular endothelial cells, enhancing inflammatory signaling in essentially all tissues. While inactivity-induced insulin resistance in skeletal muscle occurs without weight gain, the progression toward insulin resistance through inactivity can be significantly enhanced by increased circulating FFAs and TNF-α coming from expanding adipose tissue. The combination of inactivity and chronic weight gain is truly a vicious cycle resulting in increasing chronic inflammation and risks for chronic diseases.

An important aspect of physical activity reducing risks for chronic diseases is that its effects go far beyond that of simply avoiding excess risks that are associated with inactivity and calorie excess. As a result of the calcium flooding the muscle cells to stimulate muscle contractions and the metabolic production of ATP, increased production of ROSs and AMP occurs. The increase in ROSs leads to an increase in the oxidation state of muscle cells that lasts at least as long as the activity does, resulting in the oxidation of Trx, Prx, and other redox-control enzymes and subsequent increases in activity of various MAPKs. The Ca^{2+} also activates protein kinase C (PKC), calcineurin A (CnA), and Ca^{2+}/calmodulin-dependent protein kinase (CaMK) while the AMP activates AMP-dependent protein kinase (AMPK).

CnA and CaMK activate CREB and MEF2 to enhance the synthesis of PGC-1α while AMPK activates AS160 and TBC1D1 (Rab-GTPases), silent information regulator T1, and PGC-1α, with PGC-1 subsequently activating Nrf2, Nrf1, and other transcription factors. These responses lead to enhanced insulin sensitivity in skeletal muscle as well as mitochondrial biogenesis and enhanced antioxidant and redox control (via Nrf2). These same effects also occur in nonmuscle tissues, resulting in globally enhanced antioxidant and redox control, enhanced DNA repair, reduced ROS and oxidative damage, increased elimination of carcinogens, and increased sensitivity to checkpoint controls to significantly reduce risk for cancers. The activation of various protein kinases by exercise in neural tissues is also associated with activation of CREB and increased expression of BDNF, which when coupled with the enhanced mitochondrial function stabilizes nerve function and reduces risk for NDs.

In addition to the various metabolic stresses placed on cells by exercise are those that result from the physical aspects of weight-bearing movements. The mechanical stresses placed on the bones during exercise produce cellular stress responses via various stretch-activated calcium channels, integrin deformation,

and shear-induced activation of various G-proteins. As a result of these effects, increased Wnt/β-catenin signaling occurs to enhance osteogenesis. It is important to note that all of the mechanisms discussed for exercise-based prevention are very similar to those produced by various phytochemicals and mediated by essentially the same molecular mechanisms. As such, the preventive effects are dependent on the transient nature of exercise stress. Regarding osteoporosis, this concept is illustrated by the fact that in obese individuals where the mechanical stresses are constant bone strength is actually compromised.

The responses of muscle cells to the increased production of oxidants (and to calcium) during muscle contraction also include the activation of PLA2, resulting in the synthesis of proinflammatory prostaglandins, leukotrienes, and thromboxanes. In addition, the increased MAPK activities are associated with a large increase in the expression of interleukin (IL)-6. While these are traditionally considered to be proinflammatory stress responses, in the event that there is no damage and subsequent TNF-α production, the IL-6 response results in a profound anti-inflammatory effect. IL-6 mediates an induction of sIL-1r, sTNFr, sIL-1ra, and IL-10. The sIL-1r and sTNFr bind to circulating IL-1α, IL-1β, and TNF-α while the sIL-1ra binds to IL-1α and IL-1β receptors, thus attenuating proinflammatory cellular responses. The IL-10 suppresses nuclear factor κ-β and MAPKs, increases suppressor of cytokine signaling, and stimulates nonphlogistic phagocytosis, events that commonly occur during the resolution phase of inflammation. These effects only occur as a result of moderate-intensity large muscle–group exercise that is sustained for about an hour, and they can be enhanced further with even longer exercise periods. They also will not occur with damaging or exhaustive exercise, indicating that too much exercise stress can actually be counterproductive. These IL-6-mediated anti-inflammatory effects occur in addition to those of Nrf2-mediated enhanced antioxidant and redox control and represent an important additional benefit of exercise: a profound systemic anti-inflammatory and proresolution effect. While the non-IL-6-mediated effects discussed earlier will reduce risks for chronic diseases, the inclusion of 60 min/day of moderately stressful exercise is essential to maximize risk reduction.

9.1.5 Exercise Recommendations

Based on the various cellular effects of exercise, in order to obtain optimal reduction in risk for chronic diseases the recommended goal is to perform vigorous activities that are 6 METS or greater in intensity and carried out for a minimum of 1 h/day for 5 days a week when starting an exercise program. These activities include jogging/running, cycling at 10–12 mph or greater, swimming, cross-country skiing, or similar continuous vigorous activities. An example of a suggested protocol to follow is detailed in Table 9.2.

This schedule is actually designed for older or very sedentary adults. Notice that it can take almost a year to progress to the frequency and intensity that will maximize health benefits. After the 48 weeks of progressive training, the final two days of activity can be added in at the same one additional day each 6 weeks to achieve daily activity that should maximize preventive effects. There is simply no easy or fast way to do this. Unaccustomed physical activity performed for the first time results in a lot of physical discomfort, and it will take time to recover from this initial effort. It also takes time for

TABLE 9.2
Training Schedule for a Sedentary Person

	M	T	W	T	F
Week 1	~20 Minutes [a]walk/jog				
Week 2	~20 Minutes walk/jog			~20 Minutes walk/jog	
Week 3	~20 Minutes walk/jog		~20 Minutes walk/jog		~20 Minutes walk/jog
Week 4	~20 Minutes walk/jog	~20 Minutes walk/jog	~20 Minutes walk/jog		~20 Minutes walk/jog
Week 5–6	~20 Minutes walk/jog	~20 Minutes walk/jog	~20 Minutes walk/jog	~20 Minutes walk/jog	~20 Minutes walk/jog
Week 7–12	Increase the time to 30 minutes, following the same 6-week pattern. (Add 10 minutes to 1 additional day each week.)				
Week 13–18	Increase the intensity to more jogging than walking, following the same 6-week pattern (increasing the intensity on 1 additional day each week).				
Week 19–24	Increase the intensity to jogging only, following the same 6-week pattern.				
Week 25–30	Increase the intensity to running only, following the same 6-week pattern.				
Week 31–36	Increase the time to 40 minutes, following the same 6-week pattern.				
Week 37–42	Increase the time to 50 minutes, following the same 6-week pattern.				
Week 43–48	Increase the time to 60 minutes, following the same 6-week pattern.				

Note: After a vacation period, the intensity should be lowered when starting back and gradually increased to the previous level of intensity over a period of several weeks.

[a] More walking than jogging.

the physical structures of muscles and bones and the various enzyme systems to adapt to the increasing duration and intensity levels of the activity. As with the first time performing any activity, increasing intensity and/or duration also introduces discomfort, hence the 1-week recovery period between the first and second bouts of activity and the gradual increase in the frequency and intensity of the additional exercise periods. The pattern of progressively increasing activity levels that is presented here is consistent with the idea of microprogressions, which is often associated with rehabilitation of sports injuries: small gradual increases in intensity to minimize the risk of reinjury. Although sedentary individuals typically will not have such injuries, the majority will be in a very poor physical condition and sudden large increases in activity could easily lead to injury. For this reason, the microprogression approach is recommended.

Even though meeting these goals for exercise provides optimal benefits that include the profound anti-inflammatory effects of large amounts of IL-6, this does not mean that there are not significant protective benefits to lesser amounts or different types of activity. Simply being active regularly will enhance insulin sensitivity to produce a moderate reduction in risks. Such preventive effects may start to be realized after the first 4 or 5 weeks of following the suggested program, long before the 60-minutes goal is reached. This is an important concept; there are preventive benefits from the

lower levels of activity, and sustaining activity at these lower levels will reduce risks. Similar levels of protection might be provided through several short (~10 minutes or so) periods of daily low- to moderate-intensity activities such as fast walking, yard maintenance, leisurely bicycling, playing volleyball, playing baseball, or skating and through resistance-type weight-lifting and muscle endurance–training activities.

Although it does not provide maximal effects, reductions in risk are also obtained from the shorter duration (20–30 minutes) continuous, moderate-intensity to higher intensity running (or cycling, swimming, and cross-country skiing) when performed–three to five times each week. The benefits include maintained insulin sensitivity, enhanced antioxidant protection and redox control, reduced proinflammatory signaling, and possibly enhanced DNA synthesis. These benefits may start to be realized after about the 30-week time frame of the training program described in Table 9.2, and sustaining activity at this level reduces risks more than at the 5-week level.

Developing an exercise habit also entails behavioral issues that are similar to those inherent in learning a new diet. Unaccustomed exercise not only can make one uncomfortable but also is not the first thing a sedentary person will think of when it comes to spending his or her leisure time. There are plenty of sedentary activities that are much more pleasurable to participate in, and more than likely they have been participated in regularly and will be missed if not. This leads to the concept that exercise behaviors need to be learned and developed into a new daily behavior, hopefully one that will be missed if not participated in. It is pretty difficult to have feelings of missing an activity when initially there is little to no positive feedback when it is performed for the first time. For this reason, utilizing the skills of a competent personal trainer who has training in behavioral therapy as well as in exercise (and nutrition) sciences may be highly beneficial. Developing the capacity to learn to enjoy what at first is an unfamiliar and uncomfortable activity with the help of a specialist can be very helpful. The additional encouragement, empathy, expert instruction, and help with learning new behaviors that can be provided by a highly competent personal trainer, one with appropriate advanced training, can often make a difference between successfully maintaining the activity program and reverting back to a sedentary lifestyle.

One last thought on these exercise recommendations: they are made with maximal reduction in risk for chronic diseases in mind, which is after all the focus of this book. Exercise activities for maintaining optimal strength, flexibility, balance, muscle endurance, and all other physical abilities necessary for maintaining an optimal capacity for activities of daily living and for healthy aging are not to be belittled. Alternating days with 60 minutes of continuous activities with days of muscle endurance-type weight-lifting activities may optimize aging while only slightly compromising maximal prevention of chronic diseases. (Due to space limitations, however, details of training progressions for these will not be presented.) Hopefully, with properly designed clinical trials such concepts can be tested.

9.1.6 Final Analysis

Exercise clearly has the ability to initiate changes in cellular functions that profoundly reduce risk for chronic diseases, as does a nutritionally adequate and phytochemical-rich diet. These beneficial changes are initiated predominantly

through transient increases in the oxidation state of cells that then alters the patterns of activated transcription factors and subsequently the expression of various genes to enhance cell function and survival. How these recommended treatments alter epigenetics is unknown as of the time of this writing. Because hypermethylation processes appear to be initiated in part by ROS-mediated damage, it is certainly possible that the enhanced antioxidant and redox control brought about by following these recommendations will alter the hypermethylation patterns of CpG islands as well. This could conceivably increase the affinity of promoters for their transcription factors and result in greater activation of expression activities, thus further enhancing the preventive effects of phytochemicals and exercise over time. Obviously, this remains to be tested. If confirmed, however, such effects might even be passed on to progeny through inheriting the *new* hypermethylation patterns, producing progeny that may then possess an inherently greater sensitivity to the protective effects of proper diet and exercise. This would certainly be an improvement to what might be the current *American Death Spiral* of poor diet and inactivity leading to chronic disease and potentially disease-promoting epigenetic effects that might then be passed on to progeny, resulting in ever-worsening risks for disease with each subsequent generation.

Health practitioners have been educating students, patients, and the general public about the health risks associated with poor diets and inactivity and about the health benefits of proper diet and exercise for decades, with very disappointing results. The majority of Americans still do not meet the minimum recommendations for physical activity, and the proportion that does not meet the minimum activity requirements has been continually increasing during the last three decades. At the same time, the majority of the population still eats a nutritionally insufficient diet, a situation that certainly has not been improving either. Something clearly needs to change because the current approach is simply not working very well. In light of this record of failure for the majority of Americans, perhaps a much more intensive and personal approach to wellness is necessary in order to attain the type of behavior modifications that are necessary for achieving optimal health. The aforementioned recommendations appear to include a shameless plug to create more employment opportunities for personal trainers, nutritionists, and dieticians, and it is meant to emphasize the extremely difficult time that most people actually have in successfully learning a healthy and permanent new lifestyle and that most will need professional assistance. Many medical centers throughout the country have already recognized this and established wellness programs that include such services. Unfortunately, these programs are usually on a fee-for-service basis so that they are available predominantly only to those who can afford them.

At the risk of inappropriately entering the sometimes perverse world of American politics, in the final analysis the ability to successfully produce and sustain healthy alterations in behavior probably depends on changes to government policy and public education. There needs to be a universal acceptance that both diet and physical activity are far more important to health than is currently acknowledged and that without comprehensive changes in how health and wellness education and health policy are approached very little will change. The following is one example from the government side of things: the current nationwide farm-subsidy programs in America include subsidies for the production of wheat (and various other grains)

as well as feed grains for beef and other livestock, helping to keep meat prices low while little to no support is available for fresh fruit and vegetable produce. Although the subsidies are certainly not the only reason for the relatively low prices for meats and other processed foods, these policies do seem to be the exact opposite of what would be in the best health interests of the population. One result of these policies is that some of the most expensive foods in the grocery store happen to be the fruits and vegetables that Americans eat the least. In some respects, there might be little point to a public health service policy of encouraging increased consumption of fruits and vegetables when so many Americans simply cannot afford to buy them and at the same time our current economic policies ensure they are the most expensive. Perhaps, transferring all of the current subsidies to fruit and vegetable production might increase the year-round availability of fruits and vegetables at more reasonable prices, increasing the prices of meat, meat products, and highly processed grain products at the same time. Although not a panacea, such changes in policies might at least create more of a financial incentive for consumers to eat a healthier diet.

Integrating diet, nutrition, and physical activity concepts into daily health–wellness classes to help children develop a conscious focus on health and wellness behaviors from a young age also might help to avoid far greater difficulties in changing their behavior as adults. Unfortunately, to accomplish this it would take a complete overhaul of the public education and teacher education systems; currently, proper nutrition and exercise science education for public school teachers (and for many health practitioners as well for that matter) is essentially nonexistent. Without properly educated health teachers, it is highly doubtful that meaningful health and wellness education could take place anywhere.

Obviously, wholesale changes in lifestyle behaviors are necessary to significantly reduce risks for chronic diseases and reduce the more than $2.7 trillion (2011) in health spending in the United States. This will probably take a concerted effort on the part of the medical community, community and public school health educators, researchers, employers, and the government. We hope that the material in this book provides some direction on just how far we really have to go to obtain optimal reduction of the biologic-based risks for chronic diseases. The rest is up to the reader.

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SPORTS MEDICINE

Diet, Exercise, and Chronic Disease

The Biological Basis of Prevention

Exercise and diet are key factors in the etiology and prevention of chronic disease. While most books on chronic disease have a decided clinical approach, ***Diet, Exercise, and Chronic Disease: The Biological Basis of Prevention*** brings together the latest cellular- and molecular-based research on the etiology of chronic diseases and the impact of various aspects of diet and exercise on the causal mechanisms. By focusing on cellular biology, details of the integrative nature of the many different underlying factors are revealed—details that are not evident with the prevailing clinical approach to chronic disease.

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