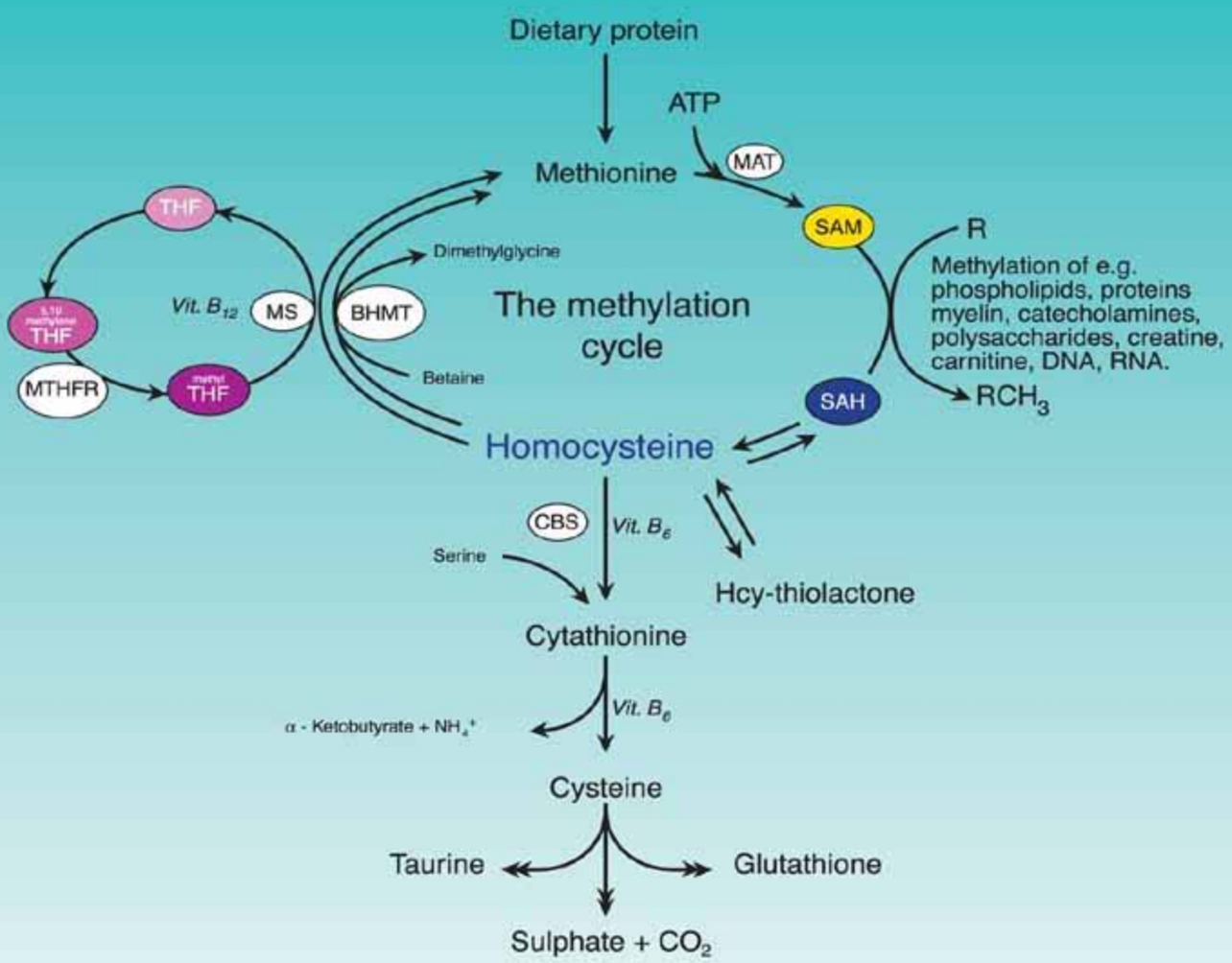
Christina Bolander-Gouaille, Teodoro Bottiglieri

Homocysteine

Related Vitamins and Neuropsychiatric Disorders

Second edition



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Preface

It is not always recognised that we face a silent epidemic of chronic neuropsychiatric disease that in many ways has as great an impact world-wide as the epidemics of acute diseases such as AIDS, tuberculosis and malaria. The World Health Organisation's Global Burden of Disease study (1997) found that psychiatric and neurological diseases together were the single largest contributors to global ill-health, measured as disability-adjusted life-years. In 1990, psychiatric diseases alone accounted for about 10% of total morbidity but this expected to rise to around 15% in 2020. The burden of chronic psychiatric disease is not confined to developed countries, but is the most important factor in all parts of the world except sub-Saharan Africa. Indeed, the developing world also faces a growing problem, as life-expectancy increases, due to age-related non-communicable diseases such as dementia.

The main contributors to global ill-health amongst psychiatric diseases are unipolar depression and dementia. By 2020, depression will be the second leading cause of disease burden in the world after ischemic heart disease. By 2040 dementia, principally Alzheimer's disease, is expected to affect at least 20 million people in Europe and the North America. Cognitive impairment in the elderly is a major risk factor for dementia; right now, every year, about two million people in Europe and North America develop cognitive impairment. The costs of dementia alone are some 20% of the health budgets of developed countries.

How can we tackle this challenge of neuropsychiatric disease? First of all, we must identify risk factors for the different brain diseases that cause psychiatric illness. One such factor for several brain diseases is homocysteine. This book is very timely for it introduces the newcomer to this important new field of research and explores the evidence for a link between homocysteine and the major neuropsychiatric diseases. The best evidence so far is for associations between moderately elevated homocysteine levels and stroke, cognitive impairment and dementia, including Alzheimer's disease.

Having identified an association between a brain disease and homocysteine, we then face the challenge of causality. Homocysteine is an established risk factor for vascular disease. Those studying homocysteine in relation to psychiatric disease thus have to consider: could the association be due to the influence of homocysteine on blood vessels in the brain? Or is it independent of vascular disease? Since any psychiatric disease is likely to have multiple causal factors, we should also consider whether homocysteine might be acting both directly on neurons and indirectly through its effects on the cerebral vasculature. Another important question is whether the association of a disease with homocysteine is due to an effect of raised homocysteine levels, or whether homocysteine is just a marker for an abnormal status of folate and/or vitamin B_{12}. From a clinical perspective, these scientific questions matter less than the answer to the question: can we prevent the psychiatric disease by lowering the level of homocysteine by administering folic acid and vitamin B_{12}? Thus the final stage in the quest for establishing a role for homocysteine in psychiatric disease will be to carry out long-term randomised controlled trials. The evidence we already have for a link between homocysteine and cognitive impairment makes these trials an urgent priority for public health.

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Contents

Introduction

The importance of homocysteine as a risk factor or risk marker is becoming a familiar concept. Since the early 1990s a constantly increasing number of studies have been published on homocysteine. It has also been the topic of four international conferences, in 1995, 1998, 2001 and 2003.

Increased plasma or serum levels of homocysteine have been shown to be an independent risk factor for cardiovascular disease, for neural tube defects and other birth defects.

Many studies have found a link between impaired homocysteine metabolism and neuropsychiatric disorders such as depression and cognitive impairment in the elderly.

There is also much focus on the association between carcinogenesis and deficiency of vitamins involved in the homocysteine metabolism, primarily folate.

Hyperhomocysteinemia is in most cases associated with low levels of folate, vitamin B_{12} and B_6. Successful lowering of elevated homocysteine levels may, in most cases, be accomplished by a simple vitamin supplementation.

Within the next few years, the results of several large homocysteine-lowering intervention studies will become available. There are strong reasons to believe that they will confirm the predicted preventive effects of homocysteine-lowering treatment not only on cardiovascular disease, but also on cognitive decline.

Supplementation with folic acid during the peri-conceptional period has already been shown to substantially decrease the incidence of neural tube defects. It may also reduce the incidence of other birth defects pregnancy complications.

Moderate elevations of homocysteine may often be caused by one or more unhealthy lifestyle factors that influence vitamin status/metabolism, such as smoking, high alcohol consumption, low nutritional intake of vitamins, high coffee consumption and lack of physical exercise. A recent large cross-sectional study indicates that a change in lifestyle factors only,

could result in a public-health-relevant homocysteine-lowering effect *(de Bree et al., 2001)*. Overweight and even stress are also associated with hyperhomocysteinemia. The diagnosis of hyperhomocysteinemia could thus be an important incentive for the patient to opt for a healthier lifestyle. Diseases and drugs are other factors that may influence the homocysteine metabolism.

The interplay between the individual genetic background and environmental factors in the pathogenesis of many disorders is currently the subject of dynamic research. During the last few years, the c DNA encoding most of the enzymes involved in the homocysteine metabolism have been elucidated. Many mutations and polymorphisms have been identified. Polymorphisms affecting the homocysteine metabolism may have a clinical impact on neuropsychiatric disorders.

In contrast to the two previous books in this series, this book primarily focuses current knowledge on the connection between disturbed homocysteine metabolism as a potential causal risk factor of neuropsychiatric disorders.

Several prospective trials have shown that low vitamin levels, even within the normal range, or homocysteine levels in the upper normal range, are risk factors for the development of dementia. Early detection of hyperhomocysteinemia is crucial for preventing neurological damage and cognitive decline. Neuropsychiatric symptoms with duration of over a year seem to be irreversible.

The book outlines various lifestyle and other factors that may impair vitamin status and thereby homocysteine metabolism, and how they may contribute to the pathogenesis of neurological and psychiatric disorders.

Some practical recommendations are also given on blood sampling procedures, handling and storage of samples to reduce pre-analytical variation. Some advice is given on interpretation of the test results and how to effectively reduce elevated homocysteine concentrations in different situations.

The book is primarily intended for the physician who wants to get a concise overview, but cannot spend too much time on studying the topic. More than 1,000 references are given for those who want to have a closer look at the primary data. In order to keep this book comprehensive some data had to be left out, such as confidence intervals and p-values. All stated differences are statistically significant. The interested reader, however, can fairly easily find additional information either in abstracts that are freely available via MEDLINE, or directly from the primary sources.

Many excellent reviews thoroughly discuss different aspects of hyperhomocysteinemia and give more details on various studies.

There is a slight overlap of the different headings. This was done intentionally in order to make each chapter independent and thus allow reading in any order. However, it is recommended to read the chapter on homocysteine metabolism first.

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What is Homocysteine?

Homocysteine was first described by Butz and du Vigneaud in 1932. They obtained the product by treating methionine with concentrated acid. This was the beginning of a most fascinating research story, which Finkelstein recently highlighted in a review *(Finkelstein JD, 2000b)*.

In 1962 homocysteine was identified in the urine of some mentally retarded children *(Gerritsen T et al., 1962*, *Carson NAJ and Neill DW, 1962)*. Several years later a severe genetic defect of cystathionine β-synthase (CBS), causing *homocystinuria* and grossly elevated plasma levels of homocysteine, was identified *(Mudd SH et al., 1964)*. Patients suffering from this genetic defect were found to have premature arteriosclerosis and frequent thromboembolisms. More than 50% of the patients had recurrent cardiovascular events and about 25% died before the age of 30 *(Gibson JB et al., 1964, Schimke RN et al., 1965)*.

In 1969 McCully described the vascular pathology in these patients, including smooth muscle proliferation, progressive arterial stenosis, and haemostatic changes. In a patient with a genetic defect of cobalamin metabolism and a patient with cystathionine β-synthase deficiency, arteriosclerosis was a common trait although thrombosis was prominent only in the latter.

Severe defects in other enzymes involved in homocysteine metabolism, like methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR) were later discovered. Common to all these defects were homocystinuria and vascular pathology, as well as mental disturbances *(Mudd SH et al., 1972, Rosenblatt DS and Cooper BA, 1990, Rozen R, 1996)*.

A defect in the conversion of cobalamin to methylcobalamin (Cbl G defect) that decreases MS activity, is reported to give symptoms resembling multiple sclerosis *(Carmel R et al., 1988a)*.

Deficient conversion of cobalamin to both methyl- and adenosylcobalamin results in white matter oedema and defects in myelination and later in white matter bulk loss *(Rossi A et al., 2001)*.

A review of these severe hereditary defects has recently been published *(Rosenblatt DS and Whitehead VM, 1999)*.

Epidemiological studies in the general population have later demonstrated an association between common moderately elevated plasma levels of homocysteine and vascular diseases, as well as pregnancy complications, neural tube defects, other birth defects, various neuropsychiatric disorders, cognitive impairment in the elderly and an increased mortality rate.

Recently, less severe, common enzyme defects, causing moderately increased homocysteine levels, have also been associated with the same clinical symptoms.

Homocysteine is a sulphur-containing amino acid that is closely related to methionine and cysteine. There are no specific base-triplets for this amino acid and homocysteine is therefore not present in naturally occurring proteins. All homocysteine found in organisms is formed during the metabolism of the essential amino acid methionine, in the methylation cycle, page 21.

Reduced homocysteine has a highly reactive free thiol group, which can participate in redox reactions and is susceptible to auto-oxidation at a physiological pH, thereby forming disulphide bonds between two homocysteine molecules (homocystine) or mixed disulphides with cysteine. Alternatively, reduced homocysteine can form disulphide bonds with proteins, for instance albumin. In the cell homocysteine is mainly present in its reduced form. The export out of the cell appears to be regulated by a presumed "reduced-homocysteine carrier" *(Blom HJ, 2000)*. Extracellularly, homocysteine is mostly found in its oxidised form, either as a disulfide or bound to proteins.

The fraction of reduced homocysteine normally only constitutes about 1% of total homocysteine in plasma, but is higher in e. g. smokers and in severe renal disease. This reactive form was recently shown to correlate closely with adverse vascular effects *(Chambers JC et al., 2000b, Chambers JC et al., 2001)*.

About 70% of plasma homocysteine is bound to albumin. The rest forms disulphides, predominantly with cysteine or as the homocysteine dimer homocystine. However, relatively little is known about the formation of different homocysteine species in vivo. The sum of all the forms is termed *total homocysteine*. Homocysteine is sometimes written homocyst(e)ine, since this term more clearly designates all the molecular species that are measured. The abbreviations "Hcy" for homocysteine and "tHcy" for total homocysteine are used in the following, where tHcy generally refers to plasma or serum levels.

Routine assays generally measure tHcy in plasma or serum, sometimes in CSF, rarely in urine. Serum concentrations are normally slightly higher than plasma levels owing to pre-analytical conditions. Analysis of the different fractions of tHcy (oxidised or reduced, free or bound) is complicated and only used for research purposes.

Over the last ten years, several assays for measuring homocysteine in plasma, serum, and CSF have been developed. A further step forward is the recent introduction of enzyme immunoassays, which now allow determination of tHcy in most routine laboratories.

Three enzymes are directly involved in the Hcy metabolism: methionine synthase (MS), betaine homocysteine methyltransferase (BHMT), and cystathionine β-synthase (CBS). Several other enzymes are indirectly involved. Vitamins B_6 and B_{12} are cofactors to these enzymes and folate is a substrate in the MS-mediated reaction.

The metabolism of Hcy is described on pages 21-33 and illustrated in fig. 2, page 23. Disturbances in this metabolism, either caused by a genetic enzyme defect or owing to deficiency of cofactor(s), normally result in cellular accumulation of tHcy, and subsequently in the circulation.

The liver and the kidney are supposed to be the most important organs for uptake and metabolism of homocysteine.

Renal excretion does not seem to be an important route of elimination. Only about 1% of the Hcy filtered by the glomeruli is normally found in the urine *(Guttormsen AB et al., 1997)*. The rest is reabsorbed and metabolised. Thus, the kidneys are Hcy-metabolising rather than Hcy-excreting *(Bostom AG et al., 1995a, Van Guldener C et al., 1998, Refsum H et al., 1998a)*.

The plasma levels of tHcy are influenced by age, gender, menopausal status and other physiological determinants, as depicted in fig. 1.

Plasma tHcy increases throughout life in both sexes. Before puberty, children of both sexes have low and similar levels (mean values of about 6 µmol/L) *(Van Beynum IM et al., 1999, Minniti G et al., 2000, Bates C et al., 2002)*. During puberty, levels markedly increase, more in boys than in girls *(Bates C et al., 2002, Osganian SK et al., 1999, Jacques PF et al., 1999b)*. At the same time, tHcy values start to show a skew distribution in populations.

Throughout life, mean tHcy increases by 3-5 µmol/L. At the age of 40-42, there is a difference of about 2 µmol/L between men and women, with mean values of about 11 and 9 µmol/L, respectively *(Bates C et al., 2002, Nygård O et al., 1995)*.

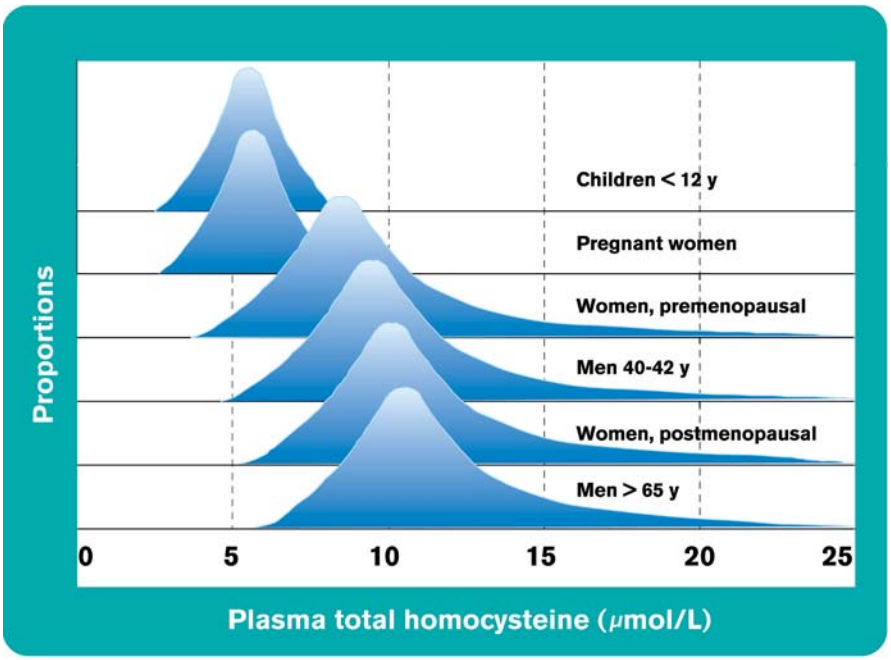

Figure 1 – Homocysteine levels in plasma and distribution related to age, sex and physiological variables. *Ueland PM et al. Determinants of plasma homocysteine. Developments in Cardiovascular medicine. 2000;230:62. Dordrecht. © 2000 Kluwer Academic Publishers. All rights reserved. Reprinted with kind Permission of Kluwer Academic Publishers.*

After the menopause, gender-related differences in tHcy diminish, but concentrations remain lower in women than in men *(Nygård O et al., 1995, Andersson A et al., 1992b*, *Morris MS et al., 2000*). The gender disparity may in part be explained by hormonal status *(Morris MS et al., 2000)*.

More relative muscle mass in men may also explain some of the difference, as the formation of creatine generates Hcy *(Brattström L, 1994, Mudd SH et al., 1995*).

Nutritional habits may also sometimes differ between the sexes *(Refsum H et al., 1996, Tucker KL et al., 1996a)* which could contribute to lower tHcy levels in women. Even psychological factors that may differ between sexes may contribute to the differences *(Stoney CM and Engebretson TO, 2000)*.

During pregnancy, tHcy concentrations are reduced by up to 50% with the lowest values in the second trimester, but levels return to previous values within 2-4 days of delivery *(Andersson A et al., 1992a, Kang S et al., 1986, Bonnette R et al., 1998, Walker M et al., 1999)*. Plasma expansion, increased metabolic rate, increased glomerular filtration and fetal Hcy metabolism may explain these variations.

The higher tHcy concentrations seen in the elderly may be a consequence of a general slowdown of the metabolism, increased prevalence of intestinal malabsorption or insufficient nutritional supply of folate, vitamins B_{12}, and B_6, reduced kidney function, and other physiological age-related changes, or be associated with diseases. Disturbed distriburion of the vitamins to deeper compartments seems to be common in the elderly. These and other factors known to increase tHcy levels are described in more detail on pages 59-108.

Nutritional and other lifestyle factors are important determinants of tHcy, and may, to a great extent, explain why mean tHcy levels vary between different populations. The levels were, for instance, found to be lower in a general French than in an Irish population. Levels in cardiovascular patients from the two countries, however, were similar *(Malinow MR et al., 1996)*.

Homocysteine Metabolism

Homocysteine is metabolised through two major pathways: transsulphuration and methylation. Normally, about 50% is re-methylated to form methionine, from which it originally derives.

The remaining 50% is converted in the transsulphuration pathway to cysteine. Homocysteine condensates with serine to form cystathionine, which is cleaved into cysteine and α-ketobutyrate.

The methylation cycle

Homocysteine is formed by demethylation of the essential amino acid, methionine, in the methylation cycle, fig. 2. Methionine is an essential amino acid and thus derived from dietary proteins. It contains a methyl group, which can be activated by conversion to S-adenosylmethionine (SAM). In every cell available methionine is partitioned between protein synthesis and the formation of SAM. The formation of SAM is mediated by adenosine triphosphate (ATP) and three isoenzymes of methionine adenosyl transferase (MAT I, II, and III). There are two genes coding for MAT, one expressed exclusively in the liver (MAT1A), the other (MAT2A) in all tissues. This MAT-mediated reaction is regulated by intracellular SAM concentrations. The liver MAT isoenzyme MAT III responds to higher concentrations. It now appears that growth factors, cytokines, and hormones regulate liver MAT and mRNA levels and enzyme activity *(Mato JM et al., 2002)*. A deficiency of MAT results in high methionine levels with low SAM levels.

Several mutations affecting MAT1A gene, encoding MATI, and III, have been identified that result in varying degrees of deficiency of the MAT I/III enzyme activity. Many affected individuals are asymptomatic, although the most severely deficient cases have neurological symptoms including demyelination.

The methyl group of the methionine moiety of SAM is activated by a positive charge of its sulphur atom. This methyl group is very reactive and can easily be transferred to a large variety of acceptor substrates, including nucleic acids (DNA and RNA), proteins, phospholipids, myelin, polysaccharides, choline, catecholamines, and a large range of small molecules.

SAM is the principal biological methyl group donor in the organism, and the only methyl donor in the CNS. It is required for numerous methylation reactions, of which over 100 have been identified. A product of all methylation reactions is S-adenosylhomocysteine (SAH), which is hydrolysed to Hcy in a reversible reaction. The reverse reaction is in fact favored. Increased tHcy levels therefore result in increased SAH levels as well. Chronic elevation in plasma tHcy, associated with vitamin deficiencies or genetic polymorphisms, may have an indirect and negative effect on cellular methylation reactions through a concomitant increase in intracellular SAH levels, as SAH competes with SAM at binding sites *(Yi P et al., 2000)*. SAM is also a necessary intermediate in the synthesis of polyamines, spermidine, and spermine.

Considering the essential role of methylation in various cellular processes, it is understandable that any alteration in the availability of SAM may have profound effects on cellular growth, differentiation, and function. This may be critical in many situations, not least in the aging brain, where neurochemical processes related to methylation may be declining, and in psychiatric and neurological diseases.

A recent review discusses the current knowledge of DNA methylation in normal cells and disease states, and how this relates directly to current understanding of the mechanisms *(Costello JF and Plass C, 2001)*.

Decreased remethylation of Hcy to methionine and SAM may, for instance, impair the methylation reactions required for normal brain function.

In most tissues, Hcy may be remethylated to methionine by the vitamin B_{12}-dependent enzyme methionine synthase (MS). A few tissues, predominantly the liver and kidneys, express in addition the enzyme betaine homocysteine methyltransferase (BHMT) that functions as an alternative pathway for the remethylation of Hcy to SAM. *However, the majority of tissues, including the CNS, are entirely dependent on the MS-mediated recycling of Hcy.*

Nitrous oxide irreversibly inactivates MS by oxidating cobalt in methylcobalamin (the active coenzyme). It thus inhibits the synthesis of SAM and has been used for experimental inactivation of this enzyme in animals to study the importance of methylation reactions for various neurological functions *(McKeever M et al., 1995a, McKeever M et al., 1995b, Scott JM et al., 1994, Weir DG et al., 1988, Weir DG et al., 1992)*.

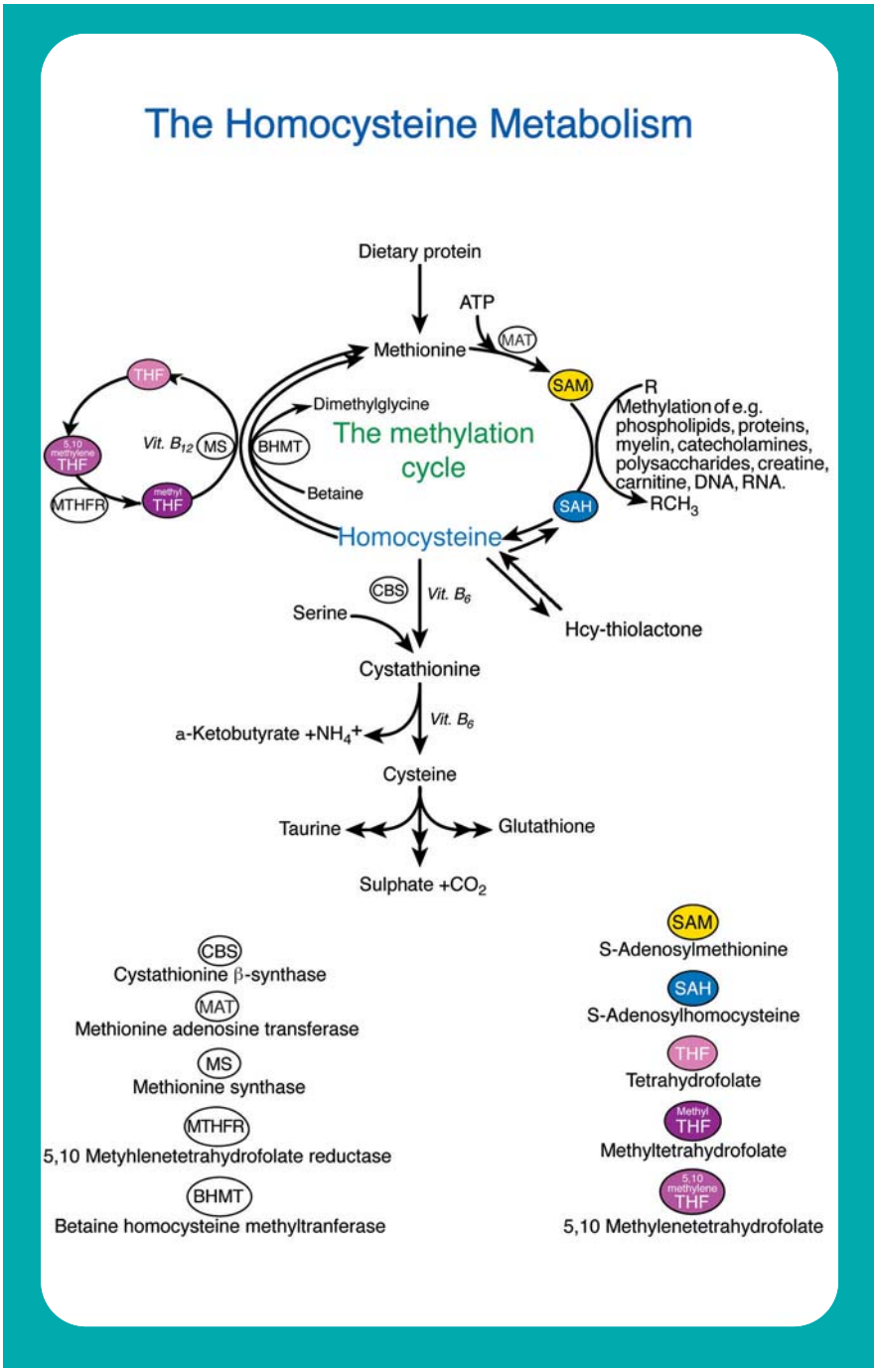

Figure 2 – The Homocysteine Metabolism.

A complicated feed-back system regulates homocysteine metabolism. If, for instance, methionine balance is negative, and in the presence of low concentrations of SAM, Hcy is primarily directed towards the remethylation pathway to form methionine by the MS-mediated reaction. Vitamin B_{12} is a cofactor and methyltetrahydrofolate (methyl-THF) a substrate in this reaction.

When tHcy concentrations increase, SAH also increases. SAH has multiple metabolic effects. It is a potent competitor to SAM at different binding sites and can therefore inhibit methylation *(Mudd SH et al., 1989)*. The SAM/SAH ratio may be used as an indicator of methylation status.

Methyl-THF is formed in a reaction catalysed by methylene tetrahydrofolate reductase (MTHFR), in which 5,10-methylenetetrahydrofolate is reduced to 5-methyltetrahydrofolate (methyl-THF). The MTHFR-mediated reaction is a rate-limiting step in the formation of methyl-THF in folate metabolism, fig. 3. This reaction is particularly important for the generation of methionine from Hcy, as methyl-THF functions as a substrate for MS. MTHFR has therefore a strong, indirect influence on Hcy remethylation *(Engbersen AMT et al., 1995)*.

Recently, knockout mice models for the MTHFR enzyme were used to study the pathogenetic mechanisms of MTHFR deficiency. Total Hcy was 1.6- and 10-fold higher in hetero- and homozygous mice, respectively, than in wild-type mice. Both hetero- and homozygous mice had, either decreased SAM, and increased SAH or both, and showed global DNA hypomethylation in brain and other tissues. Moreover homozygous mice exhibited significant growth retardation and cerebellar pathology. Abnormal lipid deposition in the proximal portion of the aorta was observed both in the older hetero- and the homozygous animals *(Chen Z et al., 2001)*.

The transsulphuration pathway

If SAM concentrations are high, the transsulphuration pathway is favoured and more Hcy is condensed irreversibly with serine to form cystathionine and – except in the CNS – cysteine by two vitamin B_6-dependent reactions, the first of which is catalysed by cystathionine β-synthase (CBS). Studies in animals have shown that inhibition of the transsulphuration pathway caused by vitamin B_6 depletion may result in both increased SAH levels and decreased levels of SAM *(Nguyen TTT et al., 2001)*.

Cysteine is a precursor of glutathione (GSH), the major cellular redox buffer. In a human hepatoma cell line approximately half of the intracellular GSH pool in human liver was calculated to be derived from Hcy *(Mosharov E et al., 2000)*.

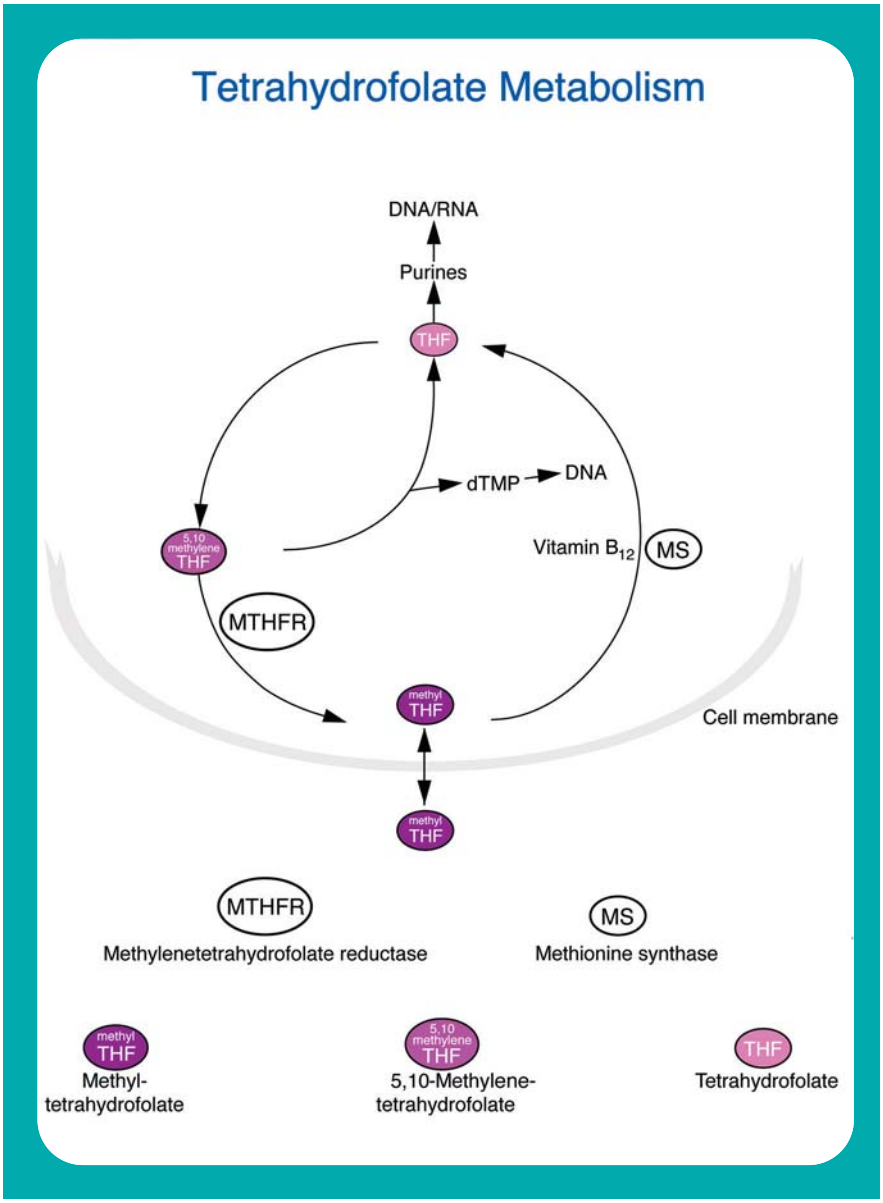

Figure 3 – The Folate Cycle.

Glutathione protects many cellular components against oxidative damage and other types of injury. It may, for instance, have protective vascular effects *(Kugiyama K et al., 2001)*. Neurones, however, lack this pathway

and are dependent upon glial cysteine for glutathione synthesis. The CNS is therefore more susceptible to oxidative stress than other tissues.

McCaddon and co-workers recently presented the hypothesis that oxidative stress may lead to oxidation of (cob(I)alamin) and thereby inhibition of the MS-mediated reaction, primarily in the CNS, thereby impairing the synthesis of SAM and increasing the levels of tHcy. Increased export from brain tissue could contribute to the mild-to-moderate elevations of tHcy found in AD. The oxidative stress would also compromise the intraneuronal reduction and activation of the vitamin, including currently available pharmaceutical forms of vitamin $B_{12,}$ to its metabolically active state. Oxidative stress would therefore render B_{12} therapy less effective *(McCaddon A et al., 2002b)*.

Glutathione interacts with vitamin E by keeping α-tocopherol in its reduced form, either by a direct reaction or by a pathway involving ascorbate *(Meister A, 1994)*. Homocysteine, on the other hand, reduces dehydroascorbic acid to ascorbic acid, leading to decreased dehydroascorbic acid availability *(Park JB, 2001)*.

The transsulphuration pathway is active primarily in the liver, kidney, small intestine, and pancreas. These tissues also have the most rapid turnover of GSH (in studies on animals). The transsulphuration pathway also directs Hcy to degradation and its ultimate removal as sulphate. Not only the transsulphuration, but also the remethylation pathway influences PML tHcy. Low levels of folate or vitamin B_{12} lead to a relatively higher increase after methionine loading *(Verhoeff BJ et al., 1998, de Jong SC et al., 1999b, Ubbink J et al., 2001)*. The importance of the remethylation pathway after methionine loading is illustrated by the results of a recent intervention study. The PML values of tHcy were reduced after treatment with 200 mg of pyridoxine. However, a combination of 0.5 mg of folic acid and 100 mg of pyridoxine given orally to hyperhomocysteinaemic vascular patients and their relatives was more effective in reducing PML tHcy concentrations *(Van der Griend R et al., 2000)*.

The conversion of Hcy into methionine and SAM may be severely blocked in some rare, genetic defects in MS or MTHFR. Studies on children with such inborn errors support the view that defective methyl group metabolism may be one of the leading causes of demyelination. Myelin basic protein normally shows (di-)methylation of arginine groups after a SAM-dependent protein methylase reaction. Brain magnetic resonance imaging has revealed demyelinated lesions that may be related to non-methylated myelin basic protein. Restoration of SAM levels is dependent on an active re-methylation cycle *(Surtees R et al., 1991)*. Patients with the above mentioned inborn errors have high levels of tHcy, low levels of

methionine, neurological symptoms, and mental retardation, as well as vascular pathology *(Mudd SH et al., 1972, Rosenblatt DS and Cooper BA, 1990, Rozen R, 1996, Carmel R et al., 1988a, McCully K, 1969)*. Low CSF levels of SAM are also found *(Surtees R et al., 1991, Hyland K et al., 1988)*.

A review on SAM and nerve regeneration was published in 1994 *(Cestaro B, 1994)*. A recent review outlines the clinical consequences of different aquired or hereditary disturbances in folate and cobalamin metabolism in children *(Rosenblatt DS and Whitehead VM, 1999)*. Two recent publications review the role of SAM as a methyl group donor *(Chiang P et al., 1996, Lu S, 2000)*.

The folate cycle

Another consequence of a block of the MS-mediated reaction is reduced recycling of methyl-THF into the pool of active folates. The regeneration of THF is critical, as it is the polyglutamated forms of THF that are acted upon by folate-metabolising enzymes (except by MS). Polyglutamation of methyl-THF proceeds much slower than that of THF.

Tetrahydrofolate is required for the formation of 5,10-methylene-THF and 10-formyl-THF, used in thymidylate and purine synthesis respectively, fig. 3. Purines are required for both DNA and RNA synthesis. Two of the carbon atoms in purine bases are derived from folate. DNA synthesis is dependent on thymidine, the formation of which requires thymidylate synthase. Thymidylate synthase is a key enzyme for cell proliferation and converts deoxyuridine monophosphate into thymidine monophosphate. This reaction involves a transfer of one carbon group from 5,10- methylenetetrahydrofolate to deoxyuridine monophosphate and is the rate-limiting step in the de novo synthesis of thymidylate.

A low MS activity, for whatever reason, leads to an accumulation of methyl-THF. Folates are "trapped" and the intracellular folate pool becomes abnormal. Moreover, intracellular folate retention may be impaired. As a consequence, serum levels of folate may be high despite an intracellular deficiency.

By the same mechanism, vitamin B_{12} deficiency may induce a secondary folate deficiency, resulting in megaloblastic anemia indistinguishable from anemia caused by primary folate deficiency. Of patients with megaloblastic anemia caused by cobalamin deficiency, 60% are expected to have low RBC folate levels, and 20% elevated serum folate levels *(Chanarin, 1979)*.

A reduced activity of MTHFR, however, that results in low intracellular levels of methyl-THF, also results in relatively higher levels of 10-formyl-THF and 5,10-methyleneterahydrofolate. DNA and RNA synthesis

may then be preserved so that megaloblastic anemia may not develop *(Kvittingen EA et al., 1997, Lalouschek W et al., 2000)*. However, this happens at the expense of SAM-dependent reactions.

The precise detail of folate metabolism is extremely complex. The folate-Hcy interrelations and potential new markers of folate status were recently studied by Lucock and co-workers *(Lucock MD et al., 1999)*. These aspects and their relation to cardiovascular disease, birth defects and other conditions are briefly summarised in a recent review *(Lucock M and Daskalakis J, 2000)*.

Enzyme defects, deficiency or disturbed distribution of the vitamins involved in the Hcy metabolism, interaction with lifestyle factors, diseases and drugs, or a combination can thus impair the methylation and the folate cycle and increase tHcy levels. The most important and frequent causes of hyperhomocysteinemia are unhealthy lifestyle, low intake of folate, vitamin B_6, and B_{12}, gastrointestinal malabsorbtion of these vitamins, impaired renal function and common polymorphisms of the MTHFR gene.

Three reviews on the pathways and regulation of the homocysteine metabolism have recently been published *(Selhub J, 1999a, Finkelstein JD, 2000a, Kruger WD, 2000b)*. The extensive review by Selhub also outlines the consequences of different enzyme defects on the regulation of the homocysteine metabolism.

The interaction between cobalamin and folate metabolism and the biochemical background to neuropathy and other deficiency symptoms are outlined in another extensive recent review *(Wickramasinghe SN, 1999)*.

Common enzyme defects

During the last few years, the enzymes, CBS, MS, MTHFR, and MAT have been cloned. Many mutations have been identified. Some are very common.

Tsai and co-workers determined different genotypes in the MS, CBS and MTHFR genes in 1,025 Americans. In this population more than 50% carried polymorphic traits influencing the Hcy metabolism *(Tsai MY et al., 2000b)*.

Enzyme defects decreasing the remethylation of Hcy, do not always lead to increased *fasting* levels of tHcy. It is sometimes necessary to perform a *post methionine loading* (PML) test to detect the defects (page 168). The enzyme system is loaded and the transsulphuration pathway in particular. If the enzymatic capacity is reduced, the increase in tHcy will be abnor-

mally high and especially in vitamin B_6 deficiency *(Ubbink J et al., 1996)* or mild CBS defects.

The prevalence and impact of mutations involved in Hcy metabolism are currently under investigation in many studies. This research will probably contribute to a better understanding of disturbances in Hcy metabolism.

These mutations are discussed in more detail in recent reviews *(Födinger M et al., 1999, Rozen R, 2000, Chamberlin M et al., 2000)*.

The C677T polymorphism of the methylenetetrahydrofolate gene

Some of the less severe enzyme defects affecting the Hcy metabolism are quite common, such as a thermolabile variant of MTHFR enzyme, first described in 1977 *(Rosenblatt DS and Erbe RW, 1977)*. A common C→T exchange in the gene coding sequence for the MTHFR enzyme at nucleotide 677 results in a alanine to valine substitution and to thermolability. The enzyme has a reduced activity in homozygous subjects, or 30-50% of normal (in vitro).

Homozygosity for the polymorphism, and to some extent heterozygosity, is associated with moderately increased fasting tHcy levels, particularly when folate status is low *(Kang S et al., 1988, Kang S et al., 1991, Engbersen AMT et al., 1995, Frosst P et al., 1995, Guttormsen AB et al., 1996, Harmon DL et al., 1996, Jacques PF et al., 1996, Kluijtmans LAJ et al., 1997, Ali N et al., 1997, Clarke R et al., 1998a, Verhoeff BJ et al., 1998, Zittoun J et al., 1998, McQuillan BM et al., 1999).* The C677T mutation may affect the composition of the intracellular folate pool. In addition it can reduce methionine and SAM concentrations.

In most populations, subjects carrying the TT genotype have mean tHcy concentrations about 25% higher than those with the CC genotype. The impact on tHcy is, however, dependent on folate status. When subjects with the TT variant were stratified according to quartiles of serum folate in a recent study, comparison of the lowest versus the highest quartile showed that there was a 48% difference in tHcy *(McQuillan BM et al., 1999)*. In a French study, women with the TT genotype had higher RBC folate levels than men, and did not show the same increase in tHcy as observed in men *(Chango A et al., 2000b)*. This may be because of higher folate intake by females and/or higher folate requirement in men for muscle creatine synthesis requiring SAM.

Although the prevalence of the C677T polymorphism is very high in, for instance, France and among Hispanic populations, tHcy is found to be relatively low in these populations *(Graham IM et al., 1997, Giles WH et*

al., 2000). A likely explanation is higher vitamin intake and possibly other environmental or genetic factors.

It has also been shown that vitamin B_{12} may be important as well in maintaining levels of THF in carriers of the C677T polymorphism. Carriage of the T allele was associated with THF levels in erythrocytes for a given B_{12} concentration, and there were genotype dependent relationships between levels of tHcy, B_{12}, methyl-THF and THF *(Lucock MD et al., 2001)*. In a study of 186 young women with the C677T and/or the A1298C (page 31) polymorphisms, tHcy was also negatively correlated with plasma vitamin B_{12} and serum folate. Total Hcy decreased highly significantly as vitamin B_{12} concentrations increased in subjects, who were heterozygous both for the C677T and the A1298C polymorphisms, whereas there was a trend in subjects homozygous for either variant *(Bailey LB et al., 2002)*.

Recent findings also indicate that MTHFR activity in homozygous subjects is dependent on riboflavin status *(Hustad S et al., 2000)*. Riboflavin is a cofactor for MTHFR, and may regulate folate distribution by affecting flavine adenine dinucleotide (FAD) dissociation kinetics *(Guenther BH et al., 1999)*.

The total concentrations of RBC folate thus vary with genotype, but are also dependent on specificity of the folate assay used *(Molloy AM et al., 1998b)*. The C677T polymorphism affects the *relative concentrations* of the different intracellular folate forms. Current research may result in determination of different intracellular folate derivatives as more reliable markers of folate status.

A reduced activity of MTHFR results in low intracellular levels of methyl-THF and high levels of formylated folate species *(Bagley PJ and Selhub J, 1998)*. The ratio of methyl-THF/total folate in RBC was 0.27 in mutant subjects, 0.66 in CT, and 0.71 in CC subjects in another recent study *(Zittoun J et al., 1998)*. A strong association between genotype and the methylated form of RBC folate with a clear dose effect was also found in another study *(Quéré I et al., 1998b)*.

Under these conditions, DNA and RNA synthesis may be preserved, particularly when folate status is high *(Stern LL et al., 2000b)*. The C677T polymorphism may then confer protective effects in connection with, for instance, survival. The allocation of folate species towards purine and pyrimidine synthesis in subjects homozygous for this MTHFR polymorphism could therefore have represented an evolutionary advantage *(Ueland PM et al., 2001)*.

A rapidly increasing number of clinical and experimental studies have, however, shown that the C677T polymorphism is overrepresented in many studies of, for instance, patients with vascular disease and cases of NTD

and other conditions, pages 151-161. The C677T polymorphism may thus affect disease susceptibility in both directions.

The prevalence of this MTHFR polymorphism shows ethnic differences. The C677T mutation is rare in Africans. The frequency is also very low in blacks living outside Africa, which has been proposed to contribute to lower tHcy in black American subjects than in white *(Estrada DA and Billett HH, 2001)*.

In Caucasians the mean prevalence of homozygous subjects is about 12% and of heterozygous subjects over 40%, but with considerable variations between geographic areas. The prevalence in the Mediterranean countries is high. The frequency of the homozygous genotype varies from 8% in Germany to 18% or more in Italy and other Mediterranean countries. The prevalence of homozygosity in whites outside Europe is 10-14%. Among a large sample of Americans it was 11.9% *(Tsai MY et al., 2000b)*.

Among Hispanics this polymorphism is very prevalent, and 21-35% of individuals are homozygous. Pooled data on several thousand Japanese show a frequency of the TT genotype of 11%. In a Chinese population the homozygote prevalence was 10.6% *(Ho CH, 2000)*. An overview of the prevalence in different geographical regions can be found in two recent publications *(Pepe G et al., 1998, Botto LD and Yang QH, 2000)*.

The reason for these ethnic differences in prevalence is uncertain. On the observation that the prevalence of this polymorphism had increased in Spain, after the introduction of folate supplementation for pregnant women was introduced, it is proposed that such a supplementation might increase the number of live-born foetuses with this mutation *(Munoz-Moran E et al., 1998, Reyes-Engel A et al., 2002)*. This hypothesis was recently supported by an open study of vitamin intervention in nulliparous, homozygous women, with a history of several spontaneous abortions *(Quéré I et al., 2001)*, and the finding of increased frequency of MTHFR polymorphisms in spontaneously aborted embryos *(Zetterberg H et al., 2002)*.

The C677T polymorphism of the MTHFR gene also increases PLM response *(Nelen WLDM et al., 1998, Verhoeff BJ et al., 1998, Candito M et al., 1999, de Jong SC et al., 1999b)*.

Other polymorphisms and interactions

Another common mutation affecting the MTHFR gene, the A1298C polymorphism, has been described in, for instance, a Dutch study *(Van der Put N et al., 1998)*, a Canadian study *(Weisberg I et al., 1998)*, a study of Jewish subjects *(Friedman G et al., 1999)*, a study of Jewish and Texan subjects *(Rady PL et al., 1999)*, and a French study *(Chango A et al., 2000a)*. In

this mutation an A→C transition at nucleotide 1298 leads to a glutamate to alanine substitution in the MTHFR protein. The frequency of this A1298C polymorphism was of the same magnitude in these populations, with a prevalence of about 8-13% of homozygosity.

This polymorphism is also associated with decreased MTHFR activity, but the influence on tHcy is smaller than that of the C677T polymorphism. However, there appears to be an interaction between the A1298C and the C677T polymorphisms. Heterozygotes for both polymorphisms – 20%, 15%, and 23.5% respectively of the Dutch, Canadian, and French study populations – resulted in a similar reduction in enzyme activity, as observed in homozygotes for the C677T polymorphism, increased tHcy, and decreased plasma folate levels. Homozygosity for both polymorphisms was not found.

Genetic variations in the methionine synthase (MS) gene have also recently been identified. One such mutation is the A2756G transition that results in aspartic acid being changed to a glycine residue *(Leclerc D et al., 1996)*. It results in trapping of methyl-THF, an effect in opposition to the C677T polymorphism, and in decreased fasting tHcy *(Tsai MY et al., 1999b, Tsai MY et al., 2000b)*. The prevalence of homozygosity of this polymorphism is about 3-5% in published studies *(Leclerc D et al., 1996, Tsai MY et al., 2000b)*. Among 1,025 Americans 32.1% were heterozygous for the A2756G transition *(Tsai MY et al., 2000b)*. It may be underrepresented among vascular patients *(Tsai MY et al., 1999b, Hyndman ME et al., 2000)*.

Some mutations may result in *increased* enzyme activity. A common mutation of the cystathionine β-synthase (CBS) gene at exon 8 (844 ins 68) increases the activity of CBS. It was first described in 1995 *(Sebastio G et al., 1995)*. Fasting tHcy and PML increase is lower in individuals carrying this variant, particularly if vitamin B_6 levels are low *(Tsai MY et al., 1999a)*.

Tsai and co-workers found that 14.9% of their sample of 1,025 Americans were heterozygous for this 68-bp insertion *(Tsai MY et al., 2000b)*. The mean prevalence of this polymorphism in a population sample from 11 European countries was about 7.5% of homozygosity, but the variation in prevalence between ethnic groups seems to be large. It was higher in the UK and the Central Europe, lower in the Baltic and the South *(De Stefano V et al., 1998)*.

The 68-bp insertion has been shown to counteract the tHcy-increasing effect of the C677T polymorphism *(De Stefano V et al., 1998)*. Interactions between the CBS 68-bp insertion and both the C677T polymorphism of the MTHFR gene, and the A2756G transition of the MS gene were also recently demonstrated *(Dekou V et al., 2001)*.

A mutation reducing the activity of human intestinal folylpoly-γ-glutamate carboxypepsidase, and affecting the absorption of folate, has recently been described. A C→T polymorphism was predicted to replace histidine with tyrosine at codon 475 in exon 13 of the catalytic region of the glutamate carboxypepsidase II gene. The presence of this H475Y variant, found in 6 out of 75 subjects, was associated with lower folate and higher tHcy levels in a Caucasian population. The enzyme activity was reduced by 53% in vitro *(Devlin AM et al., 2000)*.

Folate transport into the cell is mediated by a membrane-bound protein (FR-α) with a high binding affinity for 5-methyl-THF, and by the reduced folate carrier (RFC). A common polymorphism in RFC (80G→A) has recently been described *(Chango A et al., 2000c)*. The prevalence of homozygosity in different populations is about 25-30%. Individuals homozygous for this polymorphism and homo- or heterozygous for the C677T polymorphism had a moderately increased tHcy and also increased plasma folate levels *(Rady PL et al., 2001)*.

How can Homocysteine be Neurotoxic?

The proposed pathogenetic mechanisms, by which hyperhomocysteinemia may cause vascular damage, cognitive impairment, psychiatric and neurological complications, congenital defects, and pregnancy complications, converge.

The most commonly suggested Hcy-related toxic mechansims are oxidative injury by different mechanisms, impaired methylation, impaired DNA synthesis/repair caused by disturbed folate metabolism, and excitotoxic effects mediated by N-methyl-D-aspartate (NMDA) glutamate subreceptors. Interaction with imflammatory mechanisms, and protein homocysteinylation are other proposed mechanisms.

All these mechanisms, directly or indirectly associated with the Hcy metabolism, interact in an intricate pattern, which is far from being mapped out.

Research on the potential harmful effects of elevated Hcy levels first focused on vascular damage, which was the predominant finding in patients with homocystinuria. The vascular pathophysiological mechanisms are, however, also of relevance for neuropsychiatric damage, as vascular damage may contribute to not only dementia, but also other neuropsychiatric disorders, and there is an overlap between vascular disease and neuropsychiatric disorders.

Endothelial dysfunction and atherotrombosis may cause, for instance, CNS ischemia and neuronal hypoxia, contributing to neuropsychiatric symptoms. Stroke is the most obvious example. The proposed vascular pathophysiology is therefore also briefly summarised.

The vascular mechanisms have been extensively studied both in vitro and in vivo. In vitro studies suggest that Hcy can induce direct damage to endothelial cells, increased platelet activity, pro-coagulant effects, increased collagen synthesis, and enhanced proliferation of smooth muscle cells.

Mild hyperhomocysteinemia induced in animals, mostly by dietary means, has been accompanied by impaired vasomotor regulation and

antithrombotic function, as well as subclinical or clear atherothrombotic lesions, lipid peroxidation, enhanced neutrophil-endothelial interactions, and pronounced intimal hyperplasia. Increased systolic-diastolic hypertension, thromboembolic events, and a deterioration of the elastic structure of arteries are also described.

In humans several studies have shown that an oral methionine load acutely impairs endothelium-dependent vasodilatation (EDD), both in healthy adults *(Bellamy MF et al., 1998, Chambers JC et al., 1998*, *Chambers JC et al., 1999a*, *Chambers JC et al., 1999b*, *Schächinger V et al., 1999, Usui M et al., 1999)* and in atherosclerotic patients *(Krzanowski M, 1998*, *Schlaich MP et al., 2000)*. The dose-dependent response on tHcy levels and on brachial artery flow-mediated dilatation in healthy volunteers, found by Chambers and co-workers, is illustrated in fig. 4.

These effects have been shown to be counteracted by homocysteine-lowering treatment in placebo-controlled studies *(Bellamy MF et al., 1999*, *Woo KS et al., 1999, Chambers JC et al., 2000b, Title LM et al., 2000*, *Willems FF et al., 2002)*.

An interaction with oxidative mechanisms is supported by the finding of a protective effect of vitamin C on EDD response to methionine loading *(Chambers JC et al., 1999b)*. Vitamin C also ameliorated flow-mediated EDD in recent studies of patients with homocystinuria *(Moat SJ et al., 2001)* and in diabetic patients with coronary artery disease *(Antoniades C et al., 2001)*.

Methionine-induced pro-coagulant effect has also been documented in studies of healthy subjects and patients *(Nappo F et al., 1999, Marcucci R et al., 2000, Al-Obaidi MK et al., 2000b)*, as well as a protective effect of Hcy-reducing treatment *(Constans J et al., 1999, Undas A et al., 1999)*.

The relation between vascular risk factors and risk indicators of Alzheimer's disease (AD) was recently reviewed in two publications *(Breteler MMB, 2000, Miller JW, 2000)*. Another recent, extensive review on Hcy-related vascular damage offers over 250 references *(Durand P et al., 2001)*.

Fig. 5 outlines the interaction between vascular and other mechanisms in neuropsychiatric disorders *(Loscalzo J, 2002)*.

Oxidative stress

Neurons are extremely sensitive to attacks by free radicals. Disturbed nitric oxide (NO) activity and oxidative mechanisms are known to be involved. Findings of interaction with NO and adaptive changes in activity of different antioxidant systems strongly support that elevated tHcy represents an oxidative stress. Oxidative stress may induce various vascular and neuro-

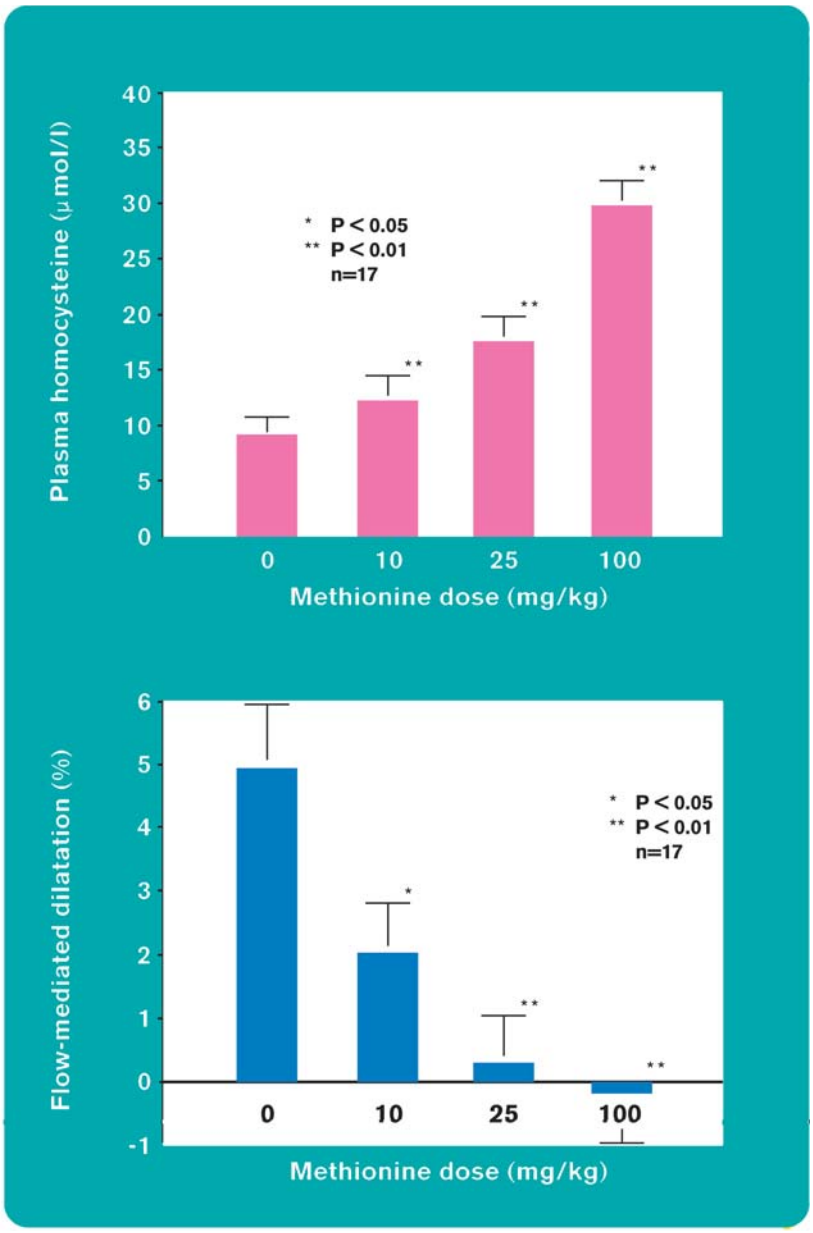

Figure 4 – Plasma homocysteine concentrations and flow-mediated dilatation (mean ± SEM, n = 17) 4 hours after fruit juice alone (vehicle) and after methionine 10, 25, 100 mg/kg. * $p < 0.05$, ** $p < 0.01$ compared with responses after fruit juice alone. *Chambers J et al. Physiological Increments in plasma homocysteine induce vascular endothelial dysfunction in normal human subjects. Arteriosclerosis and Thrombosis. 1999; 19:2922-27. © 1999 Am Heart Association Inc. Reprinted with permission.*

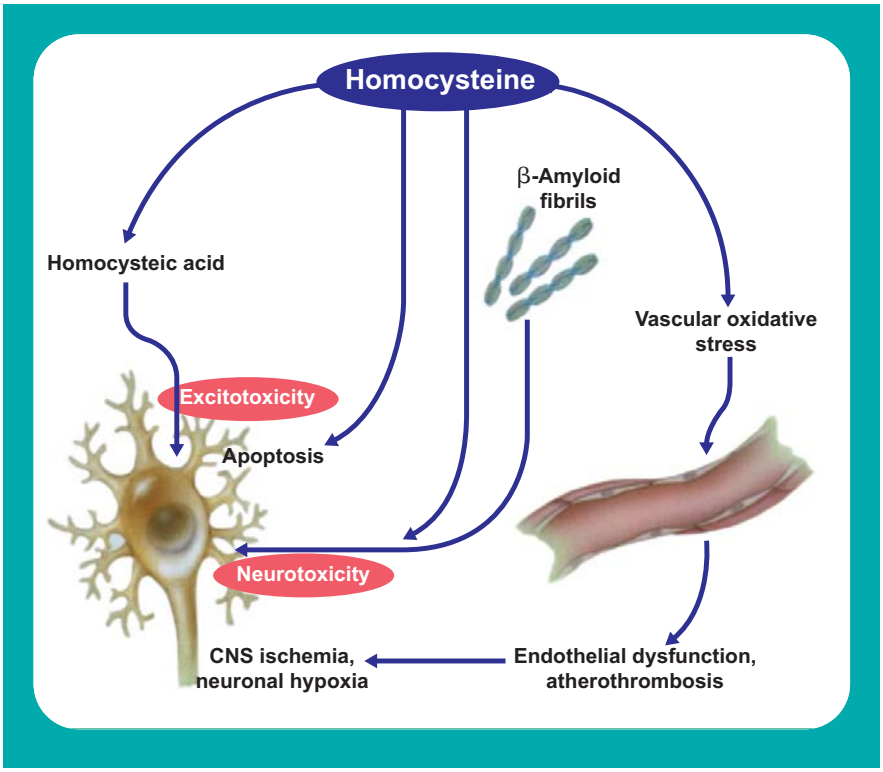

Figure 5 – Potential mechanisms by which homocysteine can cause neuronal injury. *Loscalzo J. Homocysteine and dementias. New Engl J Med, 2002; 346:466-7. © 2002 Massachusets Medical Society. All rights reserved. Reprinted with permission.*

logical damages, and seem to play a major role in aging and neurodegenerative disorders such as AD and Huntington´s disease *(Butterfield DA et al., 2001)*. There is evidence of increased oxidative damage to macromolecules in amylotrophic lateral sclerosis, Huntington´s disease, Parkinson´s disease, and AD by several mechanisms *(Beal M, 1995)*.

Both AD and PD are thought to be associated with increased microglial activity, that may lead to increased NO production. Nitric oxide can react with superoxide to form peroxynitrite, a highly damaging form of reactive oxygen species. In the CNS this molecule can be generated by microglial cells activated by pro-inflammatory cytokines or β-amyloid peptide and by neurons in three situations; hyperactivity of glutamate neurotransmission, mitochondrial dysfunction and depletion of L-arginine or

tetrahydrobiopterin. The first two situations correspond to cellular response to an initial neuronal injury, and the peroxynitrite formed exacerbates the inflammatory process. In the third situation, the generated peroxynitrite directly contributes to the initiation of the neurodegenerative process *(Torreillas F et al., 1999)*.

A recent study showed a highly significant elevation of tHcy, lipid peroxide, and NO in the plasma of patients with thrombotic cerebrovascular stroke, as compared with age- and gender-matched healthy controls. There was also a strong positive correlation between tHcy and lipid peroxide and a strong negative correlation with the plasma concentration of ascorbic acid, which points to a Hcy-related oxidative pathogenetic mechanism *(ElKossi MMH and Zakhary MM, 2000)*.

Incubation of hippocampal cultures in folate-deficient medium, or in the presence of MTX or Hcy, induced cell death and rendered neurons vulnerable to death induced by amyloid β-peptide. The investigators also found that methyl donor deficiency caused uracil misincorporation and DNA damage and greatly enhanced amyloid β-peptide toxicity. Furthermore, when maintained on a folate-deficient diet, amyloid precursor protein mutant mice, but not wild-type mice, exhibited increased cellular DNA damage and hippocampal neurodegeneration *(Kruman I et al., 2002)*.

Homocysteine may induce calcium influx and oxidative stress, and was recently shown to potentiate β-amyloid fragment induced increase in cytosolic calcium and apoptosis in human neuroblastoma cells. The effect was counteracted by vitamin E and the GSH precursor N-acetyl-L-cysteine *(Ho PI et al., 2001)*. In vitro and animal studies also suggest that various other compounds with antioxidant activity can attenuate the oxidative stress induced by β-amyloid *(Floyd RA, 1999, Pratico D and Delanty N, 2000, Meydani M et al., 2001)*.

The possible oxidative mechanisms involved in AD and their interactions are discussed in a recent article *(Christen Y, 2000)*.

Interaction with nitric oxide

Several studies have demonstrated stimulation of NO synthesis by Hcy (*Upchurch GR et al., 1997a*, *Welch G et al., 1998, Ikeda U et al., 1999, Wang S et al., 2000)*, but the interaction seems complex. Reduced release of NO from endothelial cells is also found *(Weiss N et al., 2001)*, and an inverse relation is found between plasma tHcy and NO release *(Mutus B et al., 2000, Mutus B et al., 2001)*. Two studies indicate that Hcy decreases the release of NO without affecting NO synthase (NOS) activity *(Chow K et al., 1999,*

Zhang X et al., 2000). Oxydation of sulphydryl groups in NOS may reduce the NO-generating activity. The effect may be dose-dependent and the cellular redox state seems to play an important role in the Hcy mediated suppression of the NO generation *(Zhang X et al., 2000)*.

Homocysteine-induced endothelial dysfunction and down-regulation of NO activity was attenuated by a NO donor in an in vitro study *(Preufer D, 1999)*. Pre-treatment with a NOS inhibitor, on the other hand, greatly exacerbated the sensitivity to Hcy of vascular smooth muscle cells, which were extremely responsive to angiotensin II after Hcy exposure at concentrations well below the physiological range. The study suggests that an initial effect of Hcy is a release of Ca^{2+} from intracellular compartments in vascular smooth muscle cells *(Mujumdar VS et al., 2000)*. The finding was supported by another recent study *(Unguari Z and Keller A, 2001)*.

In contrast to the effect of Hcy, a direct enhancing effect of methyl-THF on the enzymatic activity of NOS in cultured endothelial cells and in recombinant endothelial NOS was recently reported *(Stroes ESG et al., 2000)*.

Homocysteine has also been proposed to decrease dose-dependently the bioavailbility of NO by a mechanism involving glutathione peroxidase, which renders NO more susceptible to oxidative inactivation *(Upchurch GR et al., 1997b)*.

There is increasing evidence that NO plays an important role in the pathophysiology of CNS disorders such as AD, Parkinson´s disease (PD) and multiple sclerosis. Nitric oxide is thought to have a function in memory and long-term potentiation.

Cerebrovascular reactivity (CVR) has been reported to be NO-related in experimental studies. A recent study indicates that hyperhomocysteinemia may be causally associated with impairment of CVR in older subjects *(Chao CL and Lee YT, 2000)*.

A recent in vitro study indicates that NO may be involved in the modulation of signal transduction pathways by which nerve growth factor leads to increased choline acetyltransferase expression *(Kalisch BE et al., 2002)*.

At high concentrations NO is neurotoxic and may play a role in neurodegeneration.

Elevated tHcy and nitrite (a metabolite of NO) levels were found in MS patients and both L-dopa-treated and non-treated PD patients *(Qureshi GA et al., 1995, Qureshi GA et al., 1996)* . High levels of tHcy, arginine and nitrite in the CSF and a linear relation between levels of Hcy and nitrite were later found not only in patients with PD and MS, but also in patients with cerebrovascular disorders, and tuberculous meningitis, fig. 6 *(Baig S et al., 1998)*. These findings suggest that the production of nitrite is interrelated with elevated levels of Hcy.

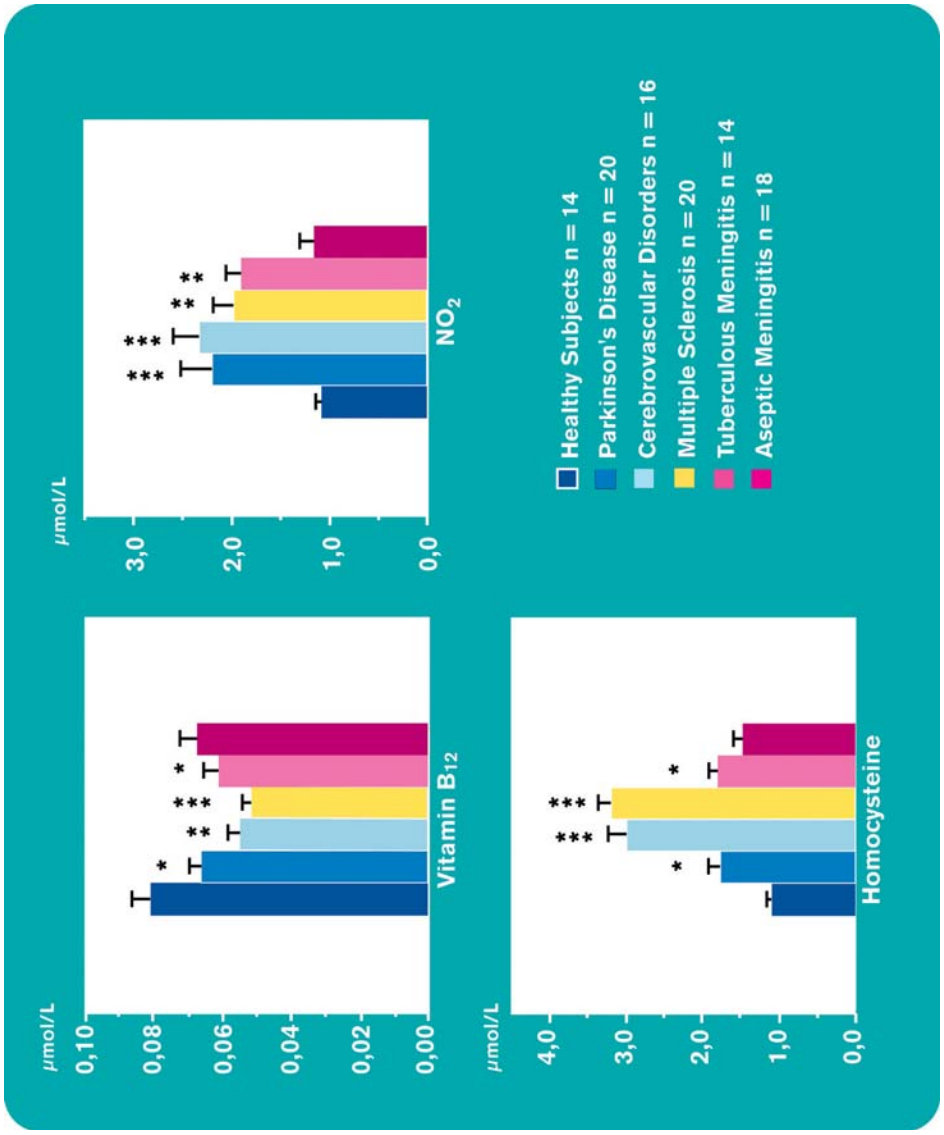

Figure 6 – CSF levels of tHcy, vitamin B_{12}, and nitrite (metabolite of nitric oxide) in neurological patients and healthy controls. The values are expressed as mean ±SEM.* $p < 0.05$, ** $p < 0.01$, *** < 0.001. *Baig S et al. The interrelation between the deficiency of vitamin B_{12} and neurotoxicity of homocysteine with nitrite in some neurologic disorders. Biogenic Amines, 1998; 14:1-14. Reprinted with permission.*

Another study showed that patients with AD or PD or with multiple system atrophy, all had lower levels of nitrate but not of nitrite, in CSF, when compared to controls *(Kuiper MA et al., 1994)*.

The publication by Baig *et al.* includes a review on this topic.

Interactions with assymmetrical dimethylarginine

Assymmetrical dimethylarginine (ADMA), is an endogenous inhibitor of NO.

A strong positive correlation between plasma levels of tHcy and levels of ADMA was seen after methionine-induced mild hyperhomocysteinemia in monkeys *(Böger RH et al., 2000a)* . The synthesis of ADMA was up-regulated by LDL cholesterol *(Böger RH et al., 2000b)*. These results were confirmed in a study of healthy humans *(Böger RH et al., 2001)*, which indicates that ADMA may mediate some Hcy-related effects. The increase of ADMA was associated with reduced activity of dimethylarginine dimethylaminohydrolase, the enzyme that degrades ADMA in another in vitro study *(Stuhlinger MC et al., 2001)*. A recent study showed that the CSF concentrations of ADMA were highly significantly lower in AD patients than in neurologically normal controls of a similar age and it decreased with impairing cognitive function *(Abe T et al., 2001)*.

Interaction with cysteine and glutathione

Cysteine is a precursor of glutathione (GSH). This endogenous intracellular antioxidant maintains the sulph-hydryl groups of proteins and other compounds in reduced form. A substantial part of the intracellular GSH pool is derived from the Hcy-dependent transsulphuration pathway *(Mosharov E et al., 2000)*. The synthesis via cysteine is dependent on two vitamin B_6-mediated reactions.

It was recently shown that vitamin B_6 can inhibit superoxide radical production, thereby reducing lipid peroxidation, protein glycosylation, and increased Na^+/K^+-ATP-ase activity induced by hyperglycosylation in human RBCs *(Jain SK and Lim G, 2001)*. Homocysteine inhibits Na^+/K^+-ATP-ase activity in animals, an effect also counter-acted by antioxidants as glutathione, cysteine and superoxide dismutase (SOD) *(Streck EL et al., 2001)*. A study in rats showed that tHcy was negatively correlated with both the cysteine and GSH levels, and that modulation of the GSH levels influenced tHcy in plasma *(Ovrebo KK and Svardal A, 2000)*.

Data from 750 cases with vascular disease and 800 controls show a U-shaped relationship between total cysteine and risk of cerebrovascular disease *(ElKhairy L et al., 2000, ElKhairy L et al., 2001)*.

Lipid peroxidation

A highly significant positive association between tHcy levels and lipoperoxidation was seen in male participants of the Antioxidant Supplementation in Atherosclerosis Prevention Study *(Voutilainen S et al., 1999)*. A recent study also showed a positive correlation in AD patients between plasma concentrations of tHcy and (E)-4-hydroxy-2-nonenal (HNE) a neurotoxic product of lipid peroxidation that is increased in the brains of AD patients. Total Hcy and HNE were higher both in plasma and CSF in AD patients than in controls *(Selley ML et al., 2002)*.

Oxidative stress induced by Hcy is indicated by an increase in malondialdehyde (MDA), a radical damage end product, and a measure of membrane lipid peroxidation. In a study of stroke patients, MDA concentrations were 89% higher and GSH levels 33% lower in CSF 48 hours after the ischaemic episode, as compared to control values *(Jovii A et al., 1999)*. A decrease in plasma antioxidant capacity after methionine loading in healthy subjects is also found *(Ventura P et al., 2000)*.

Interaction with superoxide dismutase

Increased superoxide dismutase (SOD) activity, indicating oxidative stress, has been demonstrated in several studies not only in various dementia disorders, but also in patients with PD *(Serra JA et al., 2001)*, and in neurological disorders associated with HIV infection *(Mollace V et al., 2001)*. A recent study also found that SOD activity was on the average almost 50% increased in patients with Down syndrome. Development of dementia in these patients was paralleled with decrease of SOD activity *(Torsdottir G et al., 2001)*.

A significant positive correlation between tHcy and extracellular SOD was found in a study of CVD patients *(Wang et al., 1999)*. Both the activity of SOD and that of glutathione peroxidase were also significantly elevated in the plasma of patients with inherited defects of Hcy metabolism and with heterozygous parents, when tHcy-levels were 20 µmol/L or higher *(Moat SJ et al., 2000)*. In homocystinuric patients a highly significant positive relation between extracellular SOD and tHcy was also found *(Wilcken DEL et al., 2000)*.

Exposure of rat aorta with 0-500 µmol/L of Hcy increased SOD activity, but decreased glutathione peroxidase activity in a dose-dependent manner *(Nishio E and Watanabe Y, 1997)*. Homocysteine-induced intracellular pro-

duction of superoxide and higher levels of SOD activity were also observed in a study of cultured porcine aortic endothelial cells *(Lang D et al., 2000)*.

The Hcy-mediated increase in SOD production may be inhibited by tetrahydrobiopterin (BH_4), folic acid, and methyl-THF. This inhibition may be a direct antioxidant effect or may involve increased NO production, owing to increased availability of NO-synthase co-factors, such as BH_4 *(Kredan MB et al., 1999)*.

Interactions with antioxidant vitamins

A negative correlation between plasma levels of antioxidant vitamins and the severity of cognitive impairment is demonstrated, as well as a protective effect against cognitive decline of antioxidant supplements *(Morris MS et al., 1998, Paleologos M et al., 1998, Riviere S et al., 1998, Masaki KH et al., 2000)*. The EURONUT-SENECA study showed a correlation between global cognitive performance and the plasma levels of vitamin B_{12} and folate, and also the concentrations of lycopene, β-carotene, and α–tocopherol *(Haller J, 1996)*.

Vitamin C interacts with glutathione (GSH). It increases mitochondrial GSH in GSH-deficient animals and thereby protects the mitochondria from oxidative damage *(Meister A, 1995b)*. Recycling of ascorbate from dehydroascorbate is dependent on glutathione *(May JM, 2001)*.

Vitamin C has also been found to increase NO availability in coronary atherosclerosis *(Tousoulis D et al., 1999)*.

Homocysteine reduces dehydroascorbic acid to ascorbic acid. The simultaneous oxidation of Hcy has been proposed to attenuate the deleterious effects of Hcy and explain the reported beneficial effect of vitamin C on Hcy-induced vascular lesions, and pre-eclampsia *(Park JB, 2001)*.

The clinical observations that vitamins E and C block the effects of PML hyperhomocysteinemia on coagulation and vascular endothelial function, without influencing homocysteine levels, also supports the notion that oxidative mechanisms are involved in the adverse effects of hyperhomocysteinemia *(Chambers JC et al., 1999b, Kanani PM et al., 1999, Nappo F et al., 1999, Wilkinson IB et al., 2001)*.

Various possible mechanisms by which vitamin C may prevent endothelial dysfunction are discussed in a recent review *(May JM, 2000)*.

Folic acid may also have an antioxidant effect. An experimental study recently indicated that folic acid scavenges thiyl radicals and repairs thiols *(Joshi R et al., 2001)*.

Interaction with metal ions

Several metals, among them copper, have been found to have toxic effects on nerve cells and neurobehavioural functioning. This evidence was recently reviewed *(Carpenter DO, 2001)*.

Copper is shown to increase the release of Hcy, lower the reduced fractions of thiols, and also enhance the vascular effects of Hcy in experimental studies *(Nishinaga M et al., 1993, Hultberg B et al., 1998b, Emsley AM et al., 1999, Xu D et al., 2000)*.

The reactive sulph-hydryl group of Hcy is proposed to act catalytically with ferric or cupric ions in a mixed function oxidation system to generate hydrogen peroxide, oxygen radicals, and homocysteinyl radicals. A recent study of mouse neuronal cultures showed that Hcy selectively potentiated toxicity from low micromolar concentrations of copper. The toxicity was dependent on the ability of Hcy to reduce Cu^{2+}. Homocysteine also generated high levels of hydrogen peroxide in the presence of Cu^{2+} *(White AR et al., 2001, White AR et al., 2002)*.

The presence of e.g. copper greatly enhanced the inhibitory effect of Hcy on NO-mediated relaxation in a study of isolated rat aortic rings. The effect was attenuated by SOD and catalase *(Emsley AM et al., 1999)*. Catalase also reduced the copper-dependent Hcy-induced accelerated senescence of endothelial cells *(Xu D et al., 2000)*. Copper-dependent vascular mechanisms may thus be implicated in CNS damage, but a recent study showed that also the levels of CSF-Tau protein correlated positively with the serum levels of copper and also with the serum copper/zinc ratio *(Regland B et al., 2001)*.

Another study indicates that an increased Hcy concentration causes abnormal Mg^{2+} metabolism in cerebral vascular smooth muscle cells, priming these cells for Hcy-induced atherogenesis, cerebral vasospasm, and stroke *(Li W et al., 1999)*.

Decreased synthesis of tetrahydrobiopterin

Tetrahydrobiopterin (BH_4) is a rate-limiting cofactor for the hydroxylases of phenylalanine, tyrosine and tryptophan and therefore required for the synthesis of dopamine and serotonin. Tetrahydrobiopterin also acts on specific membrane receptors to directly stimulate the release of monoamine neurotransmitters, such as dopamine and serotonin and independently of its cofactor activity. It is assumed to stimulate monoamine release by activating Ca^{2+} channels. In addition it indirectly stimulates the release of non-

monoamine neurotransmitters such as acetylcholine and glutamate, through activation of monoaminergic systems.

Dopaminergic neurons have intrinsically lower levels of superoxide than nondopaminergic neurons. Inhibiting BH_4 synthesis increased superoxide in dopaminergic neurons. Tetrahydrobiopterin decreased baseline oxidative oxygen species and attenuated induced reactive oxygen species also in fibroblasts, in which neither catecholamine nor NO production occurs. Tetrahydrobiopterin is therefore proposed also to have a specific protective antioxidative effect on dopaminergic neurons.

Oxidative stress or genetic defects may disrupt this capacity and contribute to degeneration of dopaminergic neurons in PD, for instance *(Nakamura K et al., 2001)*.

There is evidence that normal folate metabolism is required for maintaining the synthesis/recycling of BH_4. Significantly reduced levels of the metabolites of dopamine and serotonin in children with inborn errors of folate metabolism and low CSF levels of methyl-THF support this theory *(Surtees R et al., 1994)*.

The CSF levels of BH_4 have been found to be decreased in not only PD patients, but also AD patients *(Kay AD et al., 1986)*. A study of depressive patients also showed that BH_4 synthesis was reduced in female patients. There was also a correlation between plasma folate and urinary biopterin *(Coppen A et al., 1989)*.

Tetrahydrobiopterin is required for NO synthesis as a co-factor *(Thony B et al., 2000)*. A significant positive correlation between nitrite+nitrate and BH_4 in the CSF of children with various neurological diseases, including inborn errors of BH_4 metabolism has been found *(Heales SJR et al., 1999)*. Patients with BH_4 deficiency were also recently shown to have decreased concentrations of NO metabolites in the CSF *(Zorzi G et al., 2002)*.

Suboptimal concentrations BH_4 favor "uncoupling" of NOS leading to NOS-mediated reduction of oxygen and formation of superoxide anions and hydrogen peroxide. Folates and BH_4, both NOS cofactors, may have an indirect antioxidant effect *(Kredan MB et al., 1999)*. A recent study showed that folic acid, 5-methyl-THF, and BH_4 all highly significantly abolished Hcy-induced intracellular superoxide increase in vitro *(Doshi SN et al., 2001b)*. At physiological pH BH_4 scavenges a range of biologiclly active oxidants. It also interacts with ascorbic acid, which stabilises BH_4 *(Patel KB et al., 2002, Toth M et al., 2002)*. Tetrahydrobiopterin is one of the most potent naturally reducing agents.

Tetrahydrobiopterin may also have protective vascular effects of importance for neuropsychiatric disorders. An in vitro study demonstrated that both methyl-THF, and BH_4, or a combination of folic acid, methyl-THF,

and BH_4 significantly counteracted methionine-induced endothelial dysfunction. Tetrahydrobiopterin was also recently shown to counteract endothelial dysfunction during chronic alcohol consumption *(Sun H et al., 2001)* and improve endothelium dependent dilatation (EDD) in patients with heart failure *(Setoguchi S et al., 2002)*.

Recent reviews outline the function of BH_4 in the nervous system *(Bottiglieri T, 1996, Koshimura K et al., 2000)* and the vascular system *(Katusic ZS, 2001)*.

NMDA receptors and excitotoxcity

Oxidation of Hcy leads to the formation of homocysteine sulphinic acid and homocysteic acid, both shown to have potent excitotoxic effects on different subtypes of N-methyl-D-aspartate (NMDA) glutamate receptors *(Olney J, 1989, Schwartz S et al., 1990, Kubova H et al., 1995, Kim WK and Pae YS, 1996, Lipton S et al., 1997, Flott-Rahmel B et al., 1998, Kingston AE et al., 1998)*.

Celltype specific excitotoxicity was seen when human cell lines from different areas of the brain were exposed to homocysteic acid and other excitotoxic amino acids *(Parsons R et al., 1998)*.

Excessive amounts of Hcy and homocysteic acid have been reported in CSF from children with acute lymphoblastic leukemia treated with methotrexate *(Quinn C et al., 1997)*. In several cases the increase in homocysteic acid was related to the onset of seizures *(Quinn C et al., 1998)*.

A recent study in rats demonstrated that Hcy elicits a response to DNA damage in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. Homocysteine markedly increased the vulnerability of hippocampal neurons to excitotoxic and oxidative damage both in cell culture and in vivo, a mechanism that may contribute to neurodegenerative disorders *(Kruman I et al., 2000)*.

Activation of NMDA receptors produces a rise in intracellular Ca^{2+}, followed by release of cellular proteases and eventually cell death. This excitotoxic mechanism has been implicated in a wide variety of neurodegenerative and psychiatric disorders, ranging from metabolic and toxic encephalopathies to schizophrenia *(Santosh-Kumar CR et al., 1994)*.

Apoptotic cell death in the mouse retinal ganglion, induced by homocysteine and potentiated by simultaneous elevation of Hcy and glutamate, provides evidence that exitotoxic damage to neurons of the ganglion cell layer may be implicated in, for instance, diabetic retinopathy *(Moore P et al., 2001)*.

Under pathological conditions, such as ischemia or head injury, when glycine concentrations are elevated, even mildly elevated Hcy concentrations may become neurotoxic because of excessive Ca^{2+} influx and reactive oxygen generation *(Lipton S et al., 1997)*.

An interaction between excitotoxic NMDA activity and NO-related oxidative damage has been proposed in aging, neurodegenerative disease, and other neurological disorders *(Garthwaite J, 1991, Bondy S and le Bel C, 1993, Coyle J and Puttfarcken D, 1993)*. Homocysteic acid and cysteine sulphinic acid can activate NO formation by interaction with the NMDA receptors. This has also been associated with Hcy-related neurological damage *(Adachi K et al., 1998)*.

Two reviews on sulphur containing excitatory amino acids have been published *(Lipton S and Rosenberg P, 1994, Thompson G and Kilpatric I, 1996)*.

Impaired methylation

Disturbances in the activity of enzymes (MS, MTHFR, MAT) involved in the synthesis of the methyl donor SAM are associated with psychiatric disease. Hyperhomocysteinemia may be an indicator of impaired methylation capacity.

Brain tissue lacks the enzyme betaine homocysteine methyl transferase, the alternative pathway for remethylation Hcy, (page 22) and may be more sensitive to hyperhomocysteinemia and/or methyl group (SAM) depletion as a result of folate and/or vitamin B_{12} deficiency.

It is also doubtful if Hcy can contribute to the synthesis of glutathione in the CNS *(Finkelstein JD, 2000a, Molloy AM and Weir DG, 2000)*.

Cerebrospinal fluid SAM level has been determined in several studies in neuropsychiatric patients, table 1. Highly significantly decreased concentrations of SAM were, for instance, found in the CSF of patients with AD, and also in depression *(Bottiglieri T et al., 1990, 2000b)*. This observation was confirmed in a study that reported that levels of SAM were decreased by 65-85% in post mortem brain tissue from patients, as compared to controls *(Morrison L et al., 1996)*.

Conversely, treatment with SAM is shown to improve cognitive function in patients with AD *(Reynolds EH et al., 1989)*. Significant amelioration of cognition and vigilance in elderly patients with primary or secondary organic brain syndrome is also reported. The Mini Mental State Evalution (MMSE) and Sandoz Clinical Assessment Geriatric Scale (SCAG scores) improved significantly after 60 days of treatment with SAM *(Fontanari D et al., 1994)*.

		n	SAM	tHcy
Depression	Bottiglieri et al. 1990	35	⇓	–
Alzheimer's dementia	Bottiglieri et al. 1990	10	⇓	–
	Regland et al. 1990	22	–	⇑
	Morrison et al. 1996	11	⇓	–
Parkinson's disease	Pall et al. 1992	23	⇓	–
	Baig et al. 1998	20	–	⇑
MTHFR deficiency	Hyland et al. 1988	4	⇓	–
	Surtees et al. 1991	4	⇓	⇑
SACD*	Bottiglieri et al. 1994	3	⇓	⇑
HIV infektion	Surtees et al. 1990	6	⇓	–
	Keating et al. 1991	20	⇓	–
	Castagna et al. 1995	16	⇓	⇔
Multiple sclerosis	Qureshi et al. 1996	47	–	⇑
	Baig et al. 1998	20	–	⇑
Fibromyalgia/chronic fatigue syndrome	Regland et al. 1997	12	–	⇑

SACD*, Subacute degeration of the cord due to vitamin B12 deficiency; ⇓, Decreased leves; ⇑, Increased levels; ⇔, No sign.difference; –, Not determined; n, number of patients.

Table 1 – SAM and Homocysteine levels in the CSF in Neuropsychiatric Disorders.

Patients with L-dopa-treated PD have highly significantly lower levels of SAM in whole blood than have controls. Mean levels of 383 nmol/L for patients and 680 nmol/L for controls are found. The catalytic activity of MAT in erythrocytes was increased by 30% in patients, which indicates a compensatory mechanism *(Cheng H et al., 1997)*.

SAM also has an antidepressant effect. A meta-analysis of the effect as an antidepressant led to the conclusion that SAM has an effect comparable to that of standard tricyclic antidepressants *(Bressa GM, 1994)*. SAM was also recently found to be effective in depression related to PD *(Di Rocco A et al., 2000)*.

SAM may also have an indirect antioxidative effect. The administration of 50 mg/kg of SAM per day for 3 days to rats resulted in an inhibition of brain lipid peroxidation, increased total GSH production and to an increase in the mitochondrial capacity to reduce tetraphenyl tetrazolium *(Villalobos MA et al., 2000)*.

SAM-dependent carboxyl methylation is also essential for the assembly of protein (PP2A) heterotrimer formation. It was recently proposed that

the PP2A methylation system is a mechanism relating elevated tHcy with AD *(Vafai SB and Stock JB, 2002)*.

Synthesis and catabolism of catecholamines

Synthesis and catabolism of catecholamines require methyl groups, and may be affected both by low SAM and by high SAH levels.

Low 5-HT (5-hydroxytrypatamine) synthesis in the brain of folate-deficient patients and low 5-HIAA (5-hydroxyindoleacetic acid) levels in the CSF of folate- and vitamin B_{12}-deficient patients have been observed *(Botez MI et al., 1979, Botez MI et al., 1982)*. Depressive patients with high tHcy levels have also been shown to have lower CSF levels of monoamine metabolites, which points to disturbances in the turnover of serotonin, dopamine, and noradrenaline *(Bottiglieri T et al., 2000b)*, table 2a and b.

In patients with early stage AD, highly significantly reduced levels of both methionine and tryptophan, the precursor of serotonin, are also described, although the increase in tHcy was not significant *(Fekkes D et al., 1998)*.

Methylation of myelin basic protein

Methylation of myelin basic protein (MBP) plays a key role in maintaining myelin integrity and stabilizing the myelin lamellar structure. This protein is methylated at a single arginine residue (arg-107) by a protein methylase (AdoMet:protein-arginine N-methyltransferase) that uses SAM as methyl group donor.

Substantial evidence from humans and animals suggests that myelin degeneration results from methylation deficiency caused by both impaired SAM synthesis and inhibition of methylation by increased SAH levels.

It was long believed that vitamin B_{12}, but not folate, deficiency causes such demyelination. Similar syndromes are, however, described in isolated folate deficiency, and also reversibility by folic acid treatment. These studies were recently reviewed *(Green R and Miller JW, 1999)*. In a recent study of 343 consecutive neurological patients 10.5% had folate deficiency. Symptoms improved in 67% of cases after therapy with 15 mg/day of folic acid. Some of these patients had demyelinating neuropathy *(Yukawa M et al., 2001)*, characterised by paraesthesias, loss of vibratory sense, diminished reflexes, ataxia, spasticity and paraplegia.

Table 2a	Total plasma Hcy (µmol/L)	Serum vitamin B_{12} (ng/L)	RBC folate (µg/L)	CSF folate (µg/L)
Neurological controls (n=20)	6.6 (2.0)	410 (154)	373 (97)	22.3 (3.8) (n=17)
Depressives with normal tHcy (n=22) <12 µmol/L	8.2 (1.9)	392 (133)	320 (118)	21.8 (6.2) (n=15)
Depressives with high tHcy (n=24) >12 µmol/L	17.7 (5.4)[a]	334 (157)	179 (92)[a]	16.6 (8.4)[c] (n=13)
Table 2b	**CSF SAM (nmol/L)**	**CSF 5-HIAA (nmol/L)**	**CSF HVA (nmol/L)**	**CSF MHPG (nmol/L)**
Neurological controls (n=20)	163 (30) (n=16)	107 (23)	233 (49)	56.3 (18)
Depressives with normal tHcy (n=22) <12 µmol/L	160 (35) (n=15)	104 (48) (n=15)	251 (124) (n=15)	48.6 (16.1) (n=15)
Depressives with high tHcy (n=24) >12 µmol/L	129 (29)[b] (n=13)	85 (31)[e] (n=13)	165 (62)[c] (n=13)	43.5 (11.2)[d] (n=13)

Values are mean (SD). Hcy=homocysteine. Versus neurological controls: a=p<0.001; b=p<0.01; c=p<0.02; d=p<0.04; e=p<0.05.

Table 2 a, b – Homocysteine, vitamin B_{12}, folate, SAM, and CSF monomine metabolites in depression. *Bottiglieri T et al. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry, 2000; 2:228-32. ©2000 BMJ Publishing group. Reprinted with permission.*

It is of interest that SAM levels in CSF of patients with AIDS vacuolar myelopathy are reduced by as much as 69% compared to non-HIV infected persons *(Di Rocco A et al., 2002)* . The exact cause of SAM deficiency in AIDS vacuolar myelopathy is not clear, although there is a striking histopathological similarity between white matter vacuolization in AIDS myelopathy with that observed in vitamin B_{12} deficiency *(Petito CK et al., 1985)*.

Phospholipid methylation

A range of experimental studies have demonstrated a close functional relationship between one-carbon metabolism and phospholipid methylation.

A dose-dependent increase in both basal and dopamine-stimulated phospholipid methylation was induced by 5-formylTHF *(Zhao R et al., 2001)*. A recent in vitro comparison of phospholipid methylation in lymphocytes from schizophrenic patients versus control samples showed a fourfold lower methylation activity in the schizophrenia group *(Sharma A et al., 1999)*.

Lipid transport and metabolism is directly affected by choline, which also affects cell and nerve signalling. Choline is also required for the brain development of the child. Recent studies on animals have highlighted importance of choline for the development of cognitive function *(Zeisel SH, 2000)*.

The synthesis of choline is SAM-dependent, and therefore also dependent on vitamin B_{12} and folate. A study of senescence-accelerated mice indicated that a dietary supplement of phosphatidylcholine combined with vitamin B_{12} increased hippocampal protein kinase C activity and also increased 22:6n-3 in cerebral phosphatidylethanolamine and ameliorated memory *(Hung MC et al., 2001)*.

Methylation of vascular endothelium

Decreased carboxyl methylation in vascular endothelial cells, induced by clinically observed concentrations of tHcy, and inhibition of the cell cycle of these cells, point to the possibility that impaired methylation could also play a role in the pathogenesis of vascular damage. This inhibition appeared to be mediated by decreases in the carboxyl methylation indicated by a substantial increase of the SAH/SAM ratio *(Wang H et al., 1997, Lee ME and Wang H, 1999)*. Hypomethylation in atherosclerotic aortas occurred at the same level as has been reported for malignant tumours in another study *(Laukkanen MO et al., 1999)*.

Data demonstrating that production of the endogenous NO synthase inhibitor, dimethylarginine (ADMA) is regulated by SAM-dependent methyltransferase are presented *(Böger RH et al., 2000b)*.

Other data support that endothelial dysfunction may occur via inhibition of methylation reactions. It was recently shown that impaired EDD occurs in heterozygous CBS-deficient mice fed a methionine supplemented diet, and plasma tHcy was significantly correlated with liver and brain tissue SAH levels *(Dayal S et al., 2001)*.

Choline and folate are metabolically inter-related. A recent study of folate/choline depletion in healthy subjects indicated that choline is utilised as a methyl donor when folate status is low. The decrease of choline status returned to normal or higher after moderate folate repletion *(Jacob N et al., 1999)*.

Several reviews have recently been published on transmethylation defects in neuropsychiatric disorders *(Spillmann M and Fava M, 1996, Bottiglieri T, 1997, Smythies JR et al., 1997, Regland B et al., 1998, Torta R et al., 1998)*.

Interaction with inflammatory markers

Inflammatory reactions are thought to be an important contributor to neuronal damage in neurodegenerative disorders such as AD, PD, MS. It may also be implicated in depression *(Maes M et al., 1995)*.

It is known that some NSAIDs, particularly indometacin, decrease the risk and severity of both AD and vascular dementia *(Hull M et al., 2000)*. Different mechanisms, such as reduced production of IL-6 *(Bour AM et al., 2001)*, direct scavenging of NO radicals *(Asanuma M et al., 2001)*, reduction of β-amyloid protein or interferon-γ-induced NO production *(Ogawa O et al., 2000)*, or a decrease of the highly amyloidogenic Abeta 42 peptide *(Weggen S et al., 2001)* have been proposed.

An interaction of Hcy with the production of prostaglandin derivatives was first described in 1982 *(Graeber J et al., 1982)*. Since then, several studies have demonstrated an interaction between both Hcy and SAH and the production of different vaso-active prostaglandins and related eicosanoids.

Inhibition of cyclo-oxygenase activity in human endothelial cells by Hcy has been described *(Quéré I et al., 1995)*. A subsequent study showed that a folate-deficient diet enhanced the mobilisation of arachidonate from platelet phospholipids, and the subsequent formation of cyclo-oxygenase and lipoxygenase metabolites. In particular the thromboxane biosynthesis was markedly increased. Both tHcy and total GSH concentrations were highly increased in plasma from folate-depleted rats. It was suggested that these dysfunctions might be related to the loss of (n-3) polyunsaturated fatty acids, a result of Hcy-induced increased lipid peroxidation *(Durand P et al., 1996)*.

In vitro studies show that Hcy induces expression and triggers the release of cytokines such as monocyte chemotactic protein 1 (MCP-1) and IL-8 . These are specific chemotaxines for monocytes and neutrophils *(Desai A et al., 2001, Poddar R et al., 2001, Wang G et al., 2001a, Wang G et al., 2001b)*.

The addition of the superoxide scavenger superoxide dismutase (SOD) to the culture medium abolished the stimulatory effect of Hcy on MCP-1 expression *(Wang G et al., 2001b)*.

Several studies have also reported a positive association between vascular disease and elevated plasma levels of, for instance, IL-1, IL-6, IL-8, tumour

necrosis factor α (TNFα), MCP-1, and C-reactive protein. In a large clinical study, venous thrombosis was associated with elevated levels of IL-6, IL-8, and MCP-1 *(van Aken BE et al., 2000a)*.

The infiltration of monocytes into the arterial wall is one of the key events during atherogenesis. MCP-1 is a potent chemokine that stimulates the migration of monocytes into the intima of the arterial wall. Homocysteine stimulated MCP-1 expression in endothelial cells and enhanced monocyte chemotaxis in a recent study *(Sung FL et al., 2001)*. Increased levels of cytokine-induced secretion of IL-8 and MCP-1 were also observed when human aortic smooth muscle cells were exposed to pathophysiologically relevant concentrations of Hcy *(Desai A et al., 2001)*.

Homocysteine up-regulated IL-6 and IL-8 in human endothelial cells *(Hooper WC et al., 1997)*, and Hcy concentration-dependent increase in IL-6 production was seen in monocytic cells in another study *(van Aken BE et al., 2000b)*. Both tHcy and IL-6 were significant and independent predictors of aortic diameter in a study of 120 outpatients, in which the abdominal aorta was studied by ultrasound evaluation *(Rohde LEP et al., 1999)*.

Isoprostanes are prostaglandin-like compounds formed in vivo from free radical-catalysed peroxidation of arachidonic acid. In the Antioxidant Supplementation in Atherosclerosis prevention Study tHcy levels were strongly and positively associated with isoprostane levels *(Voutilainen S et al., 1999)*.

Indometacin and a thromboxane A receptor antagonist were recently shown to abolish bradykinin-induced constrictions of muscle arterioles, and aggregation of platelets in methionine-induced hyperhomocysteinemic rats *(Ungvari Z et al., 2000)* . This suggests that some of the adverse effects of Hcy, which are related to arachidonic acid metabolism, may depend on the formation of thromboxane A_2, a potent vasoconstrictor and inducer of platelet aggregation.

Total Hcy was also related to neopterin (a monocyte-derived inflammatory mediator) and endothelin-1, both in patients with disturbed glucose metabolism and in controls, which again suggests an influence of Hcy on vascular function through interference with endothelial and leucocyte function *(Gottsaker A et al., 2000)*. It was earlier shown that Hcy dose-dependently decreases the production of the vasoconstrictor endothelin 1, that is proposed to counteract endothelium-dependent vasodilatation *(Demuth K et al., 1999)*.

Pyridoxine deficiency

Vitamin B_6 is active in many other reactions than the conversion of Hcy to cysteine. It has been shown to interfere with lipid metabolism, particularly of essential fatty acids and arachidonic acid *(Cunnane SC et al., 1984, Fujimoto Y et al., 1987, Bergami R et al., 1999, Saareks V et al., 1999, Tsuge H et al., 2000)*.

Neural membrane aminophospholipids contain very high concentrations of docosahexaenoic acid, which suggests a major role in the function of the CNS *(Burdge G, 1998)*. A study in rats suggests that vitamin B_6 deficiency impairs the metabolism of (n-3) polyunsaturated fatty acids (PUFA) from alpha-linolenic acid to eicosapentaenoic acid (EPA) and particularly to docosahexaenoic acid (DHA) *(Tsuge H et al., 2000)*.

In humans, treatment with vitamin B_6 resulted in a substantial decrease of the synthesis of prostaglandin E-2, thromboxane B-2, and leukotriene E-4 *(Saareks V et al., 1999)*. Recent data from a study of human umbilical endothelial cells suggest that vitamin B_6 had a protective effect by enhancing the production of prostacyclin, thereby reducing cell injury *(Chang SJ, 1999)*.

Other proposed mechanisms

There is evidence that Hcy is converted to Hcy thiolactone by methionyl-tRNA synthetase in cultured mammalian cells *(Jakubowski H and Goldman E, 1993, Jakubowski H, 1997)*. The extent of the conversion is directly proportional to tHcy and inversely proportional to the methionine concentration *(Jakubowski H et al., 2000a)*. In cultured human cells and human serum Hcy thiolactone reacts with proteins by a mechanism involving homocysteinylation of protein lysine residues. This may lead to protein damage, which is proposed to play a role in e.g. Hcy-induced vascular damage. This pathway was found to be dominant when MS and/or CBS are inactive, either because of mutations, and/or because of deficiencies of folate, vitamin B_6, or vitamin B_{12} *(Jakubowski H, 1997, 1999 and 2000b)*.

Detoxification of Hcy thiolactone by hydrolysis to Hcy mediated by a calcium-dependent enzyme tightly bound to HDL could represent a mechanism by which protein damage may be prevented *(Jakubowski H, 2000c)*.

A study of cultured human umbilical vein endothelial cells recently revealed that Hcy alters the expression of multiple proteins *(Kokame K et al., 1998)*.

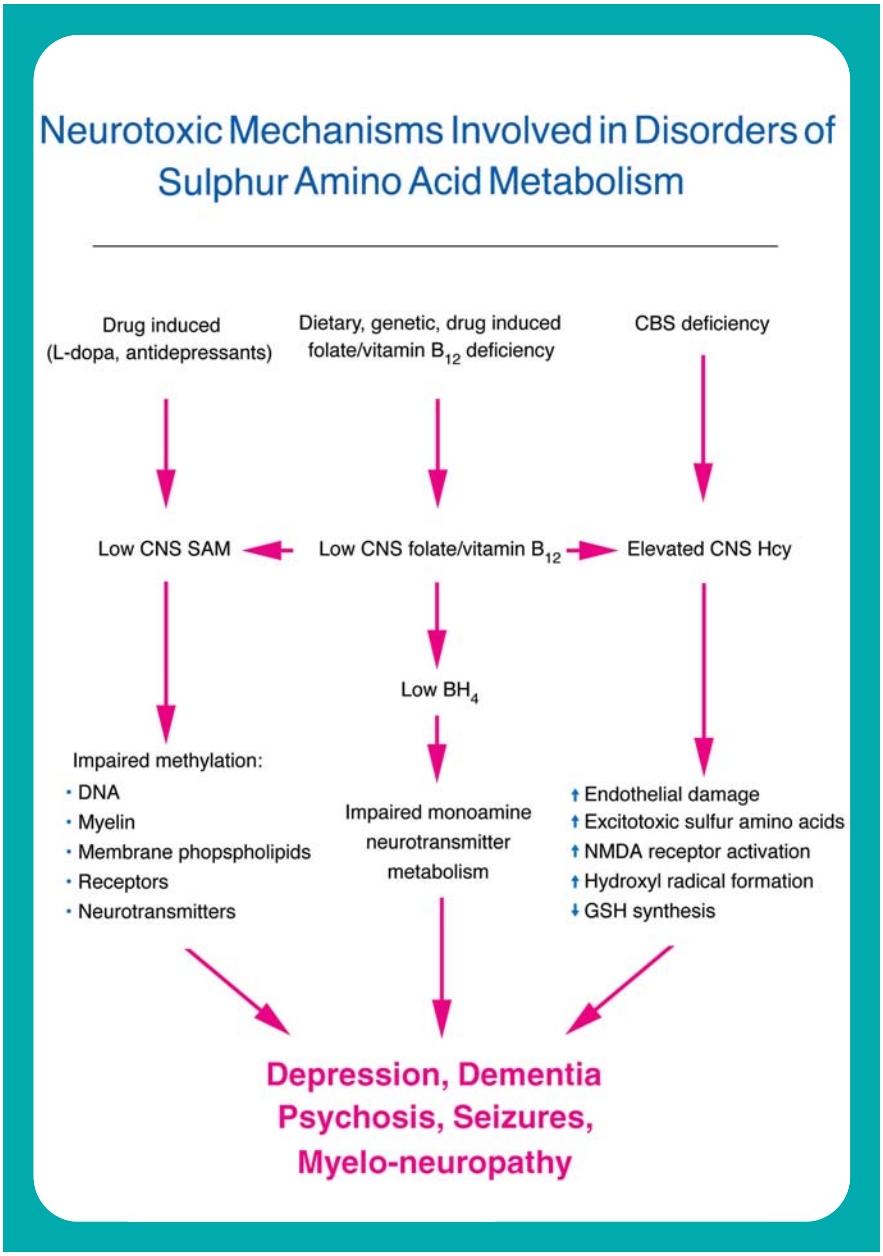

Figure 7 – Neurotoxic mechanisms involved in disorders of sulphur amino acid metabolism. SAM = S-Adenosylmethionine, BH_4 = Tetrahydrobiopterin, CBS = Cystathionine β-synthase. *© Bottiglieri T 1999. Personal communication. All rights reserved. Reprinted with permission.*

There is some data from studies in animals showing that epidermal growth factor (EGF) is involved in the signalling pathway of B_{12} and that lack of EGF contributes to neurodegenerative central neuropathy in vitamin B_{12} deficiency *(Scalabrino G et al., 2000)*.

An association between moderately increased tHcy levels in AD and the expression of cyclin E in neurons of the hippocampus was also recently reported *(Nagy ZS et al., 2000)*. Cyclin E is a marker of entry into the cell division cycle. An aberrant entry of neurons into the cell cycle might be an early step in pathogenetic processes.

An in vitro study suggests that the amyloid-beta protein may activate apoptotic pathways to cause loss of vascular smooth muscle cells in cerebral amyloid angiopathy in AD, and that Hcy increased amyloid-beta protein-induced toxicity and caspase-3 activation in a dose-dependent manner *(Mok SS et al., 2002)*.

The main proposed Hcy-related mechanisms associated with neuropsychiatric disease are illustrated in fig. 7.

The role of folates in neuro psychiatric disorders is discussed in a recent review *(Abalan F, 1999)*. Another recent review focuses the role of vitamin B_{12} and folate in brain function in the elderly *(Weir DG and Scott JM, 1999)*.

Why do Homocysteine Levels increase?

Besides vitamin deficiencies and genetic defects, there are many other causes of hyperhomocysteinemia. Lifestyle, age-related physiological changes, diseases and drugs can, directly or indirectly, disturb Hcy metabolism. Therefore, in most cases, elevated homocysteine levels are not caused by a single factor, but are the result of a cluster of factors, fig. 8.

The interactions between Hcy-increasing factors, and an overview of likely causes of hyperhomocysteinemia in 2,462 routine measurements requested by Norwegian practitioners, are discussed in a recent publication *(Schneede J et al., 2000)*.

Lifestyle factors

Several lifestyle factors may influence tHcy levels. The most important are smoking, high consumption of alcohol, and inadequate nutrition, all factors that have a considerable impact on health.

Connections between lifestyle and other factors that increase tHcy and the complications attributable to a disturbed Hcy metabolism have not yet been given much focus. However, many interesting observations support such a connection.

Smoking

In the large Norwegian Hordaland Homocysteine Study, smoking produced a shift in the distribution of tHcy towards higher values. The number of cigarettes smoked daily was one of the strongest determinants of tHcy levels *(Nygård O et al., 1995)*. In women, the increase was about 1% per cigarette smoked a day, in men it was about 0.5%. Accordingly, in the prospective cohort New York University Women´s Health Study, tHcy was 18% higher among women who smoked 20 cigarettes or more a day compared with non-smokers. Serum folate was 26% lower, although

72% of the cohort had used multivitamins during the previous year *(Kato I et al., 1999b)*. A highly significant increase in tHcy was also found in smokers in a British sample of middle-aged men. The levels highly significantly differed according to folate status *(Dekou V et al., 2001)*. Recent data from the Framingham Offspring Study also show a significant positive association with smoking in this population sample of 1,960 men and women *(Jacques PF et al., 2001)*.

Vitamin depletion

Increased tHcy and low levels of folate and/or vitamin B_6 and B_{12} in smokers have been seen in many other studies, both in healthy subjects and in patients with CVD *(Witter F et al., 1982, Vermaak H et al., 1990, Piyathilake CJ et al., 1994, Bergmark C et al., 1997, Mansoor MA et al., 1997, Christensen B et al., 1999a, Cafolla A et al., 2000)*. More than 9 cigarettes a day increased the OR of low RBC folate to 2.93 compared to non-smokers in a recent study of blood donors *(Cafolla A et al., 2000)*. Data from the European Concerted Action Project on 750 CVD patients and 800 controls showed that current smokers in both groups had higher levels of tHcy and lower levels of RBC folate and vitamins B_6 and B_{12} than non-smokers.

Smoking probably increases tHcy mainly by increasing requirement of the vitamins. Lower intake of fruit and vegetables among smokers may contribute. In a recent Danish study of 54,417 men and women, smoking was shown to be associated with lower intake of healthy food like fresh fruit, vegetables, and olive oil *(Osler M et al., 2002)*.

Enzyme induction in the liver by polycyclic aromatic hydrocarbons and increased catabolism of folate has been demonstrated in smokers *(Nakazava Y et al., 1983)*.

It has recently been proposed that increased levels of tHcy reflect an acute phase induction of pyridoxal phosphatase activity associated with increased production of IL-6. This would result in stimulation of the activity of pyridoxal phosphatase, thereby diminishing vitamin B_6 levels and reducing CBS activity *(McCarty MF, 2000b).*

Other mechanisms

A possible direct interaction between nitrous compounds from the smoke and methionine synthase (MS) is suggested by one study *(Bergman S et al., 1997)*, but smoking may also affect MS activity by impairing vitamin B_{12} status.

Cigarette smoke contains cyanide, which can be eliminated by a vitamin B_{12}-dependent mechanism. (Hydroxocobalamin is used as antidote in cyanide intoxication.) Inactivation may contribute to vitamin B_{12} inactivation in smokers.

There is also some experimental evidence that methylation reactions can be directly influenced by nicotine *(Godin C and Crooks P, 1986)*.

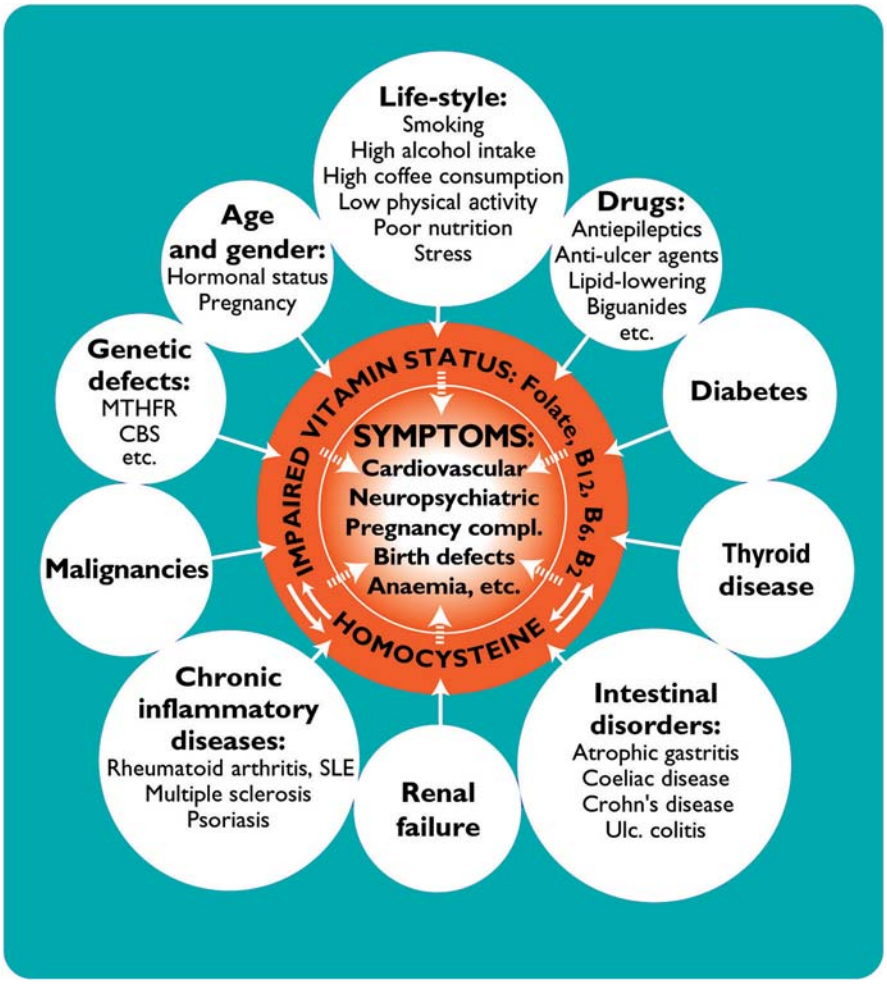

Figure 8 – Interactions between different physiological and patophysiological factors that influence vitamin status and the homocysteine metabolism, and with homocysteine-related symptoms.

Smoking also affects the redox status of Hcy, as it almost doubles the fraction of reduced Hcy *(Bergmark C et al., 1997)*. This may increase the damaging effects and constitute an additional risk factor in smokers, owing to the reactivity of the sulphhydryl portion of the free reduced form.

Cigarette smoking has also been found to lower GSH and vitamin C levels *(Banerjee KK et al., 1998)* , effects possibly associated with Hcy metabolism.

Cigarette smokers with tHcy above 12 µmol/L had a 12-fold increased risk of CVD compared with non-smoking controls in a recent study *(O´Callaghan P et al., 2002).*

Tetrahydrofolate is involved in the intracellular regeneration of tetrahydrobiopterin (BH_4), page 40. Conditions related to impaired NO activity and accelerated atherosclerosis have been associated with reduced bioavailability of BH_4. In chronic smokers, but not in non-smokers, BH_4 attenuated the blood flow response to vasodilators, and improved vasoconstrictive response to inhibition of endothelial NO synthase. The data indicate that a dysfunctional BH_4-dependent NO activity may contribute to endothelial dysfunction in smokers *(Heitzer T et al., 2000)*.

High alcohol intake

Whereas a moderate consumption of alcohol seems to be associated with lower tHcy levels *(Vollset SE et al., 1997)* chronic, high consumption produces increased levels *(Hultberg B et al., 1993a, Lambert D et al., 1993, Lambert D et al., 1997, Cravo M et al., 1996, Cravo M et al., 1997, De la Vega MJ et al., 2001)*. Serum tHcy may be about twice as high in alcoholics compared to controls, fig. 9.

The common C677T polymorphism may have a greater impact on tHcy in alcoholics. In a recent study 84.2% of heavy drinkers with the TT genotype had hyperhomocysteinemia versus 54.3% of CT carriers and 31.6% of subjects with the CC genotype *(De la Vega MJ et al., 2001).*

A postive association between ethanol levels and tHcy has also been found in acute alcohol intoxication in alcoholics *(Van der Gaag MS et al., 2000)*, but not in non-alcoholics *(Bleich S et al., 2000c)*.

Recent data from the Framingham Offspring Study show an overall significant positive association of tHcy with alcohol intake in this population sample of 1,960 males and females *(Jacques PF et al., 2001)*. Vitamin depletion may be an essential explanation.

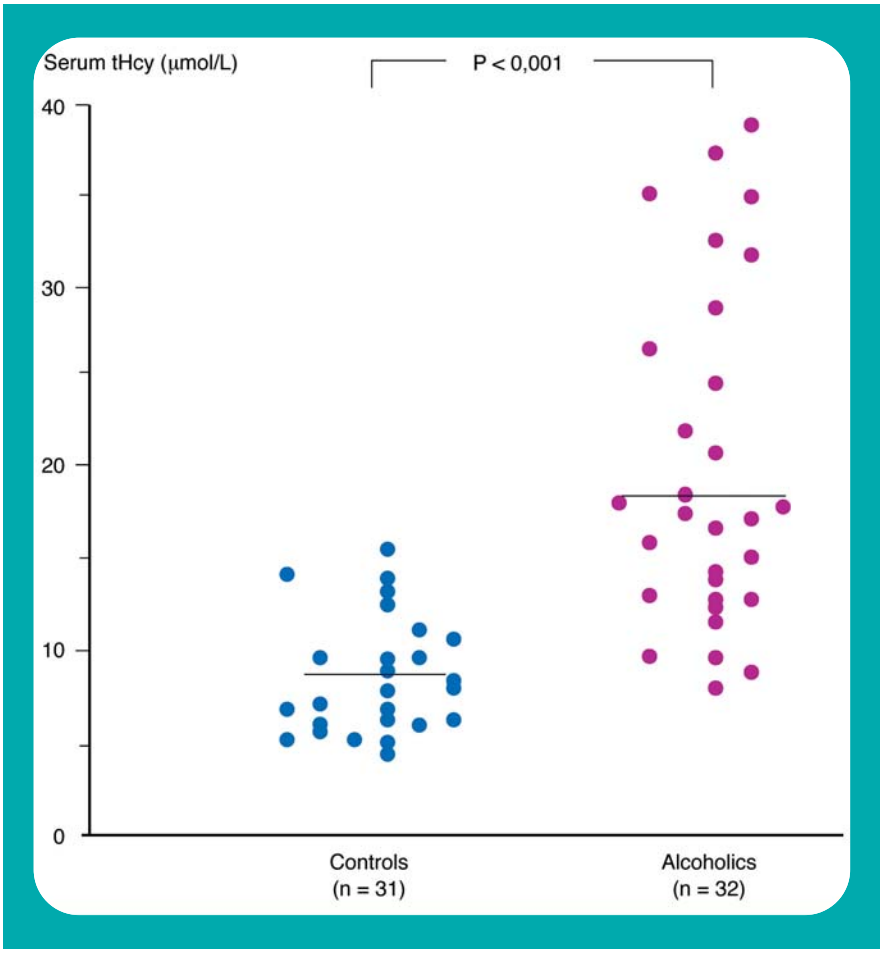

Figure 9 – Individual serum concentrations of tHcy in 32 alcoholic subjects aged 29-60 years, either active drinkers or abstinent for less than 2 weeks, and in 31 controls aged 26-63. Horizontal lines represent mean values. *Cravo M et al. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B_{12} and vitamin B_6 status. Am J Clin Nutr, 1996; 63; 16:29-35. © Am J Clin Nutr, American Society for Clinical Nutrition. Reprinted with permission.*

Vitamin depletion

High alcohol consumption is often associated with gastrointestinal disturbances, which may result in decreased absorption of vitamins, thus contributing to elevated tHcy levels. Both RBC levels of folate and serum concentrations of vitamin B_6 have been found to be lower in alcoholics than in controls *(Cravo M et al., 1996, Cravo M et al., 1997)*.

Cravo and co-workers estimated that more than 50% and 60% of alcoholics had low circulationg levels of vitamin B_6 and low RBC folate respectively, although only 18% were malnourished *(Cravo M et al., 2000)*.

The vitamin B_6 content in beer is proposed to explain two observations that tHcy does not increase after moderate consumption of beer *(Cravo M et al., 1996, Van der Gaag MS et al., 2000)*. These observations, however, were not confirmed in a third study *(Bleich S et al., 2000c)*. Beer also contains folic acid, which may contribute to lower tHcy and also explain why beer drinkers more seldom develop megaloblastic anemias than other alcoholics *(Mayer O et al., 2001)*.

Whereas RBC levels of folate are low in alcoholics, serum levels may be high. This may be a consequence of functional vitamin B_{12} deficiency although total serum levels of vitamin B_{12} (or corrinoids) are elevated in alcoholics *(Carney MWP et al., 1990, Lambert D et al., 1993, Lambert D et al., 1997, Cravo M et al., 1996, Cravo M et al., 1997)*. It has been proposed that alcoholic liver damage results in liver cell depletion of the vitamin and increased blood concentrations of vitamin B_{12}-binding proteins *(Baker H et al., 1998)*.

High serum levels of vitamin B_{12}, however, do not exclude cellular deficiency. Low CSF levels have been observed in patients with alcohol-related dementia, in spite of high serum vitamin B_{12} levels *(Van Tiggelen CJM, 1983)*.

Kanazawa and Herbert determined serum, RBC, and liver levels of total corrinoids, active vitamin B_{12}, and vitamin B_{12} analogues in 27 alcoholic patients. Compared with control subjects, the liver content of total corrinoids and active vitamin B_{12} was low in the alcoholics, although serum levels were high. Levels of inactive analogues were high in RBC of the alcoholics, but levels of active vitamin B_{12} were not *(Kanazava S and Herbert V, 1985)*.

Total analogue levels were also increased in alcoholics, and methylmalonic acid (MMA) levels were highly significantly increased compared to levels in controls in another study *(Lambert D et al., 1993)*.

A positive correlation between serum alkaline phosphatase, plasma B_{12} analogue concentration, and total corrinoids bound to haptocorrin in patients with liver cirrosis is found *(Lambert D et al., 1997)*.

These data point to an alcohol-induced inactivation of vitamin B_{12}. Current vitamin B_{12} assays may not be specific enough to distinguish active vitamin B_{12} from analogues, page 116, and high total levels may mask tissue deficiency in alcoholics.

High total corrinoid levels in serum do not exclude malabsorption of vitamin B_{12}. A dose of 5 g/kg of ethanol was shown to decrease gastric IF binding of vitamin B_{12}, and, in parallel, depress gastric GSH *(Shaw S et al., 1990)*.

A recent study in mini-pigs indicates that chronic alcohol consumption also decreases the intestinal absorption of folates. The mechanism seems to be an altered expression of the reduced folate carrier *(Villanueva JA et al., 2001)*. In addition, alcohol interferes with folate metabolism, and a relatively high alcohol intake may increase folate requirement *(Hillman RS et al., 1982)*. An in vitro study points to the possibility that superoxide, generated from the metabolism of ethanol to acetaldehyde, in the presence of xanthine oxidase, may contribute to the severity of folate deficiency in the alcoholic by cleaving folates, particularly methyl-THF *(Shaw S et al., 1989)*. Acute intravenous infusion of ethanol to fasting well-nourished volunteers caused a rapid fall in serum folate concentrations within 6 hours, which also points to mechanisms of depletion other than malabsorption *(Eichner E and Hillman J, 1973)*.

Other mechanisms

It has been proposed that alcohol interferes with the hepatic processing and/or the hepatic circulation of folate. A recent study in rats, in which chronic alcohol consumption increased tHcy and SAH and decreased hepatic SAM without any changes in vitamin levels, however, also indicated that other mechanisms affecting Hcy metabolism may be implicated *(Stickel F et al., 2000)*.

Alcohol has been reported to inhibit methionine synthase (MS) in vivo in several animal models of alcoholic liver disease *(Barak AJ et al., 1994, Barak AJ et al., 2002, Sherif F et al., 1993, Halsted CH et al., 1996)*. These studies have recently been reviewed *(Lu S, 1998)*.

Decreased MS activity may be directly caused by vitamin deficiency but also be mediated by acetaldehyde. An in vitro study has shown that this degradation product of ethanol, but not ethanol itself, inhibits the enzyme *(Kenyon S et al., 1998)*. The inactivation might be caused in the same manner as by nitrous oxide inactivation, that is, by oxidation of cobalt in the MS-bound vitamin B_{12} in an irreversible reaction.

Liver damage

Alcohol-induced damage can often be associated with a disturbed Hcy metabolism. It has been known for over 50 years that alterations of methionine metabolism occur in liver cirrosis. Intravenous methionine infusion may induce liver injury, and patients with cirrhosis often have hypermethioninemia that could be attributed to a decrease in hepatic methionine adenosine transferase (MAT) activity *(Mato JM et al., 2002)*. Other studies indicate that betaine-homocysteine methyltransferase (BHMT), which

mediates the conversion of Hcy to methionine predominantly in the liver, is also influenced by alcohol.

Elevated serum levels of cystathionine are also found in liver cirrosis, which provides indirect evidence for impairment of the transsulphuration at the level of cystathionine metabolism as well *(Look MP et al., 2000)*.

Inactivation of vitamin B_6 may be one explanation. Acetaldehyde may displace protein-bound vitamin B_6, thereby exposing the vitamin to degradation *(Lumeng L, 1978)*. The result may be a decrease in GSH synthesis, page 24. Free radicals, generated in the oxidation of acetaldehyde, can also deplete hepatic GSH. Acetaldehyde inactivation of GSH may further impair the MAT activity as GSH normally protects MAT against damage.

Withdrawal from alcohol results in decreased tHcy levels and increased levels of folate, vitamin B_{12} and B_6 within a few weeks *(Hultberg B et al., 1993a, De la Vega MJ, 2001)*.

An extensive review on hyperhomocysteinemia, deficiency of folate, vitamins B_6 and B_{12} in alcholics, and its metabolic consequences, was recently published *(Cravo M et al., 2000)*.

Inadequate nutrition

Whereas a diet inadequate in methionine is considered to be rare in Western countries, an inadequate intake of the vitamins required in the Hcy metabolism may be quite common, and is often associated with other unhealthy lifestyle factors *(Nygård O et al., 1997, Osler M et al., 2002)*.

In a study of 1,740 elderly American adults, aged 51-85 years, over 60% reported an intake of folate and other vitamins calculated to be below estimated average requirements *(Foote JA et al., 2000)*.

In another American study of 466 men, major dietary patterns were studied using the dietary information collected by food-frequency questionnaires in 1994 (before folic acid fortification). Two major dietary patterns were found. The first was characterised by a higher intake of fruits, vegetables, whole grains, and poultry, the second by a higher intake of red meat, high-fat dairy products, and refined grains.

After adjusting for potential confounders, the first pattern was significantly and positively correlated with plasma folate and inversely with tHcy. For the second pattern there was a significant positive correlations with tHcy and an inverse correlation with, for instance, plasma concentrations of folate *(Fung TT et al., 2000)*.

Data from the fifth examination cycle of the Framingham Offspring Study between 1991 and 1994 confirmed that tHcy was negatively asso-

ciated with plasma folate, vitamin B_6, and riboflavin in non-supplement users. Total Hcy was 18% lower in supplement users *(Jacques PF et al., 2001)*.

Other nutrients than vitamins may also be important for Hcy metabolism, as shown in the Hordaland study. Healthy subjects eating fish at least 3 times a week had lower tHcy levels than those eating fish less than once a month. The difference in tHcy was 11% in men and 9% in women *(Vollset SE et al., 1993)*. Fish oil, 12 g/day for 3 weeks, has also been shown to decrease tHcy significantly in hyperlipemic men *(Olszewski A and McCully K, 1993)*.

Vegetarians

A diet poor in fresh fruit and vegetables may lead to folate deficiency and increased tHcy levels. On the other hand, strict vegetarians may develop nutritional vitamin B_{12} deficiency, as only food of animal origin contains this vitamin *(Miller RD et al., 1991, Crane MG et al., 1994, Rauma AL et al., 1995, Haddad EH et al., 1999, Hokin BD and Butler T, 1999, Krajcovicova Kudlackova M et al., 2000a, Krajcovicova Kudlackova M et al., 2000b, Bissoli L et al., 2002)*.

Low vitamin B_{12} levels were observed in 78% of vegans and in 24% of (lacto) vegetarians in one study, but in none of omnivorous subjects. The vitamin B_{12} levels were 140.1 pmol/L in the vegans, 214.8 pmol/L in the vegetarians and 344.7 pmol/L in omnivores. The frequency of hyperhomocysteinemia was 53% in vegans, and 29% in (lacto-)vegetarians versus 5% in omnivores *(Krajcovicova Kudlackova M et al., 2000a, Krajcovicova Kudlackova M et al., 2000b)*.

An Italian study found hyperhomocysteinemia in 53.3% of vegetarians (31 vegans and 14 lacto-ovo-vegetarians) versus 10.3% of controls, a highly significant inverse correlation with serum vitamin B_{12} levels, and also an inverse correlation with serum folate. Mean serum vitamin B_{12} levels were 172 pmol/L in vegetarins versus 265 pmol/L in controls *(Bissoli L et al., 2002)*. Elevated tHcy may thus counteract the protective effect of a vegetarian diet on lipids.

In a study in which a 5-day self-administered food record was mailed to members of the three principal French vegetarian organisations, vitamin B_{12} intake was found to be very low, particularly in the women. It was calculated to be 0.2 µg/day for the vegans, 1.5 µg/day for the ovo-lakto-vegetarians, and 1.7 µg/day for lacto-vegetarians. More surprisingly, calculated folate intake was also particularly low for the female vegans, or 188.3 µg/day. It was 266.9 µg/day for lakto-vegetarians, and 328.8 µg/day for ovo-lakto-vegetariens *(Leblanc JC et al., 2000)*.

Certain seaweeds have been postulated to contain active vitamin B_{12} and are consumed by some vegetarians. A recent study demonstrated that 73% of the corrinoid content of raw nori (Porphyra tenera) was active vitamin B_{12}. However, in dried nori 65% of the corriniod content was inactive B_{12} analogues. These results were obtained by using paper chromatography. A current radioassay did not distinguish between active and inactive corrinoids. Inactive analogues may compete with active B_{12} and thus impair vitamin B_{12} status. When the volunteers were given 40 g of dried nori, MMA levels increased, confirming an antagonistic effect of these analogues on the vitamin B_{12} metabolism *(Yamada K et al., 1999)*.

Vitamin requirement increases during pregnancy. A vegetarian diet may therefore be particularly risky during pregnancy and lactation, as the mother may not be able to supply the fetus/infant with sufficient vitamin B_{12} *(Specker BL et al., 1988)*. The mother´s vitamin B_{12} status is crucial for the infant. It was recently shown to decrease with parity, even in non-vegetarian mothers, indicating continous depletion in the mothers *(Bjørke-Monsen ALB et al., 2001)*. The depletion could be expected to be even more marked in vegetarian mothers.

A study of 8 lactating vegetarian mothers and 11 controls showed that the milk of the vegetarian mothers did not contain enough vitamin B_{12} in 6 cases (75%), compared to 3 (27%) of the omnivorous women, although all except 3 vegetarians reported taking supplements containing 3-25 µg of vitamin B_{12}, *(Patel KD and Lovelady CA, 1998)*.

An assessment of vitamin B_{12}, folate and vitamin B_6 status in breast-fed or formula-fed neonates found more neonates with probable tissue deficiencies of vitamin B_{12} and folate than expected from serum analysis alone (15.4 versus 9.7%). Breast-fed neonates had lower vitamin B_{12} and higher tHcy levels than formula-fed infants, but the mothers´ diets are not discussed *(Minet JC et al., 2000)*.

It has been demonstrated that children consuming a strictly vegan diet have mean tHcy levels about twice as high as the levels of matched controls *(Schneede J et al., 1994)*, fig.10.

Ethnic nutritional differences

Dietary habits vary between ethnic groups. Lower RBC folate levels were, for instance, found among UK Indian and Pakistani men and women, in whom both coronary heart disease and NTD are common, than in controls from the same region *(Michie CA et al., 1998)*. In a comparison of healthy UK Indian Asian, and European men, fasting tHcy was 6% higher

in the Indian Asians. This tHcy difference was explained by lower vitamin B_{12} and RBC-folate status in the Indian Asians *(Chambers JC et al., 2000a)*.

Data from the Third National Health and Nutrition Examination Survey (NHANES) have shown that both RBC and serum folate levels are lower in African and Mexican Americans than in white Americans. However, data from the same study also showed lower age-adjusted mean geometric tHcy in Mexican American women than in African and non-

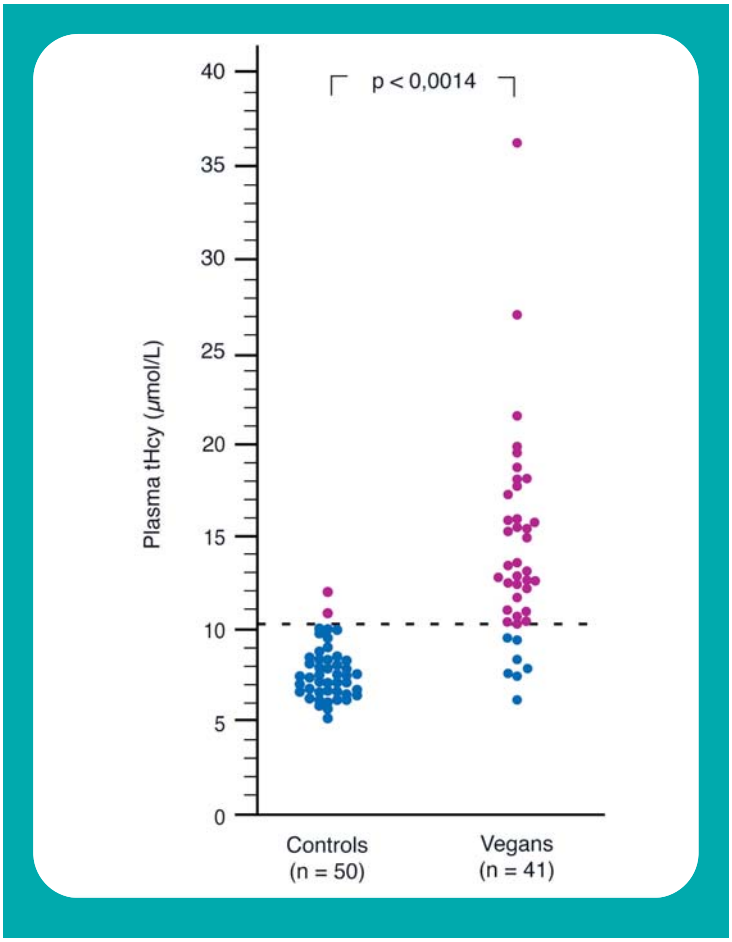

Figure 10 – Concentrations of tHcy in the plasma of macrobiotic infants (n=41) and control infants (n = 50). The horizontal dotted line represents the upper 95th percentile for tHcy in the plasma of control infants. *Schneede J et al. Methylmalonic acid and homocysteine in plasma as indicators of functional cobalamin deficiency in infants on macrobiotic diets. Pediatric Research, 1994; 36:194-201. © 1994 International Pediatric Research Foundation, Inc. Reprinted with permission.*

Hispanic white women, whereas there were no significant differences between the male ethnic groups *(Jacques PF et al., 1999b)*. Another recent study of 89 healthy black and 90 white American premenopausal women showed a higher tHcy, and significantly lower plasma folate but higher B_{12} levels in black women. After adjustment for multivitamin intake and fortified cereals, tHcy in the groups did not differ, but folate levels remained lower in the black women *(Gerhard GT et al., 1999)*.

Genetic variations, such as a varying prevalence of enzymatic polymorphisms, page 28, may play a role as well nutritional habits. A twin study has suggested that the genetic contribution to RBC folate variance is substantial *(Mitchell LE et al., 1997)*.

Ethnic and racial factors affecting vitamin B_{12} metabolism are discussed in a recent review *(Carmel R et al., 1999b)*.

Other factors

A group that risks deficiency of folate and vitamin B_{12} as well as of other nutrients, is *anorectics*, in whom highly significantly increased tHcy levels are found *(Moyano D et al., 1998)*.

Vitamins may also be destroyed by *modern food processing*. It has recently been shown that microwave heating may destroy as much as 30-40% of the vitamin B_{12} content in food *(Watanabe F et al., 1998)*. Microwave heating is also found to destroy natural folates even faster than conventional heating to the same temperature. The synthetic form, folic acid, however, was stable when heated to 100 °C *(Cooper R et al., 1978)*.

About 50% of natural folates are destroyed after conventional cooking, even of short duration. Steaming seems to result in less degradation *(McKillop DJ et al., 2001)*. Prolonged conventional heating destroys folate, vitamin B_6 and B_{12} *(Kilshaw et al., 1982)*.

High coffee consumption

In the Hordaland Homocysteine Study, in which 16,175 participants were entered, even moderate coffee consumption was found to affect tHcy levels independently of other factors that may influence tHcy. There was a marked positive correlation between coffee consumption and tHcy. In 40-42 year-old-men drinking 9 or more cups of coffee/day, the mean tHcy was 19% higher than in non-consumers. For women, the difference was 28%. Coffee drinking was associated with smoking and a lower intake of fruit and vegetables. However, the correlation between coffee consumption and tHcy was still highly significant after adjustment for these con-

founding variables. As could be expected, the combination of high coffee consumption and cigarette smoking was associated with a particularly high risk of having elevated tHcy levels *(Nygård O et al., 1997)*.

The interaction with coffee was confirmed in another observational study of 260 elderly persons, where there was a positive, significant and independent dose-response relation between coffee consumption and tHcy *(Stolzenberg-Solomon RZ et al., 1999)*. Recent data from the Framingham Offspring Study also confirm a highly significant association between coffee consumption and tHcy *(Jacques PF et al., 2001)*. Two observational studies failed to show a significant influence of coffee, although the second showed a non-significant relation *(Nieto FJ et al., 1997, Oshaug A et al., 1998)*. This may be because of a smaller sample size and/or folic acid fortification of cereals.

Two recent randomised cross-over studies also demonstrated the tHcy-increasing effect of coffee. In the first, daily consumption by 24 volunteers of one litre of paper-filtered coffee, brewed with 70 g of regular ground beans, for 3-4 weeks resulted in a highly significant increase in tHcy, or 18% higher levels than when coffee was excluded. Circulating levels of folate and vitamins B_{12} and B_6 were not affected *(Urgert R et al., 2000)*.

In the second cross-over study, one litre of unfiltered coffee (French press) was consumed daily by 64 healhty volunteers for 2 weeks. Total Hcy increased by 10% compared to other beverages, including tea, consumed for the same period. The wash-out period was 8 weeks *(Grubben MJ et al., 2000)*.

In an unblinded, controlled trial including 191 healthy, nonsmoking consumers of filtered coffee, participants were randomized to consume no coffee, 1-3 cups/day or 4 cups or more for 6 weeks. Abstention from coffee for 6 weeks resulted in a mean decrease of tHcy of 1.08 µmol/L, but also in a decrease of total cholesterol concentration of 0.28 mmol/L in participants who had been drinking on average 4 cups daily for the previous year *(Christensen B et al., 2001)*.

It is unclear to what extent the effect is caused by cholesterol-raising diterpenes present only in unfiltered coffee, and by factors present also in filtered coffee. Interestingly, vitamin B_6 concentrations were markedly lower during coffee administration than in the coffee-free periods in the study by Grubben and co-workers.

Chlorogenic acid, present in coffee, is proposed to be responsible for the increase. Other polyphenols, present in black tea, could explain the increase of tHcy associated with consumption of black tea in this study *(Olthof MR et al., 2001)*. Nygård however, found that tea consumption was associated with lower tHcy levels *(Nygård O et al., 1997)*.

A recent review discusses the interaction with coffee *(Vollset SE et al., 2000b)*.

Low physical activity

Lower tHcy concentrations have been reported from the Hordaland Homocysteine Study in middle-aged men and women, who regularly engaged in moderate physical activity. The difference was most pronounced between elderly persons with a sedentary lifestyle and those exercising daily. In this age group, the difference approached 1 µmol/L of tHcy. Exercise reduced the skewness of the tHcy distribution, and therefore seems to reduce hyperhomocysteinemia *(Nygård O et al., 1995)*. Acute exercise, however, does not seem to influence tHcy *(Vollset SE et al., 1997)*.

Psychological factors

Psychological stress has been shown to increase tHcy levels *(Stoney CM, 1999)*. Another study also showed a significant and positive relation between tHcy and hostility in both men and women. In men only, there was also a strong and positive correlation between the tendency to inhibit the expression of anger and tHcy. The authors of this study refer to two earlier cross-sectional studies, which have demonstrated an association between angiographically determined CAD and hostility and the inhibition of anger *(Stoney CM, Engebretson TO, 2000)*. Studies on the relation between stress and hostility and CVD and other diseases are discussed in this paper.

Age-related factors

Both folate and vitamin B_{12} deficiency have been shown to be very common in elderly people in many studies *(Joosten E et al., 1993a, Selhub J et al., 1993, Selhub J et al., 1999b, Nilsson K et al., 1994, Nilsson K et al., 1996, Carmel R et al., 1999a, Björkegren K and Svärdsudd K, 1999, Björkegren K and Svärdsudd K, 2001)*.

Low vitamin B_6 levels are also common *(Selhub J et al., 1993, Joosten E et al., 1993a, Folsom A et al., 1998, Bates C et al., 1999)*. Bates and co-workers studied British men and women aged 65 years or over, participating in the National Diet and Nutritional Survey 1994-5. No less than 48% of the persons living in the community and 75% of those living in institutions

had vitamin B_6 concentrations below a range considered normal *(Bates C et al., 1999)*.

An inverse relation between plasma or serum levels of tHcy and folate, vitamin B_6, and B_{12} has been found in large, epidemiological studies *(Selhub J et al., 1993, Selhub J et al., 1999b, Boushey C, 1995, Folsom A et al., 1998, Giles WH et al., 1998, Bates C et al., 1999, Jacques PF et al., 2001)*, as well as an inverse association between vitamin B_{12} levels and methylmalonic acid (MMA), a specific marker of vitamin B_{12} status.

The earlier accepted view that vitamin B_6 does not affect fasting tHcy levels has been contradicted. A significant negative correlation between fasting plasma pyridoxal phosphate, the active metabolite of vitamin B_6, and tHcy levels has been found in many studies *(Selhub J et al., 1993, Ubbink J et al., 1996*, *Robinson K et al., 1995, Robinson K et al., 1998*, *Verhoef P et al., 1996, Folsom A et al., 1998, Shultz T et al., 1998, Siri P et al., 1998*, *Bates C et al., 2002)*. Low-dose vitamin B_6 also reduced fasting tHcy effectively in healthy elderly subjects, who were folate, B_{12}, and riboflavin replete *(McKinley MC et al., 2001)*.

A much lower tHcy for any given B-vitamin concentration was found in subjects 4-18 years old than in subjects 65 years and over *(Bates C et al., 2002)*. These findings may indicate a higher vitamin requirement in the elderly. Metabolites are thus more sensitive markers of functional vitamin status than are serum levels of the vitamins, presumably because they reflect the functional interaction between these vitamins, and also take into account age-related factors influencing the cellular metabolism.

In a recent German study, tHcy, MMA and cystathionine were measured in 90 high-aged subjects (90-102 years), 92 seniors (65-75 years), and 50 younger subjects (19-50 years). Total Hcy was elevated in 62% of the high-aged subjects and 24% of seniors, and MMA in 62% and 23% respectively. Cystathionine levels were increased in 81% of high-aged subjects and 36% of seniors. The markers of intracellular vitamin deficiency showed a distinctly increased rate of deficiency, or 90% in high-aged subjects and 55% in seniors. If vitamin levels were used for diagnosis of folate and vitamins B_{12} and B_6, only 55% of high-aged subjects and 30% of seniors were deficient *(Herrmann W et al., 2000)*.

Many other studies have shown that tHcy levels increase with age, and that hyperhomocysteinemia becomes very common at the highest ages *(Pennypacker LC et al., 1992, Joosten E et al., 1993a, Joosten E et al., 1996, Joosten E et al., 1997, Selhub J et al., 1993, Selhub J et al., 1999b, Lindenbaum J et al., 1994, Nygård O et al., 1995*, *Nilsson K et al., 1996*, *Herrman W et al., 1999*, *Herrmann W et al., 2000*, *Björkegren K and Svärdsudd K, 2001)*.

In one of these studies half of the study population, a 20% random sample of persons 70 years or older in a defined geographic area, and without diseases expected to interfere with study objectives, had abnormal tHcy or MMA levels, suggesting latent or overt functional deficiency of vitamin B_{12} or folate *(Björkegren K and Svärdsudd K, 2001)*.

Inadequate nutrition and malabsorption

Inadequate nutrition with a low content of folate and vitamins B_6 and B_{12} may partly explain the increased tHcy levels at an older age, as may reduced food intake. It has been estimated that food intake decreases by about 30% at the age of 80 years *(Haller J, 1999)*. Elderly may in addition have higher requirements for several micronutrients, making them vulnerable to deficiencies that may aggravate their conditon.

Malabsorption, owing to chronic atrophic gastritis, which results in decreased acid production and other changes, is also shown to play an important role *(Jones BP et al., 1986, Krasinski S et al., 1986, Carmel R et al., 1988b, Lindstedt G et al., 1989, Nilsson-Ehle H et al., 1989, Scarlett JD et al., 1992, Joosten E et al., 1993b)*.

The absorption of vitamin B_{12} requires not only intrinsic factor (IF), but also adequate secretion of gastric acid, pancreatic enzymes, and intact B_{12}-IF receptors in the distal ileum. Particularly gastric acid secretion tends to decrease as a consequence of gastritis, impairing the gastric cleavage of the vitamin from proteins. Even a more or less asymptomatic atrophic gastritis may decrease the absorption of the protein-bound vitamin.

A recent study of healthy, 74-80-years-old Dutch subjects living at home concluded that only 28% of mild vitamin B_{12} deficiency could be explained by inadequate dietary intake or by *severe* atrophic gastritis *(Van Asselt D et al., 1998)*.

The prevalence of atrophic gastritis increases with age. It was found to be about 40% in 70-79-year-old American subjects *(Krasinski S et al., 1986)*. The gastritis may be associated with for instance Helicobacter pylori infection rather than with normal aging per se.

These factors are discussed in a recent review *(Russell RM et al., 2001)*.

Hypo- and achlorhydria may also reduce the absorption of folate. The proportion of an oral test dose of natural folates given to elderly subjects with atrophic gastritis was 31% lower than in a control group with normal gastric secretion *(Russell RM et al., 1986)*. A recent dose-finding study also showed that only the groups given folic acid supplements containing over 400 µg of folic acid had significantly lower tHcy than the placebo group.

Using multiple linear regression and each individual´s total folate intake (supplement plus diet), it was calculated that a total of 926 µg per day would be required to ensure that 95% of the elderly population would be protected from risks associated with folate deficiency *(Rydlewicz A et al., 2002)*.

Folate absorption and transport are discussed in two recent reviews *(Brzezinska A et al., 2000, Gregory JF et al., 2001)*.

There are some indications that vitamin B_6 requirements may increase with age *(Van den Berg H et al., 1999)*. It was recently proposed that increased levels of tHcy may reflect an acute phase induction of pyridoxal phosphatase activity associated with age-related increased production of IL-6. This would result in stimulation of the activity of pyridoxal phosphatase, thereby diminishing vitamin B_6 levels, and reducing cystathionine β-synthase (CBS) activity *(McCarty MF, 2000b)*. The increased cystathionine levels found in elderly by Herrmann and co-workers may be a consequence of functional vitamin B_6 deficiency *(Herrmann W et al., 2000)*.

The OR for low plasma vitamin B_6 levels for patients with AD was 12.3 compared to controls in a recent study *(Miller JW et al., 2002)*.

Low plasma vitamin C levels have also been found in Alzheimer patients, despite adequate intake *(Riviere S et al., 1998)*. Vitamin C plasma levels decreased in proportion to the severity of the cognitive impairment. Whether this is due to impaired absorption or to increased demand is not known. High intake of both vitamin C and E has also been shown to have a protective effect, page 125.

Other age-related impaired functions

As several age-related, physiological factors influence Hcy metabolism, correlation with vitamin status may be poor when subgroups are studied *(Joosten E et al., 1993a, Joosten E et al., 1997, Nilsson K et al., 1994, Nilsson K et al., 1996, Van Goor LP et al., 1998)*. Some data are presented in fig. 11 and table 3.

Age-related impairment of renal function may reduce the plasma clearance of tHcy, even if most cases of high tHcy are also associated with low vitamin concentrations *(Selhub J et al., 1999b)*. Age and serum creatinine correlate with tHcy *(Brattström L, 1994, Nilsson K et al., 1994, Nilsson K et al., 1996, Wu LL et al., 1994, Koehler KM et al., 1996, Joosten E et al., 1997)*, as well as the more sensitive marker of glomerular filtration, cystatin C *(Nordlund L et al., 1998, Bostom AG et al., 1999c)* and plasma levels of albumin *(Koehler KM et al., 1996, Bostom et al., 2000d)*.

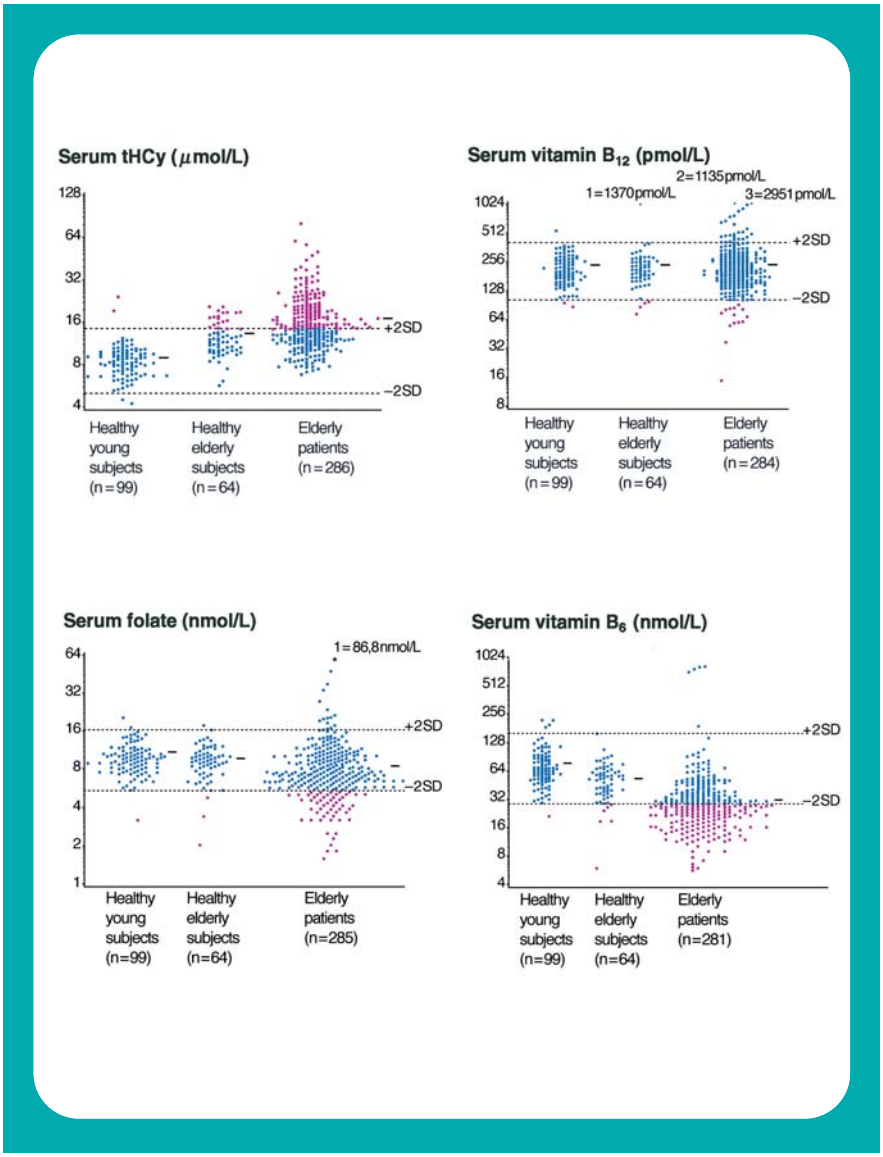

Figure 11 – Serum levels of tHcy, vitamin $B_{12,}$ folate, and vitamin B_6 in three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects. Bold lines represent the geometric mean for each group. *Joosten E et al. Metabolic evidence that deficiencies of vitamin B_{12} (cobalamin) occur commonly in elderly people. Am J Clin Nutr, 1993; 58:468-76. © Am J Clin Nutr American Society for Clinical Nutrition. Reprinted with permission.*

	Control subjects		Demented patients		Non-demented patients	
	Men n = 84	Women n = 79	Men n = 121	Women n = 174	Men n = 65	Women n = 150
Age (years)	74 +/- 7	76 +/- 7	77 +/- 8	78 +/- 10	75 +/- 14	79 +/- 8
P-tHcy- (µmol/L)	14.0 +/- 3.8	13.2 +/- 4.2	19.7 +/- 9.8c	18.6 +/- 7.4c	18.3 +/- 5.9c	18.1 +/- 7.1c
S-cobalamin (pmol/L)	245 +/- 122	268 +/- 118	232 +/- 93	254 +/- 124	276 +/- 162	278 +/- 118
B-folate (nmol/L)	353 +/- 162	352 +/- 149	285 +/- 149c	277 +/- 141c	315 +/- 199	329 +/- 212
S-creatinine (mmol/L)	93 +/- 13	79 +/- 11b	86 +/- 14d	74 +/- 16b,e	89 +/- 16	74 +/- 16b

Table 3 – Age, plasma tHcy, serum-cobalamin, blood-folate and serum-creatinine in controls and psychogeriatric patients. Means and SD are given.[a]$p < 0.001$ compared with control subjects, [b]$p < 0.001$ compared with men, [c]$p < 0.001$. [d] $p < 0.01$, [e]$p < 0.05$ compared with control subjects of the same gender. *Nilsson K et al. Hyperhomocysteinemia – a common finding in a psychogeriatric population. Eur J Clin Invest 1996; 26:853-9. © 1996, Blackwell Science. Reprinted with permission.*

A highly significant decline in CSF folate levels with age was seen in persons undergoing surgery during spinal anesthesia, fig. 12. Serum folate levels were, however, not determined *(Bottiglieri T et al., 2000a)*. Highly significantly lower CSF levels of folate in AD patients than in younger controls, and a less pronounced decline in healthy elderly subjects were, however, recently demonstrated, although there were no significant differences between the groups in serum folate levels *(Serot JM et al., 2001)*.

Isolated low CSF levels of vitamin B_{12} and/or folate in elderly demented patients have been reported in several earlier studies *(Källström B and Nylöf R, 1969, Van Tiggelen CJM, 1983, Nijst TQ et al., 1990, Regland B et al., 1992)*. Van Tiggelen and co-workers found that only 5 out of 23 demented patients had normal vitamin B_{12} levels in CSF, whereas all but 3 patients had normal serum levels of the vitamin. These findings point to a disturbed distribution associated with age and dementia.

Vitamins B_{12}, B_6, and folate are actively transported into the CNS. The protein-mediated transport and uptake of the cofactors may be affected in the elderly, because of age-related changes in blood proteins *(Scarlett JD*

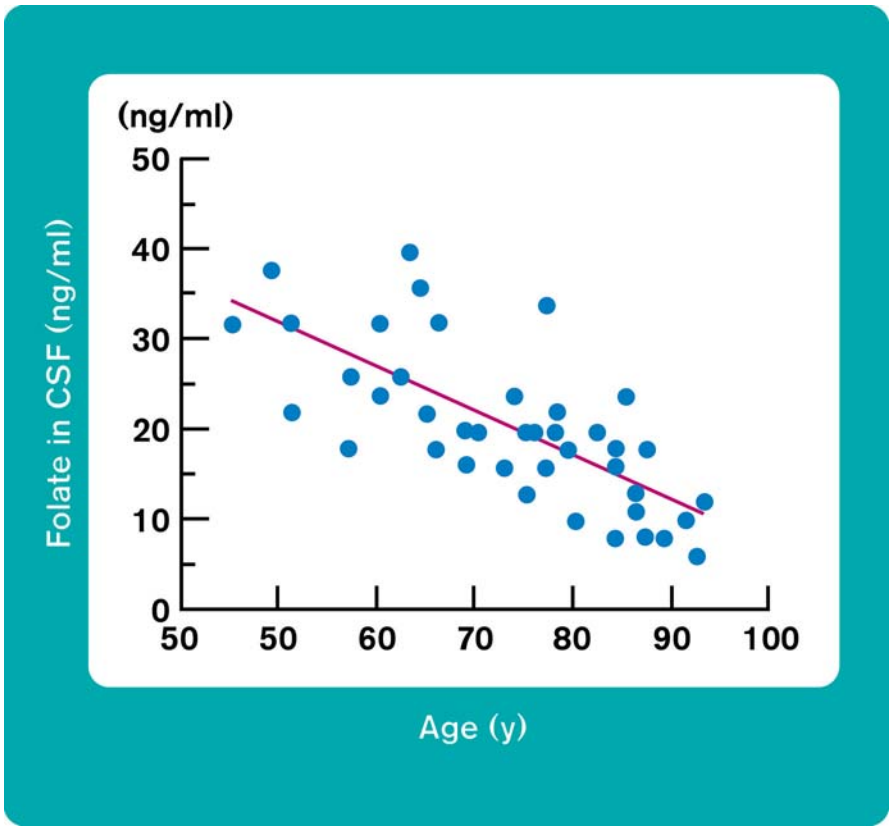

Figure 12 – Relation of age to CSF folate concentrations in elderly surgical patients. *Bottiglieri T et al. Folate in CSF and age. J Neurol Neurosurg Psychiatry, 2000; 69:562. © 2000 BMJ Publishing group. Reprinted with permission.*

et al., 1992, Metz J et al., 1996). Lower holoTC levels (the level of transport protein bound to B_{12}) have been found in both demented patients, and in elderly controls compared to younger controls, in spite of normal or even high total serum vitamin B_{12} levels *(Johnston CS et al., 1997, McCaddon A et al., 2001a, McCaddon A et al., 2002a, Smith AD et al., 2001)*.

McCaddon and co-workers recently presented the hypothesis that age-related cerebral oxidative stress may inactive cobalamin (cob (I)alamin), which is extremely vulnerable to oxidation), thereby impairing Hcy metabolism *(McCaddon et al., 2002b)*.

The affinity of vitamin B_{12} to TC could be decreased as a consequence of oxidative damage to the vitamin, which could explain high total serum B_{12}, but low holoTC concentrations and thereby impaired distribution.

Another possibility is that B_{12} homeostasis might be disrupted in AD as a result of a defect in megalin, a member of the low-density lipoprotein receptor family. Megalin mediates ileal uptake of cubilin-bound B_{12} –IF complex, and also maintains the vitamin B_{12} pool in the kidney *(Gliemann J, 1998, Christensen EI et al., 1999)*. The low density lipoprotein receptors are multifunctional. Other ligands are apolipoprotein E, amyloid precursor protein, and α_2–macroglobulin.

Genetic variations, such as the C677T polymorphism of the MTHFR gene, may also have a stronger impact on tHcy in elderly persons, and increase vitamin requirements *(Guttormsen AB et al., 1996, Clarke R et al., 1998a, Kauwell GPA et al., 2000a)* page 28. A tendency towards a negative correlation between age and CBS activity has been observed *(Nordström M and Kjellström T, 1998)*.

A greater increase in tHcy in older than in younger persons after methionine loading, and an age-dependent decrease of endothelium-dependent vasodilatation could be explained by age-related changes in the Hcy metabolism *(Chao CL et al., 2000)*. Vitamin treatment may, however, decrease tHcy, even when serum vitamin levels are within the normal range *(Nilsson K et al., 1994, Rasmussen K et al., 1996)*.

Genetic variations may also contribute to variations between elderly populations of different ethnic origin. A study of white and African American elderly disabled women showed that elevated tHcy and low serum folate were more prevalent in African than in white Americans. Vitamin B_{12} deficiency with elevation of MMA, however, was highly significantly more prevalent in elderly white than in American African women *(Stabler SP et al., 1999b)*.

In another study of American subjects above 60 years old, vitamin B_{12} deficiency was more common among white and Latin Americans than among African and Asian Americans, and more common in men than in women. Hyperhomocysteinemia associated with renal insufficiency was found to be more common in elderly African and Asian Americans than in whites Americains *(Carmel R et al., 1999a)*.

A review on the role of Hcy in age-related vascular and non-vascular diseases was recently published *(Parnetti L et al., 1997)*.

Hormonal changes

Total Hcy levels gradually increase after the menopause. Both fasting and post methionine loading (PML) concentrations increase. The levels rise faster with age in women than in men *(Andersson A et al., 1992b, Nygård O*

et al., 1995). Post methionine load tHcy in postmenopausal women even surpassed levels in men of the same age in the European Concerted Action Project study *(Verhoef P et al., 1999)*.

Total Hcy may also fluctuate during the menstrual cycle. Mean tHcy levels increased from 7.8 µmol/L in the luteal to 8.9 µmol/L in the follicular phase, in one study *(Tallova J et al., 1999)*, although no significant variation was seen during a single menstrual cycle in a smaller study *(Merkli-Feld GS et al., 2000)*.

The reason for these changes seems to be related to hormonal changes, as there is a strong negative correlation between oestradiol levels and tHcy levels in postmenopausal women *(Wouters M et al., 1995a)*. The mechanism is proposed to be an increase in kidney methionine synthase activity, or related to the transamination of methionine.

There is also a possibility that other factors, such as gender related nutritional habits *(Refsum H et al., 1996, Tucker KL et al., 1996a)* or psychological factors *(Stoney CM, Engebretson TO, 2000)* may influence tHcy and explain gender differences in tHcy.

Data from the Third National Health and Nutrition Examination Survey (NHANES) on the variation of tHcy related to age, gender, and hormonal status, however, suggest that a higher oestrogen status is associated with a decreased mean tHcy, independent of nutritional status and muscle mass *(Morris MS et al., 2000)*. So does a recent comparison between 93 pre- and 93 postmenopausal women *(Hak AE et al., 2000)*.

Hormone-replacement therapy

Oestrogen replacement therapy (HRT) was also associated with lower tHcy in NHANES III *(Morris MS et al., 2000)*. Several studies have also demonstrated a significant tHcy-lowering effect of different combined oestrogen/progestogen replacement therapies, or oestrogen therapy alone *(Van der Mooren MJ et al., 1992, Barnabei VM et al., 1999, Van Baal WM et al., 1999, Walsh BW et al., 2000, Hak AE et al., 2001, Man RYK et al., 2001, Yildirir A et al., 2002, Somekawa Y et al., 2002, Madsen JS et al., 2002)*.

The decrease is generally 5-10%. The tHcy-lowering effect seems to be more pronounced when pretreatment tHcy levels are high *(Van Baal et al., 2000)*. This may explain why the levels did not change after treatment with oral or transdermal HRT for a year, when baseline levels were as low as 8.2 and 8.7 µmol/L, respectively *(Evio S et al., 2000)*.

The initial tHcy levels were 8.8 µmol/L in another study, in which neither fasting tHcy nor folate or vitamin B_6 levels changed significantly. Post methionine loading tHcy actually increased significantly, after 2 mg

of oestradiol had been given given daily for 6 months, and 5 mg of methoxyprogesterone acetate for 10 days from the 91st day. The mean concentration of LDL cholesterol, however, declined by 20% and HDL cholesterol increased by 16% during the study period *(Berger PB et al. 2000)*.

HRT with either 17β-estradiol or conjugated equine estrogen alone or in combination with progestin for 3.5-4.5 years also significantly improved the lipoprotein profile by decreasing total cholesterol, LDL-cholesterol and apoB in another study *(Man RYK et al., 2001)*.

Androgens may enhance Hcy synthesis secondary to an increase in muscle mass, as suggested by a positive correlation between tHcy and plasma creatinine during androgen administration *(Giltay E et al., 1998a)*.

Also *raloxifene* decreases tHcy. In a placebo-controlled study of 390 healthy women, both combined HRT (conjugated equine estrogen and medroxyprogesterone acetate) and raloxifene significantly decreased tHcy. Hormone replacement therapy, but not raloxifene, increased C-reactive protein (CRP) levels by 84% in this study *(Walsh BW et al., 2000)* The CRP raising effect of conjugated equine estrogen, however, was partly counteracted by addition of medroxyprogesterone in another study *(Yildirir A et al., 2002)*. Raloxifene also reduced both tHcy and LDL and total cholesterol significantly in a third study of elderly women *(De Leo V et al., 2001)*. Long-term treatment with raloxifene reduced tHcy to the same extent as HRT, but was not as effective in reducing lipoprotein (A) in another study *(Smolders RGV et al., 2002)*.

The partial oestrogen agonist, *tamoxifen*, also reduces tHcy levels *(Lien EA et al., 1997, Cattaneo M et al., 1998a, Love RR et al., 1999)*. This interaction was recently discussed in a review *(Mijatovic V and van der Mooren MJ, 2001)*.

There is evidence that ovarian hormones, especially 17β-oestradiol, may have a cardio-protective effect, and œstrogen replacement therapy was recently shown to reverse endothelial dysfunction in postmenopausal women *(Bush D et al., 1998)*.

An in vitro study indicates that 17β-estradiol may prevent Hcy-induced endothelial cell injury by increasing the intracellular content of GSH *(Dimitrova KR et al., 2002)*.

A recent study of healthy women with regular menstrual cycles showed that treatment with ethinyloestradiol caused a shift in the balance between NO and endothelin-1 in the direction of vasodilatation *(Merkli-Feld GS et al., 2000).*

Healthy elderly men, who were were given 17β-oestradiol, showed, in addition to decreased tHcy, lowered levels of LDL cholesterol and triglyceride and an increase in HDL cholesterol *(Giri S et al., 1998)*.

Before the menopause, women appear to be protected from cardiovascular disease (CVD), but thereafter the incidence increases continuously. Increasing tHcy parallels this development.

In a prospective, nested case-control study of 28,263 postmenopausal women with no history of cardiovascular disease at baseline, there were 122 cases of CVD during a mean follow-up of 3 years. These women had higher baseline tHcy levels than controls (14.1 versus 12.4 µmol/L). Subjects in the highest quartile had a twofold increased risk of any cardiovascular event compared to the others *(Ridker PM et al., 1999)*.

Tamoxifen treatment also seems to reduce coronary heart disease *(Costatino J et al., 1997, Clarke SC et al., 2001)*. This effect may equally be related to the tHcy-reducing effect.

The effects of oestrogens on tHcy, vessel wall, cardiac function, and hemostasis are discussed in a review *(Mijatovic V et al., 1996)*.

Diseases affecting the homocysteine metabolism

Hyperhomocysteinemia in elderly patients is rarely an isolated finding. In a recent study of 600 elderly hospitalised patients it was associated with diabetes in 20%, heart failure in 30%, malignancies in 20.5% and renal failure in 48% *(Ventura P et al., 2001)* .

Gastrointestinal disorders

Various gastrointestinal disturbances resulting in atrophic changes may decrease the absorption of vitamins required for the Hcy-metabolism. The absorption of both vitamin B_{12} and folate is dependent on the secretion of gastric acid.

It is well known that the absorption of vitamin B_{12} requires both secretion of gastric acid for cleaving the vitamin from proteins and the secretion of intrinsic factor (IF). Moreover, pancreatic enzymes are required and also intact B_{12}-IF receptors in the distal ileum. A range of gastric disorders, therefore, may result in malabsorption of vitamin B_{12}.

Malabsorption of vitamin B_{12} from food, however, may arise in at least two different gastric settings, one of which involves neither gastric atrophy nor achlorhydria. Histological findings and acid and pepsin secretion may overlap considerably between individuals with normal and those with severe malabsorption *(Cohen H et al., 2000)* . Bacterial overgrowth may be considered. In some patients, malabsorption can respond to antibiotics. Folate absorption involves a complex mixture of enzymes and binding pro-

teins. The predominant folate in food is polyglutamates of 5-methyltetrahydrofolate (THF), which is readily oxidised to 5-methyl-5,6-dihydrofolate. This form may constitute 50% of total food folate. Under mildly acidic conditions (after meals) it is rapidly degraded, but can be reduced back to acid-stable 5-methyl-THF by ascorbate secreted into the gastric lumen. This may be a critical step. The formation of 5-methyl-THF monoglutamate requires the enzyme pteroyl-λ-glutamylhydrolase.

Dietary folates are absorbed in the proximal jejunum. Absorption is optimal within a very narrow pH range with a peak at pH 6.5. Folate transport into the cell is mediated by membrane-bound proteins (FR-α) with a high binding affinity for 5-methyl-THF and by mobile reduced folate carriers (RFC). Fairly common genetic defects in RFC have recently been described *(Chango A et al., 2000c, Rady PL et al., 2001)*.

Strikingly reduced folate absorption is seen in patients with atrophy of the jejunal mucosa, as compared with healthy subjects *(Botez and Bachevalier, 1981)*. A rise in pH in the jejunum, the site of folate absorption, reduces absorption. These mechanisms are outlined in recent reviews *(Gregory JF et al., 1997, Gregory JF et al., 2001, Brzezinska A et al., 2000)* .

Helicobacter pylori (HP) infection plays an important role in the development of chronic atrophic gastritis *(Kuwahara Y et al., 2000)*. HP was detected in 77 of 138 patients (56%) in a recent study of patients with vitamin B_{12} deficiency *(Kaptan et al., 2000)*. In an earlier study, antibodies against HP were found in 86% of patients with atrophic gastritis, although only 33% still had an active HP infection in the gastric mucosa *(Karnes WE et al., 1991)*.

It was recently demonstrated that coeliac patients in bipsy-proven remission and on a gluten-free diet for 8-12 years had both higher tHcy than the general population and low plasma vitamin B_6 and folate. The calculated mean daily intake of folate and vitamin B_{12} were significantly lower than in controls *(Hallert C et al., 2002)*.

It is obvious that gastrointestinal disorders other than atrophic gastritis may affect absorption of both folate and vitamin B_{12}. Surgical interventions and radiation of bladder or uterus cancer, for instance, may also damage critical portions of the intestine.

Renal failure

Under physiological conditions, non-protein-bound Hcy is subjected to glomerular filtration in the kidney, but is almost completely reabsorbed in the tubuli, and then oxidatively catabolised to carbon dioxide and sulphate in the kidney cells. Clearance is markedly reduced in the presence of renal failure *(Guttormsen AB et al., 1997)*. There is a strong, positive cor-

relation between tHcy levels and serum creatinine, and to the renal glomerular filtration rate *(Hultberg B et al., 1993b, Hultberg B et al., 1995, Chaveau P et al., 1993, Arnadottir M et al., 1996)*.

Total Hcy concentrations commonly range between 20 and 30 µmol/L in end-stage renal disease (ESRD), or 2- to 4-fold normal mean values, but there may be ethnic differences in tHcy levels associated with renal insufficiency *(Carmel R et al., 1999a)*.

A study of the methionine metabolism in ESRD patients with stable isotope, recently demonstrated that remethylation was decreased by about 30%, compared to controls, whereas transsulphuration was not significantly different *(Van Guldener C et al., 1999)*.

Another recent study of 50 ESRD patients and 40 non-dialysed patients with chronic renal disease, however, showed highly significantly increased intermediate products of the transsulphuration pathway without any change in remethylation of Hcy. Total Hcy positively and highly significantly correlated with serum concentrations of cystathionine and cysteine, but also with the marker of functional vitamin B_{12} deficiency, MMA. Of the ESRD patients, 22 received 10 mg of vitamin B_6, 6 µg of vitamin B_{12}, and 1mg of folic acid daily. These patients had lower levels of both tHcy and cystathionione *(Henning BF et al., 1999)*.

Levels of S-adenosylhomocystenine (SAH), which is a potent inhibitor of methylation reactions, page 22, were found to be higher in the RBC of chronic renal patients with chronically elevated tHcy than in either patients with vascular disease or healthy controls in a recent study *(Fu WY et al., 2000)*.

It was also recently shown that the plasma concentrations of the reactive free reduced form of Hcy was two to four times higher than normal in ESRD. This reactive form was recently shown to correlate closely with endothelial dysfunction following methionine load *(Chambers JC et al., 2000b, Chambers JC et al., 2001)*.

Two reviews on hyperhomocysteinemia in ESRD recently published *(Bostom AG and Lathrop L, 1997, Bostom AG and Culleton B, 1999)*.

The mechanisms causing hyperhomocysteinemia in renal disease are discussed in a recent article *(Van Guldener C and Robinson K, 2000)*.

Gout

A pilot study of 28 patients with primary gout, treated with allopurinol (5), antihypertensives (13), and NSAIDs (12) revealed increased tHcy compared to healthy controls (mean: 14.8 µmol/L versus 7.6 µmol/L). The underlying mechanism could be drug interactions, altered tubular excretion and/or decreased glomerular filtration. However, in this study the

tHcy levels were increased both in patients with normal and elevated serum creatinine and in patients with normal urate excretion *(Istok R et al., 1999)*. The recent observation in a study of 271 Japanese elderly men that the C677T polymorphism in the MTHFR gene increases serum uric acid, however, points to a possible influence of the homocysteine metabolism *(Zuo M et al., 2000)*. Gout is frequently associated with cardiovascular complications.

Homocysteine-related complications in renal disease

Patients with renal failure have an excess morbidity and mortality in arteriosclerotic cardiovascular disease. The incidence of myocardial infarction and stroke is five to tenfold higher in ESRD than in the general population *(United States Renal Data system, 1995, Bostom AG et al., 1995b, Bachmann J et al., 1995, Robinson K et al., 1996)*. Elevated tHcy is the commonest cardiovascular risk factor in patients with chronic renal failure *(Bostom AG et al., 1997, Bostom AG and Lathrop L, 1997)*.

In a prospective study of 167 patients with a mean 17.4-month follow-up, 33% developed cardiovascular events and 19% died. Twelve of the 31 patients died of cardiovascular causes. Total Hcy levels were higher in patients, who had cardiovascular events or died of cardiovascular causes *(Moustapha A et al., 1998)*. A 4% increase in risk for each µmol/L increase of tHcy is also calculated from a prospective study of access thrombosis in hemodialysis patients *(Shemin D et al., 1999)*.

A reduction in tHcy in patients with renal failure by daily supplementation with 15 mg of folic acid, 100 mg of vitamin B_6 and 25 µg of vitamin B_{12} was recently associated with a trend towards decreased myocardial ischemia *(Miner SES et al., 1998)*.

The association between cardiovascular complications in renal disease and tHcy was recently reviewed *(Kronenberg F, 1998)*.

Diabetes mellitus

Depending on the stage of disease, renal function, vitamin status and type of diabetes/treatment, decreased, normal, and increased tHcy concentrations may be observed.

Normal or even low levels of tHcy in type 1 diabetes may be owing to glomerular hyperfiltration *(Hultberg B et al., 1993b)*. Lower tHcy levels than normal was found in both type 1 diabetes and type 2 diabetes patients in a recent study, and tHcy was closely and independently correlated with

GFR *(Wollesen F et al., 1999)*. Similar findings were recorded in very young type 1 diabetes patients *(Salardi S et al., 2000)*, whereas normal tHcy concentrations were found in a Spanish study of young patients with type 1 diabetes *(Pavia C et al., 1999)*.

A study of elderly patients with type 2 diabetes without nephropathy or cardiovascular complications also revealed lower tHcy than in controls *(Mazza A et al., 2000)* . No significant differences between diabetic and non-diabetic pregnant women were seen for tHcy in another study *(Kaplan JS et al., 1999)*.

Conversely, both increased fasting and PML levels of tHcy have been found in many studies, particularly in type 2 diabetes.

In a study of 318 type 2 diabetes patients with duration of disease from 0-41 years, the mean fasting tHcy was highly significantly elevated compared to that of controls (12.3 µmol/L versus 10.8 µmol/L). Total Hcy was also correlated with the duration of the disease, with fasting plasma glucose and HbA_{1c} *(Passaro A et al., 2000)*.

Hultberg and co-workers have shown that type 1 diabetes patients with the lowest age at onset and with the poorest metabolic control are those most prone to a rapid increase in tHcy *(Hultberg B et al., 1997)*.

Association with renal function

Patients with nephropathy and either type of diabetes have higher tHcy levels than patients without nephropathy, but tHcy rises even with a modest deterioration of renal function, and also when vitamin status is in the low-normal range *(Chango A et al., 1996a, Munshi MN et al., 1996, Hultberg B et al., 1997, Chico A et al., 1998, Fiorina P et al., 1998a, Fiorina P et al., 1998b, Smulders YM et al., 1999b, Stabler SP et al., 1999a)*.

Elevations of tHcy are associated with increased albumin excretion, especially in patients with type 2 diabetes *(Chico A et al., 1998, Fiorina P et al., 1998a, Smulders YM et al., 1999b, Stabler SP et al., 1999a)*. A study of 61 patients with onset of diabetes before the age of 12 years, with a duration of disease longer than 7 years, and with nephropathy had higher tHcy levels, both fasting and PML, than had patients without complications, or healthy controls. The tHcy level was particularly increased in patients with albumin excretion rate over 70 µg/min *(Chiarelli F et al., 2000)*.

Vitamin status

Vitamin status in diabetic patients may be important. There is in general an inverse correlation between folate and/or vitamin B_{12} status and tHcy levels *(Hultberg B et al., 1997, Laivuori H et al., 1999, Stabler SP et al., 1999a,*

Buysschaert M et al., 2000, *Wollesen F et al., 1999)*. Higher folate levels and lower tHcy in female than in male diabetics are reported *(Salardi S et al., 2000)*.

Diabetes and pernicious anemia often coexist *(Davis RE et al., 1992, Perros P et al., 2000)*. A high prevalence of autoantibodies against gastric intrinsic factor and gastric parietal cells was found in type 1 diabetes patients without pernicious anemia in an early study *(Ungar B et al., 1967)*. Another early study showed that vitamin B_{12} influenced glucose metabolism in elderly persons, and that vitamin B_{12} could normalise glucose values *(Hadnagy C et al., 1964)* . Inactivation of vitamin B_{12} with nitrous oxide in rats also resulted in a selective depression of local cerebral glucose utilisation *(Hakim AM et al., 1983)*.

Low vitamin B_{12} levels are reported in diabetic patients, and particularly in patients treated with biguanides, page 99.

Hypothyroidism

Hypothyroid patients have higher levels of tHcy than have healthy controls and hyperthyroid patients. Green and co-workers were the first to report on an increased tHcy level in hypothyroid patients. The mean level was 18.4 µmol/L, which was highly significantly elevated, compared with the values in hyperthyroid patients, 11.0 µmol/L *(Green R et al., 1995)*.

This finding was confirmed in another study, where the mean tHcy level in hypothyroid patients was 16.3 µmol/L versus 10.5 µmol/L in healthy controls. Low levels of folate or vitamin B_{12} could not explain the raised levels of tHcy in the hypothyroid patients. Increased serum creatinine values, however, paralleled the tHcy concentrations. Serum cholesterol levels were also increased *(Nedrebø BG et al., 1998)*.

The same group showed that when thyroid hormone substitution was discontinued for 6 weeks in 17 patients, who had undergone thyroidectomy for thyroid cancer, they progressively attained a hypothyroid state. At the same time there was a parallel, progressive increase in mean tHcy (27%), serum creatinine (37%), and serum cholesterol (100%). The levels returned to the original levels within 4-6 weeks of reinitiating thyroid hormone therapy *(Lien EA et al., 2000)*. These findings were confirmed in another study of 45 hypothyroid and 26 hyperthyroid patients *(Barbé F et al., 2001)*.

Recent data from the National Health and Nutrition Examination Survey (NHANES), showed an OR relating hypothyroidism to hyperhomocysteinemia and a high total cholesterol of 4.9 and 8.0 respectively, after adjustment for age, gender, and race ethnicity, thus confirming the covari-

ation with cholesterol. Controlling for smoking, CVD history, BMI, and serum albumin did not affect these ratios *(Morris MS et al., 2001a)*.

Plasma tHcy decreased by 44% in 10 thyroidectomy patients and 4 with newly diagnosed hypothyroidism 3-9 months after initiation of replacement therapy *(Hussein WI et al., 1999)*. The tHcy-lowering effect of thyroid hormones was also confirmed in a recent prospective follow-up of 50 hypothyroid and 46 hyperthyroid patients. Creatinine and tHcy levels decreased in hypothyroid patients after substitution from 83.9 µmol/L to 69.8 µmol/L and 17.6 µmol/L to 13.0 µmol/L respectively. Levels increased in hyperthyroid patients after restoration of the euthyroid state. Plasma folate levels were lower in hypothyroid patients *(Diekman MJM et al., 2001)*.

Both fasting and PML tHcy were elevated in patients in a study of 40 patients with hypothyroidism (14 autoimmune and 26 owing to thyroidectomy) compared to healthy controls in another study. Fasting, but not PML hyperhomocysteineinemia, was normalised by thyroid hormone replacement. Multivariate analysis confirmed TSH as the strongest predictor of tHcy, independent of age, folate, vitamin B_{12}, and creatinine *(Catargi B et al., 1999)*.

Thyroid hormones markedly affect riboflavin metabolism, mainly by stimulating flavokinase and thereby the synthesis of flavin mononucleide (FAD) that serves as cofactor for enzymes involved in the metabolism of vitamin B_6, B_{12} and folate and may therefore affect the Hcy metabolism *(Hustad S et al., 2000)*.

Hypothyroidism and pernicious anemia often coexist *(Davis RE et al., 1992, Ottesen M et al., 1995, Perros P et al., 2000)*. Treatment with cyanocobalamin, however, did not have any systemic effect on thyroid function in a study of 22 patients *(Ottesen M et al., 1995)*.

Other autoimmune diseases

Rheumatoid arthritis

Hyperhomocysteinemia is common in several other autoimmune diseases.

Increased fasting levels of tHcy (by 33%) in 20 patients with rheumatoid arthritis (RA) compared to healthy controls were reported in one study. The PML values were also increased *(Roubenoff R et al., 1997)*.

In RA, both impaired gastric function, causing vitamin malabsorption, and decreased renal function could explain an elevated tHcy. ApoTC (unsaturated TC) levels were increased in a study of patients with active RA compared to levels in patients in clinical remission, whereas total serum

B_{12} levels were similar in both groups. This observation may point to deficient vitamin B_{12} distribution and cellular deficiency *(Arnalich F et al., 1990)*.

Biochemical signs of disturbed gastric function, and at the same time elevated levels of both tHcy and methylmalonic acid (MMA), the specific marker of functional vitamin B_{12} deficiency, were recently reported in patients with RA *(Pettersson T et al., 1998)*.

Folate levels are also known to be low in RA patients. Several drugs used in rheumatic diseases, particularly the folate antagonist, methotrexate, may contribute to low folate and increased tHcy levels, page 94.

Decreased plasma concentrations of vitamin B_6 in RA patients, inversely associated with markers of inflammation, are also reported *(Roubenoff et al, 1995)*. It was recently proposed that increased levels of tHcy may reflect an acute phase induction of pyridoxal phosphatase activity associated with increased production of IL-6 in chronic inflammation. This would lead to stimulation of the activity of pyridoxal phosphatase, thereby diminishing vitamin B_6 levels and reducing CBS activity *(McCarty MF, 2000b)*.

A recent case-control study of 54 RA patients showed that patients had higher tHcy than controls. Furthermore, patients with a history of cardiovascular disease had the highest mean values or 15.1 µmol/L, compared with patients without CVD history (9.9 µmol/L) and controls (8.3 µmol/L) *(Cisternas M et al., 2002)*.

Systemic lupus erythematosus

In systemic lupus erythematosus (SLE), reduced vitamin B_{12} absorption *(Molad Y et al., 1990)* and impaired kidney function *(Fijnheer R et al., 1998)* may be the cause of an observed elevation of tHcy, but a correlation with serum folate is also reported *(Petri M et al., 1996)*. Impaired DNA methylation in lupus T cells is seen *(Scott JM and Richardson BC, 1999)*.

Premature coronary heart disease is a major determinant of morbidity and mortality in patients with both RA and SLE. Elevated tHcy levels, associated with atherothrombotic events are reported in SLE patients, *(Petri M et al., 1996, Fijnheer R et al., 1998, Svenungsson E et al., 2001)*.

Psoriasis

Basal elevated tHcy levels are found in psoriatic patients, possibly as a consequence of increased dermal cell proliferation. A further increase in fasting tHcy levels also occurs after low-dose treatment with methotrexate *(Refsum H and Ueland PM, 1989)*.

HIV/AIDS

In the first studies of HIV-infected and AIDS patients, no increase in tHcy was reported, but the studies were small and therefore lack power. Highly significantly increased tHcy levels were later found in in a study of 69 HIV-infected children compared to a reference pediatric population. Folate levels were highly significantly decreased in the infected children. In patients treated with protease inhibitors tHcy was significantly higher than in patients on other retroviral treatment. There was a significant positive correlation between folate levels and the methionine/tHcy ratio, and a negative correlation between folate levels and tHcy *(Vilaseca MA et al., 2001)*.

Elevated levels of reduced Hcy, which is very reactive, are reported in in three studies of HIV infection *(Müller F et al., 1996, Aukrust P et al., 1997, Naisbitt DJ et al., 2000)*. Reduced Hcy has been proposed to contribute to the formation of reactive oxygen species, which could propagate the damaging effect of Hcy. Reduced Hcy also was recently shown to correlate closely with endothelial injury induced by methionine loading *(Chambers JC et al., 2000b)*.

A disturbed glutathione (GSH) homoeostasis in the CD4+ lymphocytes of HIV-seropositive individuals is reported *(Van der Ven AJ et al., 1998)*.

Cysteine is a major precursor to the endogenous antioxidant GSH, page 24. Levels of reduced plasma cysteine were highly significantly decreased compared to the controls in a study of 33 HIV-positive patients, although there was no difference in oxidised, protein-bound or total cysteine. Plasma from patients was less able to detoxify nitrososulphamethoxazole than control plasma *(Naisbitt DJ et al., 2000)*.

Immunological functions in diseases that are associated with cysteine and GSH deficiency may be significantly enhanced by cysteine supplementation. This was demonstrated in two placebo-controlled trials of HIV-infected patients *(Droge W and Breitkreutz R, 2000)*.

Vitamin status

In patients with HIV/AIDS the gastrointestinal microflora is often abnormal and the mucosa shows pathological changes such as atrophic villi. The production of both gastric acid and intrinsic factor (IF) may be decreased, which has been shown in many studies summarised in a recent review *(Remacha AF and Cadafalch J, 1999)*. An unexpectedly high prevalence of gastric IF antibodies is reported *(Harris PJ, Candeloro P, 1991)*.

Protease inhibition in the gastric mucosa is common in these patients and is proposed to contribute to protein-bound cobalamin malabsorption *(McCaddon A et al., 1995)*.

Folate absorption may also be impaired in HIV-infection. An oral dose of folic acid showed that the absorption was impaired irrespective of the stage of the disease, gastrointestinal disturbances and drug treatment *(Revell P et al., 1991)*.

As a consequence of these disturbances, significantly decresed levels of micronutrients, and in particular of vitamin B_{12} and RBC folate, are reported in several, but not all studies, possibly due to the small numbers of patients studied. Lower levels are generally seen in later stages of the disease. This could be expected for vitamin B_{12} as the half life may be as long as 3 years.

Distribution of vitamin B_{12} across membranes and into cells may also be disturbed. Low levels of holoTC, the B_{12} transport protein, are reported, even when total serum B_{12} levels have been normal or even high. These studies are outlined in the review by Remacha and Cadafalch.

Low vitamin B_6 levels are also reported in HIV-positive patients. Vitamin B_6 deficiency was common in a study of stage III HIV 1-infected subjects, despite adequate dietary intake. Vitamin B_6 status was significantly associated with functional parameters of immunity in these patients. Overtly deficient individuals exhibited decreased lymphocyte responsiveness to the mitogens phytohaemagglutinin and pokeweed, and reduced NK cell cytotoxicity, compared to subjects with adequate vitamin B_6 status *(Baum MK et al., 1991)*.

Tissue damage

Tissue damage may increase tHcy transiently. The reason for increased tHcy-levels after injury may be leakage from cells, but also accelerated methylation reactions in the repair processes, leading to increased formation of SAH and Hcy. Increased tHcy levels were recently found in critically ill patients admitted to hospital *(Schindler K et al., 2000)*. No data on both pre- and posttraumatic tHcy are, however, available.

After stroke, an acute phase response with an initial reduction in tHcy of about 25%, followed by an increase of about 20% during convalescence has been found *(Lindgren A et al., 1995)*. Low levels of tHcy after MI have been reported for up to 7 days *(Egerton W et al., 1996)*.

Total Hcy, with measurements obtained on admission and on days 2, 7, and 28, were, however, minimally influenced by acute phase variations in another study of patients with MI and unstable angina *(Al-Obaidi MK et al., 2001)*. The same group earlier reported that elevated admission tHcy in such patients was significantly associated with the peak troponin level, a highly specific marker of myocardial injury *(Al-Obaidi MK et al., 2000a)*.

Cancer

Hyperhomocysteinemia may be associated with carcinogenesis, but may also be secondary to the cancer. Some cancer cell lines have been found to be incapable of remethylating Hcy *(Fiskerstrand T et al., 1997)*. Hyperproliferating cells may therefore form increased amounts of Hcy, and increased tHcy levels are often found in patients with neoplastic diseases *(Wu JT et al., 1995)*.

Homocysteine might also leak from damaged tissues, particularly in advanced stages.

Other diseases

It was recently shown in 49 adults with sickle cell disease that the median tHcy in plasma was about 50% higher than in controls, in spite of similar folate and vitamin B_{12} concentrations. The difference persisted when subjects with renal insufficiency were excluded *(Lowenthal EA et al., 2000)*.

Total Hcy is also recently reported to be related to severity of lung impairment in scleroderma *(Caramaschi P. et al., 2003)*.

Drugs that interact with the homocysteine metabolism

A tHcy-increasing effect is well documented for several drugs. For some drugs decreased vitamin status, which could be expected to influence Hcy metabolism, is well documented. A few of the mentioned interactions are less well documented. Nevertheless, these are mentioned to draw attention to the possibility of avoidable side effects, as impaired vitamin status can easily be prevented. Interactions with drugs are often neglected, generally discovered by chance, and often long after the drug is marketed.

Three recent reviews give a general overview *(Alonso-Aperte E, Varela-Moreiras G, 2000, Varela-Moreiras G, 2001, Desouza C et al., 2002)*.

Nitrous oxide

Nitrous oxide rapidly and irreversibly inactivates methionine synthase (MS) by oxidising cobalt bound to its coenzyme, methylcobalamin. The outcome is impaired MS activity, decreased remethylation of Hcy, and reduced formation of SAM. Although MS is the primary target, there are also reversible changes in the other vitamin B_{12} coenzyme, adenosyl-

cobalamin, and in the activity of methylmalonyl-CoA mutase, to which it is a coenzyme *(Riedel B et al., 1999)*.

Nitrous oxide anaesthesia, even of short duration, leads to significant increases in plasma tHcy *(Ermens AAM et al., 1991, Badner NH et al., 1998)*.

Reports of neurological symptoms induced by nitrous oxide include myelo-neuropathy, impaired memory, ataxia, numbness, and other disturbances *(Meyler's Side Effects of Drugs, 1996)*. Myelopathy has been reported after less than five hours of nitrous oxide anaesthesia *(Flippo TS and Holder W, 1993)*.

Drugs that may increase homocysteine levels

In a study of 90 patients, presenting for elective carotid endarterectomy, and randomised to receive general anaesthesia with or without nitrous oxide, tHcy was highly significantly increased postoperatively in the nitrous oxide group. Total Hcy was 15.5 µmol/L after the intervention and 18.8 µmol/L 48 hours postoperatively versus 11.4 µmol/L and 11.3 µmol/L, respectively in the group not receiving nitrous oxide. Patients in the nitrous oxide group also had a significantly increased incidence of ischemia, and more ischemic events that lasted longer than 30 minutes *(Badner NH et al., 2000)*.

Elderly and debilitated patients and patients with an impaired preoperative vitamin B_{12} and/or folate status may be particularly susceptible to nitrous oxide anaesthesia, which is illustrated by many case reports.

Vitamin B_{12} supplementation was recently recommended for all patients perioperatively with all but the shortest anaesthesia with nitrous oxide *(Mayall M., 1999)*. Oral treatment with 500 µg of vitamin B_{12}, 2.5 mg of folic acid and 25 mg of vitamin B_6 for one week preoperatively prevented the increase of tHcy group after anaestesia induced with propofol, and maintained with isoflurne and nitrous oxide. The placebo-controlled study included 53 patients undergoing surgery for revision knee or hip arthroplasty *(Badner NH et al., 2001)*.

An increase in the incidence of spontaneous abortion in female dental assistants exposed to nitrous oxide has been reported *(Rowland AS et al., 1995)*. More recently the relation between occupational nitrous exposure in the second trimester of pregnancy, birth weight and gestational age at delivery was studied in the members of the Swedish Midwives Association. Exposure to the use of nitrous oxide was associated with reduced birth weight and an increase in the risk of infants being small for gestational age *(Bodin L et al., 1999)*.

This interaction and its clinical consequences are discussed in a review *(Guttormsen AB et al., 1994a)*.

Methotrexate

The folate antagonist methotrexate (MTX) is used in the treatment of malignancies, but also in conditions like psoriasis, RA, SLE, and some other autoimmune diseases. MTX impairs the activity of dihydrofolate reductase, and thereby indirectly blocks the methylation cycle. As a consequence, tHcy increases. However, the fact that folate supplementation reduces toxicity without affecting efficacy in RA patients suggests that the inhibition of the enzyme is not complete and not essential for efficacy. A

persistent increase in tHcy is observed in patients with RA treated with MTX *(Haagsma CJ et al., 1999)*.

A study of 23 children treated for cancer with MTX showed that already after 7 days of treatment the tHcy concentration in CSF was increased compared to the values in the control group. In addition, homocysteic acid and cysteine sulphinic acid, which are neurotoxic degradation products of Hcy, reached mean concentrations of 119.1 µmol/L and 28.4 µmol/L respectively in the CSF of the treatment group, whereas they could not be detected in the controls *(Quinn C et al., 1997)*.

In another study of 24 children treated with MTX, the CSF levels of both SAM and methyl-THF were highly significantly decreased *(Surtees R et al., 1998)*.

These findings are consistent with the hypothesis that MTX causes folate depletion in the CNS, leading to SAM deficiency and other disturbances that can explain the common neuropsychiatric side effects of MTX *(Meyler's Side Effects of Drugs, 1996)*.

The effects of MTX on pregnancy, fertility, and lactation, which may also be consequences of a disturbed Hcy metabolism, were recently reviewed *(Lloyd ME et al., 1999)*.

Sulphasalazine

Inflammatory bowel disease is associated with impaired absorption of vitamins, and possibly increased tissue utilisation of for instance folate.

Sulphasalazine, used in the treatment of inflammatory bowel disease, has been shown in several studies to impair the absorption of different forms of folate. This drug is also shown to interfere with folate recognition sites. These studies are discussed in a review, which also outlines other earlier studies on interactions with folate and vitamin B_{12} *(Lindenbaum J, 1983)*.

A more recent study confirms that sulphasalazine causes folate deficiency. Measurements of tHcy suggested that a substantial number of patients may have folate difficiency at the tissue level *(Krogh Jensen M et al., 1996)*.

Sulphasalazine is also used in arthritis in combination with MTX. The effect of MTX on tHcy is then further increased *(Haagsma CJ et al., 1999)*.

A recent American-Canadian study of 1,242 cases of NTD and 6,660 controls, showed an overall increased risk of NTD related to exposure of sulfasalazine during the first or second month after the last menstrual period *(HernandezDiaz S et al., 2001)*.

Antiepileptic drugs

Several antiepileptic drugs are shown to impair folate status Mental deterioration in epilepsy due to folate deficiency was reported over 30 years ago *(Neubauer C, 1970)*. Reynolds and co-workers reported a significant fall in folate concentrations in both serum and CSF in epileptic patients when compared with controls or with psychiatric patients *(Reynolds EH et al., 1972)*.

Later it was found that of 29 patients studied all but three patients showed abnormalities in one or more electrophysiological measurements. All had low folate levels in serum and CSF, these being below the normal range in 19 patients. Folate therapy reversed abnormalities in motor and sensory nerve distal latencies *(Martinez Figueroa A et al., 1980)*.

A later study in 27 patients treated with phenobarbitone and diphenylhydantoin for 3-32 years confirmed the finding of decreased serum and CSF folate levels, but serum B_{12} was increased. Increased levels of cholesterol and triglycerides were also reported *(Dastur DK and Dave UP, 1987)*.

The interaction with phenytoin, has been documented in many studies, *(Lewis DP et al., 1995).* Phenytoin seems to have a greater impact than other anti-epileptics. The impact on neuropsychiatric side effects of the drug were discussed in another article *(Froscher W et al., 1995)*.

The underlying mechanisms could be increased folate catabolism by hepatic enzyme induction. Phenytoin, phenobarbital, primidone and carbamazepine induce, whereas valproate inhibits, cytochrome 450, which may explain why the impact on vitamin status and tHcy may differ between antiepileptics. The patient´s initial folate status obviously also has an impact and may explain some inconsistencies in study results.

Reduced absorption of folate, owing to a higher gastrointestinal pH, may also play a role, as may a suggested reduced conversion of folate to active forms caused by decreased MTHFR activity *(Billings R, 1984)*.

Compared with levels in controls, mean serum folate levels were also found to be reduced in patients treated with phenobarbitone or carbamazepine, both enzyme-inducing agents, in another study. In contrast, patients treated with the non-enzyme-inducer valproate or zonisamide, had values that did not differ significantly from that of controls *(Kishi T et al., 1997)*. These results were recently confirmed. Phenytoin, phenobarbital and primidone, but not valproate, were associated with high fasting and PML levels of tHcy and low folate levels *(Apeland T et al., 2001b)*.

An impact on tHcy was first demonstrated in patients treated with phenytoin. Plasma tHcy and serum folate levels were inversely correlated in a study of 130 epileptic patients treated with different antiepileptic drugs.

The folate status was lower in patients treated for a long period *(Ono H et al., 1997)*.

The mean serum level of tHcy was 17.9 µmol/L in another study of patients treated with phenytoine alone or in combinations versus 11.9 µmol/L in the control group treated with other anticonvulsants, most often carbamazepine *(James GK et al., 1997)*.

Small children may be particularly at risk. A study of 100 children aged 2 months to 18 years receiving antiepileptic treatment (carbamazepine, valproic acid, clonazepam or clobazam) showed that tHcy was highly significantly elevated in the younger age groups as compared to controls of the same age *(Vilaseca MA et al., 1999)*.

Recent studies indicate that vitamin B_6 status may also be impaired by antiepileptic treatment. In a study of patients treated with different antiepileptic drugs, both serum folate and vitamin B_6 in plasma were lower in patients than in age- and sex-matched controls. Total Hcy over 15 µmol/L was commoner in the patients. The same result was seen when patients treated solely with carbamazepine were included *(Schwaninger M et al., 1999)*.

In another recent study, more than half of the patients receiving monotherapy with phenytion, lamotrigine, carbamazepine, or valproate had vitamin B_6 concentrations below the normal range. Of the 20 patients who received phenytoin, 17 had low plasma folate, but only 5 had low RBC folate. Valproate treatment increased the serum concentrations of vitamin B_{12} *(Tamura T et al., 2000)*. This increase could possibly be secondary to impaired folate status.

After one year of monotherapy with sodium valproate or carbamazepine, a significant decrease in both serum folate and plasma vitamin B_6 was seen in a study of young epileptic patients. There was an increase in fasting and PML tHcy in both groups of epileptics when compared to both baseline data and values from controls, even though serum B_{12} and RBC folate remained in the normal range *(Verotti A et al., 2000)*.

Both fasting and PML tHcy were increased in patients taking antiepileptic drugs that induce cytochrome 450. Patients on valproate, which inhibits cytochrome 450, had lower increase in PML tHcy *(Apeland T et al., 2000)*.

Another recent study also showed increased fasting and PML tHcy in patients treated with carbamazepine compared to controls. Both serum and RBC folate levels were decreased. Isolated PML hyperhomocysteinemia was found in some patients, which may indicate B_6 deficiency *(Apeland T et al., 2001a)*.

Not only the remethylation of Hcy, but also the transsulphuration pathway may thus be affected.

Vitamin B_6 deficiency may decrease the synthesis of cysteine, a precursor of glutathione (GSH). Hcy-related oxidative effects may also deplete GSH, page 24. Plasma levels of total GSH were recently found to be lower in patients treated with carbamazepine, phenytoin, or with multiple drugs than in controls, but total GSH concentrations did not differ in patients treated with valproic acid or phenobarbital *(Ono H et al., 2000a)*.

Decreased GSH levels may thus contribute to the oxidative adverse effects of some anticonvulsants.

Hcy-related side effects

The use of anticonvulsants is associated with many side effects that may be related to a disturbed one-carbon metabolism *(Meyler's Side Effects of Drugs, 1996)*. About 6% of infants born to mothers taking anticonvulsants are estimated to have malformations, including NTD. A further proportion shows developmental delay in later childhood.

A recent American study of 6,932 infants with various congenital defects and 8,387 control infants showed that anticonvulsants, including carbamazepine, phenytoin, phenobarbital and primidone, may increase the risk of not only NTD, but also of cardiovascular defects, oral clefts, and urinary tract defects *(Hernandez Diaz S et al., 2000)*.

The same group reported data from a recent American-Canadian study of 1,242 cases of NTD and 6,660 controls, showed an overall OR for NTD related to exposure to antiepileptics or sulfasalazine, triamterene, and trimethoprim during the first or second month after the last menstrual period of 2.8 for the whole group versus no exposure during this period. For carbamazepin the OR was 6.9 *(Hernandez Diaz S et al., 2001)*.

A recent Danish study of 235 pregnancies in women exposed to anticonvulsant drugs showed an overall OR of malformations of 2.2, and a risk of low birth weight and preterm delivery of 1.5 and 1.6 respectively, compared to 17,259 unexposed pregnancies *(Fonager K et al., 2000)*.

In another recent Danish study, singleton pregnancies in 193 women with epilepsy were compared with 24,094 singleton pregnancies in women without epilepsy. In this study too, the infants of women with drug-treated epilepsy had a lower birth weight, were shorter, and had a smaller head circumference. In epileptic women who smoked the OR of preterm delivery was increased to 3.4 compared with non-epileptic women who

also smoked *(Hvas CL, 2000)*. Smoking further impairs vitamin status, page 59.

Neuropsychiatric symptoms, mentioned above, may also be related to a disturbed Hcy metabolism.

A recent review focuses the phenytoin-folic acid interaction *(Seligman H et al., 1999)*.

Oral antidiabetic drugs

Low vitamin B_{12} levels are common in patients treated with biguanides *(Rieder HP et al., 1980)*. Up to 30% of patients treated with metformin may develop vitamin B_{12} deficiency *(Tomkin GH et al., 1971, Tomkin GH., 1973, Carpentier JL et al., 1976)*. Proposed mechanisms include alterations in small bowel motility, bacterial overgrowth, or a direct effect on absorption *(Caspary WF et al., 1977)*.

The uptake by ileal cell surface receptors of the vitamin B_{12}-IF complex is known to be a process dependent on calcium availability *(Carmel R et al., 1969)*. Metformin seems to have an effect on calcium-dependent membrane action *(Schafer G, 1976)*. It was recently shown in a controlled trial that supplementation with calcium reversed the metformin-induced diminished vitamin B_{12} absorption and holoTC depression *(Bauman WA et al., 2000)*.

The common increase in tHcy in type 2 diabetics may therefore, at least partly, be explained by drug-induced decreased vitamin B_{12} absorption/status. In a study of non-diabetic cardiovascular patients, the tHcy levels were increased in those treated with metformin 2 g and lovastatin 40 mg daily compared with patients treated with lovastatin only. The increase was 13.8% at 40 weeks of treatment. At the same time vitamin B_{12} levels decreased by 17.7% and folate by 8% *(Carlsen S et al., 1997)*.

Some increase in tHcy was seen in two other studies *(Chango A et al., 1996b, Hoogeveen EK et al., 1997)*.

Over a 5-year period, survival after a previous MI was studied in 2,395 type 2 diabetes patients on different therapeutic regimens (diet, metformin, sulphonylureas or metformin and sulphonylureas). After adjustment for variables associated with long-term prognosis, it was found that the use of metformin, alone or in combination with sulphonylureas, was associated with a significantly increased risk of all-cause mortality with an OR of 1.42, whereas the use of sulphonylureas alone was not. The lowest mortality was found in the diet-alone group *(Fisman EZ et al., 1999)*.

Levodopa

Increased levels of tHcy in parkinsonian patients treated with L-dopa and decarboxylase inhibitors are reported *(Allain P et al., 1995, Kuhn W et al., 1998, Müller T et al., 1999, Brattström L et al., 2001, Blandini F et al., 2001)*.

Patients on long-term treatment with L-dopa and decarboxylase inhibitors were found to have a higher tHcy than controls and untreated patients, or 17.3 µmol/L compared to 9.1 and 9.2 µmol/L respectively *(Müller T et al., 1999)*. In another recent study tHcy was 16.9 µmol/L in patients versus 9.3 µmol/L in controls *(Blandini F et al., 2001)*.

The probable explanation is L-dopa-induced increased turnover of SAM and formation of SAH (and Hcy). L-dopa increases methylation 4-5 times more than does dopamine *(Liu XX et al., 2000)*. L-dopa is O-methylated to 3-O-methyldopa (3-OMD). The methylation of 1,000 mg of L-dopa is calculated to consume 5 mMol of SAM. The patient would have to approximatively double his or her de novo synthesis of SAM to compensate for this increased turnover *(Brattström L et al., 2001)*.

The ratio of 3-OMD/L-dopa correlated positively with tHcy in patients in one study *(Blandini F et al., 2001)*, and tHcy correlated positively with 3-OMD in parkinsonian patients in another recent study *(Müller T et al., 2002)*.

To prevent peripheral degradation, decarboxylase inhibitors are administered with L-dopa. This increases the conversion of L-dopa to OMD, and further decreases both methionine and SAM and increases tHcy *(Müller T et al., 2001)*.

Studies on animals have verified that methylation of L-dopa leads to significant decreases in brain and liver concentrations of SAM and increases of SAH *(Daly D et al., 1997)*.

Two studies in rats showed that the L-dopa-induced decrease in SAM and increase in SAH in brain and other tissues were attenuated or prevented by COMT inhibitors *(Miller JW, 1997, Yassin MS et al., 1998)*.

A recent in vitro study showed that methionine, dimethionine, and SAM all had a protective effect against L-dopa-induced neurotoxic damage. This effect was, however, completely abolished by COMT inhibition in this study *(Werner P et al., 2001)*.

Whether COMT inhibitors reduce L-dopa-induced side effects in humans remains to be determined.

Interaction with Hcy metabolism might explain many of the side effects of L-dopa, and possibly also the increased prevalence of both ischemic heart disease and cerebrovascular disease in PD.

In a recent open preliminary study, 14 depressed PD patients, resistant to, or with intolerable side effects from other antidepressants, were treated

with SAM for 10 weeks. Two patients interrupted the treatment because of increased anxiety. Ten of the remaining 11 patients showed at least 50% improvement on the 17-point Hamilton Depression Scale *(Di Rocco A et al., 2000)*.

L-dopa has been shown to upregulate both the expression of methionine adenosyl transferase (MAT) and COMT in parkinsonian patients. This induction is proposed to explain the tolerance and weaning-off effect of L-dopa *(Zhao WQ et al., 2001)*.

Lipid-lowering drugs

Niacin

The Cholesterol Lowering Atherosclerosis Study reported increased tHcy levels in patients receiving colestipol and niacin compared with placebo.

The interaction with niacin was confirmed in the Arterial Disease Multiple Intervention Trial, which was placebo-controlled. Total Hcy increased by 17% during titration from 100 mg/day to 1,000 mg/day. Eighteen weeks after randomisation the increase from baseline was 55% in the niacin group *(Garg R et al., 1999)*.

The tHcy-increasing mechanism may vary with drug. Niacin is excreted predominantly as methylated pyridones, requiring SAM as a methyl donor. Vitamin B_6 treatment has earlier been shown to normalise altered sulphur amino acid status induced by niacin in rats, without altering the hypolipidemic effect *(Basu TK et al., 1997)*. A subsequent study in rats showed that niacin-induced hyperhomocysteinemia was accompanied by a significant decrease of not only vitamin B_6, but also vitamin B_{12} *(Basu TK et al., 2002)*.

Liver damage is a common side effect of niacin. This hepatotoxicity is proposed to reflect the high demand for methyl groups imposed by niacin catabolism, which leads to a reduction in hepatic levels of SAM *(McCarty MF, 2000a)*.

Fibrates

Fenofibrate treatment significantly increased tHcy, and also methionine and cysteine levels in two studies *(De Longeril M et al., 1999, Bisonnette R et al., 2001)*, and tHcy and cysteine in another study *(Giral P et al., 2001)*.

Vitamin levels, measured in one of the studies, were not significantly affected *(Bisonnette R et al., 2001)*. Folate supplemetation, however, is shown to prevent the increase in tHcy *(Dierkes J et al., 2001, Stulc T et al., 2001)*.

In patients with chronic renal failure treated with fenofibrate, an increase in tHcy by 56% was reported, but there was no correlation with changes in serum creatinine *(Landray MJ et al., 1999)*.

A cross-over, placebo-controlled study showed that also *bezafibrate* treatment for 6 weeks significantly increased tHcy (by 19%). Serum creatinine increased by 20%, but without any change in creatinine clearance *(Jonkers et al., 1999)*.

Both 200 mg of fenofibrate and 400 mg of bezafibrate increased tHcy (by 44% and 17.5%, respectively) in 20 patients after 6 weeks of therapy in another study. Creatinine and cystatin C increased significantly in the fenofibrate group only. No significant changes were seen in folate, vitamin B_{12}, or B_6 concentrations, which were measured solely in the bezafibrate group *(Dierkes J et al., 1999a)*. However, the same authors recently reported that 650 µg of folic acid plus 5 mg of vitamin B_6 and 50 µg of vitamin B_{12} almost completely prevented the increase in tHcy in patients treated with fenofibrate or bezafibrate, without affecting the reduction in triglycerides. The study was randomised, placebo-controlled, and double-blind *(Dierkes J et al., 2001)*.

Total Hcy, creatine and cystatin C were all raised significantly by 200 mg fenofibrate daily for 6 weeks in a recent cross-over study. In contrast, 900 mg a day of *gemfibrozil*, another fibrate that does not affect renal function, did not influence these parameters *(Westphal S et al., 2001a)*.

Statins

In contrast to fibrates, statins do not seem to increase tHcy. High-dose *simvastatin* (80 mg), on the contrary, decreased tHcy in patients with hypercholesterolemia *(Lüftjohann D et al., 2001)*. Nor did *atorvastatin* increase tHcy, or any other thiol compound significantly in another study *(Giral P et al., 2001)*. A combination of simvastatin with folic acid and vitamin B_{12} has, nevertheless, been proposed in order to increase the effectiveness of simvastatin. A non significant increased tHcy-lowering effect and reduced LDL-cholesterol was observed in a pilot study of the combination *(McMahon M et al., 2000)*.

Oral contraceptives

In contrast to hormone replacement therapy, page 80, oral contraceptives (OC) may increase tHcy, as shown in the Third National Health and Nutrition Examination (NHANES) *(Morris MS et al., 2000)*.

Inconsistencies in the reported data on changes in tHcy in women taking oral contraceptives could be explained by the observation that tHcy levels vary according to the hormonal phase of the cycle. Significantly decreased tHcy is reported in the high-hormonal phase *(Steegers-Theunissen R et al., 1992)*. In this study, lower blood levels of vitamin B_6 were found in the OC users than in the control group, both in the low and high hormonal phases, but not of vitamin B_{12} or folate. The same group, however, later found that median serum folate concentration was significantly lower 210 min after oral folate loading. Also vitamin B_{12} levels were lower in OC users *(Steegers-Theunissen R et al., 1993)*.

Two other studies found highly significantly lower B_{12} levels in OC users than in controls *(Brattström L et al., 1992, Green TJ et al., 1998)*. Serum B_{12} levels were 33% lower in OC users than in controls in the study by Green and co-workers but tHcy levels did not differ significantly. Nor was any difference found in tHcy, either fasting or PML in the study by Brattström and co-workers.

Decreased serum B_{12} levels may be caused by reduced levels of binding proteins related to hormonal status. Sixty per cent of women treated with OC had reduced levels of vitamin B_{12} binding proteins in one study *(Gardyn J et al., 2000)*.

Cyclosporin

Some studies have indicated that cyclosporin may increase tHcy in renal or heart transplant patients. In a study of 72 cardiac transplant patients, both cyclosporin and creatinine levels were significantly and independently positive predictors of tHcy *(Cole DE et al., 1998)*.

Cholestasis is a common side effect of cyclosporin. A recent study in rats suggests that SAM may protect against cyclosporin A-induced alterations in the liver, which supports an interaction with Hcy metabolism *(Galan AI et al., 1999)*.

Cyclosporin treatment alone (10 mg/kg/day) or in combination with 400 mg of SAM daily was recently compared in psoriatric patients. Hepatotoxicity and cholestasis were observed in 15 of 36 patients treated

with cyclosporin alone, whereas no case of liver toxicity was observed among the 36 patients treated with the combination *(Neri S et al., 2002)*. These observations point towards a cyclosporin-induced reduction in the remethylation of Hcy.

Increase of Hcy was confirmed in a recent study of 106 renal transplant patients. By contrast, treatment with *azathioprim* seemed to be associated with lower tHcy *(Troughton JA et al., 2001)*.

In other studies of renal transplanted patients treated with cyclosporin there was a negative correlation between tHcy and folate, and treatment with folic acid has been shown to reduce tHcy *(Arnadottir M, Hultberg B, 1997, Fernandez-Miranda et al., 2000)*.

Diuretics/antihypertensives

When blood samples from 17 hypertensive patients receiving long-term diuretic therapy and 17 hypertensive patients not taking diuretics were analysed, mean tHcy was higher and mean RBC folate lower in the patients taking diuretics. There was no significant difference in vitamins B_6 and B_{12} status *(Morrow LE et al., 1999)*.

Recent data from the Framingham Offspring Study also showed a highly significant positive association between the use of antihypertensive medication and tHcy *(Jacques et al., 2001)*. The Rotterdam Study reported a 1.2 µmol/L higher tHcy in participants who took antihypertensive drugs *(Vermeer SE et al., 2002)*.

In a study of 600 hospitalized elderly subjects, hyperhomocysteinaemia was associated with the use of diuretics in 56% of cases *(Ventura P et al., 2001)*. In a recent study 27 patients were assigned to treatment with either hydrochlortiazide (HTC) or an ACE inhibitor. At baseline and after 4-6 weeks tHcy, creatinine, folate, vitamins B_6 and B_{12} were determined. HTC raised tHcy by 28%, creatinine by 12%, and decreased folate levels by 25.8%. The ACE inhibitor did not affect any of the parameters *(Westphal S et al., 2001b)*.

Two large studies have shown that *triamterene* may increase the risk not only of NTD, but also of cardiovascular defects, oral clefts, and urinary tract defects *(Hernandez Diaz S et al., 2000)*. The same group reported data from a recent American-Canadian study of 1,242 cases of NTD, which showed an increased OR for NTD related to treatment with triamterene during the first or second month after the last menstrual period versus no exposure during this period *(Hernandez Diaz S et al., 2001)*.

Drugs that increase the gastric pH

Drugs that increase the gastric pH may reduce the absorption of vitamin B_{12} by impairing the release of protein-bound vitamin B_{12}. Long-term treatment with any drug that increases gastric pH may produce vitamin B_{12} deficiency.

Decreased vitamin B_{12} absorption is well documented for the proton pump inhibitor, omeprazole. In one of these studies, absorption of protein-bound cobalamin decreased from 3.2% to 0.9% and 3.4 to 0.4% after treatment for 2 weeks with 20 mg and 40 mg, respectively, of omeprazole daily *(Marcuard SP et al., 1994)*. A recent review calls attention to the importance of the interaction with omeprazole, particularly in the elderly *(Bradford G and Taylor C, 1999)*.

Histamine-2 receptor antagonists can also fairly rapidly induce a depletion of vitamin B_{12} *(Force RW and Nahata MC, 1992, Ruscin JM et al., 2002)*.

Histamine-2 blockers or proton pump inhibitors were taken by no less than 11.7% of the population at baseline in the Canadian Study of Health and Aging. There was an independent association between the use of these drugs and initiation of vitamin B_{12} substitution at follow-up *(Mitchell SL and Rockwood K, 2001)*.

As the half-life of vitamin B_{12} is very long, depletion develops slowly. Gradually developing deficiency symptoms may therefore be interpreted as normal age-related changes, and the interaction may not be taken into account. Neurological and other symptoms related to omeprazole-induced vitamin B_{12} deficiency are, however, reported.

An interaction between omeprazole and MTX is described. This interaction can be explained by an inhibition by omeprazole of the renal elimination of hydrogen ions, thereby blocking the active renal excretion of MTX. The result may be enhanced side effects of MTX *(Beorlegui B et al., 2000)*.

An overview of proton pump interactions was recently published *(Gerson LB and Triadafilopoulos G, 2001)*.

Antibiotics

Clioquinol

Clioquinol is a hydroxyquinoline antibiotic that has been associated with severe CNS side effects such as subacute myelo-optic neuropathy. Clioquinol has been proposed to disturb vitamin B_{12} retention through chelation of Co^{2+}. A recent study in mice showed that clioquinol decreased accumulation of vitamin B_{12} in brain and blood. Moreover, a significant decrease in SAM in brain was observed *(Yassin MS et al., 2000)*.

Trimethoprim

The antimicrobial trimethoprim acts by inhibiting dihydrofolate-reductase, and thereby causes accumulation of dihydrofolate and a depletion of THF in bacteria *(Quinlivan EP et al., 2000)*. Although the bacterial enzyme is affected 50,000 times more strongly than human dihydrofolate reductase, trimethoprim has been reported to cause megaloblastosis and pancytopenia in patients treated with this drug or with *co-trimoxazole*.

It was recently shown that 300 mg of trimethoprim twice a day for 2 weeks gave rise to a median increase in tHcy of 50% in healthy males. The increase in patients with the C677T polymorphism of MTHFR was even higher, or 191% in one homozygous subject. The median in heterozygous subjects was 79%. Plasma folate concentrations decreased from 13.5 nmol/L to 10.9 nmol/L *(Smulders YM et al., 1998, Smulders YM et al., 1998)*. The authors recommend that the practice of prescribing long-term (prophylactic) trimethoprim should be reassessed in patients with a high cardiovascular risk profile, cardiovascular disease, a history of thrombosis, or who are being treated for hyperhomocysteinemia. They also call attention to the possibility of interaction with, for instance, the C677T polymorphism of MTHFR.

A recent American study of 6,932 infants with various congenital defects and 8,387 control infants showed that trimethoprim may increase the risk not only of NTD, but also of cardiovascular defects, oral clefts, and urinary tract defects *(Hernandez Diaz S et al., 2000)*.

The same group reported data from a recent American-Canadian study of 1,242 cases of NTD which showed an overall OR for NTD related to exposure to trimethoprim during the first or second month after the last menstrual period of 4.8 versus no exposure during this period *(Hernandez Diaz S et al., 2001)*.

Isoniazid

The antituberculosis drug isoniazid reacts non-enzymatically with vitamin B_6 to form a metabolically inactive hydrazone, leading to functional vitamin B_6 deficiency, which may influence the Hcy metabolism *(Standal BR et al., 1974)*.

Other drugs

Lithium

Lithium is shown to decrease vitamin B_{12} status, but the mechanism is unclear. Based on the finding of a highly significant direct association

between lithium and cobalt concentrations in scalp hair, a role of lithium in the transport and distribution of vitamin B_{12} has been proposed *(Schrauzer G et al., 1992)*.

Lithium treated patients were recently shown to have serum levels of vitamin B_{12} that were about 20% lower than patients not treated with lithium *(Cervantes P et al., 1999)*.

Folate levels may also be low in lithium-treated patients. In 107 patients on long-term lithium, those with lower plasma folate concentration had a higher affective morbidity than those with higher folate levels, both at the time and two years earlier *(Coppen A and Abou-Saleh MT, 1982)*. No correlation between serum and RBC folate was seen in another study *(Abou-Saleh MT and Coppen A, 1989)*.

A placebo-controlled study of a daily supplement of 200 µg of folic acid added to lithium therapy showed that patients with an increase of plasma folate to 13 ng/ml or above had a 40% reduction in their affective morbidity *(Coppen A et al., 1986)*.

Homocysteine levels have not been determined.

Neuroleptics

A recent finding indicates that side effects of neuroleptics might be related to the Hcy metabolism. Patients with schizophrenia, who met the criteria for tardive dyskinesia were included in a placebo-controlled trial of vitamin B_6. Mean scores of extrapyrimidal symptom rating scale were significntly better in the vitamin group in the third week of treatment *(Lerner V et al., 2001)*.

Theophylline

Theophylline is a pyridoxal kinase antagonist in the nervous system. Patients treated with theophylline showed a significantly higher increase in post methionine loading (PML) tHcy than controls in a study of asthma patients. Treatment with 20 mg of vitamin B_6 for 6 weeks reduced PML tHcy significantly in the patients but not in the controls *(Ubbink J et al., 1996)*. Vitamin B_6 supplementation is shown to reduce significantly theophylline-induced tremor *(Bartel PR et al., 1994)*.

Erythropoietin

Treatment with erythropoietin in 49 patients with renal failure and anemia resulted in a 22% decrease in vitamin B_{12} levels, and substitution was necessary in 18% of patients in one study *(Drinovec J and Varl J, 1992)*. Erythropoietin resistance, owing to vitamin B_{12} deficiency, is described *(Zachee et al., 1992)*. Resistance to erythropoietin treatment occurs in 5-

10% of anemic haemodialysis patients, although established factors of resistance have been ruled out. Increased oxidative damage of RBC membranes has been proposed as a possible cause of resistance *(Gallucci MT et al., 1999)*. This effect might be Hcy-related.

In a recent study of haemodialysed patients, tHcy was elevated in all patients, but particularly in patients treated with erythropoietin. There was a significant negative correlation between serum erythropoietin and folate levels, and between tHcy and folate levels. Treatment with 10 mg of folic acid three times a week for 6 months resulted in significantly decreased tHcy levels. In addition, 2 of 3 patients on erythropoietin therapy could discontinue this treatment with persisting adequate haemoglobin levels after folic acid supplementation *(Korzets A et al., 2000)*.

Nitroglycerin

Studies in rats have shown that Hcy-infusion attenuates the blood pressure lowering effect of different NO donors *(Fu WY et al., 1998, Fu WY et al., 2002)*. In healthy humans, continous treatment with nitroglycerin causes nitric oxide synthase (NOS) dysfunktion, probably through reduced bioavailability of tetrahydrobiopterin (BH_4), see page 46.

A recent placebo-controlled study suggests that supplementation with 10 mg/day of folic acid prevents NOS dysfunction and nitrate tolerance in patients treated with transdermal nitroglycerin *(Gori T et al., 2001)*.

Methylprednisolone

After treatment with 1,000 mg of methylprednisolone daily in 21 patients with multiple sclerosis, a decrease of folate and vitamin B_{12} in CSF, and of serum folate was observed. The reduction of serum B_{12} was not significant. After treatment, however, all median levels of vitamin B_{12} and folate were below the reference medians *(Frequin ST et al., 1993)*.

Aspirin

Aspirin has been suggested to alter the transport of folate by competition for binding sites on serum proteins *(Alonso-Aperte E, Varela-Moreiras G, 2000)*.

Fluoxetine

Fluoxetine was recently shown to inhibit the absorption of 5-methyltetrahydrofolate in an in vitro study of rat jejunum *(Amilburu A et al., 2001)*. (Supplementation with folic acid has been shown to ameliorate the clinical response to fluoxetine, page 129).

Neuropsychiatric Conditions associated with Hyperhomocysteinemia

Numerous studies indicate that an association exists not only between vascular disease and tHcy levels, but also with Alzheimer´s dementia, depression and other neuropsychiatric disorders.

Low vitamin B_{12} and folate levels are reported in many studies of patients with cognitive disorders, and depression. In 1980 an important observation by Shorvon and coworkers was published on the neuropsychiatric complications associated with with megaloblastic anemia due to folate or vitamin B_{12} deficiency, table 4. No less than 56% of patients with affective disorders had folate deficiency, whereas peripheral neuropathy was

Neuropsychiatric findings in patients with megaloblastic anemia.

	Vitamin B_{12} deficiency (n = 50)	Folate deficiency (n = 34)
Normal	16 (32%)	12 (35%)
Organic mental change	13 (26%)	9 (27%)
Affective disorder	10 (20%)	19 (56%)
Subacute combined degeneration of the cord	8 (16%)	
Peripheral neuropathy	20 (40%)	6 (18%)
Optic atrophy	1 (2%)	

Table 4 – Summary of neuropsychiatric findings in 50 patients with vitamin B_{12} deficiency and 34 patients with folate deficiency. *Shorvon SD et al. The neuropsychiatry of megaloblastic anaemia. © Br Med J, 1980; 281:1036-8. Reprinted with permission.*

the commonest finding among B_{12}-deficient patients. Organic mental changes were just as common in both groups. Approximately one third of patients had no neuropsychiatric symptoms.

Over 20 surveys concerning the folate status in patients with psychiatric disorders have shown that as many as one third of psychiatric patient cohorts exhibited low or deficient folate levels in serum or RBC *(Alpert JE et al., 2000)*.

In 1988 Lindenbaum and co-workers published a study of patients with low vitamin B_{12} levels and various neuropsychiatric disorders, but without anemia. Out of 37 patients 36 had tHcy 3 SD above normal *(Lindenbaum J et al., 1988)*. The group subsequently reviewed the records of all patients with low serum B_{12} seen at 2 hospitals between 1968 and 1985. Neuropsychiatric symptoms that were reversible after vitamin B_{12} treatment were varied: paresthesias, leg weakness, ataxia, diminished visual acuity, memory loss, paranoid psychosis etc. *(Healton EB et al., 1991)*.

These studies stimulated further clinical research on the association between hyperhomocysteinemia and neuropsychiatric complications. Elevated levels of tHcy in plasma/serum or CSF have since been found in patients with dementia, depression, schizophrenia, Parkinson's disease, epilepsy, multiple sclerosis, fibromyalgia and chronic fatigue syndrome. Recent studies show a positive correlation between the severity of the symptoms and the levels of tHcy, page 120. There is also an extensive literature on the association between tHcy and cerebrovascular disease.

Low levels of SAM in CSF have been found in some of these disorders, table 1, page 49, implicating a role for methylation in CNS disorders.

Cognitive impairment and dementia

Vascular impact

Vascular dementia (VAD) is considered the second most common type of dementia in the elderly after Alzheimer´s disease (AD). It is estimated to account for about 15% of dementia cases *(Miller JW, 1999)*.

Subcortical vascular encephalopathy (SVE) is characterised by stepwise progressive memory deficits and cognitive decline, typical gait disorders, and incontinence. Sclerosis of small cerebral arteries and arterioles with diffuse periventricular white matter abnormalities and central lacunar lesions are seen. In a recent study such patients exhibited surprisingly high concentrations of tHcy compared to controls and even to patients with cerebral macroangiopathy. This indicates that Hcy may cause injury to small

cerebral arteries and arterioles rather than larger arteries. In addition, 63% of the SVE patients had lacunar infarctions (LI). In this study reduced levels of vitamin B_{12} and B_6 was commonly present, and the levels of tHcy correlated with the folate and vitamins B_6 levels *(Fassbender K et al., 1999)*.

A recent study of different subtypes of cerebral infarctions showed that the risk of LI was increased in the third compared to the first tertile of tHcy distribution (OR = 3.4). The risk of atherothrombotic infarctions was increased even in the second tertile (OR = 5.0), and even more in the third (OR = 7.5) *(Shimizu H et al., 2002)*.

Another recent study of 82 patients with SVE showed that these patients, in contrast to patients with cerebral large-vessel disease (n = 144), exhibited pathologically increased tHcy levels, and also lower vitamin B_6 concentrations, in comparison with controls without cerebrovascular disease *(Bertsch T et al., 2001)*.

Although the pathogenesis of AD is is multifactorial, fig. 13, AD is also often associated with atherosclerosis, cerebral microvascular abnormalities, and high blood pressure *(Launer LJ et al., 1995)*. In recent years, evidence

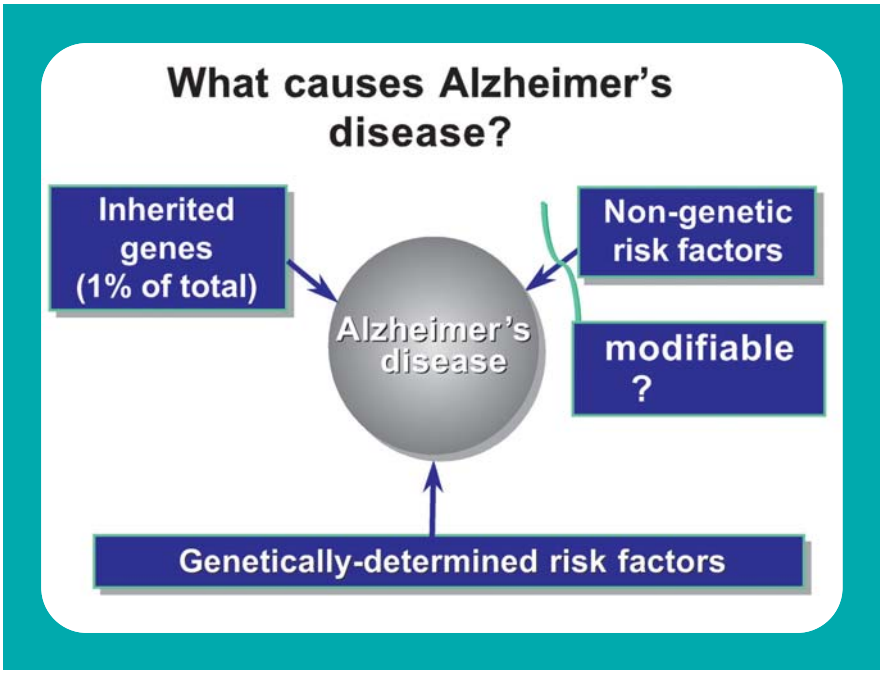

Figure 13 – Interactions in the pathogenesis of Alzheimer´s disease. *© 2002 Smith AD. Reprinted with permission.*

that vascular disease and AD may in fact be closely linked is increasing, and elevated tHcy is associated with both conditions.

Cerebrovascular disease increases the clinical symptoms of AD and there are several common risk factors. A total of 1,449 partipants in a Finnish study, aged 65-79, were re-examined after an average of 21 years of follow-up. It was found that participants with raised systolic blood pressure (≥ 160 mm Hg), or elevated serum cholesterol (≥ 6.5 mmol/L) in midlife had a significantly increased risk of AD in later life, even after adjusting for other risk factors *(Kivipelto M et al., 2001)*.

Vascular disease may contribute to the pathogenesis of AD by lowering the threshold for overt cognitive impairment and dementia caused by pathophysiological mechanisms specific to AD. When 101 patients complaining about cognitive disturbances were investigated with MMSE, laboratory investigation, brain imaging, and electroencephalography, 33% of the patients with subjective memory complaints, 45% of patients with dementia of Alzheimer type (DAT), and 62% of the patients with VAD had hyperhomocysteinemia (≥ 15 µmol/L). The expected assumption that hyperhomocysteinemia should be more prominent in VAD, and age, tau protein levels, and apoE4 alleles more prominent in DAT was not confirmed, as hyperhomocysteinemia was common in both groups, and VAD and DAT did overlap considerably *(Gottfries J et al., 2001)*.

Another recent Swedish study involving patients with early (EOAD) and late (LOAD) onset AD showed that there was no difference in tHcy or its determinants between the EOAD group and age- and sex-matched controls. In contrast, patients with VAD or mixed AD/VAD showed increased tHcy. Total Hcy was also elevated in patients with LOAD and a history of CVD compared with both AD patients without a history of CVD and with controls. These findings suggest that elevated tHcy contributes to dementia mainly through vascular mechanisms *(Nilsson K et al., 2002)*.

The relation between vascular risk factors and risk indicators of AD was recently reviewed in three publications *(Breteler MMB, 2000, Miller JW, 2000)*. A recent, extensive review on Hcy-related vascular damage offers over 250 references *(Durand P et al., 2001)*.

Association with vitamin status

Numerous studies have reported low serum and/or CSF levels of vitamin B_{12} in demented patients *(Van Tiggelen CJM, 1983, Karnaze D et al., 1987, Renvall MJ et al., 1989, Ikeda T et al., 1990, Nijst TQ et al., 1990, Regland B et al., 1990, Kristensen M et al., 1993, Clarke R et al., 1998b, Selley ML et al., 2002)*.

Frequent findings of low serum folate levels in patients with dementia are also reported in the literature *(Kristensen M et al., 1993, Ebly EM et al., 1998, Clarke R et al., 1998b, Nilsson K et al., 1999, Nilsson K et al., 2000b, Snowdon DA et al., 2000, Bottiglieri T et al., 2001, Selley ML et al., 2002)*. In fact, folate deficiency may be more prevalent than vitamin B_{12} deficiency in patients with cognitive impairment, although often overlooked.

Sometimes, isolated low RBC folate has been found *(Renvall MJ et al., 1989)*, possibly because of concomitant vitamin B_{12} deficiency as vitamin B_{12} status may influence folate distribution (and vice versa). A highly significant positive correlation between B_{12} status and RBC folate has been demonstrated *(Kristensen M et al., 1993)*.

Low serum folate was also strongly associated with postmortem athrophy in the cerebral cortex in the NUN Study *(Snowdon DA et al., 2000)*.

In the Canadian Study of Health and Aging, in which serum folate was determined in 1,171 elderly subjects, those with low folate levels were more likely to be demented *(Ebly EM et al., 1998)*.

The American National Health and Nutrition Examination Survey (NHANES) revealed significantly better results in memory tests among participants with serum folate in the upper 50th percentile compared with subjects with lower levels *(Morris MS et al., 2001b)* .

A study of 93 elderly male geriatric inpatients showed that 65.6% had low serum and/or RBC folate levels, and that patients with deficient folate status had impaired test results in the "Short Mental Status Questionnaire" *(Franchi F et al., 2001)*.

A correlation between folate status and measures of cognitive function was also recently found in a randomly selected sample of Hispanic subjects 65 years and older *(Lindeman RD et al., 2000)*.

When the associations between cognitive performance and plasma vitamin B_{12}, folate, and tHcy were tested in two cohorts of elderly subjects in the Scottish Mental Surveys of 1932 and 1947, using a range of cognitive tests, tHcy was found to be significantly higher in the older cohort. There were positive correlations between vitamin status and cognitive performance, and a negative correlation with tHcy *(Duthie SJ et al., 2002)*.

Prospective studies have indicated that low vitamin B_{12} and/or folate status increases the risk of dementia. A Swedish study, in which 370 nondemented elderly subjects (above 70 years) were followed for 3 years, showed that low baseline levels of folate and/or vitamin B_{12} were correlated with increased risk of developing dementia. Subjects with serum levels of folate of 10 nmol/L or lower *or* vitamin B_{12} levels of 150 pmol/L or

lower had a doubled risk of developing Alzheimer´s disease, diagnosed according to DSM III criteria *(Wang HX et al., 2001)*.

Data from the Canadian Study of Health and Aging, a population-based, prospective 5-year investigation of the epidemiology of dementia among 369 Canadians aged 65 years or more, has shown that there is an increased risk of vascular disease and cognitive impairment associated with low folate status. Female subjects in the lowest folate quartile at baseline had a higher risk of an adverse cerebrovascular event with an OR of 4.02 compared with subjects in the highest quartile. For male subjects the difference was not significant. In the total sample, there was a consistent trend toward poorer health and cognitive outcomes during follow-up (including mortality, cognitive decline and dementia) among those in the lowest folate quartile compared with the highest quartile *(Maxwell CJ et al., 2002)*.

Disturbed absorption and distribution of vitamins

Lack of evidence for vitamin B_{12} deficiency being related to nutrition in patients with dementia, implies that deficiency may be caused by a disorder of absorption, metabolism, or transport *(McCaddon A et al., 2000, McCaddon A et al., 2002b)*.

Malabsorption, discussed elsewhere, pages 74 and 82, is important, but it becomes increasingly evident that the transport of vitamin B_{12} to the CNS is also often disturbed.

Both vitamin B_{12} and folate are transported actively into cells and across the blood-brain-barrier. Isolated low CSF levels of vitamin B_{12}, in particular, but also folate in elderly demented patients have been reported *(Van Tiggelen CJM, 1983, Ikeda T et al., 1990, Nijst TQ et al., 1990, Regland B et al., 1992)*.

The CSF/serum ratio may vary considerably for vitamin B_{12}. Isolated low B_{12} levels in CSF may be explained by inactive B_{12} analogues interfering with the transport. A lower ratio of active vitamin B_{12}/inactive analogues has been found in neurological and demented patients *(Carmel R et al., 1988c, Regland B et al., 1990, McCaddon A et al., 2001a)*.

Lower serum levels of active vitamin B_{12} may explain lower saturation of the transport protein transcobalamin (TC) that binds active vitamin B_{12} with high specificity. One study showed that mean levels of holoTC were 52% lower in AD patients than in healthy young controls. In elderly controls holoTC levels were 47% lower than in the younger controls *(Johnston CS et al., 1997)*.

McCaddon and co-workers recently determined total vitamin B_{12} levels, holohaptocorrin, apohaptocorrin, and holoTC in serum in 23 patients with

DSM-IV criteria for primary degenerative dementia and in 18 cognitively intact age-matched control subjects. The patients had lower levels of active vitamin B_{12} than controls and a higher proportion of inactive analogues. The interrelationship between age, B_{12} analogues, and holoTC polarised patients into two distinct groups, which suggests that two different mechanisms cause cerebral vitamin B_{12} deficiency *(McCaddon A et al., 2001a)*.

A second study by McCaddon *et al.* confirmed these results. There was no difference between mean total vitamin B_{12} levels in 51 AD patients and 60 healthy age-matched controls. However, patients had a highly significantly lower holoTC *(McCaddon A et al., 2002a)*.

The recent case-control study by the OPTIMA group (Oxford Project To Investigate Memory & Aging) of 51 patients with pathologically confirmed AD and 65 controls showed no differences for geometric mean serum B_{12} levels between cases and controls. In contrast, holoTC II levels were highly significantly lower, and tHcy higher in cases than in controls, fig.14 *(Smith AD et al., 2001)*.

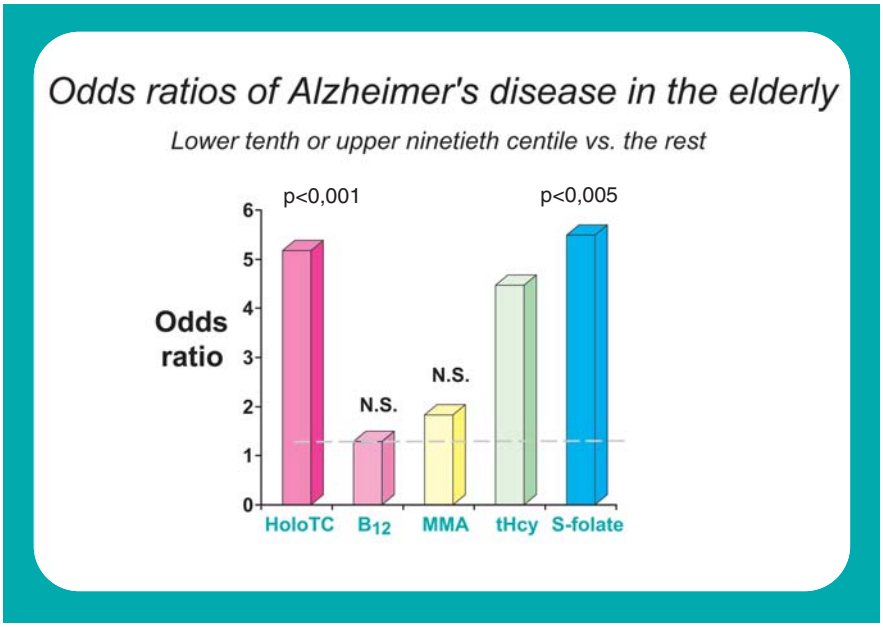

Figure 14 – Serum holotranscobalamin, tHcy, MMA, and serum vitamin B_{12} and folate in 51 patients with confirmed Alzheimer´s disease. OPTIMA (Oxford Project To Investigate Memory & Aging) *Smith AD et al. Serum holotranscobalamin and other markers of vitamin B_{12} status in confirmed Alzheimer's disease. Homocysteine Metabolism, 3rd International Conference 1-5 July 2001. Abstract 174. Reprinted with permission.*

These observations raise the question whether current radioassays are specific enough. A study in 1978 showed that in 10 commercial serum B_{12} assay kits, 51-85% of the vitamin B_{12} -binding protein was R protein, which is less specific than TC, and also binds analogues *(Kolhouse JF et al., 1978)*. Recently, a commercially available holoTC test has been introduced to European and North American markets. Evidence to date suggests that holoTC may be an early marker of negative vitamin B_{12} status *(Carmel R et al., 1988c, Herzlich BC et al., 1988, Herbert V et al., 1990, Johnston CS et al., 1997, Smith AD et al., 2001)*.

Folate is transported across the blood-brain-barrier in the form of methyltetrahydrofolate by an active transport system *(Spector R and Lorenzo A, 1975)*. The active transport into the CNS is taking place at the choroid plexus. The levels of CSF folate are typically 3-4 times higher than in serum *(Abalan F et al., 1996, Selley ML et al., 2002)*. A close relationship between serum level and CSF folate level that is maintained in the presence of folate deficiency was reported by Reynolds and co-workers *(Reynolds EH et al., 1972)*. Other investigators have reported a wide range of CSF/serum ratios in psychiatric patients *(Källström B and Nylöf R, 1969)*. Lower CSF/serum ratio have been reported in demented patients and in patients with multiple sclerosis *(Nijst TQ et al., 1990, Serot JM et al., 2001)*.

Highly significantly lower CSF levels of folate in 30 late onset AD patients compared to healthy controls 20-60 years old were found in one study *(Serot JM et al., 2001)*. Isolated low levels of CSF folate are suggestive of a specific alteration of transport. Distribution may also be disturbed by low protein binding capacity in the CSF *(Wevers RA et al., 1994)*, or inborn errors in the folate transport.

A distribution disturbance was also indicated by the findings of highly significantly increased tHcy and MMA levels, indicating tissue deficiency, in demented and non-demented hospitalised elderly subjects compared with healthy elderly subjects living at home, although neither serum folate nor B_{12} levels differed significantly *(Joosten E et al., 1997)*.

Tissue deficiency was also the proposed explanation of elevated concentrations of tHcy combined with normal serum levels of the vitamins in a study in which elevated tHcy levels were found in 88 out of 168 psychogeriatric patients. MMA levels were increased only in 29 patients. There was, however, a significant negative relation between tHcy and whole blood folate concentration *(Nilsson K et al., 1999)*.

A recent publication reviews available studies on vitamin B_{12} and folate status in relation to cognitive impairment and dementia *(Wang HX et al.,*

2002). Folate absorption and transport are discussed in two other reviews *(Brzezinska A et al., 2000, Gregory JF et al., 2001)*.

Homocysteine and MMA status

Elevated tHcy levels in plasma of patients with cognitive impairment were first reported in studies by Regland and co-workers in 1990 and Bell and co-workers in 1992. Several reports have since followed in which tHcy has been measured in addition to vitamin levels. Many of these studies are outlined in a recent review *(Selhub J et al., 2000)*.

Studies have shown that tHcy, the marker for the functional interaction between vitamin B_{12} and folate, shows a better correlation with cognitive impairment than MMA, the more specific marker for vitamin B_{12} deficiency *(Hultberg B et al., 1999b, Nilsson K et al., 1999, Nilsson K et al., 2000b, Hvas AM et al., 2001)*. These findings underscore the importance of the interaction between the two vitamins.

Hvas and co-workers found no association between MMA levels and total symptom score, total Neurological Disability score, or to the clinical manifestations of vitamin B_{12} deficiency. In this follow-up study, 432 individuals, not treated with vitamin B_{12} were examined 1-3.9 years after an initial observation of increased MMA levels *(Hvas AM et al., 2001)*.

In a study of patients with dementia and with other psychiatric disorders, the tHcy concentrations in plasma were in contrast highly significantly elevated in both patient groups in comparison with healthy controls, table 3, page 77. The patients, who had either dementia of vascular origin or a history of occlusive arterial disease, had highly significantly increased tHcy levels compared with patients without a history of vascular disease *(Nilsson K et al., 1996)*.

Blood tHcy was examined in a British study of 30 patients aged 65 years and above, who were recruited from a psychogeriatric assessment centre. All had a diagnosis compatible with the criteria for primary degenerative dementia of Alzheimer´s type. The tHcy levels were highly significantly elevated in comparison with the values in age-matched controls *(McCaddon A et al., 1998)*. Another British case-control trial within the OPTIMA project involved 164 patients with dementia. Total Hcy was higher, and both serum folate and vitamin B_{12} lower, both in the patients with dementia of Alzheimer´s type and the patients with histopathologically confirmed AD than in controls. The association with tHcy was independent of age, sex, social class, smoking, and the apoEε4 genotype *(Clarke R et al., 1998b)*.

Recently presented results from the same project confirm these associations. In a cohort of 156 community-dwelling elderly (60-90 years), tHcy were associated with lower total CAMCOG (Cambridge cognitive examination) scores.

Total Hcy, age, systolic blood pressure, and CAMCOG performance were interrelated *(Budge et al., 2002)*. There was also a significant negative association between minimal hippocampal width and tHcy in these subjects *(Williams et al., 2002)*. This group has also reported on an association between tHcy and cerebral infarction, white matter changes, and degree of athrophy. The association with white matter hypoattenuation (WMH) was calculated on the basis of 137 probable and definite AD (104 confirmed post-mortem) patients, 38 cases of other types of dementia and 279 controls, from whom a CT scan and tHcy were obtained. WMH was associated with age, dementia severity, cerebral infarcts and systolic hypertension. Total Hcy was strongly associated with severity of WMH *(Budge et al., 2000 and 2001, Hogervorst et al., 2002)*.

There was also a significant association between tHcy and cerebral infarction, white matter changes, and degree of atrophy *(Budge M et al., 2000, Budge M et al., 2001, Hogervorst E et al., 2002)*.

A recent Australian study showed a positive relationship between tHcy and lateral ventricle/brain ratios in the anterior and middle ventricular regions in 36 elderly nondemented subjects *(Sachdev PS et al., 2002)*.

No significant association between tHcy and cognitive impairment Mini Mental State Evaluation (MMSE) score either on inclusion or at follow-up was initially found in a sample of 630 subjects, 55 years and older, participants of the cross-sectional, population-based Rotterdam Study *(Kalmijn S et al., 1999)*. Recently presented data from this study, however, showed that increasing tHcy levels were associated with lower scores for global cognitive function, psychomotor speed and memory function. The effect on adverse cognitive performance was largely due to an association with tHcy levels in the upper quintile (more than 13.9 µmol/L). High tHcy was thus associated with adverse cognitive performance in non-demented elderly persons. The effect was most marked for psychomotor speed, but also present for memory function *(Prins ND et al., 2001)*.

Other recent data from the Rotterdam Study show that higher tHcy levels are associated with higher prevalence of silent brain infarction (SBI) and subcortical white matter lesions. The report is based on data from 1,077 people aged 60-90 years *(Vermeer SE et al., 2002)*.

In a Japanese study of 153 elderly aged 66 or older, 15 subjects with silent brain infarction (SBI) had highly significantly increased tHcy compared to subjects without SBI *(Matsui T et al., 2001).*

Finally, recently presented data from the prospective Framingham Offspring Cohort Study of 1,304 at baseline healthy middle-aged subjects, showed that tHcy in the highest baseline age-adjusted quartile (> 10.5 µmol/L and > 11.9 µmol/L for 50-60 years old women and men respectively) was associated with a 60 % increased prevalence of SBI and 41 % increased risk of lower total brain volume compared to subjects with lower tHcy *(Seshadri et al., 2003)*.

Predictive value of homocysteine levels

Four recent studies have shown that elevated tHcy is an independent risk factor for the later development of dementia, or a more rapid evolution of the disease, just as low folate or vitamin B_{12} levels were predictive in two studies *(Wang HX et al., 2001, Maxwell CJ et al., 2002)*.

A follow-up within the OPTIMA project showed that patients with higher tHcy at presentation showed a more rapid progression of their disease over a 3-year period. Moreover brain imaging revealed a decreased temporal medial lobe thickness, fig. 15 *(Clarke R et al., 1998b)*.

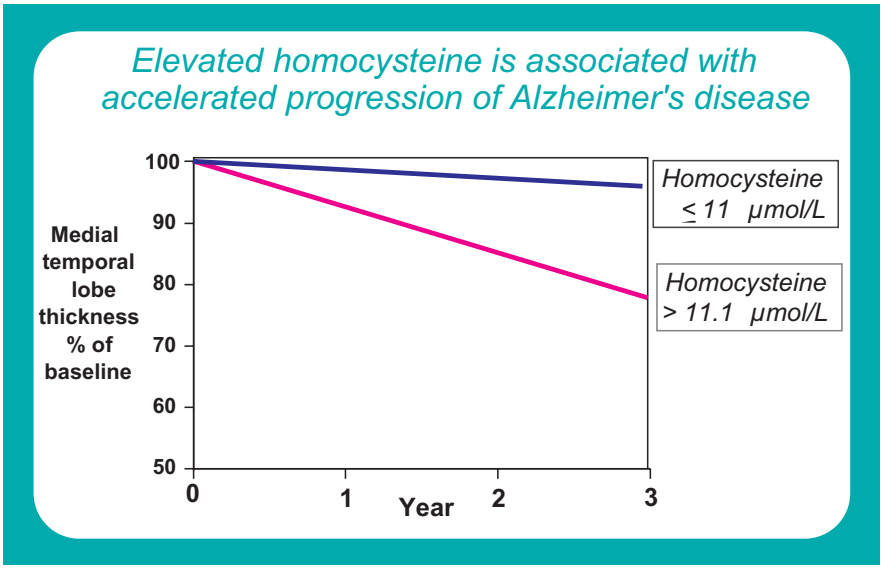

Figure 15 – Radiological evidence of disease progression, as assessed by changes in minimum medial temporal lobe thickness over a 3-year period in demented patients of Alzheimer´s type with tHcy in the lower tertile, or ≤ 11 mmol/L (n = 15) and tHcy in the upper tertiles, or > 11 mmol/L (n = 28). *OPTIMA (Oxford Project To Investigate Memory & Aging) Based on data published in: Clarke R et al. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. Clin Chemistry, 1998a; 44:1:102-07. Reprinted with permission.*

Another British study of 32 healthy elderly subjects also showed that baseline tHcy highly significantly predicted cognitive scores and rate of decline in cognitive performance at follow-up 5 years later. The association was independent of age, gender, education, renal function, vitamin B status, smoking and hypertension *(McCaddon A et al., 2001b)*.

A follow-up of a community-based sample of 98 Dutch elderly for 3 years showed that the risk of impaired cognitive function (a decline of 3 or more points of the MMSE score) was almost 4-fold for subjects with baseline tHcy within the highest quartile in comparison with subjects with values in the lowest quartile *(Van Goor LP et al., 2001)*.

Finally, samples from the members of the community-based Framingham Study (1,092 subjects with a mean age of 76 years) who were all free of dementia at baseline, were followed-up. Over a median biennial follow-up of eight years, dementia developed in 111 subjects, including 83 given the diagnosis of AD. Total Hcy greater than 14 µmol/L nearly doubled the risk of developing AD. The multivariable-adjusted OR of dementia was 1.4 for each increase of 1 SD in the log-transformed tHcy value either at baseline or 8 years earlier. For AD the OR was 1.8 per increase of 1 SD at baseline, and 1.6 per increase of 1 SD 8 years earlier, fig. 16 *(Seshadri S et al., 2002)*.

Association between degree of cognitive function and homocysteine levels

The correlation between tHcy levels and degree of cognitive dysfunction was first studied in 70 men aged 54-81. There was a highly significant association between poor spatial skills and high tHcy levels. When folate and vitamin B_{12} were entered into the regression analysis as co-variates, it became apparent that the tHcy levels made a unique contribution to the result *(Riggs K et al., 1996)*.

Data from the OPTIMA project confirm these associations. Among the 155 community-dwelling elderly, tHcy levels were associated with lower total CAMCOG scores, fig. 17 *(Budge M et al., 2000)*.

Total Hcy levels also inversely correlated with cognitive performance in a Swedish study of 336 consecutive patients attending a university-affiliated memory unit. Of patients with mild cognitive impairment no fewer than 39% had increased tHcy levels *(Lehmann M et al., 1999)*.

Another Swedish study of 147 octogenarians showed that tHcy levels above 15 µmol/L were significantly associated with decreased total life satisfaction, mood, zest for life, and with low scores for reasoning, spatial ability, and memory. Homocysteine concentrations were also significantly associated with feeling depressed, feeling cold, restlessness, impaired concentration, and loss of weight *(Jensen E et al., 1998)*.

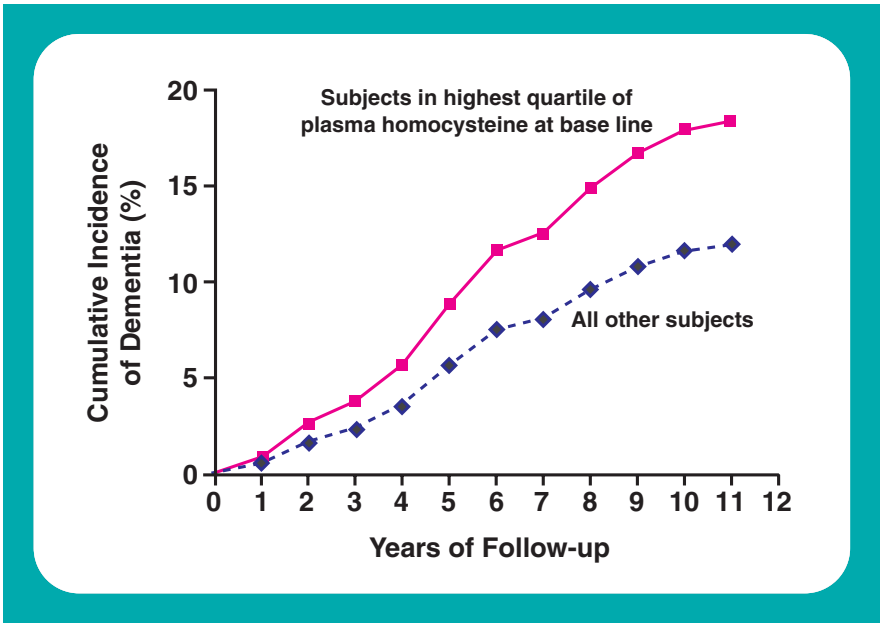

Figure 16 – Crude cumulative incidence of dementia among subjects with baseline plasma homocysteine levels in the highest age-specific quartile and among all other subjects. The 75th percentile of the plasma homocysteine level (the cut-off point for the quartile 4) was 13.2 µmol/L for subjects 65-69 years old, 13.8 µmol/L for subjects 70-74 years old, 14.5 µmol/L for subjects 74-79 years old, 16.5 µmol/L for subjects 80-84 years old, 19.3 µmol/L for subjects 85-89 years old, and 26.6 µmol/L for subjects 90-95 years old. *Seshadri S et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med, 2002; 3446:476-83. © 2002 Massachusets Medical Society. All rights reserved. Reprinted with permission.*

A third recent Swedish study showed that tHcy significantly correlated with the severity of dementia in 80 psychogeriatric patients: the Katz ADL index, the Berger scale, and the score of symptoms. Total Hcy was the only significant predictor of severity of dementia *(Nilsson K et al., 2000b)*.

In a Dutch study of 183 subjects aged 85 and above, living at home, and with MMSE scores lower than 25 were compared with sex and age-matched subjects with higher scores. The mean tHcy concentration was highly significantly increased in the group with low MMSE scores *(Van Goor LP et al., 1998)*. This group also recently presented data on 316 subjects aged 85 and above. Total Hcy levels were highly significantly raised in subjects with mental impairment. Subjects with tHcy in the highest quartile of the cohort had a an OR of 3.2 for cognitive impairment compared to subjects with values in the lowest quartile *(Van Goor LP et al., 2001)*.

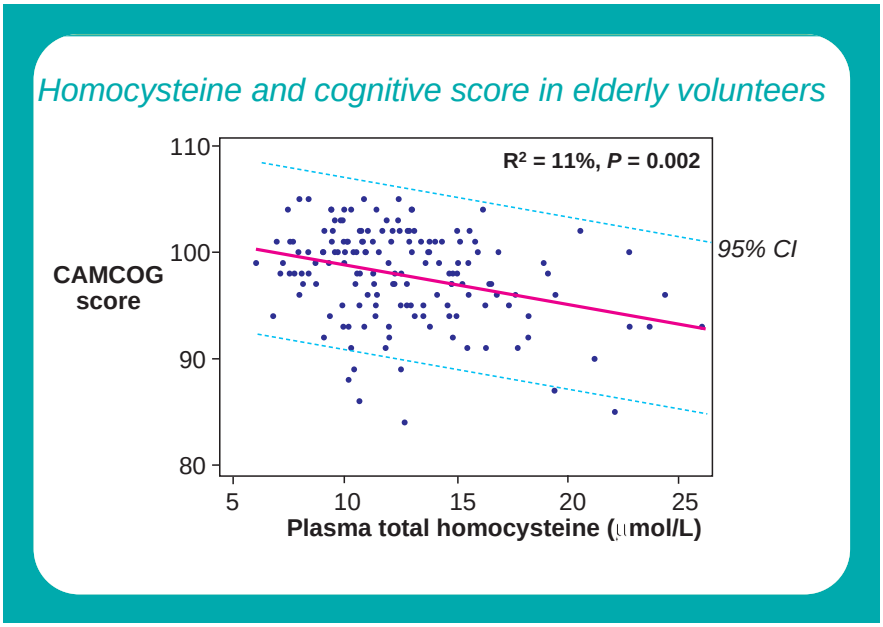

Figure 17 – Relationship between total plasma homocysteine level and the Cambridge cognitive examination (CAMCOG) score. The results are for 156 elderly community volunteers, none of whom had CAMCOG score below the cut-off for dementia of 79. The correlation p=0.002, r^2= 0.11 has not been corrected for the influence of other confounders. C.I., predicted interval for simple observations. OPTIMA (Oxford Project To Investigate Memory & Aging) *Budge M et al. Plasma total homocysteine and cognitive performance in a volunteer elderly population. Ann NY Acad Sci, 2000; 903: 407-410. Reprinted with permission.*

A significant inverse correlation between the degree of cognitive impairment, determined by MMSE, and tHcy was also found in a study of 19 patients with AD and 12 with vascular dementia *(Leblhuber F et al., 2000)*.

Finally, in the Third National Health and Nutrition Examination Survey (NHANES), both hyperhomocysteinemia and low folate status were recently reported to be associated with poor recall. Total Hcy concentrations were available for 2,243 participants aged 60 years and older (mean age 70.1 years). Folate, vitamins A, C, E, and B_{12} were determined. Paragraph delayed-recall test scores were available for 2,059. Of the subjects with low folate status, recall was poorer in those with tHcy above the 80th percentile of the distribution (13.7 μmol/L) than in those with lower concentrations. Subjects with folate levels in the upper half of the folate distribution recalled, on average, more than 4 of 6 story ideas, which

was highly significantly more than subjects with lower folate status *(Morris MS et al., 2001b)*.

These studies thus show that elevated tHcy is *more prevalent in demented patients*, and in persons with impaired cognitive performance. Levels of tHcy are also *correlated with severity of cognitive impaiement* and, most important, higher tHcy levels, even within the normal range, *confer an increased risk to develop dementia,* (as do low levels of folate and vitamin B_{12}).

A review on psychological tests, neuro-imaging, and laboratory tests, such as determination of tHcy, folate, and vitamin B_{12}, for an early diagnosis of cognitive impairment was recently published *(Gottfries CG et al., 1998)*.

Vitamin treatment in cognitive impairment

Reversible dementia is described after vitamin treatment, but treatment results are generally deceptive when cognitive impairment is severe. The reason may be that severe cerebrovascular and neruronal damage is not likely to be reversible, even if they are a consequence of deficiency of vitamin B_{12} and/or folate.

Regional cerebral blood flow has been shown to increase after vitamin B_{12} treatment in deficient patients with cognitive impairment *(Fäldt R et al., 1988, Nilsson K et al., 2000a)*. The increase was paralleled by clinical improvement *(Nilsson K et al., 2000a)*.

Experimental studies on animals have demonstrated remyelination after vitamin B_{12} and/or folate treatment. Nerve regeneration requires both the presence of SAM and of polyamines, hence both folate and vitamin B_{12} are required. Neuronal damage may regress to a certain extent, but early intervention could be expected to yield better result.

Disturbed neurotransmitter metabolism, page 50, may also be influenced by vitamin therapy. Moreover oxidative damage may be reduced as a consequence of lower Hcy-levels and increased glutathione synthesis.

Atheriosclerotic Hcy-induced lesions may not regress upon treatment but progression might be reduced *(Peterson J and Spence J, 1998, Hackham DG et al., 2000)*.

Treatment with vitamin B_{12} and/or folate has also been shown to ameliorate both cognitive and neurological disturbances in deficient patients *(Meadows ME et al., 1994, Dror Y et la., 1996, Schlichtiger U et al., 1996, Wu T and Chu N, 1996, Fioravanti M et al., 1997, Walstra GJM et al., 1997, Nilsson K et al., 2000a, Nilsson K et al., 2001, Van Asselt D et al., 2001)*. However, the

outcome is dependent both on early diagnosis and intensive treatment *(Martin DC et al., 1992, Nilsson K et al., 2000a)*.

Two open studies indicate that vitamin treatment may be effective in earlier stages of cognitve impairment. Mildly to moderately demented patients were treated with oral cyanocobalamin 1 mg and 5 mg of folic acid daily for two months. The patients were divided in two groups according to tHcy levels. Seventeen (out of 28) had tHcy of ≥20 µmol/l. Fourteen of these were classified as globally improved. These patients also improved their MMSE test scores *(Nilsson K et al., 2001)*.

The same group also treated 24 demented patients with low serum B_{12} levels with 1 mg of hydroxocobalamin intramuscularly every two days, 10 times, and thereafter once a month. The mean tHcy levels at baseline were 32 µmol/L. An increased general and focal regional cerebral blood flow was documented in 15 of the patients with mild to moderate dementia. At the same time, parameters of clinical improvement were demonstrated (MMSE and the Organic Brain Syndrome Scale) in these patients. In contrast, 9 patients who were severely demented showed no obvious clinical improvement, and no general blood flow change, although some regional flow increases were seen in sensory motor areas. The authors concluded that symptoms, such as clouding of consciouness, disorientation, and clinical fluctuation, responded to treatment, whereas the underlying condition remained essentially unchanged.

Mean baseline tHcy was 34.5 µmol/L in patients who responded to treatment and levels decreased to 17.3 µmol/L after treatment. Non-responders had initial levels of 26.5 µmol/L, and levels decreased less during treatment, or to 18.9 µmol/L. As MMA levels were normalised in both groups this may indicate concomitant folate deficiency in non responders *(Nilsson K et al., 2000a)*.

In a recent single-blind, placebo-controlled study, 16 healthy community-dwelling elderly subjects with low plasma B_{12} concentrations and no clinical cognitive impairment were treated with placebo for one month, followed by 5 months of intramuscular injections with hydroxycobalamin (1 mg weekly for 4 weeks, then 1 mg a month for 4 months).

Treatment increased vitamin B_{12} plasma levels, and decreased tHcy and MMA. The performance on the Verbal Word learning Test, Verbal Fluency and Similarities improved. Quantitative EEG showed increased fast, and decreased slow activity. Lower tHcy was related to increased EEG fast activity, and improved performance on the Verbal Word Learning Test and Similarities, and there was an association between changes on EEG and the changes in test scores *(Van Asselt D et al., 2001)*.

A positive effect on some measures of memory was seen after oral treatment with 750 µg of folic acid, 15 µg of cyanocobalamin, and 75 mg of vitamin B_6 for 35 days compared to placebo in a study of about 200 women of various ages. No effect on mood was seen *(Bryan J et al., 2002).*

No effect was observed on neurophysiological tests in a recent controlled intervention study, in which food enriched with physiological amounts of vitamins was given to frail elderly subjects for 17 weeks, although tHcy and MMA decreased *(de Jong N et al., 2001)*.

Early discovery of low vitamin status thus seems essential, and primary prophylactic vitamin substitution should be considered.

Treatment with SAM is also shown to improve cognitive function in patients with AD *(Reynolds EH et al., 1989)*. Significant amelioration of cognition and vigilance in elderly patients with primary or secondary organic brain syndrome is also reported. The Mini Mental State Evalution (MMSE) and Sandoz Clinical Assessment Geriatric Scale (SCAG) scores improved significantly after 60 days of treatment with SAM *(Fontanari D et al., 1994)*.

Vitamin B_6 has been found to have a positive effect on memory, attention and depression scores *(Tolonen M et al., 1988, Deijen JB et al., 1992, (Dror Y et al., 1996)*.

Antioxidants may have an additive effect. Five prospective trials have shown that high consumption of vitamin C and/or vitamin E was associated with a lower incidence of severe cognitive impairment, vascular dementia or AD *(Morris MC et al., 1998, Paleologos M et al., 1998, Masaki KH et al., 2000, Engelhart MJ et al., 2002, Morris MC et al., 2002)*.

The ongoing intervention studies with Hcy-lowering treatment may hopefully demonstrate a preventive effect on cognitive impairment. The Trials of Prevention of Cognitive Decline in Women include 3,445 healthy women receiving a multivitamin containing folic acid, vitamins B_6 and B_{12} or placebo. Cognitive function will be assessed at 2-year intervals.

The economic cost of dementia is already higher than that of heart disease and cancer together *(Schneider EP, 1990)*. Any intervention strategy that decreases risk or delays onset of the disease will have a substantial impact.

The UN world population projections estimate that by the year 2050 the number of people over 80 years old will approach 370 million, and the fraction of people older than 85 affected by dementia is estimated to be almost 50% *(Miller JW, 2000)*.

David Smith recently pointed out in an editorial that if vitamin treatment could stop only 10% of those with mild cognitive impairment from

developing AD, several hundred thousand persons worldwide would benefit every year *(Smith AD, 2002)*.

Homocysteine metabolism may turn out to be critical in the pathogenesis of dementia. It is discussed in relation to other pathogenetic factors in a recent review *(Nourashémi F et al., 2000)*.

A review on nutrition in general and in relation to cognition and affectivity in the elderly was recently published. Another review focuses B vitamins, cognition and aging *(Calvaresi E and Bryan J, 2001)*. Vitamin intervention is discussed in three other reviews *(Carmel R et al., 1995, Abalan F, 1999, Bassi et al., 1999)*.

Depression

Association with folate and vitamin B_{12} status

Low folate status is reported from several studies of patients with affective disorders. Carney and co-workers found that low RBC folate was far more common in depressive patients than in any other psychiatric disorder, table 5.

Folate Deficiency in Depression

	Number of patients	Red cell folate (%)	
		<200 ng/mL	<150 ng/mL
Depressed			
Endogenous	95	37 (39)	19 (20)
Neurotic	57	21 (37)	6 (11)
Total	152	58 (38)	25 (16)
Euthymic	42	7 (17)	2 (5)
Mania	32	7 (22)	3 (9)
Schizophrenia	29	5 (17)	1 (3)
Alcoholic	30	12 (40)	4 (13)
Total	285	89	35

Table 5 – Numbers of patients with red cell folate values indicating a low value (< 200 ng/ml) or severe deficiency (< 159 ng/ml). *Carney MWP et al. Red cell folate concentrations in psychiatric patients. ©J Affective Disord, 1990; 19:207-13. Reprinted with permission.*

When erythrocyte folate and vitamin B_{12} levels were measured in sucessively admitted in-patients at a District General Hospital Psychiatric Unit, and in out-patients on lithium treatment, there were 31% of patients with RBC folate below 200 ng/ml and 12% with concentrations below 150 ng/ml. The mean RBC folate was significantly lower in the depressed patients than in the euthymic, manic or schizophrenic group *(Carney MWP et al., 1990)*.

In the Canadian Study of Health and Aging of 1,171 individuals aged 65 years or older, subjects with low serum folate levels were more likely to be depressed *(Ebly EM et al., 1998)*.

Borderline low or deficient serum or RBC folate levels are found in 15-38% of depressive patients in different studies. In surveys, in which depressed patients were compared with psychiatric or non-psychiatric control subjects, depressed patients were found to have lower serum folate, methyl-THF, or RBC folate levels than all other groups, except for patients with alcoholism, who had a similar prevalence of low folate status.

In studies that failed to show an association between low folate and depression severity, there was nevertheless an inverse relationship between folate and the duration of the depressive episode, or an inverse relation between folate status and length of hospitalisation, thus with treatment outcome. Furthermore, patients with a low folate status were more severely depressed than those with folate in the normal range *(Alpert JE et al., 2000)*.

Low vitamin B_{12} levels are also reported. In older community-dwelling, disabled, non-demented participants of the Women´s Health and Aging Study, metabolically significant B_{12} deficiency (elevated MMA levels) was present in 14.9% of 478 non-depressed subjects, 17.0% of 100 mildly depressed subjects, and 22.0% of 122 severely depressed. Significant metabolic vitamin B_{12} deficiency was associated with a twofold risk of severe depression *(Penninx BWJH et al., 2000)*.

Association with homocysteine and S-adenosylmethionine

Elevated tHcy levels were seen in a study of 213 patients with major depression as compared with controls, fig. 18 *(Fava M et al., 1997)*.

Elevated tHcy levels are related to low SAM levels. An inverse correlation between plasma tHcy and the CSF levels of SAM were found in depressed patients with severe DSM III depression. Decreased SAM levels in the CSF and low folate levels both in CSF and RBC were found in patients with elevated tHcy, as well as decreased concentrations of monoamine metabolites as compared to controls, table 2a and b, page 51 *(Bottiglieri T et al., 2000b)*.

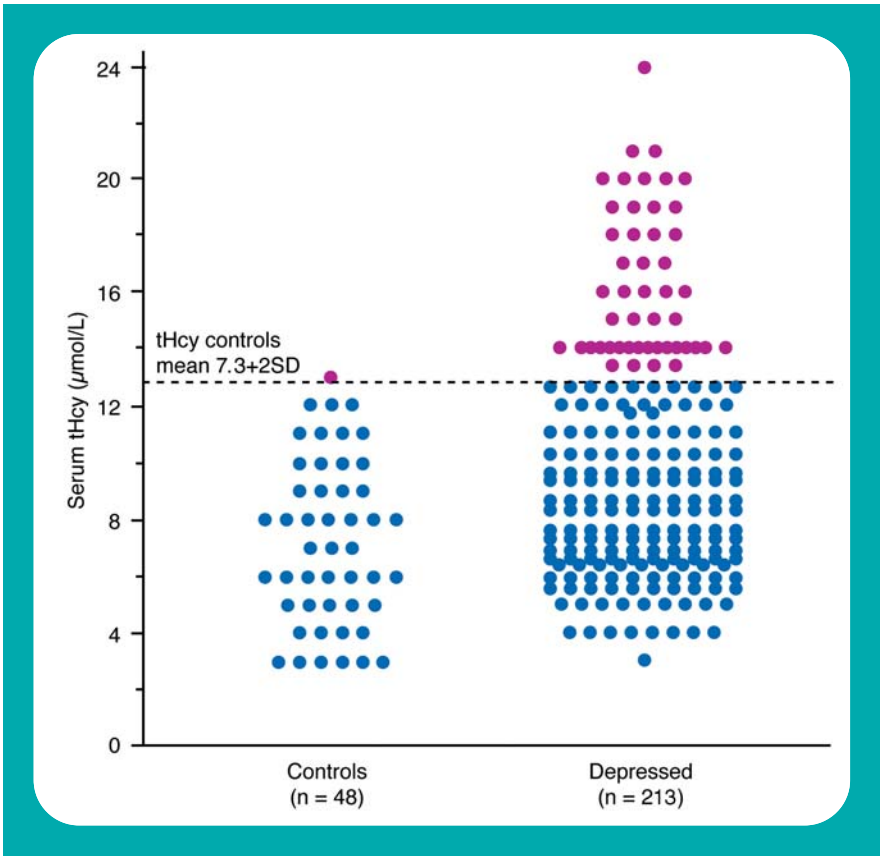

Figure 18 – Individual serum tHcy values in 213 depressed patients (mean age 39.9 years) and 48 controls (mean age 38.5 years). Dotted line represents mean tHcy in controls + 2SD. Of the depressed patients 20.5% had values above this level (13.1mmol/L). *Adapted by Bottiglieri T from data by Fava M et al. Folate, vitamin B_{12} and homocysteine in major depressive disorder. Am J Psych, 1997; 154:426-8. Reprinted with permission.*

SAM is used in some countries as a therapeutic agent in the treatment of depression. A meta-analysis of the effect of SAM as an antidepressant led to the conclusion that SAM has an effect comparable to that of standard tricyclic antidepressants *(Bressa GM, 1994)*. SAM was also recently found to be effective in depression related to Parkinson´s disease *(Di Rocco A et al., 2000)*.

Two recent reviews outline the effects of SAM in primarily psychiatric and neurological disorders, but also in internal medicine *(Bottiglieri T, 1997, Brown R et al., 2000)*.

Folate and vitamin B_{12} status in relation to response to antidepressive treatment

Over three decades ago, a study of 101 depressed inpatients receiving a variety of treatments showed that outcome was poorer for patients with low serum folate *(Reynolds EH et al., 1970)*.

In a more recent study serum folate, RBC folate, and serum B_{12} were determined at the beginning and end of a 5-week trial of desmethylimipramine in 99 consecutive unmedicated outpatients with major depressive illness. Compared with non-responders, the responders had higher serum folate levels. RBC folate showed a significant inverse correlation with the severity of depression and a significant positive correlation with age at the onset of illness. Moreover, significantly more responders than non-responders had a rise in RBC folate during antidepressant treatment in two studies. Low vitamin B_{12} levels were found at baseline in 4.9% of antidepressant responders and in 7.9% of non-responders in this study *(Wesson VA et al., 1994)*. (An adequate vitamin B_{12} status is required for the formation of intracellular folate forms.)

The change in RBC folate during anti-depressant therapy was also significantly greater in responders than in non-responders in a recent placebo-controlled study of 25 patients *(Levitt AJ et al., 1998)*.

Patients with reduced serum levels of folate were less responsive to fluoxetine treatment in another study. The OR for non-response was 2.2 in subjects with low folate levels versus patients with normal levels *(Fava M et al., 1997)*.

Folate supplementation in depression

A double-blind study of 96 patients with senile dementia and depressive symptoms, and with RBC folate within the normal range demonstrated the antidepressant effect of folate. Significant improvement of depressive symptoms, similar to the response to trazodone 100 mg/day, was achieved with 50 mg of methyl-THF daily *(Passeri M et al., 1993)*.

A placebo-controlled double-blind study later showed that administration of 15 mg of methyl-THF to patients with major depression and RBC folate less than 200 µg/L resulted in superior symptom improvement and social adjustment after 3 and 6 months *(Godfrey PSA et al., 1992)*.

Other studies have confirmed these findings. In a recent study, 127 patients with major depression were randomly assigned to receive daily doses of 0.5 mg of folic acid or placebo added to 20 mg fluoxetine.

In women, but not in men, folic acid decreased tHcy significantly. A percentage of 93.3 of the women in the folic acid group showed good therapeutic response (more than 50% reduction in Hamilton Rating Scale scores) as compared to 61.1% of the women in the placebo plus fluoxetine group, tables 6 a and b. The effect of the folic acid supplement was not significant in men, possibly because the dose of folic acid was too low *(Coppen A and Bailey J, 2000)*.

In a recent open study of 22 patients with severe depression, who had not responded to adequate treatment with SSRI after at least 4 weeks, were in addition given 15-30 mg of folic acid daily for 8 weeks. Hamilton Rating Scale (HRS-D-17) scores decreased from a mean level of 19.1 to 12.8, even though these patients had folate levels within the normal range at baseline *(Alpert JE et al., 2002)*.

A possible explanation to the enhanced effect of antidepressive drugs is restored monoamine metabolism, fig. 7, page 56 and fig. 19.

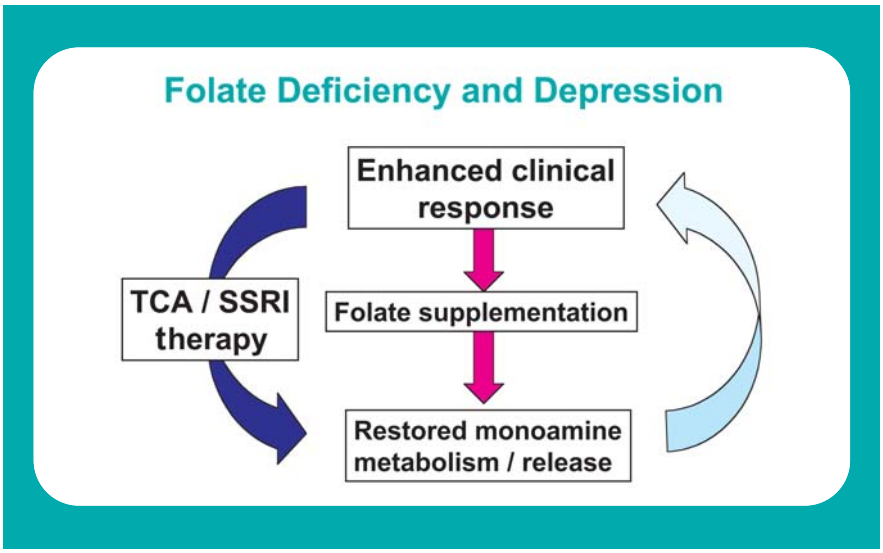

Figure 19 – Possible mechanism explaining how folate supplementation may increase the response to antidepressive drugs; tricyclic (TCA) and serotonin reuptake inhibitors (SSRI).© *Bottiglieri T. Reprinted with permission.*

Enhancement of Antidepressant Action of Fluoxetine by Folic Acid

	Fluoxetine + Placebo		Fluoxetine + Folic Acid	
	Folate (ng/ml)	Hcy (µmol/L)	Folate (ng/ml)	Hcy (µmol/L)
Women				
Baseline	4.3 ± 1.9	8.6 ± 2.3	4.0 ± 1.7	9.5 ± 3.7
Wk 10	4.3 ± 2.6	9.4 ± 4.3	12.7 ± 11.7a	7.5 ± 1.6b
Men				
Baseline	4.2 ± 1.2	9.9 ± 3.3	4.6 ± 1.7	9.7 ± 2.0
Wk 10	4.0 ± 1.4	10.2 ± 3.9	8.7 ± 3.5	9.0 ± 2.9

a = p < 0.005, b = p < 0.001

a

Enhancement of Antidepressant Action of Fluoxetine by Folic Acid

	Change in Hamilton Rating Scale at end of trial		
	Responders >50%	Non-Responders <50%	
Women			
Fluoxetine + Folic acid*	31 (93.0%)	2 (6.1%)	Chi-sq = 8.656
Fluoxetine + Placebo	22 (61.1%)	14 (38.9%)	*p<0.005*
Men			
Fluoxetine + Folic acid*	11 (61.1%)	7 (38.9%)	Chi-sq = 0.027
Fluoxetine + Placebo	14 (63.6%)	8 (36.4%)	*NS*

* Folic acid 500 µg

b

Table 6 – a) Change in Hamilton rating scale scores at week 10 in men and women. b) Plasma folate and tHcy at baseline and at week 10 in men and women treated with daily doses of 20 mg of fluoxetin with 0.5 mg of folic acid or placebo. *Coppen A and Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo-controlled trial. © J Affective Disord, 2000; 60:121-30. Reprinted with permission.*

Schizophrenia

Almost 40 years ago, Smythies presented a "transmethylation hypothesis" for schizophrenia. This hypothesis was supported by 10 clinical studies, which were reviewed in 1974 *(Cohen B et al., 1974)*.

In 1975 Freeman and co-workers described a case of homocystinuria, caused by a deficiency of MTHFR, accompanied by folate-responsive schizophrenic-like behaviour *(Freeman JM et al., 1975)*.

More recently, the DNA polymorphism-diet-cofactor-development (DDCD) hypothesis for development of schizophrenia has been presented. This hypothesis, which involves mutations of genes related to folate, vitamins B_{12}, and B_6, potentiated by maternal dietary deficiencies, was recently reviewed *(Johnson WG, 1999a)*.

Serum levels of tHcy in schizophrenia were first determined by Regland and co-workers. In this study 9 out of 20 schizophrenics had an increase in serum tHcy levels *(Regland B et al., 1995)*.

In another study of schizophrenics, tHcy levels were elevated compared to the levels of controls when folate levels were low, but no difference was seen when folate levels were high. The result indicates that an enzymatic defect could be involved *(Susser E et al., 1998)*.

Elevated tHcy levels in schizophrenics in connection with the C677T polymorphism of the MTHF gene and low folate status are described *(Regland B et al., 1997a, Arinami T et al., 1997)*, page 154.

A recent study of 193 schizophrenic patients showed a marked increase in tHcy compared to the 762 healthy controls. Mean level was 16.3 in patients versus 10.3 µmol/L in controls. The levels were particularly elevated in young male patients *(Levine J et al., 2002)*.

Multiple sclerosis

When vitamin B_{12} and folate were measured in CSF of patients with multiple sclerosis, lower median concentrations were found in patients compared to controls in one study *(Nijst TQ et al., 1990)*.

No decrease in total vitamin B_{12} levels were found in another study. However, the unsaturted binding capacity was decreased when compared to healthy controls and other neurological patients *(Kira J et al., 1994)*.

A negative correlation between serum B_{12} and progression of the disease is also reported *(Frequin ST et al., 1993)*.

Elevated plasma levels of tHcy are found in patients with multiple sclerosis, fig. 20 *(Reynolds EH et al., 1992)*.

More recently, tHcy and vitamin B_{12} levels were measured, along with neurotransmitters and other variables, in CSF of patients with multiple sclerosis. Total Hcy levels were highly significantly increased and vitamin B_{12} decreased in the CSF of MS patients compared to control values *(Qureshi GA et al., 1996)*. Increased CSF levels of tHcy in multiple sclerosis were confirmed in a later study, fig. 6, page 42 *(Baig S et al., 1998)*.

In a recent placebo-controlled, double-blind, randomised study, 138 patients were given weekly i.m. injections of 1 mg of vitamin B_{12} for 24 weeks in addition to 70 mg of lofepramine and 500 mg of L-phenylalanine or placebo twice daily. Patients improved by 2 points in the Guy´s neurological disability scale after vitamin B_{12} injections. The addition of lofepramine and L-phenylalanine added a further 0.6 points benefit *(Wade DT et al., 2002)*.

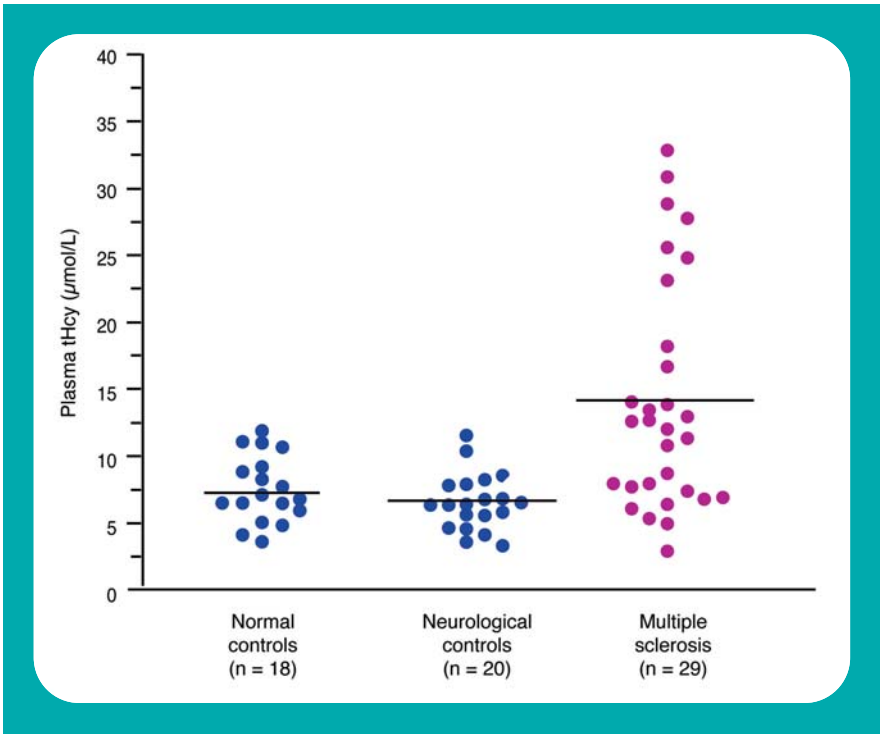

Figure 20 – Serum levels of tHcy in normal subjects, controls, neurological controls, and patients with multiple sclerosis. Horizontal lines represent mean levels. *Adapted from data in: Reynolds EH et al. Vitamin B_{12} metabolism in multiple sclerosis. Arch Neurol, 1992; 49:649-52. Presented at the 31st Scandinavian Congress of Neurology, 12-15 June, 1996. © Bottiglieri T. Reprinted with permission.*

Parkinson´s disease

Significantly elevated plasma levels of tHcy have been reported in patients with Parkinson's disease (PD) *(Kuhn W et al., 1998, Yasui et al., 2000, Müller T et al., 2001, Müller T et al., 2002)*. In one study the levels of homocysteine were elevated by 60% in levodopa-treated patients with a marked elevation occuring in patients with the MTHFR 677TT genotype *(Yasui et al., 2000)*.

Levodopa is O-methylated to 3-O-methydopa (3-OMD) by catechol-O-methyltransferase (COMT), a reaction that requires SAM and generates SAH. SAH is subsequently metabolized to Hcy. One group has reported that plasma tHcy is significantly elevated in PD with higher 3-OMD *(Müller T et al., 2002)*. These authors suggested that the increase in plasma tHcy as a result of levodopa treatment might induce vascular disease.

Another group has recently confirmed that plasma tHcy levels are significantly higher in patients treated with levodopa (mean $\pm$ SD; 16.1 + 6.2 µmol/L), compared to patients not on levodopa (12.2 $\pm$ 4.2 µmol/L, $p < 0.0001$). Furthermore, this study showed that patients, whose plasma tHcy levels were in the highest quartile ($\geq$ 17.7 µmol/L) had an increased prevalence of coronary artery disease. The OR was 1.75 compared to patients with lower tHcy *(Rogers et al., 2003)*.

These findings have implications for the treatment of PD in patients at risk for vascular disease, and potentially for those at risk of dementia and depression as well.

Elevated mean levels of tHcy were also found in a recent study of 24 patients with dystonia, as compared with controls (19.3 µmol/L versus 13.9 µmol/L), with a significant trend towards an association between the severity of dystonia and tHcy *(Müller T et al., 2000)*.

There is one report on elevated CSF tHcy and significantly lower CSF vitamin B_{12} in Parkinson's disease compared to controls, fig. 6, page 42 *(Baig S et al., 1998)*.

Stroke

The prevalence of hyperhomocysteinemia (> 15 µmol/L) is less than 5% in a general population, but as high as 50% in patients with stroke *(Hankey GJ and Eikelboom JW, 2001)*. Stroke in these patients is accompanied by a higher rate of cerebral microangiopathy and multiple infarctions than in other stroke patients *(Evers S et al., 1997)*. A recent study showed

a highly significant elevation of tHcy, lipid peroxide, and NO in the plasma of patients with thrombotic cerebrovascular stroke, as compared with age- and gender-matched healthy controls. There was also a strong positive correlation between tHcy and lipid peroxide and a strong negative correlation with the plasma concentration of ascorbic acid, which points to a Hcy-related oxidative pathogenetic mechanism *(ElKossi MMH and Zakhary MM, 2000)*.

Prospective studies

Nearly all cross-sectional and case-control studies have shown a strong and significant positive association between tHcy levels and cerebrovascular disease.

Most of the prospective studies have also shown significant evidence that elevated tHcy is a risk factor, although the association is less pronounced than in cross-sectional and case-control studies.

During prospective studies subjects may change their lifestyle so that risk factors in general are modified. Changes in lifestyle factors are important predictors over tHcy-changes over time *(Nurk E et al., 2001b)*. Homocysteine levels are not always measured repeatedly.

A significant association with tHcy was found in 5 prospective studies for stroke *(Bots ML et al., 1999b, Perry IJ et al., 1995, Bostom AG et al., 1999a, Ridker PM et al., 1999, Aronow WS et al., 2000)*. No significant association was found in 2 prospective studies *(Alfthan G et al., 1994, Verhoef P et al., 1994)*.

In the study by Perry, of 5,661 middle-aged men from UK were followed for up to 12.8 years. The 141 men who suffered a stroke had a higher mean tHcy than age-matched controls, and the adjusted OR for stroke was 2.8 for the highest versus the lowest quartile of tHcy *(Perry IJ et al., 1995)*.

Data from the Framingham Heart Study, on 1,947 men and women with a mean age of 70 at baseline, show that the OR for stroke increased directly with baseline tHcy levels after adjustment for other risk factors. Mean follow-up was 9.9 years. The adjusted OR was 1.82 for subjects with baseline tHcy levels in the upper quartile compared with the lowest quartile *(Bostom AG et al., 1999a)*.

Aronow and co-workers investigated the association between tHcy and different risk factors for atherothrombotic brain infarction (ABI) in 500 American elderly men and women. After a follow-up of 31 months tHcy, folate and vitamin B_{12} levels were found to be the strongest independent predictors of recurrency, together with prior ABI, hypertension and diabetes mellitus *(Aronow WS et al., 2000)*.

The prospective First National Health and Nutrition Examination Survey (NHANES I) including 9,764 US men and women aged 25-74 years, and free of vascular disease at baseline showed an inverse correlation between stroke and calculated folate intake. Over an average of 19 years of follow-up 926 incident stroke events were diagnosed. Participants in the highest quartile of folate intake had a 21% lower risk than participants within the lowest quartile *(Bazzano LA et al., 2002)*.

A meta-analysis of studies on tHcy and stroke/cerebrovascular disease was recently published. Data from 8 cross-sectional and 4 longitudinal studies with comparable populations were included in the analysis. The overall weighted OR for disease for subjects with a concentration of tHcy above the 95th percentile for the controls was 4.12 for the cross-sectional studies and 3.74 for the longitudinal studies. For all 12 studies it was 3.97. The results from 5 excluded studies all pointed to a positive relation between hyperhomocysteinemia and cerebrovascular disease *(Møller J et al., 2000)*.

A very rigorous meta-analysis of studies including 1,113 stroke patients was recently published. This meta-analysis also showed a significant positive association between tHcy and stroke in all age groups studied and independent of smoking, cholesterol and blood pressure. The risk association was stronger for stroke than for other vascular diseases *(Clarke et al., 2001)*.

Intervention studies

Intervention with vitamins, using intermediate vascular end points, such as changes in carotid artery intimal-media thickness, effects on flow-mediated endothelial responses, on thrombin-antithrombin complexes and prothrombin fragments 1+2 support that Hcy-lowering therapy is effective. Results from some clinical intervention studies have also been presented.

The effect of 5 mg of folic acid and 1 mg of vitamin B_{12} given daily for 8 weeks to 59 men with CHD was investigated in a double-blind, placebo-controlled study. Flow mediated EDD improved significantly in the active group, fig 21. The improvement correlated closely with the decrease of free reduced Hcy *(Chambers JC et al., 2000b)*.

Treatment of normohomocysteinemic and hyperhomocysteinemic subjects with folic acid for 12 months increased the levels of nitric oxide end products by 121%, the vasodilatory response to acetylcholine by 124%, the ischemia-mediated hyperemic response in the microcirculation by 60%, and in the forearm vasculature by 47% *(Holven KB et al., 2001)*.

Several more studies confirm a positive effect on EDD.

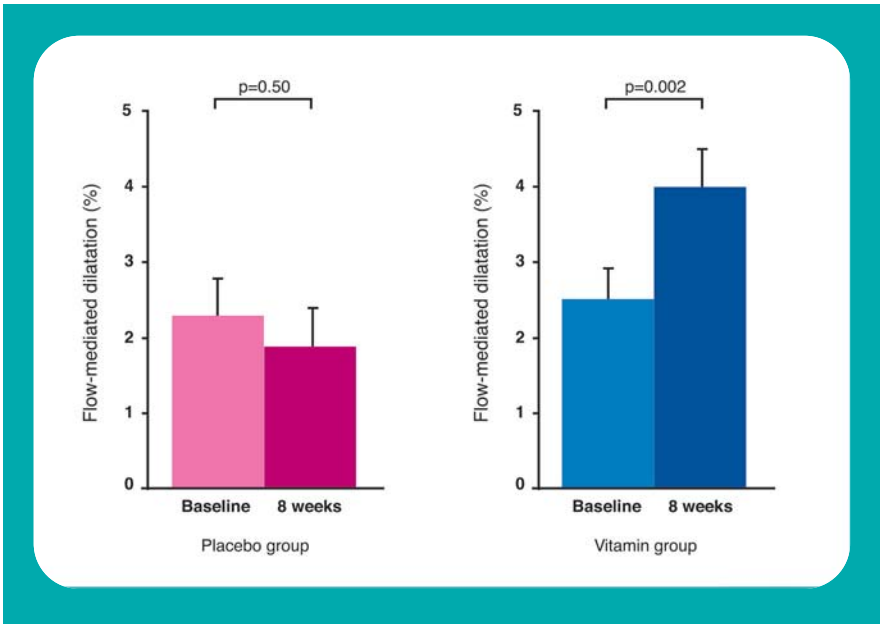

Figure 21 – Flow-mediated dilatation (mean ± SEM) at baseline and at 8 weeks after B vitamin/placebo in patients with established coronary heart disease.
Chambers J et al. Improved vascular endothelial function after oral B vitamins. An effect mediated through reduced concentrations of free plasma homocysteine. Circulation. 2000; 102:2479-83. © 2000 Am Heart Association Inc. Reprinted with permission.

Total Hcy, fasting and PML, and soluble thrombomodulin was measured in 18 patients with previous arterial or venous vascular disease. At baseline, all had hyperhomocysteinemia after methionine loading (PML), but only 2 had fasting hyperhomocysteinemia. After three months of daily supplementation with 5 mg of folic acid and 250 mg of vitamin B_6, PML tHcy had decreased by 34%, and there was a significant decrease in fasting soluble thrombomodulin *(Constans J et al., 1999)*.

The rate of progression of carotid plaque formation was highly significantly reduced after treatment with folic acid and vitamins B_6 and B_{12} in an open study of patients with tHcy exceeding 14 µmol/L and unexplained progression of atherosclerosis, *(Peterson J and Spence J, 1998)* fig. 22.

This finding was confirmed in a subsequent study of 51 patients with tHcy exceeding 14 µmol/L and 50 patients with lower levels. In the patients with the higher tHcy levels, therapy with 2.5 mg of folic acid, 25 mg of vitamin B_6, and 0.25 mg of vitamin B_{12} daily decreased the rate of progression of carotid plaque highly significantly or from 0.21 ± 0.41 cm^2/year before to 0.049 ± 0.24 cm^2/year after vitamin

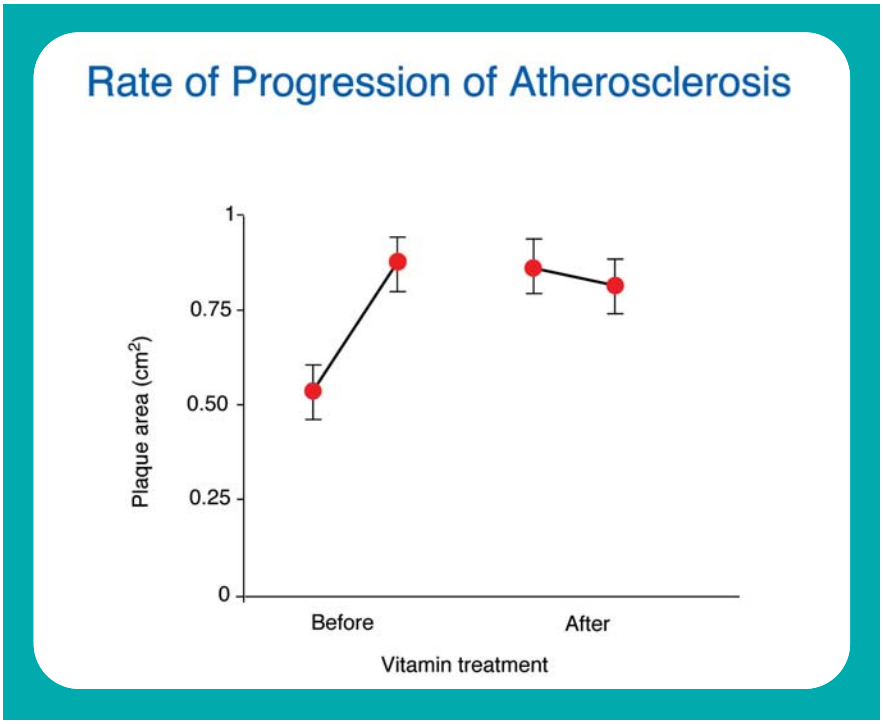

Figure 22 – Rate of progression of atherosclerosis before and after vitamin treatment in 38 patients with plasma tHcy ≥14 µmol/L. Mean follow up was 4.4 years. Initial treatment was 5 mg folic acid daily, followed by 2.5 mg folic acid, 25 mg pyridoxine, and 250 mg vitamin B_{12}. *Peterson J and Spence J. Vitamins and progression of atherosclerosis in hyperhomocyst(e)inemia. The Lancet, 1998; 351:263. © 1998 by The Lancet Ltd. Reprinted with permission.*

therapy. Among patients with tHcy below 14 µmol/L, the decrease was also significant, or 0.13 ± 0.24 cm^2/year before to 0.024 ± 0.29 cm^2/year after vitamin treatment, a result, which in combination with epidemiological evidence, suggests that levels over 9 µmol/L should be treated *(Hackham DG et al., 2000)*.

Bleeding time and the formation of thrombin was assessed in 17 hyperhomocysteinemic subjects (aged 22-60 years), of whom 11 had symptomatic atherosclerotic vascular disease. Blood was collected at 30-second intervals from an incision on a forearm. Thrombin-antithrombin III complexes and prothrombin fragments 1+2 were determined. All the tests were performed before and after an oral treatment with 5 mg of folic acid and 300 mg of vitamin B_6 daily, and 1 mg of vitamin B_{12} given intramuscularly weekly for 8 weeks. Median fasting tHcy decreased from 20 to 10 µmol/L. Plasma levels of fibrinogen, prothrombin, and antithrombin III and activity of proteins C, S,

and factor VII showed no changes. Thrombin-antithrombin III complexes and prothrombin fragments 1+2 in peripheral venous blood, however, fell significantly. Compared with pretreatment values, significantly less thrombin was produced during the first 3 minutes of bleeding after vitamin therapy. Bleeding time increased by about 60 seconds *(Undas A et al., 1999)*, fig. 23.

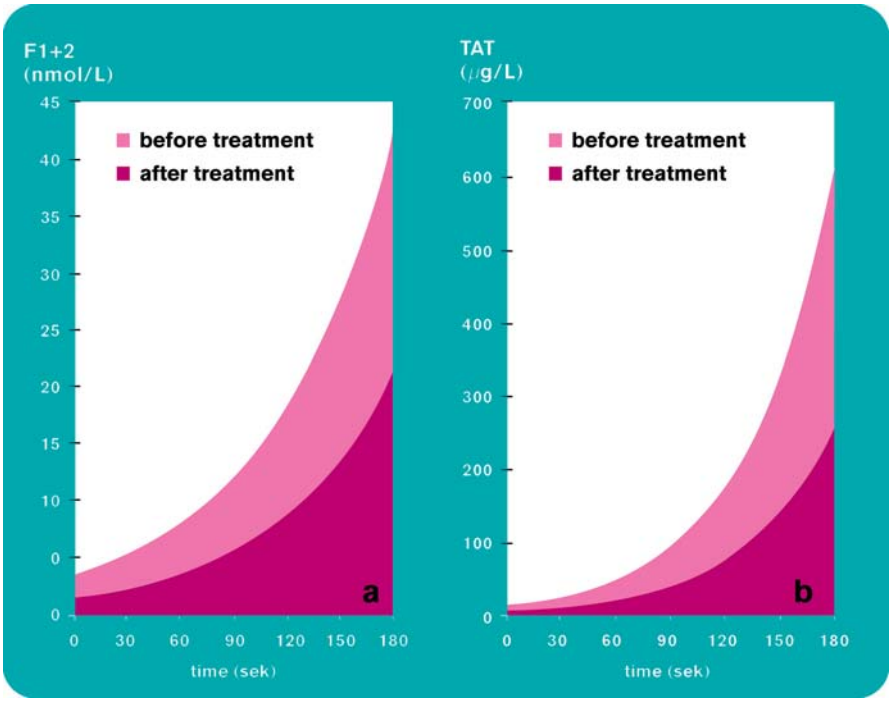

Figure 23 – Kinetics of thrombin generation before and after vitamin treatment assessed by a mathemathical model using prothrombin fragment 1 +2 (F1 +2) and thrombin-antithrombin III complexes (TAT) concentrations. Darker areas indicate the total amount of thrombin generated during the first 180 seconds of bleeding after vitamin supplementation. *Undas A et al. Treatment of hyperhomocysteinemia with folic acid and vitamins B_{12} and B_6 attenuates thrombin generation. Thrombosis Res, 1999; 95:281-8. © 1999 Elsevier Science Ltd. Reprinted with permission.*

Large-scale studies of prophylactic vitamin supplementation

About 15,000 patients are currently enrolled in trials on stroke prevention. An overview of these studies was recently published *(Clarke R, 2000)*.

The VITATOPS (Vitamins to prevent Stroke) study aims to recruit and follow up 8,000 patients, and provide a reliable estimate of the safety and

effectiveness of homocysteine-lowering vitamin supplementation in reducing recurrent vascular events among patients with stroke or transient ischemic attacs of the brain or eye.

There is reason to believe that the results of these studies will confirm the predicted effect of reduced Hcy-levels, even though there is also some reason for concern. Most of the studies are secondary prophylaxis studies, where therapy is given after a first episode of stroke. It is quite likely that these patients may modify their lifestyle during the study towards a healthier one, which may reduce the impact of Hcy and thus the power of the study.

Furthermore, folic acid fortification programmes can be expected to influence results. (The VITATOPS study is, however, conducted in countries with no current folic acid fortification.) Moreover, compliance with the placebo-controlled scheme, which implies the use of no other vitamin supplements by these patients, who have all been informed of the expected beneficial effects of the vitamins, is far from evident. Finally, primary prophylaxis might be more efficient than secondary, where significant vascular damage is already present.

Several reviews on hyperhomocysteinemia in vascular disease have been published *(Refsum H et al., 1998b, Cattaneo M et al., 1999, Guba SC et al., 1999, Nygård O et al., 1999, Hankey GJ and Eikelboom JW, 2001)*.

Other neuropsychiatric disorders

Fibromyalgia and chronic fatigue syndrome

Increased concentrations of tHcy and low levels of vitamin B_{12} in CSF were found in 12 patients, who met the criteria for both fibromyalgia and chronic fatigue syndrome, fig. 24. There was a significant positive correlation between the tHcy levels in CSF and a negative correlation with vitamin B_{12} levels in CSF and fatiguability *(Regland B et al., 1997b)*.

Optic neuropathy

Recent studies have identified hyperhomocysteinemia as a possible risk factor for optic neuropathy. Two patients (out of 12) with non-arteritic anterior ischemic optic neuropathy before the age of 50, were first reported to have tHcy levels of 16.1 µmol/L and 29.5 µmol/L respectively *(Kawasaki A et al., 1999)*.

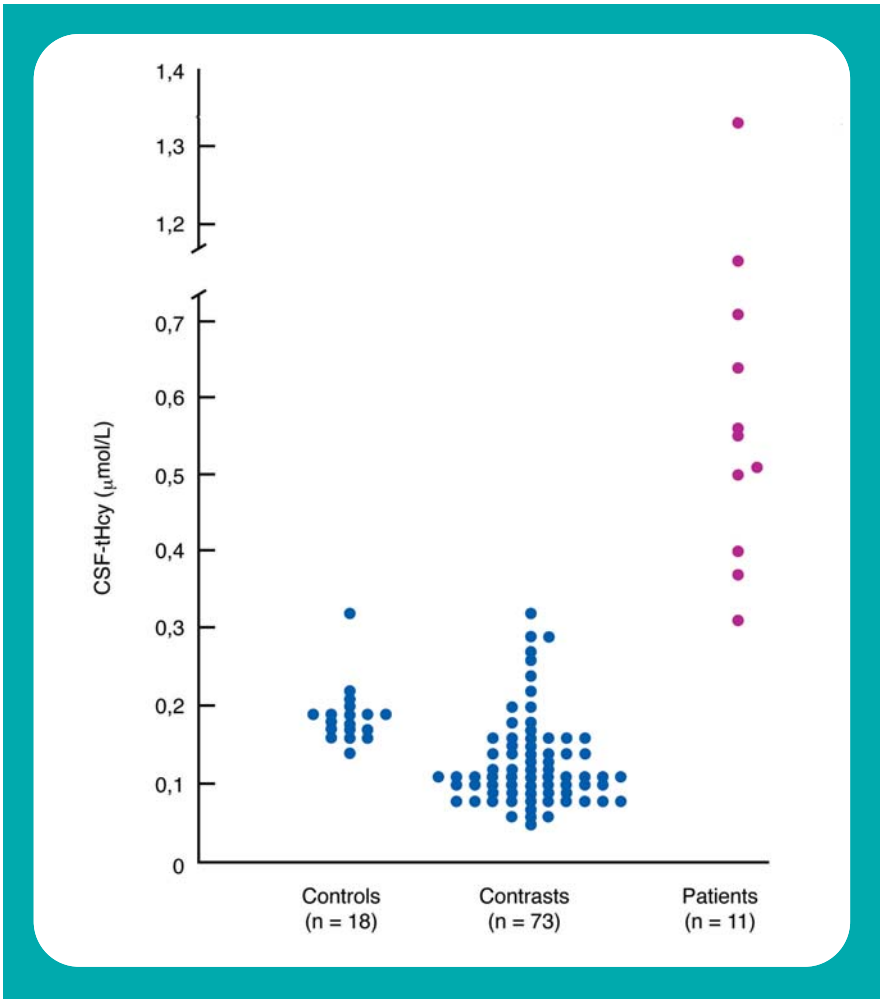

Figure 24 – Scatter plots of tHcy in the CSF of 11 female patients with fibromyalgia/chronic fatigue syndrome and of corresponding values in 18 healthy age-matched controls and a contrast group of 73 mentally healthy individuals. *Regland B et al. Increased concentrations of homocysteine in the cerebrospinal fluid in patients with fibromyalgia and chronic fatigue syndrome. © Scand J Rheumatolgy, 1997; 26:301-7. Reprinted with permission.*

A retrospective study of 84 cases later supported that hyperhomocysteinemia is a risk factor for non-arteritic anterior ischemic optic neuropathy and central retinal artery occlusion *(Pianka P et al., 2000)*.

Another retrospective study of 87 cases disclosed that elevated tHcy is an independent risk factor for both retinal vein, retinal artery, and branch retinal vein occlusion with an OR of 2.85 *(Cahill M et al., 2000).*

Finally, a case-control study of 74 patients with central retinal vein occlusion showed that hyperhomocysteinemia was present in 55% of the patients with bilateral disease, 30% of patients with ischemic occlusions, and 31% of cases with severe visual loss *(Vine AK, 2000)*. Another case-control study of 59 patients with nonarteritic ischemic optic neuropathy also showed that tHcy was significantly higher in patients than in controls (11.8 µmol/L versus 9.8 µmol/L). The OR for patients with tHcy exceeding the 95th percentile of control tHcy levels was 5.8. Mean plasma folate was also lower in patients than in controls *(Weger M et al., 2001)*.

No difference in frequencies of MTHFR C677T genotype between groups was seen in this study, but an increased risk of retinal vein occlusion in carriers of the C677T genotype of the MTHFR gene was found in another study *(Salomon O et al., 1998)*.

Long-term use of vitamin B_{12} and folate were recently found to have a strong protective influence on cortical cataract in the Blue Mountains Eye Study *(Kuzniarz M et al., 2001)*.

Migraine

Based on the observation that hydroxocobalamin may have clear scavenging properties against nitric oxide, an open trial to look at a possible preventive effect of hydroxocobalamin on migraine was recently performed. Hydroxocobalamin was administered intranasally to 19 patients. A reduction of at least half of the migraine attacks was reported by 10 of the patients *(Van der Kuy PHM et al., 2002)*.

In an editorial the rationale for use of vitamin B_{12} in migraine patients is discussed *(Montagna P, 2002)*.

Migraine is also associated with the C677T polymorphism of the MTHFR gene, page 158.

Neuropsychiatric disorders in the newborn

Vitamin deficiency and hyperhomocysteinemia in the newborn may be quite common, page 68. Clinical signs of vitamin B_{12} deficiency may not be apparent until months after delivery and may, if overlooked, result in neurological damage of the newborn, sometimes irreversible *(Graham IM et al., 1992, Navér L et al., 1995, Renault F et al., 1999)*. The article by Renault and co-workers comprises a review on the subject.

A follow-up of older children/adolescents (aged 10-16 years), who were given vegan food up to the age of 6 years, followed by lacto-vegetarian or omnivorous food showed that of 48 subjects, 31 still had a vitamin B_{12}-

deficiency according to their MMA levels. Moreover, a significant negative relation between vitamin B_{12} deficiency and fluid intelligence test scores was observed. Control subjects, fed omnivorious food from birth onward, performed better on most psychological tests than did macrobiotic subjects *(Louwman MWJ et al., 2000)*.

Neuropsychiatric complications associated with diabetes, alcoholism and HIV/AIDS

Diabetic complications

Diabetes is often accompanied by vascular and neurological complications, and hyperhomocysteinemia is commoner in patients with, than in those without such complications. One study showed that patients with type 1 diabetes had highly significantly increased mean tHcy levels, both fasting and PML, compared with controls (12.0 µmol/L versus 7.7 µmol/L). They also had a higher prevalence of late complications: nephropathy, retinopathy, neuropathy, and macroangiopathy compared with patients with tHcy within the normal range *(Hofmann MA et al., 1997, Hofmann MA et al., 1998)*.

It was recently shown that basal NO production was lower in diabetic patients than in controls, and there was an inverse relation between plasma tHcy and basal platelet NO release *(Mutus B et al., 2000, Mutus B et al., 2001)*. Disturbed NO metabolism may contribute to many of these complications, page 40.

Cardiovascular complications

Highly significantly elevated levels of tHcy were observed in type 2 diabetes patients with vascular complications, as compared with patients without such complications *(Araki A et al., 1993)*. A subsequent study of 250 elderly diabetic patients confirmed that patients with symptomatic and asymptomatic cardiovascular disease (CVD) had higher tHcy than those without CVD. Total Hcy was also associated with the presence or number of small infarct-like lesions on MR images *(Araki A et al., 1999)*.

Patients with type 1 diabetes with tHcy above the normal range (26 out of 75) had highly significantly increased plasma levels of thrombomodulin in one study. They also had a higher prevalence of macroangiopathy, or 57% versus 33% of patients with tHcy within the normal range *(Hofmann MA et al., 1997, Hofmann MA et al., 1998)*.

In another study of 122 type 2 diabetes patients with chronic complications 31% had elevated tHcy levels. The prevalence of macroangiopathy and CVD was higher in this group than in the patients with normal tHcy *(Buysschaert M et al., 2000)*.

A study of 318 type 2 diabetes patients with a duration of disease from 0-41 years also showed a higher mean tHcy in patients with a history of CVD. Each increase of tHcy by 1 µmol/L was associated with an OR of 1.45 for CVD *(Passaro A et al., 2000)*.

Total Hcy was also a stronger risk factor for CVD in subjects with type 2 diabetes than in non-diabetic subjects among 631 Caucasians (aged 50-75 years) in the cross-sectional Hoorn study *(Hoogeveen EK et al., 1998)*. It was also a stronger risk factor for death *(Hoogeveen EK et al., 2000b)*.

A study of 250 elderly diabetic patients showed that tHcy was associated with the presence or number of small infarct-like lesions on MR images, and tHcy significantly and negatively correlated with cognitive test results *(Araki A et al., 1999)*.

Hyperhomocysteinemia and the endocrine system and the implications for atherosclerosis and thrombosis are discussed in a recent paper *(Fonseca V et al., 1999)*.

Retinopathy

Increased prevalence of retinopathy was found in hyperhomocysteinemic type 1 diabetics. The prevalence of retinopathy 69% versus 51% in patients with normal tHcy *(Hofmann MA et al., 1997, Hofmann MA et al., 1998)*.

Another study of patients with type 1 diabetes, showed that moderate hyperhomocysteinemia (>10 µmol/L) was commoner in the patients with retinopathy than in patients without this complication *(Vaccaro O et al., 1997)*. In a subsequent larger study, 69 consecutive outpatients with type 1 diabetes were assigned to one of 3 groups: without retinopathy, with nonproloferative diabetic retinopathy, and with proliferative diabetic retinopathy (PDR). Total Hcy was higher in patients with, than in patients without PDR. Total Hcy progressively increased with a significant lineal trend from the stage of no retinopathy to PDR. The plasma levels of folate and vitamin B_{12} were comparable in the 3 groups *(Vaccaro O et al., 2000)*.

Both fasting and PML tHcy was increased in 63 patients with proliferating retinopathy compared to 54 matched controls in another Italian study of young diabetics *(Chiarelli F et al., 2000)*.

In a smaller case-control study mean tHcy was 13.9 µmol/L in 25 type 1 diabetics with severe retinopathy in cases versus 10.4 µmol/L (n.s.) in 24 patients with no or minimal retinopathy *(Agardh E et al., 2000)*.

Data from the cross-sectional Hoorn study, revealed that the OR for retinopathy was 3.44 for patients with type 2 diabetes and tHcy higher than 16 µmol/L after adjustment for age, sex, hypertension and glycosylated hemoglobin *(Hoogeveen EK et al., 2000a)*.

Neuropathy

A higher than expected prevalence of vitamin B_{12} deficiency in diabetic patients with neuropathy, and a dramatical improvement of neurological symptoms after treatment with hydroxocobalamin i.m. was reported already in the 60s *(Khan MA et al., 1969)*.

Patients with type 1 diabetes and with tHcy above the normal range had highly significantly increased prevalence of neuropathy 57 versus 41% compared with patients with tHcy within the normal range in recent studies *(Hofmann MA et al., 1997, Hofmann MA et al., 1998)*.

Another study of type 2 diabetes patients showed that a 5 µmol/L increase in tHcy was associated with a 2.3-fold increased risk of diabetic neuropathy *(Ambrosch A et al., 1998)*.

The relation with diabetic autonomic neuropathy, but not sensory motor peripheral neuropathy, was also recently found to be independently associated with tHcy in a large prospective American study of diabetic complications *(Cohen JA et al., 2001)*.

It was also recently shown that vitamin B_6 can inhibit superoxide radical production, thereby reducing lipid peroxidation and protein glycosylation, and increase $(Na^{++}K^{+})$-ATPase activity induced by hyperglycosylation in human RBCs *(Jain SK and Lim G, 2001)*. This mechanism could help to alleviate the oxidative damage by oxygen species, which may partly be generated by elevated Hcy concentrations.

In a study of type 2 diabetics, elevated tHcy were efficiently lowered by treatment with vitamin B_6 and folic acid *(Baliga BS et al., 2000)*. Interestingly, hyperinsulinemia has been shown to inhibit the vitamin B_6-dependent CBS-reaction in rat liver cells *(McCarty MF, 2000c)*.

Uncontrolled Japanese studies of vitamin B_{12} treatment of patients with diabetic neuropathy suggest a positive effect, which, however, must be confirmed in controlled studies.

Alcohol-related neuropsychiatric complications

There is growing evidence that chronic alcoholism is associated with a derangement in the sulphur amino acid metabolism. Folate, vitamin B_{12}

and B_6 levels are low and tHcy increased in alcoholism, page 62. A correlation between neurological alcohol-related symptoms and functional markers of vitamin B_{12} deficiency is found *(Gimsing P et al., 1989)*.

Many of the neuropsychiatric complications may be related to a disturbed Hcy metabolism. The degradation product of ethanol, acetaldehyde, has been associated with in particular cardiovascular complications in alcoholism, but may also contribute to neuropsychiatric complications, as it interferes with homocysteine metabolism, page 65.

Alcohol withdrawal seizures

In a recent study, patients with chronic alcoholism, who suffered from withdrawal seizures, had mean tHcy levels of 84.7 µmol/L on admission compared to 30.2 µmol/L in patients, who did not develop seizures *(Bleich S et al., 2000a)*. There was also a significant correlation between tHcy and blood alcohol concentration and malondialdehyde (MDA, a radical damage end product) on admission *(Bleich S et al., 2000b)*. It has been suggested that tHcy levels may be a valuable tool for predicting withdrawal seizures *(Kurth C et al., 2001)*.

Alcohol-induced hyperhomocysteinemia may cause neuronal cell damage by stimulating NMDA receptors as well as by producing free radicals. These mechanisms may contribute not only to withdrawal seizures, but also to pathological changes such as brain shrinkage *(Bleich S et al., 2000d, Bleich S et al., 2003)*.

Cognitive disturbances and depression in alcoholism

The distribution of the vitamins to the CNS may be disturbed in alcoholic patients, which may further increase the impact of vitamin deficiency. Van Tiggelen and co-workers found that CSF levels of vitamin B_{12} in demented alcoholic patients were subnormal despite normal or even high serum levels *(Van Tiggelen CJM et al., 1983)*. Correlation between vitamin levels and neuropsychiatric symptoms in alcoholics may therefore be poor.

Alcoholics had RBC folate levels in the same range as depressive patients in one study, page 126 *(Carney MWP et al., 1990)*. Low folate levels are also associated with depression in alcoholics *(Merry J et al., 1982, Abou-Saleh MT and Coppen A, 1989)*.

HIV/AIDS-related neuropsychiatric disorders

Both cognitive and neurological disorders are common in HIV/AIDS. The pathogenesis of these disorders is unknown, but may be related to meta-

bolic abnormalities rather than to direct HIV infection. There is a striking similarity between AIDS-related myelopathy and the vacuolar myelopathy observed in vitamin B_{12} deficiency *(Di Rocco A and Simpson DM, 1998)*.

Reduced concentrations of SAM in CSF, indicating disturbed methionine metabolism, have been found in three independent studies of HIV-infected patients, one in children with congenital HIV infection *(Surtees R et al., 1990)*, and two in adults *(Keating NJ et al., 1991, Castagna A et al., 1995)*.

A suppression of vitamin B_{12}-dependent methionine synthase (MS) would result in a decreased synthesis of methionine and SAM. Low levels of SAM may also, by feed-back, reduce the synthesis of cysteine and glutathione (GSH), leaving the nervous system more vulnerable to oxidative stress. Impaired methionine metabolism and DNA replication have been proposed as pathogenetic mechanisms in the AIDS dementia complex *(Regland B and Gottfries CG, 1992)*.

It has been suggested that vitamin B_{12} may play an important role in cellular immunity. Methylcobalamin given to vitamin B_{12}-deficient patients and controls resulted in an increase in lymphocyte counts in both groups. Moreover, the CD4/CD8 ratio and suppressed NK cell activity improved, and CD3 (-) CD16 (+) cells increased after treatment *(Tamura J et al., 1999)*.

Homocysteine and SAM and neuro-psychiatric complications

In 20 HIV-infected patients, with or without overt clinical signs of myelopathy, the mean SAM concentration in the CSF was highly reduced and the mean SAH concentration highly significantly elevated compared with the values in controls *(Keating NJ et al., 1991)*.

The levels of SAM, methionine, tHcy and GSH in serum and CSF were recently determined in 15 HIV-infected patients with myelopathy, and 13 patients without myelopathy. Values were compared with the levels in a non-infected reference population. Patients with myelopathy had a highly significant decrease of SAM compared to both patients without myelopathy and non-infected controls. Infected patients without myelopathy, however, also had lower levels than non-infected controls. Serum levels of methionine were also lower in patients with myelopathy than in non-infected controls *(Di Rocco A et al., 2002)*.

Administration 800 mg/day of SAM intravenously for 14 days to 6 HIV-infected patients produced significant increases in both SAM and GSH in CSF *(Castagna A et al., 1995)*.

A correlation between vitamin B_{12} and/or folate status and neuropsychiatric parameters is reported in several studies. Low serum concentra-

tions of vitamin B_{12} have also been associated with the faster progression of HIV-1 disease and higher mortality rate in many studies. These findings support an abnormality of the transmethylation.

A study of protein methylation of postmortem cortical brain from HIV-positive patients, however, suggested a relative hypermethylation *(Goggins M et al., 1999)*.

Mechanisms relating to the methionine metabolism, which may contribute to explain neuropathy, cerebral atrophy, dementia, and myelopathy in AIDS, are discussed in a review, which bridges evidence of cytokine activation, myelinotoxic and neurotoxic events with abnormal methylation *(Tan SV and Guiloff RJ, 1998)*.

A review on vitamin B_{12} as HIV-I integrase inhibitor and on its associated clinical effects was recently published *(Mathé G, 1999)*.

Vascular complications

Disturbed methylation and oxidative mechanisms could contribute to atheriosclerotic lesions found in young HIV-positive patients *(Tabib A et al., 1992, Valdes E et al., 1996)*. HIV infection is also associated with increased risk of stroke, particularly cerebral infarction in young patients.

Intervention studies

Clinical results of treatment with vitamin B_{12} have generally been relatively deceptive. It is possible that this may be because of the use of cyanocobalamin that has a lower affinity to the transport protein TC than hydroxocobalamin.

Hydroxocobalamin, methylcobalamin, and adenosylcobalamin, but not cyanocobalamin, were shown to inhibit HIV-I infection of normal human blood monocytes and lymphocytes *(Weinberg JB et al., 1995)*. (Injections of hydroxocobalamin has been shown to result in higher relative levels of the active co-enzymes than injections of cyanocobalamin *(Gimsing P et al., 1982)*. The authors recommend that vitamin B_{12} should be considered potentially useful agents for the treatment of HIV-1 infection, so much the more as high tissue levels may be achieved without any toxicity.

Positive results on performance were reported in 14 HIV-infected patients with autoantibodies to IF, and serum B_{12}-levels less than 300 pg/ml, when 2 mg of hydroxocobalamin was given intramuscularly three times a week together with 2 mg of folic acid daily *(Harris PJ, Candeloro P, 1991)*.

Reversibility of AIDS-associated dementia is also described after daily injections of 1 mg vitamin B_{12}, but derivative is not indicated *(Herzlich BC and Schiano TD, 1993)*.

Several open studies of methionine, the precursor of SAM, in the treatment of AIDS-related myelopathy and dementia also indicate an effect *(Di Rocco A et al., 1996a, Di Rocco A et al., 1996b, Di Rocco A et al., 1998, Dorfman D et al., 1997)*.

In a pilot study of oral treatment with methionine of AIDS-associated vacuolar myelopathy, 7 out of the 9 patients, who completed the study, showed a clinical and electrophysiological improvement *(Di Rocco A et al., 1998)*.

A longitudinal study of vitamin B_6 status in 88 HIV-infected patients, stages II-IV, showed a significant decline in psychological distress with normalisation of vitamin B_6 over an 18-month period *(Shor-Posner G et al., 1994)*.

Clinical Impact of Enzyme Defects

Over 40 mutations of the methylenetetrahyrodrolate reductase gene (MTHFR) are identified, other than the common C677T polymorphism. In the cystathionine β-synthase (CBS) gene, over 90 mutations have been identified so far. A few of these, often fairly common mutations, increase the enzyme activity, and thereby reduce tHcy levels. Others reduce the activity and may have a synergistic tHcy-increasing effect with, for instance, the C677T polymorphism.

The polygenic influence on tHcy of the C677T polymorphism of MTHFR, the A2756G transition of the vitamin B_{12}-dependent methionine synthase (MS), and the CBS 844 ins-68 variant was recently studied in 1,025 American individuals. Overall, more than 50% of all individuals carried polymorphic traits in this study, which predisposed them to either higher or lower tHcy levels *(Tsai MY et al., 2000b)*.

The association between these mutations and disease have primarily focused vascular disease and teratogenicity, but some studies have also revealed associations with neuropsychiatric disease. Disturbances in the activity of enzymes involved in the synthesis of SAM: MS, MTHFR and methionine adenosine transferase (MAT), and also recently CBS, have been associated with psychiatric disease and cognitive disorder.

It is likely that the C677T polymorphism, for instance, may increase the risk and severity of disease, and particularly in the presence of other risk factors. On the other hand, adequate vitamin status and other lifestyle factors may abolish the impact of the polymorphism. Most Italian and Spanish studies on the association between this polymorphism and disease are negative, which may be explained by a high folate status in Italy.

An overview of the metabolic significance, risks and impact on folate requirement of the C677T polymorphism was recently published *(Bailey LB et al., 1999)*.

Ueland and co-workers recently discussed various biological implications of this polymorphism in another review *(Ueland PM et al., 2001)*.

Cognitive impairment

The fact that elderly and cognitively impaired patients may have high tHcy-levels in spite of normal blood concentrations of folate and vitamin B_{12} *(Joosten E et al., 1993b, Joosten E et al., 1997, Nilsson K et al., 1994*, *Nilsson K et al., 1996*, *Van Goor LP et al., 1998)* is proposed to be, at least partly, a consequence of enzymatic defects. The common C677T polymorphism may, for instance, have a stronger impact on tHcy in elderly persons with a poor vitamin status (*Guttormsen AB et al., 1996*, *Clarke R et al., 1998a, Kauwell GPA et al., 2000a*).

Enzyme defects may be overrepresented in demented patients. In a recent study of 143 patients with vascular dementia, and 122 patients with cerebral infarction, the prevalence of elevated tHcy was higher in both groups than in the controls. However, a significantly higher prevalance of the TT genotype of the C677T mutation was found only in demented patients (25.2% versus 12% in controls), and the TT genotype was significantly associated with the risk of vascular dementia in hyperhomocysteinemic patients only with an OR of 4.13 *(Yoo JH et al., 2000)*. This result again points to vitamin status as an important factor.

Other studies, however, have found no association with dementia. In a Dutch population-based study, the C677T genotype was determined in 365 subjects aged 85 years and over and 250 subjects aged 18-40 years. The frequency of the C677T polymorphism was significantly lower in the elderly, but the difference in genotype distribution was only present in men. No deleterious effect of the C677T polymorphism was observed in the women aged 85 and over *(Heijmans BT et al., 1999)*. Of the participants in this study, 171 participants without dementia at baseline were re-examined after a median of 4 years. No patient with the TT genotype had developed dementia *(Gussekloo J et al., 1999)*.

Three recent Italian case-control studies showed no difference in C677T prevalence between demented patients and controls *(Brunelli T et al., 2001*, *Postiglione A et al., 2001*, *Bottiglieri T et al., 2001)*. One of these studies, in which 74 AD patients were included, showed that the prevalence of the TT genotype was 18% of patients and 20% of controls. The duration of disease, however correlated positively with tHcy, and negatively with plasma folate and vitamin B_{12} levels *(Postiglione A et al., 2001)*. In the study by Bottiglieri and co-workers moderate to severe hyperhomocysteinemia was associated with significantly lower MMSE scores.

A British study of 60 pairs of demented and non-demented patients and an Isreli study of 177 demented patients and 82 controls also failed

to show any association with the C677T polymorphism *(Tysoe C et al., 1997, Pollak RD et al., 2000)*.

One could speculate that the very old age may constitute a selection of subjects with some protective factor, which compensates for the enzyme defect.

Environmental or lifestyle factors, which may vary between sexes, may interact other polymorphisms so that the homocysteine metabolism is preserved.

In a recent Swedish study of patients with Alzheimer´s disease, the association with apoEε4 was studied. Of 140 patients 75% had at least one apoEε4 allele. Of these 15% were also homozygous, and 34% heterozygous for the C677T polymorphism. The frequency of this polymorphism in those lacking apoEε4 was higher, or 23% and 48.5% respectively. The numbers of apoEε4 and C677T alleles correlated significantly with the serum folate levels, but in opposite directions. This indicates that also apoEε4 may be related to the one-carbon metabolism *(Regland B et al., 1999)*.

A decreased MAT activity as may the RBC of demented patients was reported in 1995 *(Gomes-Trolin C et al., 1995)*. Studies of brain tissue from demented patients later provided evidence of significant alterations in brain MAT activity, depending on the vitamin B_{12} status *(Gomes-Trolin C et al., 1996)*.

Depression

Normal fasting levels of tHcy, but significantly increased postprandial levels found in depressive patients, as compared to controls could indicate some enzymatic defect *(Candito M et al., 1997)*.

A Japanese study of the prevalence of the C677T polymorphism in 32 patients with major depression showed that the TT genotype was more common in patients than in controls, or in 28% versus 12% *(Arinami T et al., 1997)*. The polymorphism was, however, not overrepresented in 143 patients with unipolar or bipolar depression in another Japanese study *(Kunugi H et al., 1999)*.

Patients with late-onset depression, but not with early-onset disorders, had higher prevalence of homozygous or heterozygous forms of the C677T polymorphism in another study. The prevalence was 74% versus 48% in early-onset depression *(Hickie I et al., 2001)*.

Folate status, which may modulate both the impact of the polymorphism, and treatment response in depression, page 129, was not reported in these studies.

Schizophrenia

An overrepresentation of the C677T polymorphism was found in a Swedish study of 11patients with schizophrenia-like psychosis and hyperhomocysteinemia. Seven were homozygous for the defect, and one was heterozygous *(Regland B et al., 1997a)*.

In the Japanese study by Arinami and co-workers on 297 schizophrenics, the TT genotype was also highly significantly more frequent in patients, or present in 21% versus 12% in controls *(Arinami T et al., 1997)*. A study of 56 Caucasian mothers, fathers, and affected children also supported the association *(Wei J and Hemmings GP, 1999)*.

A recent Canadian study of two groups of schizophrenics, selected on the basis of their response to neuroleptics (43 excellent responders and 62 and non-responders), replicated the findings of a significant association between schizophrenia and the C677T polymorphism. The TT genotype was, however, found significantly more often only in responders. The 677T allele was present in 61.3% of responders versus 36.3% of non-responders. The prevalence of the T allele did not differ between controls and non-responders *(Joober R et al., 2000)*.

No significant differences in tHcy or distribution of the C677T polymorphism were, however, observed in a recent Spanish study of 210 schizophrenic in-patients compared to hospitalised controls *(Virgos C et al., 1999)*. Nor was the polymorphism overrepresented in 343 patients with schizophrenia in another Japanese study *(Kunugi H et al., 1999)*.

Folate status, which may modulate the impact of the polymorphism, and treatment response, was not reported in these studies.

Alterations in MAT activity in brain tissue of schizophrenics has been seen, but these might be induced by medication *(Gomes-Trolin C et al., 1998)*.

Parkinson´s disease

Enzyme defects may also increase the impact of drug interactions and thereby have an indirect influence.

A recent Japanese study showed that tHcy levels were increased in 60% of 90 L-dopa-treated patients with Parkinson´s disease, and that patients homozygous for the C677T polymorphism had the most marked elevation. In this group tHcy and folate levels were inversely correlated *(Yasui K et al., 2001)*.

Additional data from this group *(Yasui K et al., 2000)* and other recently reported data *(Kuhn W et al., 2001)* lend firm support that the TT poly-

morphism is a significant factor resulting in hyperhomocysteinemia in L-dopa-treated patients. Homocysteine monitoring may therefore be of particular value for PD patients with this polymorphism.

Epilepsy

A recent Japanese study of 90 epileptic patients showed that the frequency of the C677T polymorphism was higher in patients with symptomatic or cryptogenic epilepsy (with a central nervous system defect or developmental delay) than in subjects without epilepsy *(Ono H et al., 2000b)*.

Down syndrome

Increased susceptibility to trisomy 21

Down syndrome is attributed to the presence of an extra copy of chromosome 21. The extra chromosome derives from the mother in 95% of cases and is caused by abnormal chromosome segregation during meiosis. Advanced age at conception is a risk factor for Down syndrome, but most children with Down syndrome are born to mothers younger than 30. Other risk factors are not well known. Recent studies suggest a link with the Hcy metabolism.

The C677T polymorphism of the MTHFR gene

The frequency of the C677T polymorphism was studied in 57 children with Down syndrome, in their mothers, and in 50 age-matched control women. Mutant allele frequency was commoner in the case mothers than in the controls. Of the case mothers, 14% were homozygous for the polymorphism compared to 8% of the controls. The polymorphism conferred a 2.6-fold increase in risk of having an affected child. A significant increase in tHcy and a higher ratio of tHcy/methionine, independent of C677T genotype, were observed in the mothers of children with Down syndrome. Methotrexate cytotoxicity was greater in lymphocytes from the mothers of children with Down syndrome, which is consistent with a reduced ability to adapt to folate deprivation *(James SJ et al., 1999)*.

An Italian study, however, did not find an increased risk of Down syndrome in mothers carriers of the T allele *(Stuppia L et al., 2002)*.

Other enzyme defects and interactions

A subsequent larger study by James and co-workers confirmed the association with the C677T polymorphism and also revealed an association with a common A→G polymorphism at position 66 of the cDNA sequence of the methionine synthase reductase gene (MTRR) (A66G).

DNA samples from 157 mothers of children with Down syndrome and 144 control mothers were evaluated. The C677T polymorphism was commoner in Down syndrome mothers than in controls with an OR of 1.91. In addition, the homozygous MTRR A66G polymorphism was independently associated with a 2.57-fold increase in estimated risk, and the combination was associated with a greater risk than was the presence of either mutation alone with an OR of 4.08 *(Hobbs et al. 2000)*.

This finding was supported by a study of 48 mothers who had given birth to a child with Down syndrome, and 192 control mothers. In this study the A66G polymorphism was more common in mothers of children with Down syndrome, although no increase in tHcy was recorded. The MTHFR C677T polymorphism alone was not significantly associated with Down syndrome in this smaller study. However, mothers, who had the TT genotype and the MTRR GG genotype had an almost 3-fold risk of having an affected child *(Oleary VB et al., 2002)*.

These studies suggest that factors involving both genotypes and nutrition may underlie susceptibility to trisomy 21. This is a totally new approach that will certainly be pursued.

Possible impact of polymorphisms on mental performance

The C677T polymorphism of the MTHFR

The CBS gene is located on chromosome 21. Individuals affected by Down syndrome often have an increased cystathionine-β-synthase (CBS) activity, or about 165% of its normal activity. More homocysteine may therefore be directed towards the transsulphuration pathway, which may result in reduced remethylation of Hcy and hence decreased levels of methionine and SAM, page 21. This mechanism has been proposed to be a pathogenetic mechanism in Down syndrome *(Regland B and Gottfries CG, 1992)*.

The hypothesis was supported by a recent study. Plasma levels of tHcy, methionine, SAM and SAH were all significantly decreased in 42 children with Down syndrome compared to 35 normal children. Plasma GSH was also significantly decreased indicating increased oxidative stress, which may be due to the overexpression of superoxide dismutase gene, also on chromosome 21. The addition in vitro to lymphoblastoid cells of methionine,

folic acid, vitamin B_{12}, thymidine, or dimethylglycine improved the metabolic profile *(Pogribna M et al., 2001)*.

A recent study of 60 patients with Down syndrome showed that when patients were separated according to degree of mental retardation, tHcy was 13.4 µmol/L and folate levels 12 pmol/L in those with severe retardation (n = 26) versus 8.6 µmol/L and 15.2 pmol/L, respectively, in those with mild or moderate retardation (n = 17) *(Anello G et al., 2001)*.

Heterozygosity for the reatively common CBS 844 ins-68 variant, in which the enzyme activity is somewhat increased, was recently shown to be significantly underrepresented in children with a high IQ *(Barbaux S et al., 2000)*.

Decreased remethylation also impairs the folate metabolism, page 27, and patients have a decreased tolerance to the folate antagonist, MTX *(James SJ et al., 1999)*.

Stroke

Many studies of the association between the C677T polymorphism, alone or combined with other mutations, and stroke have been published. In general, tHcy levels are significantly elevated in stroke patients compared with controls, and a significant relationship between the C677T polymorphism and both folate status and elevated tHcy is found.

A correlation between the 677TT genotype, low plasma SAM levels, low folate levels, and high tHcy levels are found in patients with stroke *(Papagiannopoulos M and Lalouschek W, 1999)*, and a meta-analysis of 16 studies recently supported that the TT genotype may have an influence on susceptibility to ischemic stroke *(Kelly PJ et al., 2002)*.

However, a statistically increased prevalence of the polymorphism is often not demonstrated, possibly due to fairly small samples.

In studies of premature stroke in children or young adults, a significantly increased frequency is, however, often found *(Nowak-Gottl U et al., 1999, Cardo E et al., 2000,Prengler M et al., 2001, Assanelli D et al., 2002)*.

It is possible that the association is dependent on type of event, and interactions with other polymorphisms may interfere.

Silent brain infarction, but not symptomatic subcortical infarction was associated with the C677T polymorphism in one study (*Notsu Y et al., 1999)*. Spontaneous cervical artery dissection in young adults, but not atherothrombotic stroke, was associated with the C677 polymorphism in another *(Pezzini A et al., 2002)*. A significantly increased risk for stroke was

found for the T allele, but not for for carotid stenosis in third study *(Topic E et al., 2001)*.

Other neurological disorders and complications

Optic neuropathy

An increased risk of retinal vein occlusion with an OR of 1.9 for TT carriers genotype was demonstrated in a study of 102 patients with retinal vein occlusion. Hypertension and a family history of stroke were other risk factors *(Salomon O et al., 1998)*.

A subsequent study of 59 patients with nonarteritic ischemic optic neuropathy showed that tHcy was significantly higher in patients than in controls patients and healthy controls, but no increased frequencies of MTHFR C677T genotype between groups was demonstrated in this smaller study *(Weger M et al., 2001)*. Diabetes may be another additional risk factor.

Diabetic retinopathy

Hyperhomocysteinemia is common in patients with diabetic complications, page 143. Abnormal post methionine loading (PML) results, indicating an enzyme defect or vitamin B_6 deficiency, were found in an early study in 7 out of 18 type 2 diabetes patients and particularly in patients with macrovascular disease *(Munshi MN et al., 1996)*. Enzyme defects may be overrepresented among patients with diabetic complications.

The C677T polymorphism of the MTHFR gene

The TT variant of the C677T polymorphism was recently found in 18.1% of 94 diabetic patients, but only in 10.6% of 1,180 controls (*(Ho CH, 2000)*.

A significantly higher prevalence of the C677T polymorphism was also found in Japanese type 2 diabetes patients with diabetic retinopathy than in patients without this complication *(Neugebauer S et al., 1997)*. Another study suggests that the polymorphism is a strong risk factor for accelerating arterial wall thickening in type 2 diabetes patients *(Arai K et al., 1997)*.

The prevalence of the C677T polymorphism was higher in Italian type 1 diabetes patients with proliferative diabetic retinopathy (PDR) than among patients without PDR. Out of 10 patients with PDR, 7 carried the TT genotype versus 6 out of 34 in the group without retinopathy. Total Hcy

progressively increased with a significant lineal trend from the stage of no retinopathy to PDR *(Vaccaro O et al., 2000)*.

In another recent study of type 2 diabetes, patients with serum folate less than 15.4 nmol/L, and who were hetero- or homozygous for the C677T polymorphism, had a significantly increased incidence of diabetic nephropathy *(Shpichinetsky V et al., 2000)*.

Migraine

A Japanese study of 74 patients with migraine (22 with aura and 52 without aura) the incidence of the homozygous genotype for the C677T genotype of the MTHFR gene was significantly higher in patients than in controls, or 20.3% versus 9.6%. In patients with aura migraine the prevalence was 40.9% *(Kowa H et al., 2000)*. Homocysteine levels are not reported.

Alcohol-related neuropsychiatric complications

Alcoholics carrying the C677T polymorphism have significantly increased tHcy levels. The prevalence of hyperhomocysteinemia among TT genotype carriers was 84.2%, among CT carriers 54.3% and among CC cariers 31.6% in a recent study *(De la Vega MJ et al., 2001)*.

The impact of this polymorphism on alcohol-related complications has not yet been studied, but an impact on e.g. withdrawal seizures could be expected, considering the impact of hyperhomocysteinemia for alcohol-related complications, page 145.

Interaction with drug treatment

Antiepileptics

Depletion of active folate forms through inhibition of polyglutamation by antiepileptics, page 96, may be modulated by the MTHFR genotype, and it was recently reported that the homozygous C677T polymorphism of the MTHFR gene significantly increased tHcy in epileptic patients receiving anticonvulsants.

These patients may have an increased folate requirement *(Yoo JH and Hong SB, 1999)*. Folate deficiency was found in 44.4% of homozygous patients treated with anticonvulsants versus 0% in heterozygotes and patients without the polymorphism in another study. Hyperhomo-

cysteinemia was also commoner among homozygous patients *(Ono H et al., 2002)*.

In a study of 57 infants with malformations, whose mothers had taken anticonvulsants and 152 controls, it was also shown that the C677T polymorphism during pregnancy was significantly associated with fetal anticonvulsant syndrome in the offspring *(Dean JCS et al., 1999)*.

Levodopa

A Japanese study showed that tHcy levels were increased in 60% of 90 L-dopa- treated patients with Parkinson´s disease, and that patients homozygous for the C677T polymorphism of MTHFR had the most marked elevation. The tHcy and folate levels were inversely correlated in this group *(Yasui K et al., 2000)*. Additional data from this group *(Yasui K et al., 2001)* and other recently reported data *(Kuhn W et al., 2001)* firmly support that this polymorphism is a significant factor for hyperhomocysteinemia in L-dopa-treated patients. Homocysteine monitoring may therefore be of particular value for PD patients with this polymorphism.

Sulfasalazine

In rheumatoid patients treated with MTX or sulfasalazine, tHcy concentrations are also higher in patients with the TT genotype of the C677T polymorphism than in patients with the CC genotype *(Haagsma CJ et al., 1999)*. The presence of the TT or CT genotype was associated with an increased risk of discontinuing MTX treatment because of adverse events (OR = 2.01) among rheumatiod arthritis patients *(van Ede AE et al., 2001)*.

Other drugs

A preliminary report suggests that essentially all cancer patients experiencing severe toxicity from the drug combination MTX, cyclophosphamid, and fluorouracil, possessed the TT genotype *(Toffoli G et al., 2000)*. An increased treatment-related toxicity has also been reported in bone marrow transplant patients treated with MTX for graft-versus-host disease prophylaxis in patients homozygous for this polymorphism *(Ulrich CM et al., 2001)*. Increased MTX in vitro sensitivity was also determined for pediatric acute lymphoblastic leukemia *(Taub JW et al., 2002)*.

In hypercholesterolemic children treated with cholestyramine, only children with one or two T alleles had a significant increase in tHcy *(Tonstad S et al., 1998)*.

Carriers of the C677T polymorphism require higher vitamin doses for lowering of tHcy. Vitamin treatment in postmenopausal women, homozygous for the C677T polymorphism, did not decrease tHcy levels to the same degree as demonstrated for women without this polymorphism *(Brown CA et al., 1999)*.

Another study of postmenopausal women showed that tHcy was significantly higher in the low-folate group than in the high-folate group only in women with the homozygous mutant. Hormone replacement therapy, however, decreased tHcy in both groups *(Somekawa Y et al., 2002)*.

A 4-week administration of 15 mg of tetrahydrofolate daily in a study of C677T homozygous patients with early onset thrombotic events, markedly lowered tHcy, but the response correlated with vitamin B_{12} levels, which indicates that the polymorphism may also increases vitamin B_{12} requirements *(D´Angelo A et al., 2000)*.

When and how to check Homocysteine Levels?

When should hyperhomocysteinemia be suspected?

Table 7 and fig. 25 give an overview of factors that increase tHcy and indicate the levels that are to be expected in various conditions. The commonest reasons for elevated tHcy are functional vitamin deficiency owing to low intake, deficient absorption, decreased enzymatic activity, interactions with drugs or lifestyle factors, and increased requirement caused by diseases.

There are some diseases, symptoms, and situations in clinical practice that are commonly associated with vitamin B_{12} and/or folate deficiency. Hyperhomocysteinemia is an early marker of cellular, *functional* deficiency of these vitamins. In contrast, assays of serum/plasma levels of vitamin B_{12} or folate do not mirror intracellular status. Determination of RBC folate is considered to be a better measure of tissue stores, but serious questions have been raised about the sensitivity and specificity of the isotopic methods used *(Lindenbaum J et al., 1995)*. Determination of different folate forms would probably confer advantages.

Elderly patients often have an inadequate consumption of dietary micronutrients in addition to atrophic changes in the gastric mucosa, which further reduce the supply. Various other physiological functions affecting Hcy metabolism may decline with age (page 72). This population in particular is thus at risk of developing hyperhomocysteinemia. Early stages of Hcy-related neuropsychiatric disease can easily be overlooked or attributed to normal age-related changes. If untreated, these symptoms may become irreversible within a year *(Martin DC et al., 1992)*. Early diagnosis of a disturbed Hcy metabolism is therefore essential.

In women with previous recurrent spontaneous abortions or other pregnancy complications, hyperhomocysteinaemia may indicate a treatable disorder.

Although there are not yet any results of large-scale controlled intervention studies that confirm that Hcy-lowering therapy does change, for

	Effect
Genetic factors	
Homozygosity for CBS defects	↑↑↑
Homozygosity for MTHFR defects	↑↑↑
Cobalamin mutations (C, D, E, F, G)	↑↑↑
Down's syndrome	↓
Thermolabile MTHFR	↑
Heterozygosity for CBS defects [1]	↑
Heterozygosity for MTHFR defects	↑
Physiologic determinants	
Increasing age	(↑)
Male sex	(↑)
Renal function, reduced GFR	(↑)
Increasing muscle mass	(↑)
Lifestyle determinants	
Vitamin intake	↓
Smoking	(↑)
Coffee consumption	(↑)
Ethanol consumption	↑↓
Physical activity	↓
Clinical conditions	
Folate deficiency	↑↑
Vitamin B12 deficiency	↑↑↑
Vitamin B6 deficiency1	↑
Renal failure	↑↑
Hyperproliferative disorders	↑
Hypothyroidism	↑
Drugs	
Folate antagonists (methotrexate)	↑
Vitamin B12 antagonists (nitrous oxide)	↑↑
Vitamin B6 antagonists1	↑
AdoHcy hydrolase inhibition	↓
Antiepileptic drugs	↑
Contraceptives, hormone therapy	↓
Aminothiols (acetylcysteine, penicillamine)	↓
Others (L-dopa, cholestyramine, niacine)	↑

Table 7 – Determinants of plasma tHcy. (↓) = Reduction of the total homocysteine level; (↑) = increase within normal reference range; ↑, ↑↑, ↑↑↑ = moderate (15-30 µmol/L), intermediate (30-100 µmol/L), and severe hyperhomocysteinemia (>100 µmol/L), respectively. [1] In subjects with vitamin B_6 deficiency or mild defects in CBS, the fasting total homocysteine level is usually normal but the postmethionine load level is increased. *Refsum H et al. Homocysteine and cardiovascular disease. Ann Rev Med 1998; 49:286-94. Reprinted with permission from the Annual Rewiew of Medicine, © 1998, by Annual Reviews.*

instance, the outcome of cardiovascular disease, there is certainly enough scientific evidence to justify recommendations to modify lifestyle factors in order to decrease tHcy concentrations. This is especially true, as such recommendations are well within the scope of generally accepted lifestyle guidelines for patients at risk of cardiovascular disease. Changes in lifestyle factors are important predictors over tHcy-changes over time *(Nurk E et al., 2001b)*.

Patients´ motivation to change their lifestyle may be prompted by the awareness of having a modifiable risk factor of vascular disease and other diseases and complications.

Hyperhomocysteinaemia may be suspected in any of the conditions discussed in this book. The most important conditions are listed in table 8.

Blood Sampling

Sample handling is of critical importance. Careless handling can result in artificially high levels of tHcy. Blood cells continuously form Hcy, which is exported into the plasma. The increase is about 10% per hour at room temperature. The sample should therefore be put on ice immediately to slow down the process, and plasma should be separated out as soon as possible. This is also the reason why tHcy should not be measured in serum, because the tHcy concentration will increase by 5-10% during the time required for completion of coagulation before centrifugation.

If EDTA is used as anticoagulant, blood should be centrifuged immediately after sampling. After centrifugation, plasma is stable for up to 3 days at room temperature and for several weeks at 0-2ºC *(Ueland PM et al., 1993)*.

Fluoride partially inhibits further formation of Hcy in blood cells *(Ubbink J et al., 1992)*, but seems to cause osmotic dilution through hemodialysis. Fluoride therefore initially causes a decrease in tHcy levels, followed by a subsequent increase *(Hughes M et al., 1998a, Hughes M et al., 1998b)*. Recent studies indicate that acidic citrate may be a preferable anticoagulant, as it stabilises blood samples for 4-6 hours in room temperature *(Willems H et al., 1998, Salazar JF et al., 1999)*. Mean concentrations in these two media may, however, vary significantly, so that different reference values may be needed *(Salazar JF et al., 1999)*.

Although significant changes in plasma concentration are unlikely to occur within the first two hours of blood sampling *(Møller J and Rasmussen K, 1995)*, it is preferable to separate out the plasma immediately.

Patients and special events/symptoms in which hyperhomocysteinaemia should be suspected

Elderly complaining of:
- Vertigo
- Asthenia
- Loss of weight
- Impaired memory

Neurological and psychiatric symptoms:
- Numbness in hands and feet
- Ataxia
- Impaired reflexes
- Confusion
- Irritation
- Concentration difficulties

Dementia
Depression
Chronic fatigue syndrome
Fibromyalgia
Symptoms of anemia:
- Tiredness
- Apathy
- Effort dyspnoea

Adverse reactions to anaesthesia/surgery
After resection of the stomach or ileum
Gastrointestinal disease:
- Atrophy of the mucosa
- Gluten-induced enteropathy
- Crohn´s disease

Inadequate nutrition
Vegans/vegetarians
Autoimmune diseases:
- Diabetes
- Rheumatoid arthritis
- Hypothyroidism

Renal disease
Previous pregnancy complications
Previous cardiovascular events
Spontaneous thrombosis
Anaesthesia with nitrous oxide
Patients taking drugs affecting Hcy
Lens luxation
Vitiligo
Hair loss
Marfanoid appearance

Table 8

Timing of food ingestion before the sampling may also be important. A protein-rich meal can increase the tHcy levels significantly, particularly the free fraction.

After a protein-rich evening meal, tHcy is found to start to rise after about 3 hours, and a maximum increase of 15-20% is reached within 6-8 hours *(Guttormsen AB et al., 1994b, Nurk E et al., 2001a)*. The values slowly return to those measured before the meal (within 12-20 hours). In contrast, after an ordinary continental breakfast, tHcy tends to decrease during the following 1-4 hours *(Guttormsen AB et al., 1994b)*. There may be some circadian variations in tHcy, with the lowest values in the morning *(Bremner WF et al., 2000)*.

Vitamin status influences the response to a methionine-rich diet. No corresponding changes in fasting tHcy were seen in young normohomocysteinemic men, despite changes in methionine intake of up to 100% during one week, when vitamin levels were adequate *(Ward M et al., 2000)*.

It is, however, generally recommended that the subject should be fasting at the time of blood sampling, but more important, attention should be paid to sampling time in relation to a protein-rich meal. Rasmussen and co-workers recommend a light meal in the evening before blood collection that should take place no longer than 3 hours after an ordinary continental breakfast *(Rasmussen K et al., 2000)*.

It is also important to standardise the posture of the patients at sampling, as the position of the subject may have a much greater effect than the assay imprecision.

About 80% of Hcy is bound to albumin, and the albumin concentration is lower in the supine than in the sitting position. This may contribute to orthostatic changes in tHcy. After 30 min of supine rest, levels were up to 29.9% lower than levels measured in upright position in a recent study, but there was only a weak correlation with plasma albumin, which may indicate that other mechanisms may also be implicated. Mean levels were 19% or 2.1 µmol/L lower in the supine position *(Thirup P and Ekelund S, 1999)*.

In another study, in which subjects were seated for 15 minutes before sampling, the mean decrease was only 6.3% with a maximum change of 15.3%. Changes correlated significantly with the change in albumin in this study *(Rasmussen K et al., 1999)*. Rasmussen and co-workers recommend that blood collection is avoided in a supine position, as most patients are ambulatory.

Mean within-person changes in fasting tHcy were in the same order in 5 studies, or between 7.0% and 9.4% *(Cobbaert C et al., 1997, Garg K et al., 1997, Clarke R et al., 1998a, Rasmussen K et al., 1999, Rossi E et al., 1999)*, whereas it was somewhat higher or 15.2%, in another recent study *(Thirup P and Ekelund S, 1999)*.

When tHcy levels were checked at a longer interval (mean 63 days), both fasting and PML varied 15% to 23% in a study of normo- and hyperhomocysteinemic individuals. The variability in this study was significantly correlated to folate status, and especially in normohomocysteinemic subjects *(Van den Berg H et al., 1999)*.

Total Hcy may fluctuate during the menstrual cycle. Mean tHcy levels increased from 7.8 µmol/L in the luteal phase to 8.9 µmol/L in the follicular phase, in a study of 15 premenopausal women *(Tallova J et al., 1999)*. No significant variation was seen, however, in a later study of 10 women *(Merkli-Feld GS et al., 2000)*.

As intra-individual short-term concentrations are fairly constant, a single measurement may characterize the average concentration reasonably well in clinical practice *(Garg K et al., 1997, Clarke R et al., 1998a, Tsai MY et al., 2001)*. The variation may be large enough, however, to confound findings in clinical trials. Repeated measurements would more accurately assess an individual´s tHcy status.

The use of serum instead of plasma may also lead to an overestimation of tHcy by 10%, which may compromise comparisons of studies *(Pfeiffer CM et al., 1999)*.

Standardisation of tHcy assays is underway to exclude between-method and between-laboratory variations.

Seasonal variation with about 2 µmol/L higher mean levels during the summer is recently reported *(Bates C et al., 2002)*. The explanation might be inactivation of vitamin B_{12} and folate by UV light.

An extensive, recent review gives an excellent overview of questions related to tHcy measurements in clinical practice *(Rasmussen K et al., 2000)*.

Methionine loading

Methionine loading is a stress test of the Hcy metabolism and mirrors the physiological postprandial changes in tHcy. The test consists of oral administration of methionine 100 mg/kg, often dissolved in orange juice. Baseline tHcy is determined. Post-methionine loading (PML) values are generally measured after 4 or 6 hours. Attempts to introduce two-hour measurements after loading as clinical routine have failed, as the PML results were not reliable *(Cattaneo M et al., 1998a)*. However, a recent study indicated that measurement after 3 hours had almost equal discriminating power compared to the 6-h test *(De Jonge R et al., 2001)*.

The PML values are usually about 2-3 times higher than the fasting tHcy levels, table 9, page 172.

Methionine loading is mainly a test of the vitamin B_6-dependent transulphuration pathway. Mild CBS defect and vitamin B_6 deficiency increase the PML response *(Ubbink J et al., 1996)*. In connection with increased PML, tHcy vitamin B_6 status should therefore be determined.

However, pathological methionine loading tests are also encountered in connection with MTHFR polymorphisms *(Nelen WLDM et al., 1998, Verhoeff BJ et al., 1998, Candito M et al., 1999, de Jong SC et al., 1999b)*. Low levels of folate or vitamin B_{12} also lead to a relatively higher increase in PML, which shows that remethylation is also of importance *(Verhoeff BJ et al., 1998, de Jong SC et al., 1999b, Ubbink J et al., 2001)*.

Other genetic factors may also contribute to increased PML tHcy *(de Jong SC et al., 1999b)*.

The methionine loading test could be particularly valuable in patients with previous complicated pregnancy pregnancy outcome, or with hereditary or other risk factors for vascular disease, and who have normal fasting tHcy levels, but also in other conditions, in which enzyme defects may be overrepresented, pages 151-161.

Determination of fasting tHcy alone failed to identify over 40% of subjects with methionine intolerance in one study *(Bostom et al., 1995c)*. In the European Concerted Action Project, 27% of subjects at risk were discovered only after PML *(Graham IM et al., 1997)*.

In a recent prospective study, subjects with premature arterial disease and their first-degree relatives were tested fasting and PML. Hyperhomocysteinaemia was found in 33% of the entire sample. Of the hyperhomocysteinemic persons, 55% were diagnosed only after methionine loading *(Van der Griend R et al., 1998)*. Similar results were found in a recent Dutch study *(Keijzer MBAJ et al., 2001)*.

Plasma levels of free Hcy *(Chambers JC et al., 2001)* or SAH *(Kerins DM et al., 2001)* may be more sensitive markers than tHcy of CVD and possibly other diseases, but assays are not available for clinical practice.

An overview of questions relating to tHcy assessment was published by Refsum and co-workers in 1997.

How to interpret the Test Results and how to handle Hyperhomocysteinemia?

Reference ranges

Each laboratory uses a central 95% reference range based upon results from a general population. The reference limits are generally within the range of 5 and 13-15 µmol/L.

The distribution of tHcy is, however, skewed towards higher levels in the general, presumed healthy, population, fig. 1, page 18. Table 9 shows the values, fasting and PML, for men and women in different age groups, that were found in the 800 controls of the European Concerted Action Project on Homocysteine and Vascular Disease *(Refsum H et al., 1998b)*.

Whether Hcy reference intervals should be based on such a skewed distribution is currently under debate. A non-optimal supply of cofactors, negative lifestyle factors, as well as age-related changes and enzymatic defects may be responsible for this skewness. This was illustrated by data from 11,941 subjects in the Hordaland study. The skewness almost disappeared when non-smokers with low coffee consumption and a high folate intake were studied separately. The median concentration of tHcy in these subjects was 3.0-4.8 µmol/L lower than in the rest of the population *(Nygård O et al., 1998)*.

Supplementation with vitamin B_6, B_{12}, and folate in elderly subjects resulted in a decreased upper reference limit from 21 µmol/L to 13 µmol/L in another study. The lower reference limit was reduced from 6.8 to 5 µmol/L after vitamin supplementation *(Joosten E et al., 1996)*.

Rasmussen and co-workers have calculated reference intervals based on samples from 235 subjects before and after folate supplementation *(Rasmussen et al., 1996)*. From these data, they proposed the following age and gender-specific central 95% intervals:

Subjects aged under 30 years:
4.6-8.1 µmol/L

Women aged 30-59 years:
4.5-7.9 µmol/L

Subjects aged over 60 years:
5.8-11.9 µmol/L

Men aged 30 - 59 years:
6.3-11.2 µmol/L

Percentile µmol/L	2.5	5	10	20	50	80	90	95	97.5
All (n = 800)									
Fasting	5.9	6.4	7.0	7.7	9.5	12.1	13.4	15.7	18.5
PML	17.4	19.2	21.5	24.2	29.9	38.0	43.7	51.3	58.1
Increase	10.4	11.6	13.1	15.4	19.8	27.3	31.6	37.2	42.8
Men < 45 year (n = 232)									
Fasting	6.3	6.8	7.2	7.8	9.6	11.9	12.9	15.8	18.8
PML	17.3	19.0	20.9	23.8	29.6	39.8	47.5	56.4	60.9
Increase	10.4	11.4	12.3	15.2	19.3	28.2	34.1	39.1	44.7
Women < 45 year (n = 135)									
Fasting	4.8	5.3	6.0	6.6	8.1	10.4	12.6	13.6	16.9
PML	16.0	16.6	17.9	20.0	26.0	34.1	40.2	44.7	48.2
Increase	8.9	9.9	11.3	13.1	17.8	23.9	30.1	32.1	34.9
Men ≥45 year (n = 338)									
Fasting	6.8	7.2	7.8	8.5	10.3	12.6	13.7	15.9	18.6
PML	21.0	22.0	23.6	25.7	31.0	38.0	42.9	50.3	57.9
Increase	12.8	13.8	14.9	16.2	20.6	26.6	30.1	35.5	41.7
Women ≥45 year (n = 95)									
Fasting	5.8	6.3	6.7	7.1	9.1	11.4	12.9	13.7	15.1
PML	16.8	19.6	21.5	24.0	31.2	36.9	42.2	50.1	54.2
Increase	10.1	12.0	13.7	15.3	21.4	28.2	33.3	40..3	43.2

Table 9 – Fasting, postmethionine load (PML) and increase in plasma tHcy level after loading in the control group of the European Concerted Action Project on homocysteine and vascular disease. *Refsum H et al. Homocysteine and cardiovascular disease. Ann Rev Med 1998; 49:286-94. Reprinted with permission, from the Annual Rewiew of Medicine, © 1998 by Annual Reviews.*

These reference intervals coincide well with a non-differentiated reference interval calculated by using subjects´ responses to appropriate vitamin supplementation and applying a mathematical prediction model, which gave a reference interval of 4.9-11.7 µmol/L *(Ubbink J et al., 1995)*.

It is important to be aware of these age and gender-dependent variations in "normal" concentrations, as *conventionally defined upper reference*

limits could be misleading. Mean levels in different patient groups are often in the range 12-14 µmol/L, that is, generally under the defined upper reference limit.

A special group is pregnant women, whose tHcy levels are considerably lower than those of non-pregnant women. In a recent study the mean tHcy level was 5.6 µmol/L during the first, 4.3 µmol/L during the second, and 3.3 µmol/L during the third trimesters *(Walker M et al., 1999)*.

The Nutrition Committee, American Heart Association, recently indicated values higher than 10 µmol/L, as a cut-off value for patients with augmented risk status such as malnutrition, malabsorption syndromes, hypothyroidism, renal failure, or with a family history of premature cardiovascular disease, and for patients taking drugs *(Malinow MR et al., 1999)*.

Interpretation

The high prevalence of hyperhomocysteinemia in a presumed healthy population may surprise the clinician.

Hyperhomocysteinaemia is often subdivided into moderate (15-30 µmol/L), intermediate (30-100 µmol/L), and severe (more than 100 µmol/L) *(Kang S et al., 1991)*.

If these criteria are applied, up to 10% of the general population can be expected to have moderate, about 1% intermediate, and 0.02% severe hyperhomocysteinaemia, depending on the population tested *(Nygård O et al., 1995)*. Even higher prevalences may be expected, if the subjects to be tested for tHcy are selected according to the criteria listed in table 8. Of all the samples sent by general practitioners for tHcy analysis at the Haukeland University Hospital, Norway, over 30% show hyperhomocysteinaemia (Schneede, personal communication). If reference limits as those proposed by, for instance, Rasmussenand co-workers should be used the prevalence would obviously increase further.

The most common causes of hyperhomocysteinemia will differ in the three groups.

Unhealthy lifestyle, poor nutrition, the C677T polymorphism of the MTHFR gene combined with low folate status, isolated folate deficiency, renal failure, diseases with increased cell proliferation, and drug effects predominate as causes of *mild hyperhomocysteinemia*.

The commonest causes of *intermediate hyperhomocysteinemia* are the C677T polymorphism combined with folate deficiency, moderate vitamin B_{12} deficiency, severe folate deficiency, and severe renal failure.

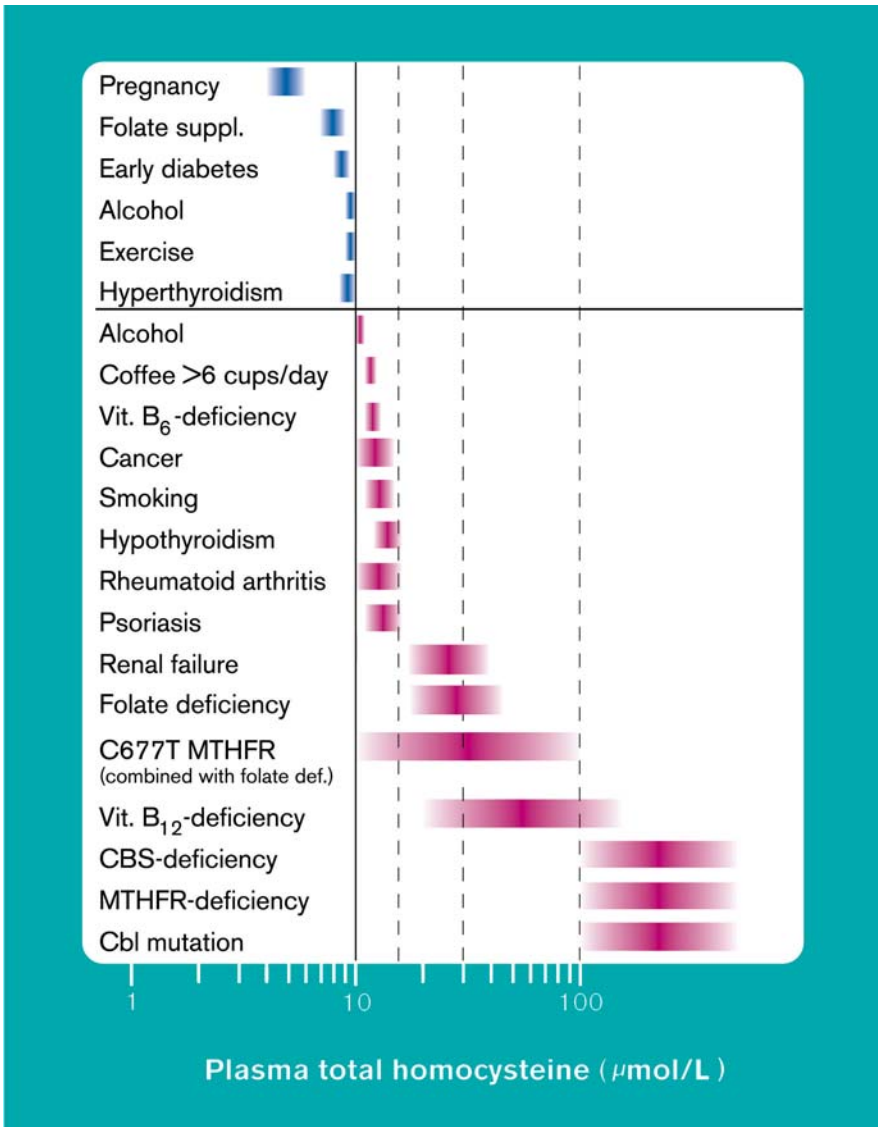

Figure 25 – Schematic representation of the effect from an isolated determinant on the expected mean homocysteine level. Normal value for homocysteine is defined as 10 µmol/L, and factors that reduce or increase homocysteine are sorted and separated by the horizontal line.The width of the bar does not indicate the range of homocysteine values but rather the uncertainty of the estimate, related to the extent or severity of disease or variable response. The estimates are not based on published data, but rather reflect an overall impression of the authors. *Ueland PM et al. Determinants of plasma homocysteine. Developments in Cardiovascular medicine. 2000; 230:62.*

Severe hyperhomocysteinemia is in most cases caused by severe vitamin B_{12} deficiency or by severe CBS defect.

Intervention

The tHcy assay is a sensitive tool for early diagnosis of disturbed remethylation and transsulphuration, *irrespective of the causes*. A high concentration of tHcy without known underlying diseases should, however, prompt further investigation. Most important, renal failure, gastrointestinal disease including malignancies and coeliac disease with malabsorption of cofactors, poor nutrition, myeloproliferative disorders and thyroid disease must be ruled out.

Many factors may contribute to high tHcy

From about 20% up to 40% of western adult populations smoke and up to 20% of these populations drink enough alcohol to influence vitamin status. Most people consume coffee, many of them excessively. A large proportion of the elderly have disturbed gastrointestinal function. About 10% of any Caucasian population are homozygous and 40% heterozygous for the C677T polymorphism of the MTHFR gene.

Elevated vitamin B_{12} levels in serum, found in alcoholics, does not exclude tissue deficiency, page 64. Concomitant elevated tHcy and also MMA, indicating tissue deficiency, are common. Serum folate may be high, but often decreases when vitamin B_{12} substitution is given, which indicates tissue distribution. Conversely, in folate deficiency, serum B_{12} may decrease when folate substitution is given.

A number of factors thus influence tHcy levels. It seems obvious that the cause of hyperhomocysteinemia is, in most cases, multifactorial.

Before any intervention, one should look for and – if possible – eliminate causes of hyperhomocysteinemia. In many cases, a change in lifestyle may be enough to lower tHcy levels. It has, for instance, already been demonstrated that high tHcy levels caused by high alcohol intake of rapidly decrease after alcohol consumption is stopped *(Hultberg B et al., 1993a)*.

Cessation of smoking improves the lipid profile, decreases thrombotic tendency, reduces vascular endothelial damage, and improves insulin sensitivity *(Eagles C and Martin U, 1998)*. Many of these effects may be owing to improved homocysteine metabolism.

Modification of dietary pattern, with increased consumption of fruit and vegetables, and reduced consumption of saturated and total fat con-

sumption, may also have substantial effects on tHcy, as shown in an 8-week intervention study *(Appel LJ et al., 2000)*.

Just as there are interactions between the tHcy-elevating factors, so is there an interplay between tHcy-reducing factors. Even if many of the underlying causes cannot be modified (diseases, enzyme defects), tHcy levels can still be lowered by improving lifestyle and vitamin intake.

Modification of the dietary pattern may have substantial effects on folate and tHcy, and in particular a switch to a diet rich in fruit, vegetables, and low-fat dairy product and a low content of saturated and total fat *(Appel LJ et al., 2000, Broekmans WMR et al., 2000)*. Dairy products seem to protect against B_{12} deficiency *(Tucker KL et al., 2000)*.

The new American Dietary Reference Intakes for folate, were recently reported by the National Academy of Science. For adults aged 19 years and over, the RDA is 400 µg/day of dietary folate equivalents; for lactating and pregnant women, an additional 100 and 200 µg /day respectively are recommended *(Bailey LB, 2000)*.

A recent study, in which urinary folate catabolites were measured during pregnancy and in control women, showed a progressive increase in these metabolites and peak in the third trimester. From these results, the authors recommend that the dietary allowance of folate should rise to 430 µg during the second and 540 µg during the third trimester *(Higgins JR et al., 2000)*.

NTDs are among the most serious birth defects. About 4,000 pregnancies are affected each year in the USA. Up to 70% of these cases could be expected to be prevented if women consumed 400 µg of folic acid daily for at least one month prior to pregnancy and during the first trimester *(Am Public Health Association, 2000)*.

Folic acid is, in general, absorbed more efficiently than is dietary folate.

Folic acid food fortification programmes

Several countries have opted for fortification of cereals with folic acid, among them USA and Canada, primarily to reduce the risk of NTD. In 1996, the FDA issued a regulation requiring all enriched grain products to be fortified with folic acid – 140 µg per 100 g. The process was essentially complete by mid-1997.

It was recently reported that the fortification increased folic acid intake by a mean of 190 µg/day in nonsupplement users, and the prevalence of individuals with a daily intake below the estimated average requirement decreased from 48.6% before fortification to 7.0% after fortification. The

increase of folic acid intake was approximatievly twice as large as previously projected *(Choumenkovitch SF et al., 2002)*. The actual folate fortification was earlier estimated to lower tHcy as much as an increase of consumption of folate rich food *(Riddell LJ et al., 2000)*.

Impact on folate status in pregnancy

Young women consuming fortified food had a higher folate intake and RBC folate than had women who did not consume fortified food. When fortified food was excluded from the diet of young women for 12 weeks, the RBC folate decreased significantly, or from a mean of 881 nmol/L to 770 nmol/L *(Cuskelly et al., 1999)*.

The increase resulting from fortification is predicted to have a significant, but not optimal, effect in preventing NTD. Persons needing a folate supplement most, do not necessarily choose enriched food, and the amount supplied has been calculated to be inadequate in many cases *(Malinow et al., 1998b)*.

Two national food consumption surveys in the USA were recently updated to reflect folate intake as a result of the fortification. The findings suggested that 68-87% of females of child-bearing age still had intakes below current standards *(Lewis CJ et al., 1999)*.

Based on a study of 1,136 American mothers of infants with major malformations, the conclusion was drawn that even with folic acid fortification, women of child-bearing age should be advised to take supplements containing folic acid on a daily basis *(Werler MM et al., 1999)*.

Nevertheless a 19% reduction of NTD birth incidence following the fortification of the US food supply is recently reported *(Honein MA et al., 2001)*. In Canada the frequency of NTD was recently reported to have decreased from 1.13 to 0.58 per 1,000 pregnancies since fortification was implemented *(Ray et al. 2002).*

Some countries have preferred to opt for programmes promoting folate supplementation for women of child-bearing age. Although folic acid supplements are very effective in optimising folate status, they may not necessarily be the most efficient strategy for decreasing the incidence of NTD as compliance is uncertain. Moreover, treatment aiming at reducing NTD and other malformations or pregnancy complications *must be given before pregnancy*, as the neural tube closes already three weeks after conception. At that stage, the woman may not even be aware of the pregnancy. Other organs are formed soon after.

Effects of folic acid fortification in other population groups

Of 1,106 middle-aged and older subjects in the Framingham Offspring Study, 350 were seen after fortification had begun, the controls were seen before. Among the 350 subjects who did not use vitamin supplements the mean plasma folate concentration increased significantly, or from 4.6 to 10.0 ng/ml from the baseline visit to the follow-up visit. The prevalence of low plasma folate levels decreased from 22.0 to 1.7% and the mean tHcy concentration decreased significantly from 10.1 to 9.4 µmol/L *(Jacques PF et al., 1999a)*.

Data from Kaiser Permanente members show a continuous increase in median serum folate from 11.7 ng/ml in 1996 to more than 20 ng/ml in 1999 *(Lawrence JM et al., 2000)*. A parallel inverse trend in tHcy levels was found in a large number of specimens between September 1997 and August 1999 at another American clinical reference laboratorium *(Komaromy-Hiller G and Nuttall KL., 1999)*. Cessation of habitual ingestion of fortified breakfast cereal was also recently shown to result in a significant increase in tHcy *(Malinow MR et al., 2000)*.

Two US national food consumption surveys, updated to reflect folate intake as a result of the fortification, suggest that the majority of the general population, with exception for pregnant women, meet or surpass the new estimated average requirement *(Lewis CJ et al., 1999)*, but there are some reasons for concern.

Risk of masking vitamin B_{12} deficiency

Apart from the risk of inadequate supply, particularly during pregnancy, there is another concern about fortification. Large-scale food enrichment with folic acid alone might camouflage a vitamin B_{12} deficiency, and possibly delay its diagnosis and treatment *(Tucker KL et al., 1996b, Rothenberg SP., 1999)*.

Recently presented data from the Framingham offspring study, covering 2,999 subjects aged 26-83, showed that 39% of subjects had B_{12} concentrations below 258 pmol/L, 17% had levels below 185 pmol/L, and 9% had levels below 148 pmol/L. Supplement users were significantly less likely than non-supplement users to have low levels. Food choice substantially influenced vitamin B_{12} levels *(Tucker KL et al., 2000)*.

In April 1998, the Institute of Medicine recommended adults 50 years of age or older to consume 2.4 µg of synthetic vitamin B_{12} daily (*Institute of Medicine,* 1999).

Vitamin supplementation

Vitamin supplementation may normalise metabolite levels even when serum vitamin levels are within the normal range *(Nilsson K et al., 1994)*, or even in the high range *(Faurschou M et al., 2000)*. Metabolic, environmental, and genetic factors can make individual nutrient requirements different from the estimated needs calculated from population-based data.

A placebo-controlled study of 151 patients with ischemic heart disease, in which different doses of folic acid were given, indicated that a dosage of folic acid of 0.8 mg daily is necessary to achieve a maximum reduction in tHcy *(Wald DS et al., 2001)*. Current fortification levels supply only a small proportion of this dose.

Several studies, however, have shown that combinations of folate with vitamin B_{12} and/or vitamin B_6 more efficiently lower tHcy than folate alone. Such combinations have been shown to normalise tHcy levels in most cases, irrespective of the causes of the hyperhomocysteinemia, and both in patients and in healthy individuals *(Ubbink J et al., 1993, Brattström L., 1996, Malinow MR et al., 1997, Bronstrup A et al., 1998, Den Heijer M et al., 1998, Woodside JV et al., 1998, Lobo A et al., 1999, Mansoor MA et al., 1999, Van der Griend R et al., 1999)*.

Different doses and combinations have been used. An overview of randomised trials was published by the Homocysteine Trialists' Collaboration (1998). The conclusion was that a daily supplementation with 0.5-5 mg of folic acid and about 0.5 mg of vitamin B_{12} would be expected to reduce tHcy levels by about 25-30% in a typical population, or for instance from about 12 µmol/L to 8-9 µmol/L. However, the optimal combination should rather be individual than general, as many factors interact.

An overview of published and ongoing homocysteine lowering clinical trials was recently published *(Clarke R, 2000)*.

Folate levels are often normal or high in vitamin B_{12} deficiency. When vitamin B_{12} is given, serum folate may decrease markedly, thus indicating increased formation of active, intracellular folates. Folate supplementation may be required.

As many as one-third of mutations in a gene result in the corresponding enzyme having a decreased binding affinity for a co-enzyme, resulting in a lower rate of reaction. Such genetic defects can be compensated for, completely or partially, by the administration of high doses of the vitamin *(Ames BN et al., 2002)*.

In subjects homozygous for the common C677T polymorphism of the MTHFR gene, tHcy levels can usually be normalised, if higher doses are given *(Guttormsen AB et al., 1996, Malinow MR et al., 1997)*.

PML hyperhomocysteinemia is often a consequence of some enzyme defect.

Vitamin B_6 alone may then decrease PML tHcy significantly, but not fasting tHcy levels. The reduction in PML was, however, smaller after 200 mg of pyridoxine only, than after a combination of 0.5 mg of folic acid and 100 mg of pyridoxine in a recent study of 117 hyperhomocysteinemic vascular patients and their relatives *(Van der Griend R et al., 2000)*.

Vitamin supplementation in the elderly

A recent study in elderly women with moderate folate depletion (118 µg/day for 7 weeks), followed by repletion with different folate doses showed that tHcy was normalised within 7 weeks with a daily dose of 415 µg of folate provided as a combination of diet and folic acid, but not with 200 µg daily *(Kauwell GPA et al., 2000b)*.

Combined folate and vitamin B_{12} depletion is common, particularly in the elderly. A recent intervention study, in which 69 subjects, 70 years or older, with a serum B_{12} less than 300 pmol/L, tHcy of 15 µmol/L or higher, or MMA values of 0.37 µmol/L or more were first treated with vitamin B_{12} alone, showed that folate supplementation was often required as well, to normalise tHcy, in spite of normal vitamin B_{12} and folate levels in the majority of cases *(Björkegren K and Svärdsudd K., 1999)*.

Neuropsychiatric symptoms as a consequence of vitamin B_{12} depletion, which develops very slowly, or of inadequate B_{12} utilisation often require intensive treatment with vitamin $B_{12,}$ and reversibility is dependent on early diagnosis. Therefore, if there are indications of vitamin B_{12} deficiency – symptoms, low vitamin B_{12} levels, gastrointestinal atrophy, heredity, **etc.** – it is important that a diagnosis is made and that efficient therapy is given.

As folic acid is reduced to THF without the need for vitamin B_{12}, fortification/supplementation with folic acid alone may be risky, as folic acid supplements to vitamin B_{12}-deficient patients may correct megaloblastic changes, but allow neurological symptoms to progress.

Vitamin supplementation in renal disease

Renal function is an important determinant of fasting tHcy *(Bostom AG et al., 1999b)*, but elevated tHcy levels cannot be reduced efficiently by peritoneal dialysis.

Vitamin treatment, however, can substantially reduce tHcy, as shown in many studies. Folic acid alone, but also combinations with vitamin B_6 and sometimes vitamin B_{12} have been used *(Arnadottir M et al., 1993,*

Bostom AG et al., 1996 (Dierkes J et al., 1999a, Suliman ME et al., 1999, Tremblay R et al., 2000).

Folic acid in doses from 1 to 15mg daily reduces tHcy significantly, although in about 50% of the cases levels remain above 15 µmol/L *(Van Guldener C and Robinson K., 2000)*.

A randomised, double-blind study of 121 haemodialysis patients, showed that daily oral treatment with 30 or 60 mg of folic acid for 4 weeks was not more effective in lowering tHcy than 15 mg of folic acid daily *(Sunder-Plassmann G et al., 2000)*.

Vitamin B_{12} status in renal disease has received less attention than folate. In ESRD the remethylation rate of Hcy is reduced, page 83, which points to a possible critical role for vitamin B_{12}. Supplementation with vitamin B_{12} alone in ESRD was recently studied. One mg of cyanocobalamin was given intravenously once a week for 4 weeks to patients with serum B_{12} levels below 180 pmol/L. All patients had elevated levels of tHcy and MMA before supplementation. After supplementation, tHcy and MMA decreased by 35% and 48% respectively. Serum vitamin B_{12} levels increased significantly, whereas serum folate levels decreased 41%, which illustrates the close interaction between vitamin B_{12} and folate distribution and metabolism *(Dierkes J et al., 1999b)*.

Another recent study found a decrease in tHcy of 19.2% after daily treatment for one month with a multivitamin containing 1 mg of folic acid. A further decrease of 16.7% was attained when 1 mg of vitamin B_{12} daily was added for 4 weeks. No significant additional effect on tHcy was achieved when the daily folate dose was increased to 20 mg *(Manns B et al., 2001)*.

Lower tHcy levels, predialysis and postdialysis, were recently found in patients routinely treated with 1mg of folic acid orally a day plus 1 mg of vitamin B_{12} intravenously a month than in patients treated with daily oral doses of 6 mg of folic acid alone. In the vitamin B_{12} group the mean predialysis and postdialysis levels were 18.2 µmol/L and 10.6 µmol/L versus 23.4 µmol/L and 14.5 µmol/L *(Hoffer LJ et al., 2000)*.

Pyridoxine deficiency is very common in renal failure, and was present in nearly 40% of 168 chronic dialysis patients in a recent study *(Tremblay R et al., 2000)*.

A combination of intravenous doses of 50 mg of folic acid weekly, and 250 mg of pyridoxine three times a week given to 37 haemodialysis patients during a mean of 11.2 months decreased the mean tHcy from 37.3 µmol/L at baseline to 12.3 µmol/L. Out of 37 patients, 29 had normalised tHcy (mean 9.8 µmol/L). No adverse effects were observed *(Touam M et al., 1999)*.

Chronic renal failure is characterised by specific alterations in the lipoprotein metabolism. When haemodialysis patients were given 300 mg

of vitamin B_6 for 4 months and then folic acid 5 mg daily for 4 months, serum total cholesterol and LDL cholesterol decreased significantly after treatment with vitamin B_6, and increased thereafter. There were no correlations between tHcy and vitamin B_6 levels. Folic acid decreased tHcy significantly *(Arnadottir M et al., 1993)*.

However, mean total cholesterol, LDL cholesterol, and triglyceride concentrations also decreased significantly in patients on continous ambulatory peritoneal dialysis, when they were given a supplement of 5 mg of folic acid orally for 4 months. Total Hcy levels decreased 33%. The effect was more marked in these patients than in the haemodialysis patients included in the study *(McGregor D et al., 2000)*.

An oral dose of 15 mg of methyl-THF a day for 2 months lowered tHcy, increased methionine levels in plasma, and at the same time increased both RBC levels SAM and SAH, indicating increased conversion of Hcy to methionine and SAM and increased methylation *(Perna AF et al., 1999)*.

Although vitamins lower tHcy in renal patients, no improvement on the endothelial function of Hcy-lowering treatment has been demonstrated so far. In a double-blind, placebo-controlled study of 100 patients with predialysis renal failure, no significant differences in endothelium-dependent dilatation, combined nitrite/nitrate concentrations, or plasma von Willebrand factor were seen when 5 mg of folic acid was given orally for 12 weeks, in spite of a decrease in tHcy (15.1 µmol/L versus 20.1 µmol/L in the placebo group) *(Thambyrajah J et al., 2000)*.

No major effect on carotid artery stiffness was seen in another study after a 12-week treatment with 5 mg of folic acid daily, with or without 4 mg of betaine, and of 1 or 5 mg of folic acid thereafter for 40 weeks in 41 chronic dialysis patients *(Van Guldener C et al., 2000)*.

A possible future alternative/complementary approach to hyperhomocysteinemia in renal patients might be N-acetyl-cysteine, which was recently reported to increase the renal clearance of Hcy (and cysteine), supposedly by displacing Hcy from plasma binding sites forming mixed disulphides *(Ventura P et al., 1999)*.

In isolated vitamin B_{12} deficiency, tHcy should be normalised within a few weeks after treatment has begun. If not, a concomitant folate and/or vitamin B_6 deficiency may exist. To make sure that the treatment is adequate, the tHcy concentrations should be checked a couple of weeks after initiation of treatment and, if necessary, the treatment should be adjusted. *The tHcy assay is thus both a diagnostic tool and an instrument for following up and optimising treatment.*

Abbreviations

AD	Alzheimer´s disease
ADMA	Assymetrical dimethylarginine
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
BHMT	Betaine-homocysteine methyltransferase
CAD	Coronary artery disease
CAMCOG	Cambridge cognitive examination
CBS	Cystathionine β-synthase
CHD	Coronary heart disease
COMT	Catechol O-methyl transferase
CSF	Cerebrospinal fluid
CVD	Cardiovascular disease
CVR	Cardiovascular reactivity
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EDD	Endothelium-dependent vasodilatation
EPA	Eicosapentaenoic acid
FA	Folic acid
FAD	Flavine adenine dinucleotide
GSH	Glutathione
HbA_{1c}	Glycated haemoglobin
Hcy	Homocysteine
tHcy	Total homocysteine
5-HIAA	5-Hydroxyindole acetic acid
5-HT	5-hydroxytryptamine
holoTC	Holotranscobalamin (transcobalamin bound to cobalamin)
HP	Helicobacter pylori
HRT	Hormone replacement therapy
HVA	Homovanillic acid
IHD	Ischaemic heart disease
IMT	Intima medial thickness

MAT	Methionine-adenosyl transferase
MBP	Myelin basic protein
MDA	Malondialdehyde
MMA	Methylmalonic acid
MMSE	Mini mental state evaluation
MHPG	3-Methoxy-4-hydroxyphenyl glycol
MS	Methionine synthase
MTHFR	Methylene-tetrahydrofolate reductase
MTRR	Methionine synthase reductase
NHANES	National Health and Nutritional Examination Survey
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
N_2O	Nitrous oxide
NTD	Neural tube defect
OPTIMA	Oxford project to investigate memory and aging
OR	Odds ratio
PA	Pernicious anemia
PD	Parkinson´s disease
PML	Post methionine loading
PTCA	Percutaneous transluminal coronary angioplasty
PUFA	Poly-unsaturated fatty acids
RBC	Red blood cell
RCF	Red cell folate
RDA	Recommended dietary allowance
RNA	Ribonucleic acid
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SOD	Superoxide dismutase
SSRI	Serotonin reuptake inhibitors
SVE	Subcortical vascular encephalopathy
TC	Transcobalamin
TCA	Tricyclic antidepressives
THF	Tetrahydrofolate

All stated differences are statistically significant (p values < 0.05-0.001). Highly significant indicates p-values < 0.001.

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