Homocysteine, the New Marker of Disease Risk – An Overview

a report by
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Introduction

Butz and du Vigneaud first described homocysteine in 1932. This sulphur-containing amino acid is closely related to methionine and cysteine. Homocystinuria, a condition in which the homocysteine levels in blood and urine are very high, is caused by some severe enzyme defects. This condition was found to be associated with premature occlusive cardiovascular disease (CVD) and with mental retardation.

In 1969, McCully described the vascular pathology, including smooth muscle proliferation, progressive arterial stenosis and haemostatic changes found in such patients. A large number of epidemiological, case–control and longitudinal studies have since demonstrated an association between moderately elevated homocysteine levels in the plasma or serum and pregnancy complications, neural tube defects, other birth defects, various neuropsychiatric disorders, cognitive impairment in the elderly and an increased mortality rate, in addition to vascular diseases. Research within the field has been very active during the last decade. About 1,000 scientific reports are now published annually.

This research has been made possible by the development of accurate methods for measuring homocysteine in plasma and serum. The recent introduction of enzyme immunoassays is a further step forward. Automated methods using standard immunoassay equipment have been introduced.

The One-carbon Metabolism

Homocysteine is an intermediate product of the one-carbon metabolism. All homocysteine found in mammals is formed during the metabolism of methionine in the methylation cycle (see *Figure 1*). Dietary methionine is used either for protein synthesis or the formation of S-adenosyl-methionine (SAM), which contains a very reactive methyl group. This is transferred to a large variety of acceptor substrates, including nucleic acids (deoxyribonucleic acid (DNA) and ribonucleic acid), proteins, phospholipids, myelin, polysaccharides, choline, catecholamines and a large number of small molecules. SAM is the principal biological methyl group donor in the organism and the only donor in the central nervous system (CNS). S-adenosyl-l-homocysteine (SAH) is hydrolysed in a reversible reaction to homocysteine, which can be recycled to methionine and SAM or directed toward the transsulphuration pathway.

Three enzymes are involved directly in this metabolism: methionine synthase (MS); betaine homocysteine methyltransferase; and cystathionine β-synthase (CBS). Vitamin B_{12} is a co-factor to MS and vitamin B_6 to CBS. Methyl tetrahydrofolate (methylTHF) is a substrate in the MS-mediated reaction. This reaction is also critical for the formation of the active folate forms required for purine and thymidine synthesis and thus for DNA synthesis and repair.

The majority of tissues, including the CNS, are entirely dependent on methyl groups derived from the MS-mediated recycling of homocysteine. This reaction is indirectly regulated by the activity of methyl-enetetrahydrofolate reductase (MTHFR), as this enzyme mediates the formation of methylTHF. This enzyme therefore has a strong, indirect influence on the remethylation of homocysteine.

Normally, about 50% of formed homocysteine is re-methylated. The remaining homocysteine is converted in the transsulphuration pathway to cysteine in two reactions requiring vitamin B_6 as a co-factor. This pathway is important for the synthesis of glutathione. Glutathione protects many cellular components against oxidative damage. The transsulphuration pathway also directs homocysteine to degradation and removal via the urine.

It is understandable that impaired homocysteine turnover may have strong effects on cellular growth, differentiation and function. This may be critical in many situations, not least in the ageing brain with declining neurochemical processes, in psychiatric and neurological diseases and for the rapidly growing foetus and infant.

Genetic Variations Modifying Activity of Implicated Enzymes

A rapidly increasing number of variations of the genes regulating the enzymes that are involved in the one-carbon metabolism have been identified lately. Their impact on homocysteine-related disorders has become a field of very active research. About 200 studies are published on just one of these enzymes, a polymorphism resulting in a thermolabile form of MTHFR, leading to a reduction in enzyme activity. It increases mean homocysteine levels by about 25%. The impact is dependent on vitamin status, particularly folate. This polymorphism is present in its homozygous form in about 10% of the white US and most European populations, but the frequency varies substantially geographically and with ethnicity. The frequency is about 30% in Mexico and about 20% in Italy, whereas it is very low in black populations.[1]

Many Factors Contribute to Increased Homocysteine Levels

Increased levels of plasma homocysteine may be caused by several factors such as enzyme defects, deficient or disturbed distribution and/or increased catabolism of the co-factor(s). Mean homocysteine levels increase throughout life by 3µmol/L to 5µmol/L and the level is higher in men than in women.

There is often a cluster of factors leading to hyperhomocysteinemia (see *Figure 2*). The most frequent causes are an unhealthy lifestyle, a low intake of vitamins, gastrointestinal (GI) malabsorption of these vitamins and enzymatic defects and drug interactions.

Smoking is strongly associated with increased homocysteine levels.[2,3] The effect of smoking on homocysteine levels may be enhanced by high alcohol intake, coffee consumption and inadequate nutrition. Lack of physical exercise, obesity and even stress are associated with elevation of homocysteine levels.

Unhealthy lifestyle factors may therefore offer the main explanation for a moderately increased homocysteine level. This is illustrated by data from the Norwegian Hordaland study. When non-smokers with low coffee consumption and high folate intake were studied separately, the median concentration of homocysteine was 3.0–4.8µmol/L lower than in the rest of the population[2].

The higher concentrations of homocysteine seen in the elderly may be caused by lifestyle factors in combination with malabsorption owing to common atrophic gastritis, reduced metabolism, reduced kidney function and other physiological

Figure 1: Homocysteine Metabolism

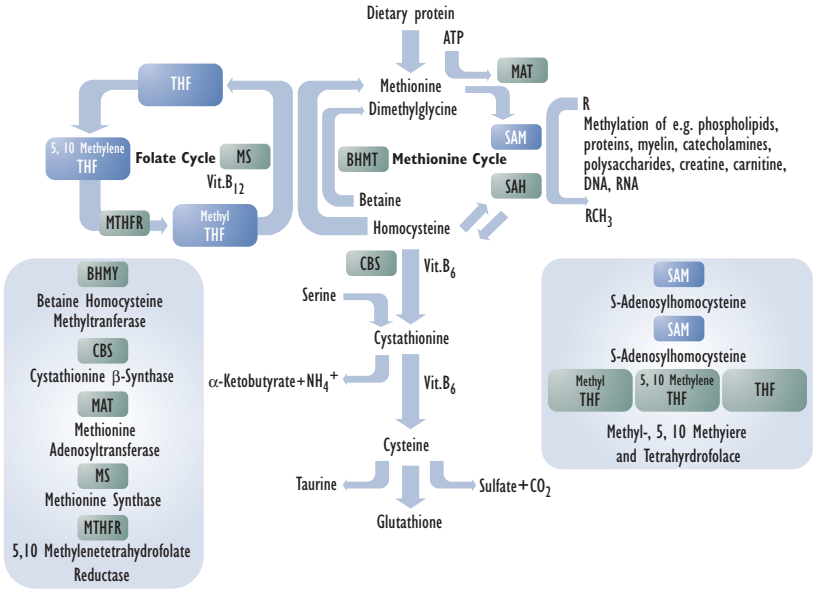
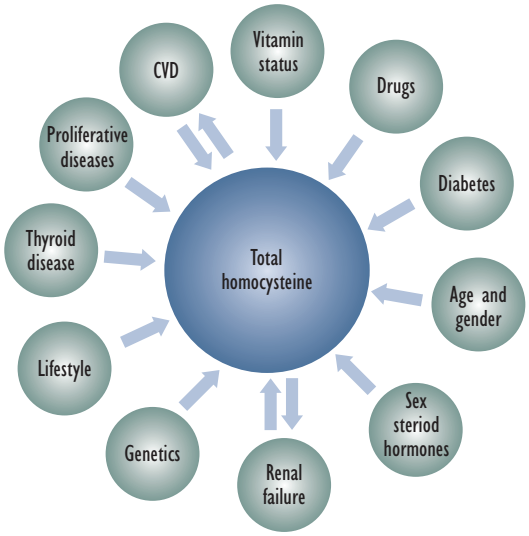


Figure 2: Physiological Determinants of Total Homocysteine Levels



age-related changes. Moreover, many drugs interact with homocysteine metabolism causing a reduction in the absorption of co-factors or an increase in the rate of catabolism of the vitamins. Various autoimmune diseases, such as diabetes, hypothyroidism, rheumatoid arthritis and vitiligo, are associated with elevated homocysteine levels.

An individual's genetic make-up, lifestyle, diseases, drugs they are taking and age-related factors thus determine homocysteine levels.

A Disturbed One-carbon Metabolism May Cause Disorders by Several Mechanisms

Homocysteine levels mirror the function of the one-carbon metabolism. Increased homocysteine levels indicate an impaired methylation capacity. It has also

Table 1: Patients in which Hyperhomocysteinemia can 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
  - Impaired memory
- **Dementia**
- **Depression**
- **Chronic fatigue syndrome**
- **Fibromyalgia**
- **Symptoms of anaemia:**
  - Tiredness
  - Apathy
  - Effort dyspnoea
- **Adverse reactions to anaesthesia/surgery**
- **After resection of the stomach or ileum**
- **Gastrointestinal diseases:**
  - 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 homocysteine levels**
- **Lens luxation**
- **Vitiligo**
- **Hair loss**
- **Marfanoid appearance**

been proposed that homocysteine causes oxidative injury by various mechanisms and interaction with N-methyl-D-aspartate receptors.

These effects may contribute to both vascular and neurological damage. Impaired DNA synthesis and repair caused by disturbed methylation and folate metabolism may promote carcinogenesis and contribute to birth defects.

Vascular Disease

More than 100 epidemiological, case–control and longitudinal cohort studies have established that even mild hyperhomocysteinemia both predicts and precedes the development of cardiovascular morbidity and mortality and is an independent risk factor for cardio- and cerebrovascular diseases.

Homocysteine-lowering treatments showing improvement of cardiovascular reactivity and coagulation factors support the fact that such therapy should be effective. A recent placebo-controlled study found that coronary endothelial function improved significantly in patients with coronary artery disease and homocysteine levels are greater than or equal to 16µmol/L after treatment with folic acid and vitamin B_{12}[4].

Results from the Swiss Heart Study, a placebo-controlled homocysteine-lowering study, showed that homocysteine-lowering therapy improves the outcome after percutaneous coronary intervention both in larger and smaller arteries.[5,6] Reduced progression of atherosclerosis in renal transplant recipients treated with a combination of folic acid and vitamins B_6 and B_{12} was also reported.[7]

Several recent meta-analyses have shown significant associations between CVD and total plasma/serum homocysteine levels both for prospective and retrospective trials and an association with the MTHFR C677T polymorphism,[8–11] although the calculated magnitude of the impact varies. These analyses are complicated because several of the established risk factors, e.g., smoking and high alcohol intake, cause increased homocysteine levels.

All studies agree that the risk associated with the established risk factors is strongly increased by concomitant, elevated homocysteine levels. New data from The European Concerted Action Project, presented at the 4th International Homocysteine conference in 2003, demonstrated for instance that the risk is much higher in smokers who have elevated homocysteine levels, increased total cholesterol, low-density lipoprotein, triglycerides and apolipoprotein B.

Within the next few years, results from other large on-going intervention trials are expected. A positive result from these studies may have an immense impact in the future and the monitoring of reductions in homocysteine levels as a result of vitamin supplementation and lifestyle changes is likely to be common.

Cognitive Impairment and Neuropsychiatric Disorders

During recent years, a lot of data has been generated on the association between elevated homocysteine levels and neuropsychiatric disorders.

A significant association between homocysteine and dementia has been found in several recently

reviewed studies.[12] It was demonstrated that elevated homocysteine is related to the damage of cerebral tissues in elderly people.[13,14] Prospective studies have shown that higher baseline homocysteine levels in elderly people are associated with a more rapid progression of dementia.[15,16] The longitudinal Framingham Study of elderly subjects, all free of dementia at baseline, showed that the risk of developing dementia was increased in subjects with higher baseline homocysteine levels.[17] Reduction of homocysteine levels has been shown to have a positive impact on cognitive performance in the elderly with mild cognitive impairment and to increase regional cerebral blood flow. Early intervention seems crucial, however. Severe underlying neuronal and vascular damage is hardly likely to regress, although studies in animals suggest the possibility of reversing neuronal damage.

Increased homocysteine is also common amongst depressed patients.[18,19] New data from the Norwegian Hordaland Study showed there was a 90% incidence of increased homocysteine levels, greater than 15µmol/L, in depressed patients. The C677T polymorphism of the MTHFR gene was also associated with the increased risk.[20] Several earlier studies have shown that patients with low folate status are more severely depressed than patients with folate in the normal range.[21] A placebo-controlled intervention study of patients with major depression showed that the lowering of homocysteine, by adding folic acid to fluoxetine, significantly improved the therapeutic response.[22] A new report indicates that the therapeutic effect of both an SSRI (sertraline) and a tricyclic antidepressant (nortriptyline) is dependent on baseline red blood cell folate, even though all folate levels were in the normal range. A higher folate status predicted a better outcome and the association was strongest for the sertraline group.[23]

Elevated levels of homocysteine have also been found in cases of schizophrenia, multiple sclerosis, Parkinson's disease and fibromyalgia/chronic fatigue syndrome.

Neural Tube Defects, Other Birth Defects and Pregnancy Complications

Low intracellular levels of folate in mothers of children affected by neural tube defects (NTD) were already reported in 1976.[24] Decreased levels of both vitamin B_{12} and folate were later found in a large study of mothers of babies with NTD.[25] Many studies have now shown that increased maternal homocysteine is associated not only with NTD but also with other malformations such as orofacial clefts, heart defects, spontaneous abortion, low birth weight and other pregnancy complications.[26]

Several placebo-controlled trials have shown that homocysteine-lowering therapy reduces the frequency of NTD both in women with and without a previous NTD-affected pregnancy.[27] Treatment has to begin before pregnancy, as NTD defects are formed very early in pregnancy. The folic acid fortification of grain in the US and Canada has led to a decreased frequency of NTD.[28,29]

When Should Homocysteine Levels be Checked?

Elderly people are at particularly high risk of developing homocysteine-related disorders. Moreover, early stages of neuropsychiatric diseases can easily be overlooked or attributed to normal, age-related changes. If left untreated, these symptoms may become irreversible within a year.[30] Patients with symptoms that might be related to, or have, any condition that may predispose subjects to hyperhomocysteinemia should therefore be checked (see *Table 1*).

Women with previous NTD pregnancies, recurrent spontaneous abortions, or other pregnancy complications should be checked, as these complications may be homocysteine-related and avoidable.

There is also a general consensus that patients at risk for, or with, premature vascular disease should be tested. These patients often display unhealthy lifestyle factors associated with increased homocysteine levels. Motivation to improve their lifestyle may be increased by the awareness of having a modifiable risk factor of vascular disease and other diseases and complications.

Sampling and Handling of Samples

Timing of food ingestion before the sampling can be important. A small meal will not influence homocysteine levels in healthy people, but intake of a protein-rich meal can increase levels significantly. After a protein-rich evening meal, levels start to rise after about three hours. A maximum increase of 15% to 20% is reached within six to eight hours. The values return to those measured before the meal within 12–20 hours.

Careless handling of samples can result in artificially high levels, as blood cells continuously form homocysteine that is exported into the plasma. The increase is about one micrometre per hour at room temperature; therefore plasma should be separated out as soon as possible. Meanwhile, the sample should be put on ice to slow down the process.

An extensive review gives an overview of questions related to homocysteine measurements in clinical practice.[32]

Normal Homocysteine Levels

Some homocysteine is always present in the organism. Plasma levels are low during childhood but increase thereafter and are higher in boys than in girls. At the age of 40–42, there is a difference of about 2µmol/L between men and women, with mean values of about 11µmol/L and 9µmol/L respectively. The gender disparity may be explained by hormonal status, greater muscle mass in men and gender-related lifestyle differences.

After the menopause, these differences diminish, but concentrations remain lower in women.[32] Pregnant women have homocysteine levels that are considerably lower than non-pregnant women, supposedly owing to a larger plasma volume, an increased metabolic rate and glomerular filtration.

The currently used reference range is generally 5–15 µmol/L. This interval is based on values from a supposedly healthy reference population of different ages with varying lifestyles. An individual's homocysteine level should therefore be interpreted keeping in mind the patient's age, gender, and symptoms.

The Nutrition Committee of the American Heart Association recently indicated values higher than 10µmol/L as a cut-off value for patients with increased risk due to malnutrition, malabsorption syndromes, hypothyroidism, renal failure, or a family history of premature CVD; and for patients taking drugs.[33] This might also be a reasonable cut-off value for the elderly complaining of, for example, memory disturbances. This limit may be too high for women with previous pregnancy complications.

How to Lower Homocysteine Levels

Before any intervention, one should look for, and if possible eliminate, causes of hyperhomocysteinaemia. In many cases, a change in lifestyle may be enough to normalise levels. Changes in lifestyle are shown to lead to substantial changes in homocysteine levels over time.[34]

Vitamin supplementation may normalise metabolite levels even when serum vitamin levels are within the normal or high range. Even when, for example, genetic factors, renal impairment or age-dependent factors underlie the increase, vitamin supplementation may decrease homocysteine levels. Individual requirements may differ from the estimated levels calculated from population-based data.

An overview of randomised homocysteine-lowering trials was recently published.[35] The conclusion was that supplementation with folic acid and vitamin B_{12} would be expected to reduce homocysteine levels by about 25% to 30% in a typical population, depending on the concentrations used.

Combined B vitamin depletion is common, particularly in the elderly. In isolated vitamin B_{12} deficiency, homocysteine levels should be normalised within a few weeks after B_{12} treatment has begun. Otherwise, it may be that a concomitant folate and/or vitamin B_6 deficiency exist.

Is the Risk Factor Homocysteine Here to Stay?

There is hardly any doubt that homocysteine determination will become a tool used increasingly for risk assessment. At the same time it appears highly likely that current reference limits will evolve from being based statistically on values from a presumed healthy population, toward a more normative base where the norm is an optimal level. Current upper reference limits would then decrease (in the same way they have done for cholesterol). There is also likely to be a focus on lifestyle factors interacting with the homocysteine turnover and on oxidative interactions.

Results from on-going intervention trials involving over 70,000 subjects will hopefully indicate the extent to which lowering homocysteine levels will affect the occurrence, recurrence, or progression of potential homocysteine-related diseases; however, there is some concern about these studies. One reason is that folic acid fortification has been introduced in, for example, the US and Canada, which reduces the power of the studies. Furthermore, it is difficult to guarantee that these studies are really placebo-controlled, as patients have been informed about the possible effects of vitamins and lifestyle.

Another problem is that the duration of the studies may be too short. Attention was recently drawn to the fact that diseases associated with nutrient factors may develop over a very long period. Both short- and long-latency deficiency states of calcium and vitamin D have been demonstrated.[36] Some of the consequences of hyperhomocysteinemia may become apparent fairly soon, whereas for others it will be after a long latency period. This might be the reason why the association between homocysteine and morbidity was found to be far stronger in an elderly population than in a younger, in the Norwegian Hordaland Study. These considerations do not render homocysteine to be a less important risk marker, although it will be some time before its final role can be determined. ■

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