Biological and Environmental Determinants of Plasma Homocysteine

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ABSTRACT **This article gives an overview over common physiological, lifestyle, and pathological conditions that may modulate the homocysteine status. The interplay of several environmental factors, including age, gender, nutrition, smoking, and coffee consumption and physical activity with commonly used drugs and prevalent diseases are described. In most cases, an abnormal homocysteine status is not caused by a single factor alone but often is the result of combined effects. We address these frequently found "clusters" of homocysteine-modulating factors. Finally, we give an overview of likely causes of hyperhomocysteinemia found in an authentic material. This material is based on 2462 routine measurements of plasma total homocysteine carried out at the Haukeland University Hospital. The data represent the total number of combined homocysteine and methylmalonic acid determinations, requested by general practitioners in Norway during February 1998.**

Keywords: **Homocysteine, hyperhomocysteinemia, environmental factors, methylmalonic acid, lifestyle**

During the last two decades, elevated homocysteine concentration in blood—denoted hyperhomocysteinemia—has been identified as a prevalent and strong risk factor of cardiovascular occlusive disease, as well as of venous thromboembolism.[1] Interpretation of hyperhomocysteinemia is often difficult, because plasma total homocysteine (tHcy) status is determined by an interaction of a variety of inherited and acquired factors, as well as lifestyle.

In this article, we will present an overview of the interaction between common inherited, physiological, pathophysiological, and lifestyle determinants, which all are related to homocysteine (Hcy) status (Fig. 1). The effects of commonly used drugs will also be described. Notably, to date most articles describe the determinants of tHcy in selected patient groups or normal controls. Here we describe authentic, nonselected material of tHcy analyses requested by general practitioners in Norway. Using these data obtained from more than 2400 routine measurements of plasma tHcy performed at the laboratory of clinical chemistry, Haukeland University Hospital (Bergen, Norway), we will present a survey of the most likely causes of hyperhomocysteinemia in such

Objectives
Upon completion of this article the reader should be able to: 1) list some of the physiological factors that influence plasma levels of homocysteine, 2) summarize some of the disease entities that have an impact, and 3) recognize some of the environmental factors.

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Disclosure
Statements have been obtained regarding the author's relationships with financial supporters of this activity. There is no apparent conflict of interest related to the context of participation of the author of this article.

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0094–6176,p;2000,26,03,263,280,ftx,en;sth00642x

a material, which clinical chemists are dealing with in daily routine work.

AGE AND GENDER

In a general population, tHcy concentrations show a positive skew distribution.[2] Plasma tHcy increases throughout life in both sexes[1]; however, higher tHcy concentrations are measured in men than in women.[3] A similar distribution and age- and sex-related changes was seen among the 2462 homocysteine analyses carried out at the laboratory of clinical chemistry, Haukeland University Hospital, which will be referred to as the "Haukeland material" (Figs. 2 and 3).

In early childhood, tHcy levels in boys and girls are similar (about 5 μmol/L). The levels show a marked increase, particularly in boys, during the course of puberty, and plasma tHcy concentration increases to about 6 to 7 μmol/L.[2,4,5] From this age on, sex differences develop and the distribution becomes skewed, as in adult populations.[4] From puberty to old age, mean tHcy increases (about 3 to 5 μmol/L) in both sexes.[3,6]

In adults, the plasma tHcy levels are usually about 1 to 2 μmol/L higher in men than in women. In the Norwegian Hordaland cohort, the geometric means were 10.8 μmol/L in 5918 healthy men and 9.1 μmol/L in 6348 women age 40 to 42 years.[3] Similar median values were measured in the Haukeland material in the corresponding age groups (40 to 45 years) (Fig. 3).

Sex-related differences are explained by the effects of sex steroids on tHcy.[7–10] In addition, increased tHcy concentrations in men may be the result of a comparatively higher homocysteine production that may be related to differences in the creatine-creatinine synthesis.[11,12] However, after menopause tHcy concentrations in men and women approach each other.[3,8,13]

During pregnancy, there is a substantial reduction (by about 50%) of tHcy.[14–16] These differences are unlikely to be related to folate status alone[17] and not restricted to pregnant women taking folic acid supplementation, as shown in a recent study.[16] Total Hcy decreases between the first and second trimesters and thereafter remains stable throughout the rest of the pregnancy but returns to normal concentrations within 2 to 4 days postpartum.[18] It has been speculated that the lowered tHcy concentrations could be due to fetal uptake of maternal Hcy.[19]

Interestingly, we found an increased number of women with elevated tHcy and the vitamin B_{12} marker methylmalonic acid (MMA) among women in the age groups 21 to 25 and 26 to 30 years in the Haukeland material. Among these subjects, 13 to 15% had tHcy and MMA concentrations above the upper reference limits, whereas in the age groups 16 to 20 years and 31 to 40 years, respectively, only 7 to 10% had elevated concentrations (data not shown). These data could indicate an increased prevalence of impaired B_{12} status among women in childbearing age.

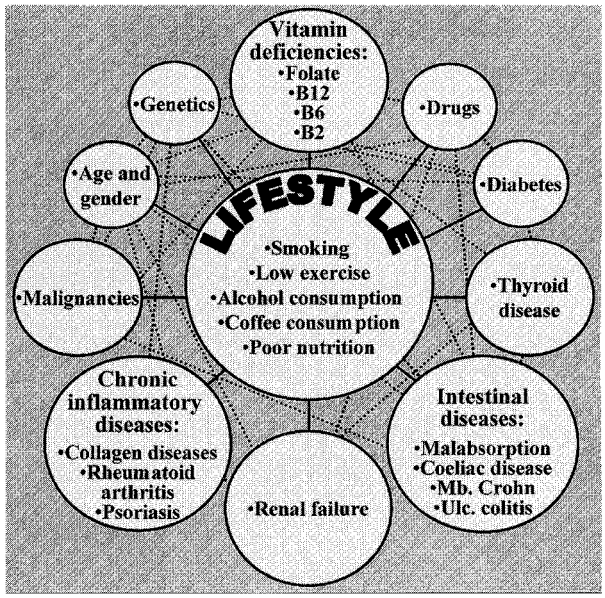

FIG. 1. Physiological and pathophysiological factors that modulate plasma total homocysteine. Dashed lines indicate common combinations ("clusters") of risk factors for hyperhomocysteinemia. Solid lines indicate interrelationship with lifestyle factors.

In the elderly, an increasing number of subjects will exhibit hyperhomocysteinemia.[20] The age-dependent increase may be attributed to deterioration of renal function[12,21] and impaired folate status.[22,23] Moreover, an increasing prevalence of cobalamin deficiency among the elderly may play an important role.[24–28] Cobalamin deficiency often develops because of malabsorption, which has been related to the aging of the gut.[29] Notably, in the Haukeland material, more than 30% of the subjects > 70 years old had tHcy concentrations > 15 μmol/L (data not shown).

In conclusion, tHcy gradually increases with age, and higher tHcy concentrations in the elderly may be explained by an interaction of variety of factors (see Fig. 1). The most important age-related conditions are suboptimal vitamin status,[30] impaired renal function,[31] dietary insufficiencies,[23] and intestinal malabsorption.[32] Moreover, an increased number of chronic conditions, like malignant and rheumatic diseases, among the elderly may also contribute to impaired folate status.[30]

GENETICS

Cystathionine ß-synthase (CBS) deficiency in homozygous form causes homocystinuria and is associated with extremely elevated tHcy levels. This inborn error of metabolism is, however, rare, with a frequency of 1:58,000 to 1:1,000,000 in newborns and a worldwide birth prevalence of 1:300,000.[33] On the other hand, het-

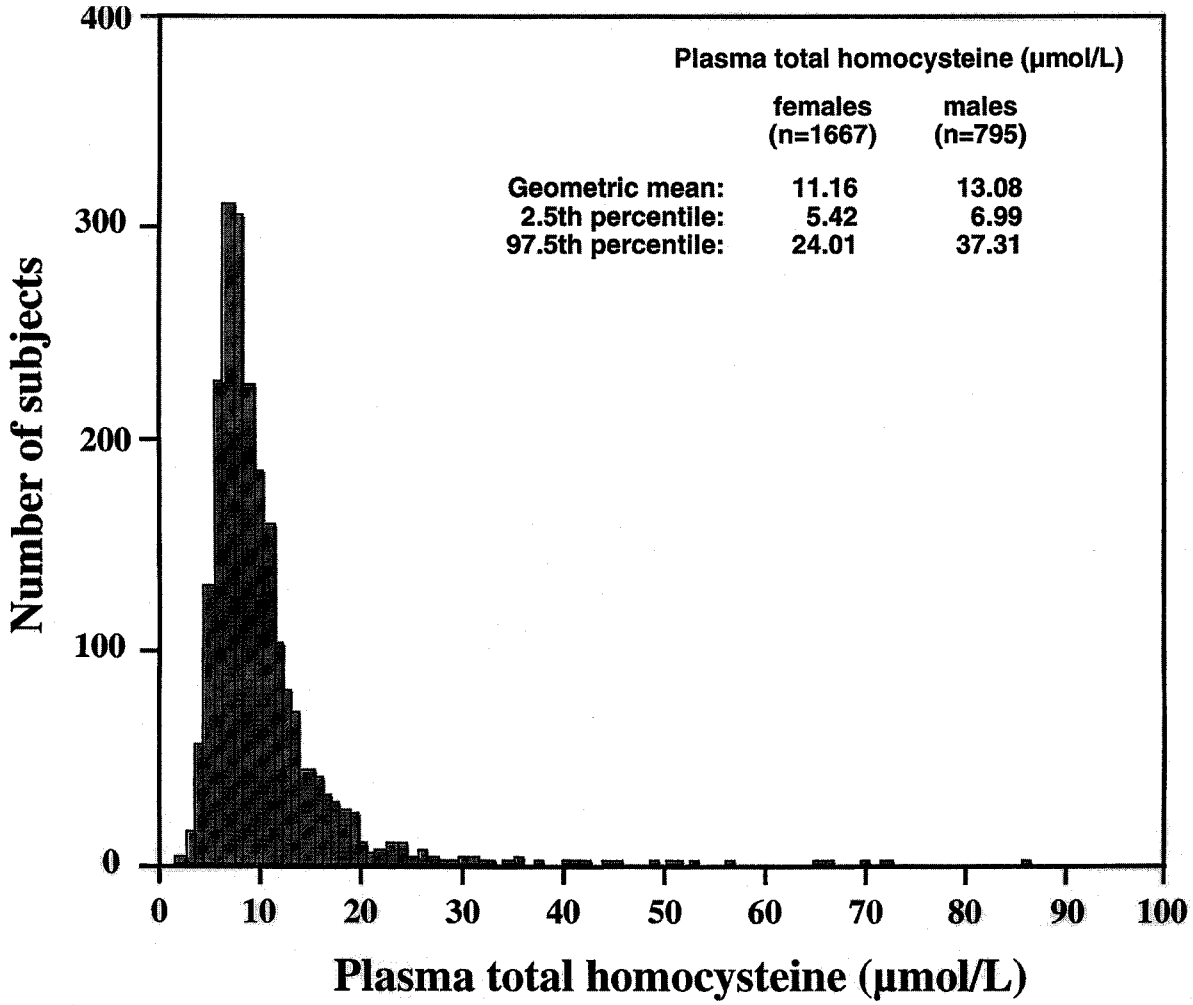

FIG. 2. Distribution of plasma total homocysteine in 1667 women and 795 men age 1 to 100 years (geometric mean 55.69). The data are from tHcy analyses requested from the Haukeland Hospital by Norwegian general practitioners during February 1998.

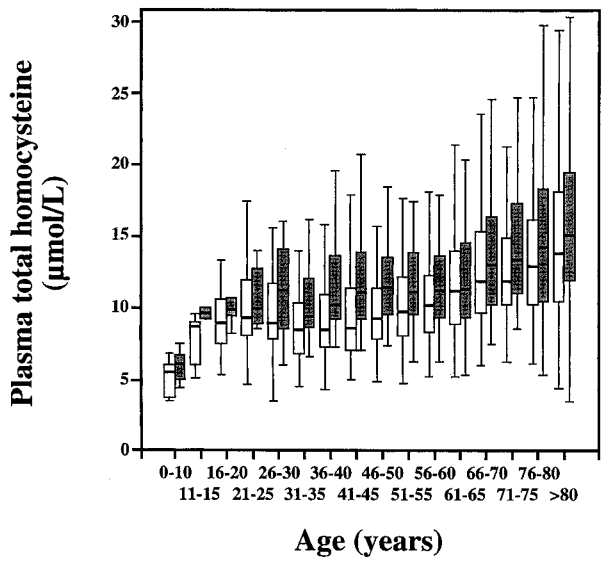

FIG. 3. Boxplot depicting the median plasma total homocysteine in the different age groups of the Haukeland material. The height of the boxes represents the interquartile range; the lines indicate the extreme values. Women are represented by open boxes; men by gray-shaded boxes.

erozygosity for the CBS deficiency was regarded to be a major cause of elevated post–methionine loading plasma homocysteine more than 30 years ago.[34] The prevalence of heterozygosity for this mutation in the general population is less than 1%,[11] and fasting levels in these individuals seem to be normal or only slightly elevated.[35,36] Early findings indicated that heterozygosity for CBS deficiency might be a major cause of hyperhomocysteinemia in vascular patients,[37] but this assumption could not be confirmed.[38] Post loading tHcy seems to be partly a genetic trait.[39] However, the frequency of carriers of CBS deficiency alone cannot explain the high incidence of hyperhomocysteinemia, neither in healthy[3] nor in vascular populations.[40]

The common C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene has been established as an important genetic determinant of elevated fasting tHcy.[38] Homozygosity for this polymorphism (TT genotype) predisposes to intermediate hyperhomocysteinemia.[41] The phenotypic expression of this genotype is, however, strongly related to folate status. TT subjects with adequate folate levels usually have nor-

mal tHcy levels, whereas TT subjects with low folate status usually exhibit hyperhomocysteinemia. For instance, we observed that in presumed healthy subjects with fasting tHcy concentrations > 40 μmol/L, the vast majority showed the TT genotype. When excluding subjects with cobalamin deficiency, 92% were found to be homozygous for the T-allele compared with approximately 10% in the general population.[42] Moreover, children with TT genotype and low serum folate have higher tHcy levels than controls with CC genotype have.[5] In addition, the MTHFR activity is likely to be modulated by riboflavin (vitamin B_2).[43] These examples indicate a strong interaction between genetic factors, lifestyle, and vitamin status (see Fig. 1).

LIFESTYLE

Several lifestyle factors are important determinants of homocysteine status in the general population. Lifestyle factors may interact with essentially any of the other homocysteine determinants depicted in Figure 1. In most cases, the homocysteine status is thus the result of an interaction between genetic, physiological, and environmental factors.

There are close relations between homocysteine and methionine metabolism.[44] A dynamic interaction between reduced, oxidized, and protein-bound forms of Hcy, cysteine, and cysteinylglycine in human plasma after methionine and homocysteine loading has been demonstrated.[45,46] It might thus be argued that high methionine intake could influence fasting as well as post–methionine load tHcy. Total Hcy increases by about 14% within 8 hours after a protein-rich meal.[47] However, neither the tHcy response after loading[48,49] nor fasting tHcy[50] seem to be related to the daily dietary methionine or protein intake. On the contrary, recent reports suggest that high dietary protein[51] or methionine intake[52] may in fact decrease fasting tHcy. However, food with high methionine content often is rich in cobalamin. The lower tHcy concentrations could thus be related to differences in cobalamin status rather than direct effects of increased methionine intake. This assumption is corroborated by the observation that elevated tHcy is uncommon in subjects with high consumption of meat.[53] However, in the Hordaland study, a reduction from normal to subnormal levels was usually attributable to intake of folic acid supplements.[54]

Folic acid supplementation seems to be more efficient in lowering tHcy than folate derived from food is.[55,56] A recent meta-analysis of intervention studies demonstrated that increasing the folic acid supplement dose to above 0.5 mg/d would not result in a further reduction of the tHcy concentration.[57] The effectiveness of folate supplementation seems to reach a plateau at about 0.4 mg/d.[56] It has been reported that 0.2 to 0.4 mg/d are sufficient to maintain a positive folate homeostasis and thereby optimal homocysteine remethylation in healthy men and women.[58,59] A similar dose-response relationship has recently been observed for folic acid–fortified cereals, demonstrating maximal tHcy lowering effect between 0.5 and 0.665 mg folic acid per 30 g cereal.[60]

The tHcy concentrations in most adult populations show a positive skew distribution.[3] There are consistent reports that tHcy is reduced and values approach normal distribution both after folic acid supplementation and in subgroups with adequate vitamin status.[61–63]

Low dietary folate intake and other lifestyle factors that affect plasma tHcy are closely interrelated (see Fig. 1).[54] Examination of the bioavailability of food folates is complicated,[64,65] and folate pharmacokinetics are still not fully understood.[66] Thus, considerable uncertainty about sufficient dietary intake exists. Recommended daily allowances for this vitamin have been changed several times.[67] In addition, folate absorption and demands may be influenced by other factors, the most important being high alcohol intake, coffee consumption, and smoking.[68–71] Individuals may often be exposed to a combination of adverse factors.[72] Moreover, an interaction between these factors and genetic predisposition seems to be present.[73] Hence, the use of functional tests, like tHcy, for assessment of functional folate status is required.

Higher levels of tHcy have been demonstrated in smokers than in nonsmokers.[74–76] There is a strong dose-response relationship between the number of cigarettes and tHcy levels, independent of age and sex.[3] This relationship was even seen in subjects with high folate intake.[54] Several explanations for this effect of smoking exist. Smokers have lower blood folate values compared with nonsmokers.[77–79] However, low red blood cell (RBC) folate values in smokers could partly be due to an analytical artifact.[80] Smokers generally consume a less healthy diet containing fewer vegetables and more fat than nonsmokers do.[81,82] Especially, heavy male smokers have lower nutritional vitamin intake and use supplements less frequently.[83] Moreover, smokers have reduced intake and blood levels of other vitamins involved in homocysteine metabolism, including vitamins B_{12}[84] and B_6.[85,86] In addition, tobacco smoke contains abundant free radicals that confer oxidative stress and thereby may affect redox status of thiols,[87] including homocysteine.[88]

Coffee consumption was among the strongest lifestyle determinants of tHcy in the Hordaland homocysteine cohort.[89] Individuals drinking more than 6 cups per day had mean tHcy levels that were 2 to 3 μM higher than in those who did not drink coffee. Others could not verify a relation between tHcy and moderate coffee consumption.[90] A recent study, however, demonstrated tHcy elevation in the elderly consuming 4 or more cups daily.[51] Coffee consumption is known to be associated with unhealthy lifestyle and poor nutrition.[81,91,92] However, because the consumption of decaffeinated coffee

did not have an effect on tHcy, caffeine may play a mechanistic role.[72,89] The caffeine effect may be related to its influence on kidney function.[93] Another possibility is interference with vitamin B_6 function, as reported for another xanthine, theophylline,[36] but such a mechanism would imply an effect on post–methionine load tHcy concentrations rather than on fasting levels.

Exercise was a weak but significant determinant of tHcy in the Hordaland cohort.[3,94] The difference in tHcy between subjects with sedentary lifestyle and those doing exercise on a daily basis was most prominent in the elderly (65 to 67 years old). In this age group, it approached 1 μmol/L. Exercise reduces the skewness of the tHcy distribution curve and therefore seems to lower tHcy in subjects with hyperhomocysteinemia.[3] However, acute exercise does not seem to have an effect.[95]

Alcohol consumption exhibits a complex effect on homocysteine. In the Hordaland study, the relation between tHcy levels and long-term alcohol consumption forms a weak U-shaped curve that reached its nadir at 14 alcohol units per week.[3] This relation was most prominent among smokers. Higher alcohol intake increases tHcy.[94] Plasma tHcy shows a transient increase during acute alcohol intoxication in alcoholics.[96] A direct inhibition of methionine synthase by acetaldehyde[97] could possibly explain this phenomenon. Chronic alcoholism seems to be associated with hyperhomocysteinemia.[98] This may be explained by impaired folate, vitamin B_{12}, or vitamin B_6 intake.[98] Malabsorption[68,99–101] may play an important role and is most prominent in malnourished alcoholics.[71]

The influence of obesity on tHcy is unclear. A correlation between body mass index and homocysteine could not be demonstrated.[102,103] However, both folate and vitamin B_{12} deficiencies have been observed after gastric surgery for obesity,[104–107] during the course of which hyperhomocysteinemia may evolve.

In the Hordaland study, a lifestyle profile was constructed to investigate the combined effects of the three major modifiable tHcy determinants: folate intake, smoking, and coffee consumption.[54] Subjects with a contrasting lifestyle had a difference of 3 to 5 μmol/L in tHcy. Furthermore, tHcy was essentially normally distributed in a population characterized by a healthy lifestyle profile.[54]

Among the 18,043 subjects investigated in the Hordaland homocysteine study, only 67 (0.4%) had tHcy equal to or higher than 40 μM.[42] As already mentioned, the vast majority of these subjects were homozygous for the MTHFR polymorphism. However, these subjects also showed lower plasma folate and cobalamin levels, lower intake of vitamin supplements. Moreover, they consumed more coffee and were frequently (60%) smokers. Because the prevalence of the MTHFR polymorphism in the general Norwegian population is about 10%, these results illustrate the strong additional influence of lifestyle factors on the tHcy concentration.[72]

VITAMIN DEFICIENCIES

Folate and cobalamin deficiencies are the most common causes of moderate to severe fasting hyperhomocysteinemia.[108,109] The incidence of these deficiencies increases with age.[110] In most cases, cobalamin deficiency is the result of a malabsorptive disorder,[27] whereas folate deficiency more frequently is explained by poor diet, overcooking, use of certain drugs, or excessive alcohol intake. Impaired vitamin B_{12} status is often combined with folate deficiency, and the same pathophysiological mechanisms are often involved.[29,32,111–113] There is also a tight metabolic interrelationship between these two vitamins,[114,115] and deficiency of one vitamin may affect the status of the other. Although vitamin B_6 is required in the two sequential reactions in which Hcy is converted into cysteine, vitamin B_6 deficiency normally does not result in elevated fasting tHcy but increases post–methionine load tHcy.[116] Isolated nutritional vitamin B_6 deficiency is considered rare,[117] and low-dose vitamin B_6 supplementation is not believed to reduce fasting tHcy.[118] However, other reports indicate that fasting plasma tHcy is negatively correlated with both intake and serum levels of not only folate and cobalamin but also vitamin B_6.[50,119] Moreover, vitamin B_6 deficiency might be more common than anticipated earlier, especially in the elderly,[120] and B_6 supplementation may have a preventive effect on cardiovascular disease, possibly independent of homocysteine.[90,121–123]

Combined deficiencies of the previously mentioned vitamins are commonly found and display an interrelationship with other determinants of homocysteine status (Fig. 1). For instance, a variety of drugs may impair vitamin absorption or function (see later). The severity of clinical vitamin deficiency may be modified by genetic predisposition, such as the MTHFR polymorphism.[124] Gastrointestinal diseases will often result in impaired vitamin absorption.[111] In pernicious anemia, there is a predisposition toward other autoimmune diseases, such as hypothyroidism, that may affect homocysteine status.[125–127] Renal diseases may be associated with increased vitamin loss or demand,[128] and increased demands have also been reported in chronic inflammatory diseases, cancer, and thyroid disease.[129–132]

Increased tHcy has repeatedly been reported after cardiac transplantation.[133,134] The elevated tHcy concentrations found in heart transplant recipients probably have multiple causes,[135] including the use of immunosuppressive drugs such as cyclosporine[136] and azathioprine[137] (see next paragraph) and renal glomerular dysfunction. However, impaired function

of folate, vitamin B_6,[138] and possibly vitamin B_{12}[134] may represent important contributory factors.

DRUGS

A variety of drugs may affect tHcy levels. The literature on the interaction between homocysteine and drugs was reviewed earlier[139–141] and summarized recently.[142] Therefore, the drug effects on homocysteine will be mentioned only briefly here.

Most drug effects are conferred by interaction with absorption or metabolism of folate, cobalamin, or vitamin B_6. The antifolate drug methotrexate (MTX) inhibits the dihydrofolate reductase and thereby depletes cells for reduced folates. Homocysteine rises within hours after high-dose infusions used for cancer therapy. This effect is reversed by high doses of folic acid.[143,144] In psoriasis, patients are treated with considerably lower doses of MTX (10 to 25 mg weekly). Here, tHcy increases more slowly over a period of several days.[145] In rheumatoid arthritis, even lower doses of MTX are used, and elevated tHcy seems to develop over a period of 3 to 12 months.[146,147] In these patients, folic acid (5 to 27.6 mg/wk) improves folate status and prevents the MTX-induced hyperhomocysteinemia without affecting the therapeutic efficacy.[147] In addition, folate supplementation may alleviate other adverse effects of long-term MTX treatment[148] and possibly prevent premature cardiovascular disease in rheumatoid patients.[147]

Several anticonvulsive drugs may cause hyperhomocysteinemia through interference with folate metabolism.[141,149,150] These adverse effects are probably induced by a depletion of liver folate stores through inhibition of polyglutamation[151] and may be modulated by the MTHFR genotype.[152]

Homocysteine also increases during therapy with niacin in combination with the bile acid sequestrant colestipol. The latter agent may interfere with folate absorption.[153] Notably, in children treated with another bile resin, cholestyramine, elevation of tHcy was largely confined to subjects carrying TT or CT genotype of the MTHFR gene.[154]

Plasma tHcy increases within hours in patients exposed to the anesthetic gas nitrous oxide.[155–157] The increase reflects irreversible oxidation of cob(I)alamin, which is formed as a transient intermediate of the methionine synthase reaction.[158] In addition, the enzyme methionine synthase itself is irreversibly inactivated.[159] Both mechanisms may be responsible for the bone marrow and central nervous system side effects observed after prolonged nitrous oxide exposure. The deleterious effect of nitrous oxide on methionine synthase can be aggravated by high levels of folate but may be alleviated by methionine loading before anesthesia.[156]

In contrast to the rapid increase in tHcy observed during nitrous oxide exposure, a slow increase over months to years is expected during prolonged intake of drugs interfering with cobalamin absorption. Such interference with cobalamin absorption has been described for cholestyramine,[160] histamine H_2-receptor antagonists,[161] omeprazole,[162–165] and the antidiabetic metformin.[166–169] However, an increase in tHcy has hitherto been measured only in patients using cholestyramine[154,170] and metformin.[171–174] These latter two drugs may also affect folate absorption.

Several drugs interfere with the function of vitamin B_6. A common mechanism involves inhibition of pyridoxal kinase.[175] Elevated levels of Hcy in plasma or urine have been reported following treatment with azauridine,[176] isoniazid,[177] niacin,[178] and theophylline.[36]

The effect of sex steroid hormones on tHcy is indicated by gender differences in tHcy level and by the observation of low tHcy levels in premenopausal women[6,8] and during pregnancy.[14,15] Inconsistent data have been published on changes in plasma tHcy of women taking oral contraceptives.[179] Replacement therapy containing estrogen in postmenopausal women decreases plasma tHcy within 6 to 12 months of treatment.[180] Estrogen treatment also reduces tHcy of healthy men[181] and men with prostatic carcinoma,[179] whereas short-term treatment of healthy men with supraphysiological doses of testosterone is without effect.[9] A positive correlation between tHcy and plasma creatinine levels during androgen administration[182] suggests that androgens act by enhancement of creatinine-Hcy synthesis secondary to increase in muscle mass. Moreover, sex hormones and contraceptives may impair folate,[183] cobalamin,[184] and vitamin B_6 status,[185] which may predispose to hyperhomocysteinemia.

The immunosuppressive drug cyclosporine A (CyA) increases plasma tHcy. Renal transplant patients receiving CyA have significantly higher tHcy than do untreated renal transplant recipients.[186] Hyperhomocysteinemia also develops in cardiac transplant patients, and high tHcy is predicted by both serum creatinine and serum CyA concentration,[136] suggesting that the CyA effect is at least partly independent of renal function.

RENAL FAILURE

Apart from folate and cobalamin deficiency, renal failure[187–189] is the most frequent clinical cause of hyperhomocysteinemia. Although the pathogenesis of hyperhomocysteinemia in folate and cobalamin deficiency is well-described, little is known about the basis of hyperhomocysteinemia in chronic renal failure. Possible mechanisms are decreased renal homocysteine excretion, impaired renal metabolism or inhibition of extrarenal homocysteine metabolism by uremic toxins, or generally reduced B vitamin status in renal failure.[190] In rat kidneys, a substantial renal homocysteine uptake and metabolism were demonstrated by measuring arteriove-

nous amino acid differences along with simultaneous determination of renal plasma flow, urine flow, and urinary homocysteine concentration.[191] Others, however, could not verify these findings in humans with normal renal function, in whom no net renal extraction of homocysteine was found.[192] Thus, impaired extrarenal homocysteine metabolism could be the most important contributor to elevated tHcy concentrations found in renal patients.

Improved folate status is considered one of the major modifiers of hyperhomocysteinemia in renal failure.[193,194] A considerable reduction of tHcy may be achieved by folate supplementation, given either alone[195] or in combination with other B vitamins.[196] The role of vitamin B_{12} status in renal failure is more uncertain. Some recommendations on vitamin supplementation in end stage renal disease patients do not include vitamin B_{12}.[188] It has been claimed that hemodialysis patients are vitamin B_{12} replete.[197,198] However, recent investigations indicate that vitamin B_{12} alone might be almost as effective as folic acid in reducing tHcy, at least in renal patients with low B_{12}-levels.[199]

MALIGNANCIES AND CHRONIC INFLAMMATORY DISEASES

Elevated tHcy is frequently found in benign and malignant diseases associated with a large burden of proliferating cells such as acute lymphoblastic leukemia,[200] psoriasis,[145] and some chronic inflammatory diseases. In these conditions, there could be an increased export of Hcy by rapidly dividing cells.[201] The increased tHcy-export might be explained by an intracellular redistribution of the folate pool in favor of DNA synthesis and at the expense of Hcy remethylation. In addition to folate, impaired vitamin B_6 status may play a role.[202]

In rheumatoid arthritis, data on tHcy levels are somewhat controversial. Recent findings indicate, however, that hyperhomocysteinemia is common in these patients. A combined influence from vitamin deficiencies (vitamin B_6, B_{12}, and folate) and the MTHFR polymorphism may be responsible for the tHcy elevations.[148,203–205] Elevated fasting tHcy levels have also been reported in patients with severe and long-standing rheumatoid arthritis combined with impaired cobalamin absorption and function.[206]

In rheumatoid patients not receiving MTX, one small study reported an elevated postload tHcy level, which may possibly be attributable to impaired vitamin B_6 status often seen in rheumatoid arthritis.[207]

Moreover, patients with rheumatoid arthritis are often immobilized and undergo variable and extensive drug treatment, including sulphasalazine,[208] D-penicillamine,[209] or low-dose therapy with MTX.[146] Sulphasalazine and in particular MTX have antifolate effects causing elevated Hcy levels, whereas D-penicillamine could even reduce tHcy.[210] On the other hand, short-term low-dose MTX treatment may not effect homocysteine status.[146,207]

Moreover, chronic inflammatory bowel disease may predispose to hyperhomocysteinemia (see section on *Intestinal Diseases*), which could be causally related to thromboembolic complications in these patients.[211] Again, inflammatory bowel diseases combined with the thermolabile MTHFR C677T variant could further increase tHcy.[212]

ENDOCRINE DISORDERS

In type I diabetes, hyperhomocysteinemia only occurs at advanced stages and is often accompanied by elevated creatinine or macroalbuminuria. Elevated tHcy may be attributable to impaired renal function,[213–215] but marginal folate deficiency[216] and cobalamin deficiency[217] may also play a role. In both type 1 and type 2 diabetes, elevated fasting[218–220] or post–methionine load tHcy[219,221] are associated with macroangiopathy, whereas a relation between tHcy and microangiopathy[219,222,223] or microalbuminuria[224,225] has been demonstrated in some but not all[214] studies. Subnormal tHcy has been reported in subjects with type I diabetes with normal creatinine[226] and in nondiabetic hyperinsulinemic subjects.[172] Low tHcy may be due to the glomerular hyperfiltration observed in early diabetes[227] or may possibly be a metabolic effect of high insulin levels. The latter possibility is in agreement with elevated tHcy in insulin-resistant subjects[228] and with reduction of tHcy by insulin, as demonstrated during hyperinsulinemic-euglycemic clamp.[229] In the latter study, tHcy reduction was only observed in normal subjects but not in type II diabetes.[229] These findings suggest that insulin resistance may also impede the tHcy reducing effects of insulin in these patients. Mild Hcy elevations are observed in type II diabetes patients treated with metformin.[171,172] Interestingly, although the antidiabetic drug metformin is thought to interfere with B_{12} absorption,[168] long-term metformin treatment resulted only in tHcy elevations but not in increased concentrations of the B_{12} marker MMA.[171] Moreover, folate administration counteracts the Hcy increasing effect of metformin and even increases serum levels of vitamin B_{12}.[173]

Total Hcy has recently been reported to be moderately elevated in hypothyroidism and low in hyperthyroidism.[230] This finding may be related to the influence of thyroid function*. However, an impaired riboflavin status[231] and folate status,[232] reduced glomerular filtration rate (GFR),[233,234] or alterations in creatinine synthesis[235] could also be important. Elevated tHcy concentration may be normalized by L-thyroxine replacement therapy.[236,237]

*On metabolic turnover.

INTESTINAL DISEASES

A number of gastrointestinal conditions and diseases may cause elevated tHcy concentrations. Again, the pathogenesis of hyperhomocysteinemia is complex. However, it is likely that deficiency of vitamin B_{12} or folate, or both, because of various malabsorption syndromes is the predominating cause.[238]

Elevated tHcy levels and thrombotic complications have been described in patients with chronic inflammatory bowel diseases, such as ulcerative colitis[239] and Crohn's disease.[240–242] Vitamin B_{12} and folate deficiencies are reported both in these diseases and in celiac disease.[238,243–249] Vitamin deficiencies in inflammatory bowel disease are likely to be of multifactorial genesis.[244,245] These factors include inadequate diet,[244] gastrointestinal surgery,[245,250,251] bacterial overgrowth,[252,253] drug-induced chronic hemolysis,[208] and malabsorption.[254–257] Moreover, increased jejunal surface pH may impair folate absorption.[258,259]

Gastrointestinal surgery in general may cause malabsorption of vitamin B_{12} and folate and may thereby increase tHcy levels. These surgical procedures comprise gastric surgery,[104–107,260–264] different kinds of pouches,[265,266] and after colectomy or ileostomy.[250,251,267] However, homocysteine was only measured in three of the previously mentioned studies.[107,263,264] All of them demonstrated elevated tHcy concentrations.

Intestinal bacterial overgrowth with anaerobic bacteria is another cause of vitamin B_{12} malabsorption.[217,253,268,269] Bacterial overgrowth is frequently found in the elderly.[29,270]

Pelvic and abdominal radiotherapy often result in gastrointestinal dysfunction with chronic diarrhea, increased stool frequency, and faster small bowel and whole gut transit. This may result in a malabsorption of bile acids and vitamin B_{12}[271–278] and even folate.[273] In some cases antibiotic treatment in addition to vitamin supplementation may be warranted if intestinal bypass surgery is combined with radiotherapy.[271]

The importance of adequate folate status in chronic inflammatory bowel disease is underlined by recent investigations demonstrating an increased risk of colon cancer.[279–286] A possible prevention of colon cancer by folate supplementation has already been proposed.[287]

COMMON CAUSES OF HYPERHOMOCYSTEINEMIA IN THE HAUKELAND MATERIAL

We evaluated the results of 2917 MMA analyses performed at the Laboratory of Clinical Biochemistry, Haukeland University Hospital during February 1998. In 2462 subjects, results from concomitant tHcy analyses were available. At the time of the study, the Haukeland University Hospital was the only laboratory in Norway that offered routine determination of MMA. In more than 99% of cases, these analyses were requested by general practitioners. This material thus gives an authentic picture of the spectrum of tHcy concentrations encountered in a routine laboratory in Norway.

This is a nonselected material demonstrating the total of all combined tHcy and MMA analyses performed during 1 month. The investigated subjects do not represent a normal population. This material is based on analysis of tHcy in 1667 women and 795 men; the median age was 61.0 (range, 2 to 100) and 66.0 (range, 1 to 100) years, respectively. The tHcy values showed a positive skewness (Fig. 2). The overall geometric mean was 11.75 μmol/L, and the geometric means for women and men were 11.16 μmol/L and 13.08 μmol/L, respectively. In the age group 61 to 65 years (n = 162, 87 women and 75 men), geometric means were 11.49 μmol/L and 12.74 μmol/L, respectively. These values and the relative sex differences are similar to those found in the oldest age group (65 to 67 years) in the Hordaland Homocysteine Study,[3] in which 11.04 μmol/L and 12.27 μmol/L were measured.

Total Hcy and MMA were requested for certain indications (Table 1). Furthermore, the population predominantly consisted of elderly people (Fig. 4). In addition, the frequency distributions of the number of tHcy requests in relation to age were different. In women, the distribution curve showed three distinct peaks (Fig. 4). The second peak could be related to menopause, whereas the first peak in the late twenties may reflect increased demand for tHcy testing in women of childbearing age. In contrast, tHcy is only seldom measured in young men. The number of tHcy requests in men gradually increases between the age of 20 and 70 and culminates at the age of 75 (Fig. 4).

In 37% of cases, the general practitioners supplied comments on the request forms (Table 1). More than 75% of combined tHcy and MMA analyses were requested on three indications: low cobalamin values, control after vitamin supplementation, and neuropsychiatric symptoms.

Table 2 gives an overview of the results of the 2464 combined tHcy and MMA analyses carried out at Haukeland University Hospital during February 1998. Cut-off values used for MMA and tHcy were 0.26 μmol/L and 15.0 μmol/L, respectively. Except for age, gender, and comments from the physicians requesting the analysis, additional clinical informations or results from other laboratory tests and final clinical diagnosis were not available in the majority of cases. Neither was information of the final clinical diagnosis accessible.

In cobalamin deficiency, both tHcy and MMA are usually elevated. Total Hcy concentrations between 20 and 80 μmol/L and MMA values between 0.30 and 5.0

μmol/L are typically encountered.[288,289] In contrast, only tHcy is usually increased in folate deficiency. Thus, MMA can differentiate between folate and cobalamin deficiencies, which constitute, in addition to renal insufficiency, the three major causes of hyperhomocysteinemia.[290] Renal insufficiency is the only clinical condition causing elevated MMA concentrations.[291] In patients showing normal MMA but elevated tHcy concentrations, renal insufficiency, as a cause of hyperhomocysteinemia, is unlikely and vice versa.

The classification of the different diagnostic categories in Table 2 is based on the following assumptions. Slight elevations of tHcy concentrations ranging from 1 to 4 μmol/L above the upper reference limits are often related to unhealthy lifestyle.[54] Moderately increased tHcy (15.1 to 30.0) and MMA within normal range (≤ 0.26 μmol/L) (category A) indicate impaired folate status or unhealthy lifestyle, or both, but is unlikely to be caused by renal insufficiency. We know from the Hordaland Study that most subjects with intermediately elevated tHcy (30.1 to 100.0) and normal MMA (category B) were homozygous for the MTHFR T-allele, often combined with reduced folate status.[42] Severe hyperhomocysteinemia and normal MMA is indicative of a genetic defect, most likely a CBS deficiency (category C). Elevated MMA (> 0.26 μmol/L) and moderately elevated tHcy (15.1 to 30.0 μmol/L) (category D) are indicative of B_{12} deficiency if renal insufficiency can be excluded.[290] Elevated MMA and intermediate or severe increase in tHcy concentrations (> 30.0 μmol/L) (categories E and F) are indicative of severe B_{12} deficiency.

Based on these assumptions, the following conclusions may be drawn: In the Haukeland material, hyperhomocysteinemia (> 15.0 μmol/L) was found in approximately 24% of cases. About 56% of all elevated tHcy values may be explained by unhealthy lifestyle or folate deficiency, or both. In 31% of cases, moderate B_{12} deficiency or renal disease, or both, may be responsible for hyperhomocysteinemia. Severe B_{12} deficiency was the likely cause of hyperhomocysteinemia in 6% of cases. A combination of the TT genotype of the MTHFR polymorphism and impaired folate status could possibly explain about 7% of hyperhomocysteinemia in this material (category B).

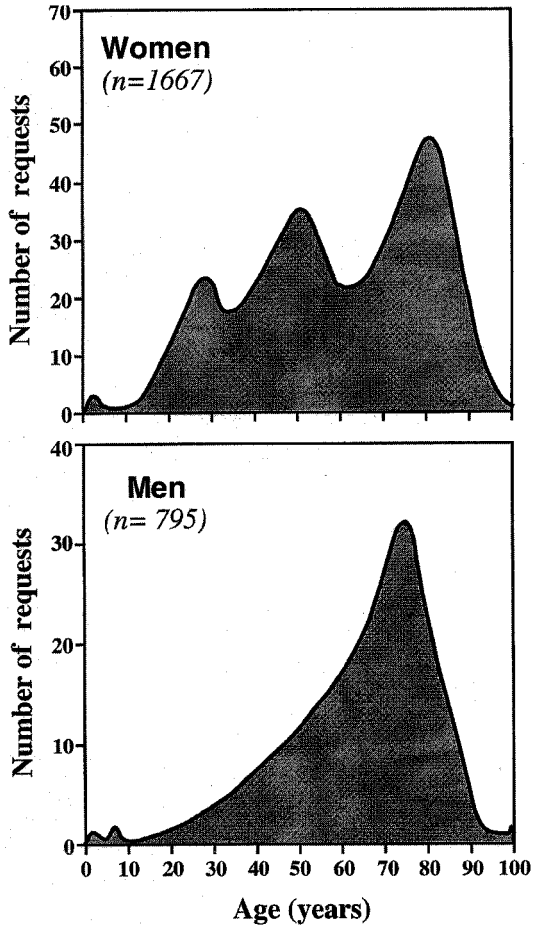

FIG. 4. Frequency distribution of homocysteine requests according to age and gender. Women are represented in the upper panel; men in the lower.

TABLE 1. Requesters' Comments on the Clinical Indications for Ordering Plasma tHcy and MMA and Portion of Elevated Metabolites in Each Category

Requester's Comment	*Frequency, n (%)*	*Elevated tHcy, > 15 μmol/L, in %*	*Elevated MMA, > 0.26 μmol/L, in %*
Anemia	68 (6.3)	26	29
Neurological symptoms	244 (22.7)	24	16
Elevated Hcy	3 (0.3)	67	33
Low cobalamin	323 (30.0)	25	24
Low folate	14 (1.3)	36	14
Treatment control	248 (23.0)	23	17
Cardiovascular disease	45 (4.2)	29	31
Gastrointestinal symptoms	40 (3.7)	8	10
Other comments	91 (8.5)	19	21
Total	1076 (100)		

TABLE 2. Overview of Combined Analysis of Total Plasma tHcy and MMA in the Haukeland Material

MMA Interval (μmol/L)	*Homocysteine Interval (μmol/L)*				
	0–15.0	*15.1–30.0*	*30.1–100.0*	*> 100*	*Sum*
			n (%) Category (%)†*		
≤ 0.26	1611 (65.4)	328 (13.3) A‡ (56.5)	39 (1.6) B‡ (6.7)	0 (0) C‡ (0)	1978 (80.3)
> 0.26	270 (11.0)	180 (7.3) D‡ (31.0)	33 (1.3) E‡ (5.7)	1 (0.04) F‡ (0.2)	484 (19.7)
Sum	1881 (76.4)	508 (20.6)	72 (2.9)	1 (0.04)	2462 (100.0)

*Percent of total number of analyses

†Percent of all cases with hyperhomocysteinemia

‡The letters represent the different categories for the causes of hyperhomocysteinemia: A, folate deficiency and/or unhealthy lifestyle, high age; B, MTHFR deficiency (combined with folate deficiency); C, severe inborn error; D, B_{12} deficiency and/or renal insufficiency; E, F, B_{12} deficiency

SUMMARY AND CONCLUSION

Total plasma Hcy concentrations are the result of multiple genetic, physiological, and pathophysiological factors, in which folate and cobalamin deficiencies, lifestyle, age-related phenomena, and renal insufficiency represent the most important determinants.

Because tHcy also depends on gender and menopausal status, age- and gender-specific reference intervals should be established. Moreover, possible short-term changes of tHcy after acute events, such as after myocardial infarction[292,293] and stroke,[294] must be taken into account when interpreting tHcy concentrations in the individual case.

Moderate elevations of tHcy concentrations of 1 to 4 μmol/L above the adjusted upper reference limits are often related to unhealthy lifestyle factors, such as smoking, coffee and alcohol consumption, insufficient nutrition, and lack of exercise. Most of these factors are modifiable and normalization of tHcy concentrations could be an incentive to improve lifestyle.

Intermediate to severe hyperhomocysteinemia is in many cases the result of certain "clusters" of risk factors. Some diseases, such as renal failure, hypothyroidism, and conditions with increased burden of proliferating cells, can predispose to the development of vitamin deficiencies, which may affect homocysteine status (Fig. 1). In some cases, it may be advisable to test for coexistence of several determinants. As an example, in hypothyroidism due to autoimmune thyroiditis the patient should also be monitored for pernicious anemia.[295]

Finally, the extent of tHcy elevation in itself and simultaneous MMA values may give important clues to identify the causes of hyperhomocysteinemia.

ACKNOWLEDGMENTS

The valuable assistance of Sigrunn Kjaerstad, member of the staff of the Laboratory of Clinical Chemistry, Haukeland University Hospital (Bergen, Norway), is highly appreciated.

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