The Hordaland Homocysteine Study: A Community-Based Study of Homocysteine, Its Determinants, and Associations with Disease[1]

Helga Refsum,*†**[2] Eha Nurk,† A. David Smith,* Per M. Ueland,**‡ Clara G. Gjesdal,†† Ingvar Bjelland,# Aage Tverdal,§ Grethe S. Tell,‡†† Ottar Nygård,**‡ and Stein E. Vollset‡†††

*Oxford Centre for Gene Function, University Laboratory of Physiology, University of Oxford, Oxford, U.K., †Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, Norway, **Institute of Medicine, University of Bergen, Bergen, Norway, ‡LOCUS for Homocysteine and Related Vitamins and ††Department of Public Health and Primary Health Care Institute of Medicine, University of Bergen, Bergen, Norway, #Department for Child and Adolescent Mental Health Services, Haukeland University Hospital, Bergen, Norway, and §Norwegian Institute of Public Health, Oslo, Norway

ABSTRACT The Hordaland Homocysteine Study (HHS) is a population-based study of more than 18,000 men and women in the county of Hordaland in Western Norway. The first investigation (HHS-I) took place in 1992–93, when the subjects were aged 40–67 y. In 1997–99, a follow-up study (HHS-II) of 7,053 subjects was carried out. In this large population, plasma levels of total homocysteine (tHcy) are associated with several physiologic and lifestyle factors and common diseases. Increasing age, male sex, smoking, coffee consumption, high blood pressure, unfavorable lipid profile, high creatinine, and the MTHFR 677C > T polymorphism are among the factors associated with increased tHcy levels; physical activity, moderate alcohol consumption, and a good folate or vitamin B-12 status are associated with lower tHcy levels. Subjects with raised tHcy levels have increased risk of cardiovascular morbidity, cardiovascular and noncardiovascular mortality, and are more likely to suffer from depression and from cognitive deficit (elderly). Among women, raised tHcy levels are associated with decreased bone mineral density and increased risk of osteoporosis. Women with raised tHcy levels also have an increased risk of having suffered from pregnancy complications and an adverse pregnancy outcome. Significant associations between tHcy and clinical outcomes are usually observed for tHcy levels >15 μmol/L, but for most conditions, there is a continuous concentration–response relation with no apparent threshold concentration. Overall, the findings from HHS indicate that a raised tHcy level is associated with multiple clinical conditions, whereas a low tHcy level is associated with better physical and mental health. J. Nutr. 136: 1731S–1740S, 2006.

KEY WORDS: • *homocysteine* • *folate* • *cobalamin* • *vitamin B-12,* • *methylenetetrahydrofolate reductase* • *blood analyses* • *epidemiology* • *humans* • *risk factors* • *chronic diseases* • *mortality* • *middle-aged* • *aged* • *cohort studies* • *prospective studies* • *cross-sectional studies*

In 1991, a collaboration was established between the National Health Screening Service and the University of Bergen. This collaboration resulted in the Hordaland Homocysteine Study (HHS),[3] which is a large population-based study of 18,044 subjects living in Hordaland County in Western Norway (**Fig. 1**). The majority of the subjects belong to 2 different age groups. The "younger group," aged 40–42 y (n = 12,595) at the first examination, were recruited from the entire county. The "older group" included subjects aged 65–67 y (n = 4766) and were from the city of Bergen and its surroundings (1). The first investigation was carried out in 1992–93 (HHS-I), and, in 1997–99, there was a follow-up study of participants living in Bergen and its surroundings (HHS-II). The recruitment in HHS-I and HHS-II is depicted in **Figure 2**.

This review briefly summarizes the main findings related to plasma total homocysteine (tHcy) in HHS and discusses how these findings contribute to our understanding of the factors that determine the level of tHcy and of the relationships between plasma tHcy levels and common diseases.

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the conference "The Fifth Workshop on the Assessment of Adequate Intake of Dietary Amino Acids" held October 24–25, 2005 in Los Angeles. The conference was sponsored by the International Council on Amino Acid Science (IACAAS). The organizing committee for the workshop and guest editors for the supplement were David H. Baker, Dennis M. Bier, Luc Cynober, Yuzo Hayashi, Motoni Kadowaki, and Andrew G. Renwick. Guest editors disclosure: all editors received travel support from ICAAS to attend the workshop.

[2] To whom correspondence should be addressed. E-mail: helga.refsum@physiol.ox.ac.uk.

[3] Abbreviations used: BMD, bone mineral density; CVD, cardiovascular disease; HHS, Hordaland Homocysteine Study; MTHFR, methylenetetrahydrofolate reductase; tHcy, total homocysteine.

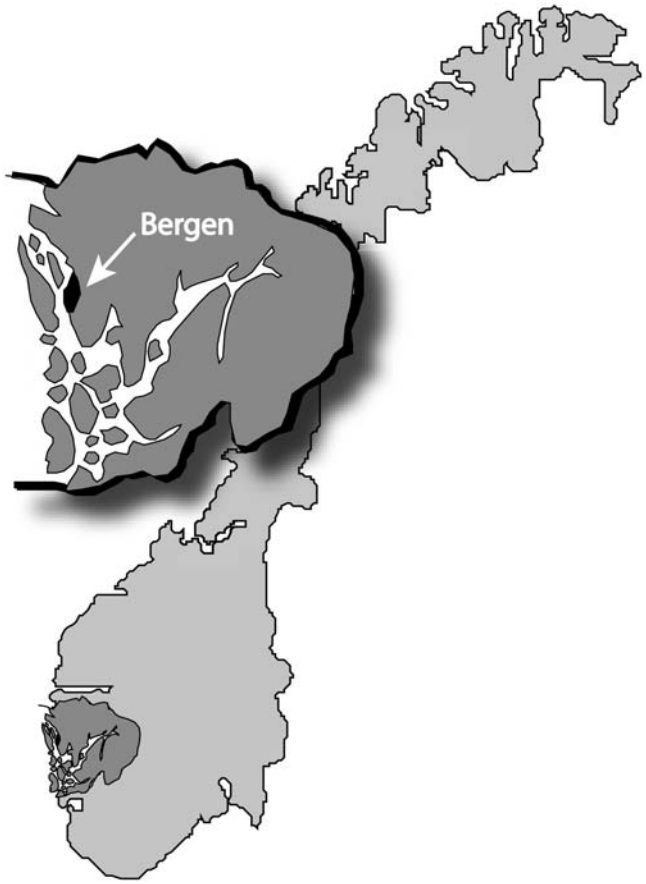

FIGURE 1 The county of Hordaland is located on the western coast of Norway. From Ueland et al. (1) and reprinted with permission of AOCS. Originally published in *Lipids* 36, Suppl (2001), S33.

Data collection

In HHS-I, participants underwent the standard examinations of the National Health Screening Service (2). This included measurement of height, weight, and blood pressure. The participants completed questionnaires focusing on lifestyle factors, dietary habits and risk factors for cardiovascular disease (CVD). Nonfasting blood samples were collected for measurement of serum lipids and for preparation of EDTA plasma and packed blood cells. In the follow-up study, HHS-II, essentially the same variables were included, but more extensive data were obtained on diet (3,4). It also included data on bone mineral density (BMD), cognitive function, and symptoms of depression and anxiety in subsets. In HHS-II, measurements were made of serum levels of creatinine and HDL and LDL cholesterol. Details about data collection have been reported (5–9). Measurements of tHcy, total cysteine, folate, and vitamin B-12 have been performed in all samples in HHS-I and -II, and polymorphisms related to 1-carbon metabolism, including *MTHFR* 677C > T and 1298A > C, have been determined in all subjects. To date, HHS is the largest cohort on tHcy and its related markers.

Determinants of plasma total homocysteine levels

Lifestyle factors and CVD risk factors in HHS. Data from HHS-I have been published in a series of papers regarding lifestyle, CVD risk factors and tHcy levels (5,10–12). The concentration-response relationships between several factors

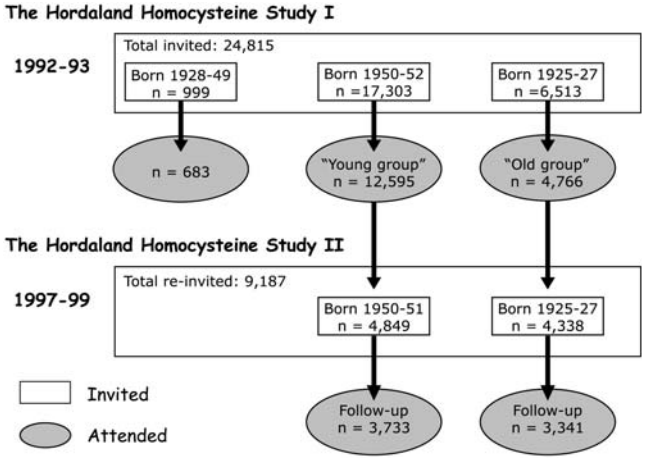

FIGURE 2 Recruitment into the Hordaland Homocysteine Study. The cohort was established in 1992–1993 and included 18,044 community-dwelling individuals born in 1925–1952. The "younger group" was recruited from the entire county of Hordaland, the older group was recruited from the city of Bergen and 3 neighboring suburban municipalities, whereas the group born 1928–1949 was a 2% random sample from the city of Bergen. In 1997–1999, all living cohort members born in 1925–1927 or 1950–1951 and residing in the city of Bergen or the neighboring suburban municipalities were invited to a second survey.

and tHcy are shown in **Figure 3**. The first paper from HHS-I was published in 1995 (5). The data confirmed that tHcy is higher in men than in women and that it increases with age. More surprising were the observations of an inverse relation to physical activity and a positive but relatively weak association to blood pressure and total cholesterol. However, the most important finding was a dose-dependent relation between the number of cigarettes smoked per day and the tHcy level; it was present in all age and gender groups and remained strong after adjustment for potential confounders. Thus, in this paper it was reported that elevated tHcy levels were more frequently observed in men, and, in the older age group, in subjects that were physically inactive, who were smokers, and who had higher blood pressure and higher cholesterol levels. It was the first paper to demonstrate that an elevated tHcy level reflects the overall cardiovascular risk profile (5).

A novel and unexpected finding in the HHS-I was the strong concentration-dependent association between intake of coffee and tHcy levels (11). Coffee seemed predominantly to affect lower tHcy levels, which is markedly different from the effect of low vitamin status and smoking, both of which lead to a complete shift of the tHcy distribution to higher tHcy values (11,13).

In 1993, Hultberg et al. (14) reported that tHcy was elevated in alcoholism. However, it was HHS-I that first reported that a moderate intake of alcohol was associated with reduced tHcy levels (10). The association was weak and significant only in smokers. In the younger group, the association was U-shaped; tHcy declined until an intake of 14 alcohol units per week; then it started to rise again.

Analyses of the HHS-I database have shown that sex, age, folate intake, smoking status, and coffee consumption are the strongest determinants of tHcy concentration in the general population (12). The combined effect of the 3 modifiable factors was larger than the effect of each factor alone. A lifestyle characterized by low folate intake, smoking, and coffee consumption was associated with a high median tHcy concentration and a marked skewness toward high tHcy values, whereas in nonsmoking subjects eating a diet rich in folate and

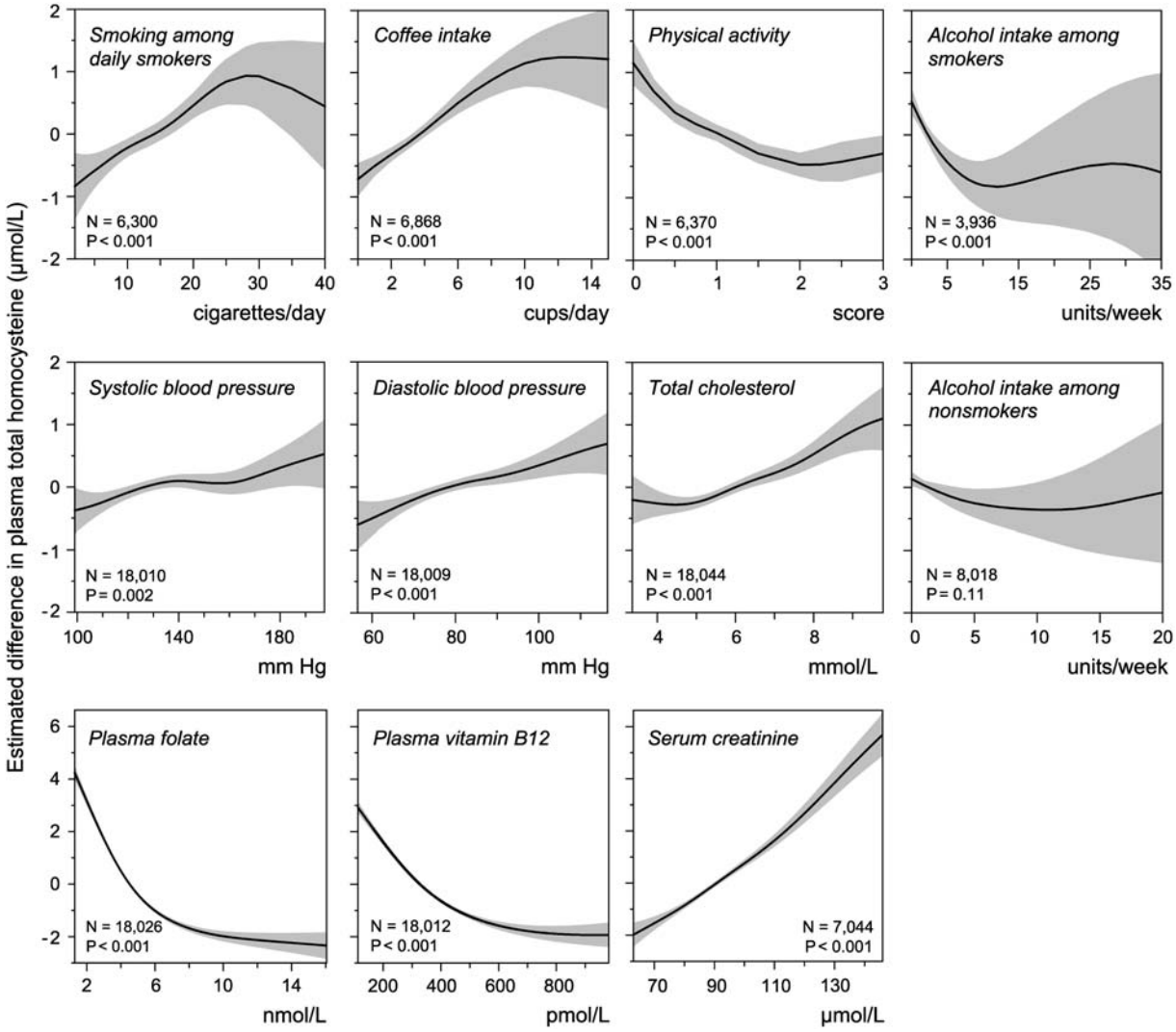

FIGURE 3 Factors associated with total homocysteine in the Hordaland Homocysteine Study. Dose– or concentration–response relationships between various factors and tHcy obtained by generalized additive logistic regression adjusted for age and gender. Shaded areas represent 95% confidence intervals. The *P*-value has been obtained by linear regression analyses, adjusted for age and gender. For the continuous independent variables, the results shown are confined to the 0.5 to 99.5 percentiles of the variable. The reference value for tHcy is the value associated with the mean value of the independent variable for all subjects. For coffee intake, physical activity, and serum creatinine levels, the data are from HHS-II, whereas for all the other variables, the data are from HHS-I. For further description of scores for alcohol intake and physical activity, see Vollset et al. (10) and Sirnes et al. (131).

drinking <1 cup of coffee per day, the tHcy values were almost normally distributed, and the median concentration was 3 to 5 μmol/L lower (12). Thus, solely a change in lifestyle may have a stronger tHcy-lowering effect than use of high-dose folic acid (15). Later analyses showed that lifestyle changes, such as higher folate intake and smoking cessation, are associated with a decline in tHcy levels (16).

In HHS-I, it was found that the *MTHFR* 677C > T polymorphism was extremely common among subjects with tHcy $\geq$40 μmol/L (73% having the TT genotype), and these subjects also had lower plasma folate and vitamin B-12 levels, were more frequently smokers, and drank more coffee (17). In the subjects who remained hyperhomocysteinemic for an average of 2 y, an uncontrolled intervention study (17) showed for the first time that a low-dose folic acid supplement of 0.2 mg per day efficiently lowers moderately raised tHcy levels. A higher dose was, however, necessary in some individuals to obtain normal tHcy levels (17). Controlled clinical trials have later confirmed that 0.2 mg folic acid per day efficiently lowers tHcy, whereas a higher dose is required to obtain a maximal tHcy-reducing effect (15).

Comments on determinants of tHcy. Age and sex are among the most consistent and strong determinants of tHcy in adults: in 1985, it was shown that premenopausal women have lower homocysteine levels than men and postmenopausal women (18), and several of the early studies reported age and/or gender differences (19). However, before the HHS, there was only 1 large-cohort study, and that was confined to subjects over 67 y of age (20). Later cohort studies have shown that tHcy concentrations are higher in men than in women after the age of 10 y and that there is a gradual increase throughout life (21–24). The reasons for the higher tHcy concentrations at older ages are not well understood, although changes in renal function are certainly involved. Higher tHcy concentrations in men than in women may be explained by differences in muscle mass, hormone and vitamin status (24).

The observed associations between tHcy and total cholesterol and blood pressure in the HHS-I (Fig. 3) (5) have been

confirmed in other studies (25–27). The reasons for the associations are unclear. Treatment with drugs affecting blood pressure or cholesterol does not have a consistent effect on tHcy (28), and statins have minimal effect on tHcy despite their efficiency in reducing cholesterol, whereas fibrates cause a significant increase (28,29). Thus, the association of raised tHcy with blood pressure and cholesterol is confusing. An alternative explanation is that tHcy affects the levels of these CVD risk factors. Although there are no data suggesting that homocysteine directly influences total cholesterol levels, there is evidence that homocysteine can affect endothelial function (30), and homocysteine-lowering therapy has been associated with lowering of blood pressure (31).

Before HHS-I, there were conflicting data on whether smoking affected tHcy levels (32–34). The dose-dependent association observed in HHS-I (Fig. 3) (5) has since been demonstrated in other large cohorts (26,35–38). The reversibility of the effect is uncertain: 1 study showed that short-term cessation of smoking does not change tHcy levels (39), whereas another suggested that cessation, but not a reduction in smoking, decreased tHcy levels (40). Thus, it is possible that the tHcy–smoking relation is not, or is only partly, dependent on the smoking itself but rather reflects other behavioral traits in smokers. An alternative explanation is that smoking has a long-lasting effect on tHcy.

The inverse relation between tHcy and physical activity seen in data from HHS-I (5) or HHS-II (Fig. 3) is not consistently found in other studies (35,37,38,41). In HHS-I, an influence of BMI was observed: subjects with the lowest BMI had the strongest inverse association between exercise and tHcy. In those with the highest BMI, the association was in the positive direction (5). BMI itself was not independently associated with tHcy (5). Interestingly, intense exercise seems to cause an increased tHcy level (42), whereas regular exercising is associated with lower tHcy levels (38,43). It remains to be shown whether regular physical activity directly influences tHcy or whether the inverse association observed in some studies reflects an overall healthier lifestyle.

The coffee effect on tHcy in HHS-I (11) and also in HHS-II (Fig. 3) was unexpected, and it was possible that the association could have been a result of residual confounding with other lifestyle factors. However, other observational studies confirmed the association (36,37,44), and intervention studies have now demonstrated that coffee raises tHcy levels (45,46), an effect that is probably mediated by chlorogenic acid (47) and caffeine (48), which are coffee constituents. Notably, the effect of coffee becomes apparent within hours after intake (48,49), whereas the duration of its effect remains to be determined.

Regarding moderate alcohol intake and tHcy, the larger-cohort studies have shown conflicting results (27,35,36), possibly because other factors seem to modify the effect of alcohol. In both HHS-I (10) and HHS-II (Fig. 3), the alcohol effect on tHcy was present only in smokers, whereas in a large Dutch cohort, the effect was stronger in those with low folate levels (35). An interaction between folate status and the *MTHFR* 677C > T polymorphism may further complicate the picture (50). Finally, the effect of alcohol on tHcy may depend on the type of beverage: spirits and possibly wine consumption may increase tHcy concentrations, whereas beer seems to have no effect or even reduced tHcy levels (27,37,51,52).

Other factors that influence tHcy levels in the general population include diet, in particular folate intake, blood levels of folate, vitamin B-12, and betaine, renal function, and the *MTHFR* 677C > T polymorphism (24,53–56). Some of these associations are depicted in Figure 3 (lowest panel) and **Figure 4**. Although these factors are important determinants of tHcy, they are not discussed here because their effects on tHcy levels have not yet been studied in detail in the general HHS population.

Homocysteine and the risk of disease

The HHS has contributed to our knowledge about associations between moderately raised tHcy levels and several common diseases (**Fig. 5** and **Fig. 6**). As with all association studies, the proof of causality requires intervention trials with tHcy-lowering treatment, usually by B-vitamin treatment, to see if the risk of the disease is reduced. Few such studies have been completed. However, it is established that the extremely high tHcy levels observed in homocystinuria lead to serious complications and often early death (57). In homocystinuria secondary to cystathionine β-synthase deficiency, lowering of tHcy by B vitamins and betaine can prevent most of the complications (58,59). This result suggests that markedly raised tHcy levels are harmful and that the effect can be prevented by lowering tHcy. Studies in the HHS on associations between moderately raised levels of tHcy and some common diseases are reviewed below.

Pregnancy complications and birth defects. Coupling data from HHS-I to data collected by the Medical Birth Registry of Norway has allowed analysis of possible associations between tHcy and pregnancy complications, adverse pregnancy outcome, and birth defects (60) (Fig. 5). Data were available from 5883 women in the age groups 40–42 y, with records of 14,492 pregnancies. This is the largest study to date on the relation between tHcy and these conditions. It should be noted that ~80% of the pregnancies occurred >10 y before blood samples were collected. Despite this shortcoming in design, it was found that raised tHcy concentrations were associated with increased risk of preeclampsia, prematurity, very low birth weight, stillbirth, and placental abruption. Also neural tube defects and clubfoot in the offspring were significantly associated with

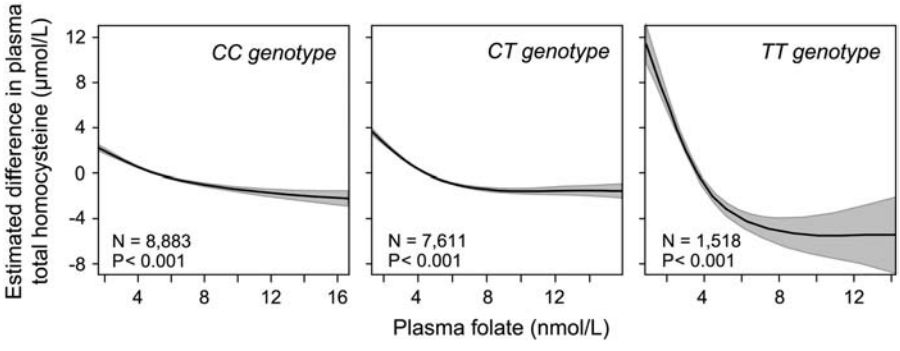

FIGURE 4 Association between plasma folate and total homocysteine according to the *MTHFR* 677C > T genotype. The concentration–response relation is obtained by generalized additive regression adjusted for age and gender. Shaded areas represent 95% confidence intervals. The *P*-value has been obtained by linear regression analyses, adjusted for age and gender. The results shown are confined to the 0.5 to 99.5 percentiles of the plasma folate concentration in each genotype. The reference value for tHcy is the value associated with the mean plasma folate level for all subjects within the genotype category. The data are from HHS-I.

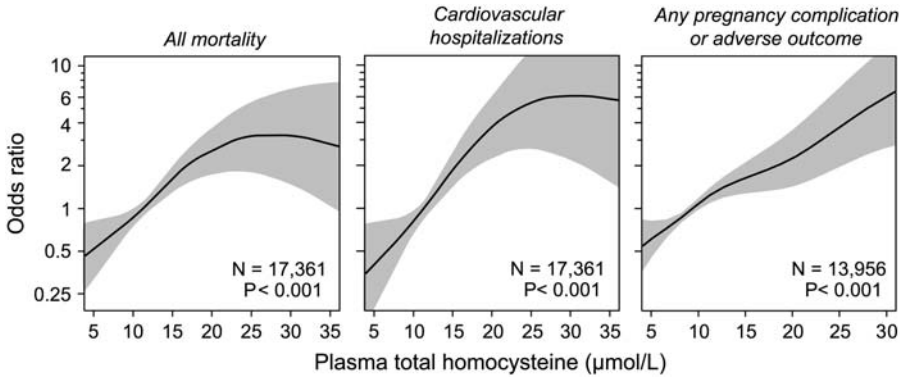

FIGURE 5 Association between plasma total homocysteine and various clinical outcomes. The concentration–response relation is obtained by generalized additive regression adjusted for age and gender, except for any complication or adverse outcomes of pregnancy (adjusted for maternal age and parity). Shaded areas represent 95% confidence intervals. The *P*-value has been obtained by linear regression analyses, using the same adjustments as for generalized additive regression models. The results shown are confined to the 0.5 to 99.5 percentiles of the plasma tHcy concentration in each panel. An odds ratio of 1 corresponds to the mean plasma tHcy level for all subjects. Plasma levels of tHcy are from HHS-I.

plasma tHcy in the mother (60). Further investigations, using the same data set, showed that maternal *MTHFR* 677C > T polymorphism was a risk factor for placental abruption and intrauterine growth restriction (61).

Numerous articles have been published on the association among tHcy, pregnancy, and the possible harmful effect of elevated tHcy; see reviews (62–65). Elevated tHcy is often looked on as being a marker of low folate status, although more recent studies indicate that homocysteine itself may act as a teratogen (62–64).

Although increased folic acid intake in the periconceptual period certainly reduces the risk of neural tube defects and some other birth defects (64), the effect of folic acid on adverse pregnancy outcome and pregnancy complications remains to be determined. Folic acid fortification does not seem to have had a measurable effect on risk of preeclampsia (66), and folic acid intervention in the early part of pregnancy does not reduce the risk of miscarriage (67). However, folic acid may reduce the risk of low birth weight (68) and pregnancy-associated hypertension (69). In West Africa, the use of micronutrient supplements including folic acid was associated with increased birth weight (70), and in HIV-positive Tanzanian women, the use of multivitamins reduced the risk of hypertension during pregnancy (71). Thus, at least in certain subgroups, homocysteine-lowering therapy may be beneficial.

Mortality and cardiovascular disease. The associations between tHcy and risk of mortality or subsequent hospitalization for CVD in the HHS-I cohort are depicted in Figure 5. So far, the association between tHcy measured in HHS-I and risk of mortality has been based on mortality data up to February 1997 (72,73), corresponding to a median follow-up time of only 4.1 y, and relatively few deaths. Nevertheless, in the older age group, a strong concentration-dependent association was observed between tHcy and overall mortality (72). With subjects with tHcy <9 μmol/L as reference, mortality was 3.6 times higher in those with tHcy ≥20 μmol/L. The association with tHcy was apparent both for cardiovascular and for non-cardiovascular mortality. The association, independent of cause of death, was strongest in older subjects with increased risk of CVD, such as angina, previous stroke, myocardial infarction and hypertension. Notably, 33% of the elderly group in HHS-I belonged to this high-CVD-risk population. Subjects with tHcy <9 μmol/L in the high-risk group had 2.9% mortality, whereas those with tHcy ≥20 μmol/L had 21% mortality (over a period of 4 y) (72).

In a study that was confined to cardiovascular mortality in both the older and the younger age groups in the HHS-I cohort, no significant associations were found between baseline tHcy levels and CVD deaths in the younger age group, and only a weak association in the older group without history of CVD or hypertension at baseline. In contrast, in the older group with history of CVD or hypertension at baseline, those with tHcy ≥20 μmol/L had a 3-fold greater risk of cardiovascular mortality compared those with tHcy <9 μmol/L (73). In another study from the same region in Norway, confined to patients with coronary artery disease, those with tHcy

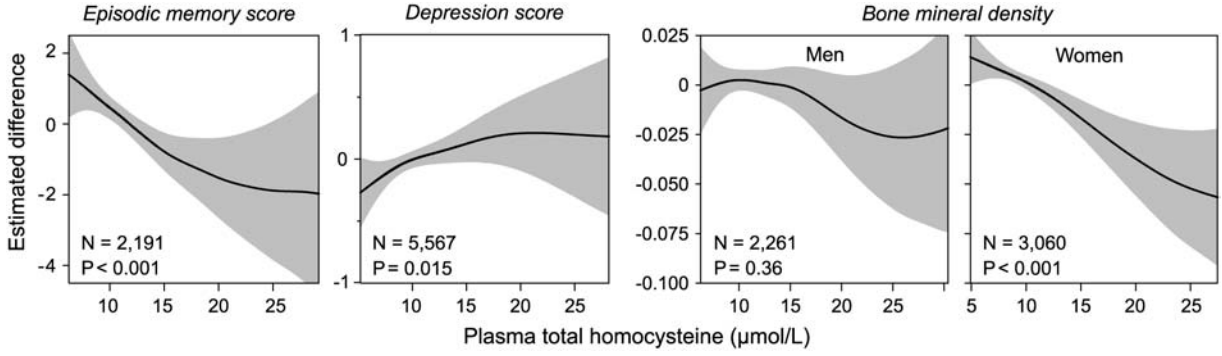

FIGURE 6 Association between plasma total homocysteine and various scores reflecting episodic memory, depressive mood, or bone mineral density. The concentration–response relationships are obtained by generalized additive regression. Depression score (HADS-D) and episodic memory score (Kendrick Object Learning Test score) are adjusted for age and gender. Bone mineral density measured by densitometry of the hip is adjusted for age. Shaded areas represent 95% confidence intervals. The *P*-value has been obtained by linear regression analyses, using the same adjustments as for generalized additive regression models. The results shown are confined to the 0.5 to 99.5 percentiles of the plasma tHcy concentration in each panel. The reference value for the outcome variable is the value associated with the mean value of tHcy. The data are from HHS-II.

<9 μmol/L had 3.8% mortality, whereas patients with ≥20 μmol/L had 27% mortality over a period of 4.6 y (74), i.e., quite similar to the high-risk group in HHS.

The HHS database was used to investigate the association between tHcy levels from HHS-I and the risk of hospitalization for CVD (73). After a mean follow-up time of 5.3 y, 3.7% of the participants in the younger group and 16.8% of those in the older group had been hospitalized for CVD-related conditions. The risk of hospitalization increased with tHcy levels in a concentration-dependent manner, but it was significant only in the older age group (65–67 y at baseline), and, as for mortality, the risk was strongly dependent on preexisting CVD risk factors. Indeed, in those aged 40–42 y and without CVD or hypertension at baseline, there was no association between tHcy and hospitalization (73).

Similar observations on tHcy and mortality have been made in other large-cohort studies. Most of these have focused on CVD-related mortality, and they usually report that preexisting CVD risk markedly increases the association between tHcy and mortality (75–80). Elevated tHcy concentrations are, however, not a strong risk factor for mortality in relatively young subjects free of baseline CVD (81). Neither is tHcy a particularly strong risk factor for cardiovascular events in subjects free of CVD at the time of tHcy measurement (82). Thus, it can be concluded that in young or middle-aged adults without particular risk factors for CVD, tHcy is not a strong risk factor for CVD events, for hospitalization because of CVD events, or for death in general.

Meta-analyses on the relation between tHcy and CVD suggest that tHcy is a risk factor for venous thrombosis (83,84) and for coronary heart disease and stroke (82,84). Notably, the *MTHFR* 677 TT genotype is associated with a small increase in risk for heart disease (85) and venous thrombosis (83), consistent with its tHcy-increasing effect. The genetic studies do not share the same potential sources of error as the prospective studies because they represent a natural process of randomization (Mendelian randomization) (86). Thus, these results support the hypothesis that impaired folate metabolism or high tHcy levels are causally related to increased risk of CVD (84,85). However, the clinical significance of the genetic data has recently been questioned (87).

The key question is whether a raised level of tHcy causes CVD or whether it is simply a surrogate marker of, for example, poor lifestyle or impaired renal function. Many tHcy-lowering trials have been initiated to answer this question (15). Smaller trials with B vitamins in CVD patients show conflicting results (15). For instance, 1 study showed that a combination of folic acid and vitamin B-12 and B-6 decreased the incidence of major adverse events after percutaneous coronary intervention (88), whereas another study showed that a similar combination of B vitamins, but with lower vitamin B-12 dose, increased the risk for in-stent restenosis (89).

Among larger intervention trials (15), results are available from 2 studies that investigated the effect of folic acid, vitamin B-12, and high doses of vitamin B-6: VISP included 3680 stroke patients from North America and Scotland treated for 2 y (90); NORVIT included 3479 Norwegian patients with acute myocardial infarction followed for 3.5 y (91). Neither trial showed a benefit of combined B-vitamin intervention on events or deaths, although a reexamination of the data from VISP showed a 21% reduction in CVD events and mortality in a subgroup defined by their vitamin B-12 status at baseline (92). A third large-scale trial, CHAOS, was prematurely terminated because it would lack power to demonstrate any effect (15), and recently a Norwegian study, WENBIT, with similar treatment intervention as NORVIT, was stopped because of lack of compliance among the participants following media reports of the NORVIT study.

Folic acid fortification in the United States has reduced tHcy levels in the population (93). Thus, if raised tHcy is a risk factor for mortality, this should be revealed by changes in mortality rate. In 1 study, it was estimated that ~13,000 deaths from stroke have been prevented each year in the United States since fortification was started (94). However, in another study, it was concluded that the effects of fortification on mortality were negligible in patients who had coronary disease (95).

Experimental research shows that high levels of homocysteine may be toxic to the blood vessels. It causes endothelial dysfunction, accelerates thrombin formation, inhibits native thrombolysis, promotes lipid peroxidation through free radical formation, and induces vascular smooth muscle proliferation and monocyte chemotaxis (30,96–98). In particular, the human studies on vascular reactivity and the animal models relating homocysteine to atherosclerosis provide evidence that raised homocysteine levels may be harmful (30,97). Despite the epidemiologic and experimental studies, the current status of tHcy in CVD risk assessment is equivocal: tHcy is a powerful prognostic marker of mortality and CVD events in patients with preexisting CVD risk factors, but the evidence is not sufficient to conclude that moderately raised homocysteine causes CVD.

Anxiety and depression. In HHS-II, nearly 6000 subjects responded to a questionnaire that included the Hospital Anxiety and Depression Scale (6). There was no significant association between tHcy and anxiety, but there was a weak concentration–response relation between tHcy and depression score (Fig. 6) and risk of depression (6). The association was strongest for those with tHcy >15 μmol/L; they had 2-fold higher risk of having depression compared with subjects with tHcy <9 μmol/L. In addition, it was observed that those with the *MTHFR* 677 TT genotype had a 70% higher risk of depression compared with the CC genotype (6).

Although the relation between folate status and depression has been addressed since the 1960s (99), studies that include measurements of tHcy as well as folate and vitamin B-12 started in the 1990s (100,101). In recent years, the associations of tHcy and related B vitamins with depression have been studied in larger cohorts. Most of these studies found an association between depression and tHcy or B-vitamin status, but, often, the relation was weak and sometimes disappeared in multivariate analyses (102–107)

To date, there are no data from large-scale intervention studies, but available evidence from open trials and some few double-blind placebo-controlled clinical trials suggests that folic acid may have a potential role as a supplement to other treatments for depression (99,108).

Cognitive impairment. The HHS-II also assessed cognition in 2189 elderly subjects in relation to tHcy at baseline and tHcy determined 6 y later, i.e., when the cognitive tests were done (7). Episodic memory scores, using the Kendrick Object Learning Test, were inversely related in a concentration-related manner to the tHcy levels at both time points (Fig. 6). A fall in tHcy, or a rise in serum folate, over 6 y (i.e., from HHS-I to HHS-II) was associated with a higher memory score, whereas a rise in tHcy or a fall in folate was associated with a lower memory score.

There is now a considerable body of evidence showing an association of moderately raised tHcy levels with dementia and with cognitive impairment (109–112). Cognitive impairment in the elderly is associated with shrinkage of the brain. Notably, raised tHcy is associated with more rapid shrinkage of the brain in patients with dementia (113), and cross-sectional studies in

community-dwelling elderly have shown that elevated levels of tHcy are associated with smaller size of several regions of the brain (114,115). Experimental studies indicate that homocysteine is neurotoxic, which could possibly account for its association with brain atrophy and with cognitive impairment (116). Altogether, the epidemiologic and experimental findings are consistent with a causal role for high homocysteine or low folate in cognitive impairment in the elderly, but results from tHcy-lowering trials are needed before this conclusion can be drawn.

Bone mineral density. HHS-II examined the association of BMD of the hip with plasma tHcy, folate, and vitamin B-12. BMD of the hip was measured in 2268 men and 3070 women, aged 47–50 and 71–75 y (8). Plasma levels of tHcy were inversely related to BMD in women but not in men (Fig. 6). Likewise, the risk of osteoporosis among subjects with tHcy >15 μmol/L compared with tHcy <9 μmol/L was 2.8 (95% CI 1.6–5.0) for elderly women and not significant for elderly men.

Our study adds to the increasing evidence that plasma tHcy is inversely associated with bone health (8). It has been speculated that moderately elevated tHcy levels could contribute to osteoporotic changes (117), based on the fact that osteoporosis is a common phenomenon in homocystinuria (57). In a Japanese study of postmenopausal women, *MTHFR* 677TT genotype was found to be associated with BMD (118). In 2004, there were 2 reports showing that tHcy is a risk factor for osteoporotic fractures in men and women (119,120). The association between tHcy and the risk of fracture appeared to be independent of BMD (119). These reports have been followed by other studies on tHcy and B vitamins and their relation to BMD or osteoporosis (121–123). Perhaps the most notable report is an intervention study in a Japanese stroke population that indicated that combined treatment with folate and vitamin B-12 reduced the risk of a hip fracture in elderly stroke patients (124).

It remains unclear whether the association between tHcy and BMD, osteoporosis and fractures is mediated directly by homocysteine itself or if tHcy is just a marker. Disturbed cross-linking of collagen has been detected in homocystinurics (125), and treatment of such patients with vitamin B-6 seems to delay development of osteoporosis (58). High tHcy and low vitamin B-12 concentrations are significantly associated with high levels of markers of bone turnover (126), and relations have been reported between tHcy and markers of bone resorption (127). Recent in vitro studies suggest that homocysteine disturbs osteoblast function (128) and leads to increased osteoclast activity (129). Thus, there exist plausible mechanisms for linking homocysteine to bone metabolism and turnover.

Implications

Raised tHcy levels are associated with a plethora of serious clinical manifestations, starting at conception and ending with death. However, questions remain. Is homocysteine per se responsible for the effects? If so, is it possible to define a range of "safe" tHcy levels that can be used as a basis for advising people on how they should obtain or retain "safe" tHcy levels?

Overall, the epidemiologic studies, including HHS, provide convincing evidence that elevated tHcy levels are associated with increased risk of disease. Most of these studies suggest that a significant association is observed for tHcy levels above 12–15 μmol/L. However, the findings in the larger studies suggest that the relation between tHcy and disease is usually concentration-dependent, with a gradual increase in risk from the low-normal to above-normal range for tHcy (Fig. 5). Experimental data suggest that it is biologically plausible that homocysteine could cause damage and impair normal cellular and physiologic functions (97,98,130). Folic acid intervention reduces the risk of neural tube defects (64), but whether this effect is caused by a lowering of tHcy remains an open question. In relation to other conditions related to homocysteine, such as pregnancy complications and adverse outcome, CVD, osteoporosis, psychiatric disorders, and cognitive impairment, results from vitamin intervention trials are sparse, incomplete, and conflicting. Thus, currently it is not appropriate to recommend the use of B-vitamin supplements to lower tHcy levels with the intention of protecting against adverse effects of homocysteine.

However, based on HHS and other studies, it is possible to conclude that there are significant and sometimes very strong relationships between high levels of tHcy and disease and between low levels of tHcy and health. Even if raised tHcy might not be a direct cause of disease, it is a prognostic marker of serious chronic disorders and death. Raised tHcy levels are related to a poor lifestyle, and a change in lifestyle will also change the tHcy levels (16). Thus, for those with raised tHcy levels, the best approach is to inform them of the risks and to recommend a healthy lifestyle. A healthy lifestyle not only will lower tHcy levels but, more importantly, will promote health irrespective of whether homocysteine is a cause or a marker of disease.

LITERATURE CITED

1. Ueland PM, Nygard O, Vollset SE, Refsum H. The Hordaland Homocysteine Studies. Lipids. 2001;36:S33–9.
2. Bjartveit K, Foss OP, Gjervig T, Lund-Larsen PG. The cardiovascular disease study in Norwegian counties. Background and organization. Acta Med Scand Suppl. 1979;634:1–70.
3. Andersen LF, Solvoll K, Johansson LR, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. Am J Epidemiol. 1999;150:75–87.
4. Nes M, Frost Andersen L, Solvoll K, Sandstad B, Hustvedt BE, Lovo A, Drevon CA. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. Eur J Clin Nutr. 1992;46:809–21.
5. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland PM, Kvåle G. Total plasma homocysteine and cardiovascular risk profile.The Hordaland Homocysteine Study. JAMA. 1995;274:1526–33.
6. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. Arch Gen Psychiatry. 2003;60: 618–26.
7. Nurk E, Refsum H, Tell GS, Engedal K, Vollset SE, Ueland PM, Nygaard HA, Smith AD. Plasma total homocysteine and cognition in the elderly. The Hordaland Homocysteine Study. Ann Neurol. 2005; 58:847–57.
8. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Tell GS. Plasma total homocysteine and bone mineral density. The Hordaland Homocysteine Study. Arch Intern Med. 2006;166:88–94.
9. Vikse BE, Vollset SE, Tell GS, Refsum H, Iversen BM. Distribution and determinants of serum creatinine in the general population: the Hordaland Health Study. Scand J Clin Lab Invest. 2004;64:709–22.
10. Vollset SE, Nygård OGK, Ueland PM, Refsum H. The Hordaland homocysteine study: Lifestyle and and plasma homocysteine in Western Norway. In: Graham I, Refsum H, Rosenberg IH, Ueland PM, editors. Homocysteine metabolism. From basic science to clinical medicine. Norwell, MA: Kluwer Academic Publishers; 1997. p. 177–82.
11. Nygård O, Refsum H, Ueland PM, Stensvold I, Nordrehaug JE, Kvale G, Vollset SE. Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. Am J Clin Nutr. 1997;65:136–43.
12. Nygård O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr. 1998;67:263–70.
13. Refsum H, Nygard O, Kvale G, Ueland PM, Vollset SE. The Hordaland homocysteine study: the opposite tails odds ratios reveal differential effects of gender and intake of vitamin supplements at high and low plasma total homocysteine concentrations. J Nutr. 1996;126:1244S–8S.
14. Hultberg B, Berglund M, Andersson A, Frank A. Elevated plasma homocysteine in alcoholics. Alcohol Clin Exp Res. 1993;17:687–9.
15. Clarke R. Homocysteine-lowering trials for prevention of heart disease and stroke. Semin Vasc Med. 2005;5:215–22.
16. Nurk E, Tell GS, Vollset SE, Nygard O, Refsum H, Nilsen RM, Ueland PM. Changes in lifestyle and plasma total homocysteine: the Hordaland Homocysteine Study. Am J Clin Nutr. 2004;79:812–9.

17. Guttormsen AB, Ueland PM, Nesthus I, Nygård O, Schneede J, Vollset SE, Refsum H. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥40 micromol/liter). The Hordaland Homocysteine Study. J Clin Invest. 1996;98:2174–83.

18. Boers GH, Smals AG, Trijbels FJ, Fowler B, Bakkeren JA, Schoonderwaldt HC, Kleijer WJ, Kloppenborg PW. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med. 1985;313:709–15.

19. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. Clin Chem. 1993;39:1764–79.

20. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA. 1993;270:2693–8.

21. Jacques PF, Rosenberg IH, Rogers G, Selhub J, Bowman BA, Gunter EW, Wright JD, Johnson CL. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 1999;69:482–9.

22. Must A, Jacques PF, Rogers G, Rosenberg IH, Selhub J. Serum total homocysteine concentrations in children and adolescents: results from the third National Health and Nutrition Examination Survey (NHANES III). J Nutr. 2003;133: 2643–9.

23. Russo GT, Friso S, Jacques PF, Rogers G, Cucinotta D, Wilson PW, Ordovas JM, Rosenberg IH, Selhub J. Age and gender affect the relation between methylenetetrahydrofolate reductase C677T genotype and fasting plasma homocysteine concentrations in the Framingham Offspring Study Cohort. J Nutr. 2003;133:3416–21.

24. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem. 2004;50:3–32.

25. de Bree A, van der Put NM, Mennen LI, Verschuren WM, Blom HJ, Galan P, Bates CJ, Herrmann W, Ullrich M, et al. Prevalences of hyperhomocysteinemia, unfavorable cholesterol profile and hypertension in European populations. Eur J Clin Nutr. 2005;59:480–8.

26. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988–1994. Stroke. 1998;29: 2473–7.

27. Ganji V, Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2003;77: 826–33.

28. Dierkes J, Westphal S.Effect of drugs on homocysteine concentrations. Semin Vasc Med. 2005;5:124–39.

29. Milionis HJ, Papakostas J, Kakafika A, Chasiotis G, Seferiadis K, Elisaf MS. Comparative effects of atorvastatin, simvastatin, and fenofibrate on serum homocysteine levels in patients with primary hyperlipidemia. J Clin Pharmacol. 2003;43:825–30.

30. Moat SJ, McDowell IF. Homocysteine and endothelial function in human studies. Semin Vasc Med. 2005;5:172–82.

31. Stehouwer CD, van Guldener C. Does homocysteine cause hypertension? Clin Chem Lab Med. 2003;41:1408–11.

32. Genest JJ, Jr., McNamara JR, Salem DN, Wilson PW, Schaefer EJ, Malinow MR.Plasma homocyst(e)ine levels in men with premature coronary artery disease. J Am Coll Cardiol. 1990;16:1114–9.

33. Taylor LM, Jr., DeFrang RD, Harris EJ, Jr., Porter JM.The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease. J Vasc Surg. 1991;13:128–36.

34. Mansoor MA, Bergmark C, Svardal AM, Lonning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. Homocysteine and peripheral vascular disease. Arterioscler Thromb Vasc Biol. 1995;15:232–40.

35. de Bree A, Verschuren WM, Blom HJ, Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am J Epidemiol. 2001;154:150–4.

36. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr. 2001;73:613–21.

37. Husemoen LL, Thomsen TF, Fenger M, Jorgensen T. Effect of lifestyle factors on plasma total homocysteine concentrations in relation to MTHFR(C677T) genotype.Inter99 (7). Eur J Clin Nutr. 2004;58:1142–50.

38. Chrysohoou C, Panagiotakos DB, Pitsavos C, Zeimbekis A, Zampelas A, Papademetriou L, Masoura C, Stefanadis C. The associations between smoking, physical activity, dietary habits and plasma homocysteine levels in cardiovascular disease-free people: the 'ATTICA' study. Vasc Med. 2004;9:117–23.

39. Tonstad S, Urdal P. Does short-term smoking cessation reduce plasma total homocysteine concentrations? Scand J Clin Lab Invest. 2002;62:279–84.

40. Stein JH, Bushara M, Bushara K, McBride PE, Jorenby DE, Fiore MC. Smoking cessation, but not smoking reduction, reduces plasma homocysteine levels. Clin Cardiol. 2002;25:23–6.

41. Mennen LI, de Courcy GP, Guilland JC, Ducros V, Bertrais S, Nicolas JP, Maurel M, Zarebska M, Favier A, et al. Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. Am J Clin Nutr. 2002;76:1279–89.

42. Herrmann M, Schorr H, Obeid R, Scharhag J, Urhausen A, Kindermann W, Herrmann W. Homocysteine increases during endurance exercise. Clin Chem Lab Med. 2003;41:1518–24.

43. Konig D, Bisse E, Deibert P, Muller HM, Wieland H, Berg A. Influence of training volume and acute physical exercise on the homocysteine levels in endurance-trained men: interactions with plasma folate and vitamin B12. Ann Nutr Metab. 2003;47:114–8.

44. Stolzenberg-Solomon RZ, Miller ER 3rd, Maguire MG, Selhub J, Appel LJ. Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. Am J Clin Nutr. 1999;69:467–75.

45. Grubben MJ, Boers GH, Blom HJ, Broekhuizen R, de Jong R, van Rijt L, de Ruijter E, Swinkels DW, Nagengast FM, Katan MB. Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial. Am J Clin Nutr. 2000;71:480–4.

46. Urgert R, van Vliet T, Zock PL, Katan MB. Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers. Am J Clin Nutr. 2000;72:1107–10.

47. Olthof MR, Hollman PC, Zock PL, Katan MB. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. Am J Clin Nutr. 2001;73:532–8.

48. Verhoef P, Pasman WJ, Van Vliet T, Urgert R, Katan MB. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. Am J Clin Nutr. 2002;76:1244–8.

49. Slow S, Miller WE, McGregor DO, Lee MB, Lever M, George PM, Chambers ST. Trigonelline is not responsible for the acute increase in plasma homocysteine following ingestion of instant coffee. Eur J Clin Nutr. 2004;58: 1253–6.

50. Chiuve SE, Giovannucci EL, Hankinson SE, Hunter DJ, Stampfer MJ, Willett WC, Rimm EB. Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. Am J Clin Nutr. 2005;82:155–62.

51. Mennen LI, de Courcy GP, Guilland JC, Ducros V, Zarebska M, Bertrais S, Favier A, Hercberg S, Galan P. Relation between homocysteine concentrations and the consumption of different types of alcoholic beverages: the French Supplementation with Antioxidant Vitamins and Minerals Study. Am J Clin Nutr. 2003;78:334–8.

52. van der Gaag MS, Ubbink JB, Sillanaukee P, Nikkari S, Hendriks HF. Effect of consumption of red wine, spirits, and beer on serum homocysteine. Lancet. 2000;355:1522.

53. van Guldener C, Stehouwer CD.Homocysteine and methionine metabolism in renal failure. Semin Vasc Med. 2005;5:201–8.

54. Gellekink H, den Heijer M, Heil SG, Blom HJ. Genetic determinants of plasma total homocysteine. Semin Vasc Med. 2005;5:98–109.

55. Ueland PM, Holm PI, Hustad S. Betaine: a key modulator of one-carbon metabolism and homocysteine status. Clin Chem Lab Med. 2005;43:1069–75.

56. Verhoef P, de Groot LC. Dietary determinants of plasma homocysteine concentrations. Semin Vasc Med. 2005;5:110–23.

57. Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GH, Bromberg IL, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet. 1985;37:1–31.

58. Yap S, Naughten E. Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. J Inherit Metab Dis. 1998;21:738–47.

59. Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, Walter JH, Howard PM, Naughten ER. Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. Arterioscler Thromb Vasc Biol. 2001;21:2080–5.

60. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, Monsen AL, Ueland PM. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr. 2000;71:962–8.

61. Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. Am J Med. 2004;117: 26–31.

62. Brauer PR, Tierney BJ. Consequences of elevated homocysteine during embryonic development and possible modes of action. Curr Pharm Des. 2004;10: 2719–32.

63. Ueland PM, Vollset SE. Homocysteine and folate in pregnancy. Clin Chem. 2004;50:1293–5.

64. Daly S, Cotter A, Molloy AE, Scott J. Homocysteine and folic acid: implications for pregnancy. Semin Vasc Med. 2005;5:190–200.

65. Mignini LE, Latthe PM, Villar J, Kilby MD, Carroli G, Khan KS. Mapping the theories of preeclampsia: the role of homocysteine. Obstet Gynecol. 2005;105: 411–25.

66. Ray JG, Mamdani MM. Association between folic acid food fortification and hypertension or preeclampsia in pregnancy. Arch Intern Med. 2002;162: 1776–7.

67. Gindler J, Li Z, Berry RJ, Zheng J, Correa A, Sun X, Wong L, Cheng L, Erickson JD, et al. Folic acid supplements during pregnancy and risk of miscarriage. Lancet. 2001;358:796–800.

68. Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall MH. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. Paediatr Perinat Epidemiol. 2005;19:112–24.

69. Hernandez-Diaz S, Werler MM, Louik C, Mitchell AA. Risk of gestational hypertension in relation to folic acid supplementation during pregnancy. Am J Epidemiol. 2002;156:806–12.

70. Kaestel P, Michaelsen KF, Aaby P, Friis H. Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. Eur J Clin Nutr. 2005;59:1081–9.
71. Merchant AT, Msamanga G, Villamor E, Saathoff E, O'Brien M, Hertzmark E, Hunter DJ, Fawzi WW. Multivitamin supplementation of HIV-positive women during pregnancy reduces hypertension. J Nutr. 2005;135:1776–81.
72. Vollset SE, Refsum H, Tverdal A, Nygard O, Nordrehaug JE, Tell GS, Ueland PM. Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. Am J Clin Nutr. 2001;74:130–6.
73. Nurk E, Tell GS, Vollset SE, Nygard O, Refsum H, Ueland PM. Plasma total homocysteine and hospitalizations for cardiovascular disease: the Hordaland Homocysteine Study. Arch Intern Med. 2002;162:1374–81.
74. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997;337:230–6.
75. Bostom AG, Silbershatz H, Rosenberg IH, Selhub J, D'Agostino RB, Wolf PA, Jacques PF, Wilson PW. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Arch Intern Med. 1999;159:1077–80.
76. Kark JD, Selhub J, Adler B, Gofin J, Abramson JH, Friedman G, Rosenberg IH. Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. Ann Intern Med. 1999;131: 321–30.
77. Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott JM. Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. Arch Intern Med. 1998;158:862–7.
78. Virtanen JK, Voutilainen S, Alfthan G, Korhonen MJ, Rissanen TH, Mursu J, Kaplan GA, Salonen JT. Homocysteine as a risk factor for CVD mortality in men with other CVD risk factors: the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. J Intern Med. 2005;257:255–62.
79. Zylberstein DE, Bengtsson C, Bjorkelund C, Landaas S, Sundh V, Thelle D, Lissner L. Serum homocysteine in relation to mortality and morbidity from coronary heart disease: a 24-year follow-up of the population study of women in Gothenburg. Circulation. 2004;109:601–6.
80. Blacher J, Benetos A, Kirzin JM, Malmejac A, Guize L, Safar ME. Relation of plasma total homocysteine to cardiovascular mortality in a French population. Am J Cardiol. 2002;90:591–5.
81. de Bree A, Verschuren WM, Blom HJ, Nadeau M, Trijbels FJ, Kromhout D. Coronary heart disease mortality, plasma homocysteine, and B-vitamins: a prospective study. Atherosclerosis. 2003;166:369–77.
82. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA. 2002;288:2015–22.
83. Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost. 2005;3:292–9.
84. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ. 2002;325:1202.
85. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002;288:2023–31.
86. Minelli C, Thompson JR, Tobin MD, Abrams KR. An integrated approach to the meta-analysis of genetic association studies using Mendelian randomization. Am J Epidemiol. 2004;160:445–52.
87. Lewis SJ, Ebrahim S, Smith GD. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005;331:1053.
88. Schnyder G, Roffi M, Flammer Y, Pin R, Hess OM. Effect of homocysteine-lowering therapy with folic acid, vitamin B12, and vitamin B6 on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. JAMA. 2002;288:973–9.
89. Lange H, Suryapranata H, De Luca G, Borner C, Dille J, Kallmayer K, Pasalary MN, Scherer E, Dambrink JH. Folate therapy and in-stent restenosis after coronary stenting. N Engl J Med. 2004;350:2673–81.
90. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA. 2004;291:565–75.
91. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med. 2006;354:1578–88.
92. Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin Intervention For Stroke Prevention trial: an efficacy analysis. Stroke. 2005;36:2404–9.
93. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med. 1999;340:1449–54.
94. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, Friedman JM. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation. 2006;113:1335–43.
95. Anderson JL, Jensen KR, Carlquist JF, Bair TL, Horne BD, Muhlestein JB. Effect of folic acid fortification of food on homocysteine-related mortality. Am J Med. 2004;116:158–64.
96. Splaver A, Lamas GA, Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. Am Heart J. 2004;148:34–40.
97. Wilson KM, Lentz SR. Mechanisms of the atherogenic effects of elevated homocysteine in experimental models. Semin Vasc Med. 2005;5:163–71.
98. Jacobsen DW, Catanescu O, Dibello PM, Barbato JC. Molecular targeting by homocysteine: a mechanism for vascular pathogenesis. Clin Chem Lab Med. 2005;43:1076–83.
99. Paul RT, McDonnell AP, Kelly CB. Folic acid: neurochemistry, metabolism and relationship to depression. Hum Psychopharmacol. 2004;19:477–88.
100. Bell IR, Edman JS, Selhub J, Morrow FD, Marby DW, Kayne HL, Cole JO. Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. Acta Psychiatr Scand. 1992;86:386–90.
101. Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B12, and homocysteine in major depressive disorder. Am J Psychiatry. 1997;154:426–8.
102. Penninx BW, Guralnik JM, Ferrucci L, Fried LP, Allen RH, Stabler SP. Vitamin B(12) deficiency and depression in physically disabled older women: epidemiologic evidence from the Women's Health and Aging Study. Am J Psychiatry. 2000;157:715–21.
103. Sachdev PS, Parslow RA, Lux O, Salonikas C, Wen W, Naidoo D, Christensen H, Jorm AF. Relationship of homocysteine, folic acid and vitamin B12 with depression in a middle-aged community sample. Psychol Med. 2005;35: 529–38.
104. Tiemeier H, van Tuijl HR, Hofman A, Meijer J, Kiliaan AJ, Breteler MM. Vitamin B12, folate, and homocysteine in depression: the Rotterdam Study. Am J Psychiatry. 2002;159:2099–101.
105. Tolmunen T, Hintikka J, Voutilainen S, Ruusunen A, Alfthan G, Nyyssonen K, Viinamaki H, Kaplan GA, Salonen JT. Association between depressive symptoms and serum concentrations of homocysteine in men: a population study. Am J Clin Nutr. 2004;80:1574–8.
106. Morris MS, Fava M, Jacques PF, Selhub J, Rosenberg IH. Depression and folate status in the US Population. Psychother Psychosom. 2003;72:80–7.
107. Ramos MI, Allen LH, Haan MN, Green R, Miller JW. Plasma folate concentrations are associated with depressive symptoms in elderly Latina women despite folic acid fortification. Am J Clin Nutr. 2004;80:1024–8.
108. Bottiglieri T. Homocysteine and folate metabolism in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29:1103–12.
109. Smith AD. Homocysteine, B vitamins, and cognitive deficit in the elderly. Am J Clin Nutr. 2002;75:785–6.
110. Morris MS. Homocysteine and Alzheimer's disease. Lancet Neurol. 2003;2:425–8.
111. Troen A, Rosenberg I. Homocysteine and cognitive function. Semin Vasc Med. 2005;5:209–14.
112. Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Porcellini E, Licastro F. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr. 2005;82:636–43.
113. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol. 1998;55:1449–55.
114. Williams JH, Pereira EA, Budge MM, Bradley KM. Minimal hippocampal width relates to plasma homocysteine in community-dwelling older people. Age Ageing. 2002;31:440–4.
115. den Heijer T, Vermeer SE, Clarke R, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MM. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain. 2003;126:170–5.
116. Kruman II, Kumaravel TS, Lohani A, Pedersen WA, Cutler RG, Kruman Y, Haughey N, Lee J, Evans M, Mattson MP. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. J Neurosci. 2002;22:1752–62.
117. Brattstrom LE, Hultberg BL, Hardebo JE. Folic acid responsive postmenopausal homocysteinemia. Metabolism. 1985;34:1073–7.
118. Miyao M, Morita H, Hosoi T, Kurihara H, Inoue S, Hoshino S, Shiraki M, Yazaki Y, Ouchi Y. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. Calcif Tissue Int. 2000;66:190–4.
119. van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, van der Klift M, de Jonge R, Lindemans J, de Groot LC, Hofman A, Witteman JC, et al. Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med. 2004;350:2033–41.
120. McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples LA, Kiel DP. Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med. 2004;350:2042–9.
121. Golbahar J, Aminzadeh MA, Hamidi SA, Omrani GR. Association of red blood cell 5-methyltetrahydrofolate folate with bone mineral density in postmenopausal Iranian women. Osteoporos Int. 2005;16:1894–8.
122. Morris MS, Jacques PF, Selhub J. Relation between homocysteine and B-vitamin status indicators and bone mineral density in older Americans. Bone. 2005;37:234–42.
123. Dhonukshe-Rutten RA, van Dusseldorp M, Schneede J, de Groot LC, van Staveren WA. Low bone mineral density and bone mineral content are associated with low cobalamin status in adolescents. Eur J Nutr. 2005;44:341–7.
124. Sato Y, Honda Y, Iwamoto J, Kanoko T, Satoh K. Effect of folate and mecobalamin on hip fractures in patients with stroke: a randomized controlled trial. JAMA. 2005;293:1082–8.
125. Lubec B, Fang-Kircher S, Lubec T, Blom HJ, Boers GH. Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. Biochim Biophys Acta. 1996;1315:159–62.
126. Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B12 status relate to bone turnover

markers, broadband ultrasound attenuation, and fractures in healthy elderly people. J Bone Miner Res. 2005;20:921–9.

127. Herrmann M, Kraenzlin M, Pape G, Sand-Hill M, Herrmann W. Relation between homocysteine and biochemical bone turnover markers and bone mineral density in peri- and post-menopausal women. Clin Chem Lab Med. 2005;43: 1118–23.

128. Sakamoto W, Isomura H, Fujie K, Deyama Y, Kato A, Nishihira J, Izumi H. Homocysteine attenuates the expression of osteocalcin but enhances osteopontin in MC3T3–E1 preosteoblastic cells. Biochim Biophys Acta. 2005;1740:12–6.

129. Herrmann M, Widmann T, Colaianni G, Colucci S, Zallone A, Herrmann W. Increased osteoclast activity in the presence of increased homocysteine concentrations. Clin Chem. 2005;51:2348–53.

130. Kuo HK, Sorond FA, Chen JH, Hashmi A, Milberg WP, Lipsitz LA. The role of homocysteine in multisystem age-related problems: a systematic review. J Gerontol A Biol Sci Med Sci. 2005;60:1190–201.

131. Sirnes E, Sodal E, Nurk E, Tell GS. [Occurrence of musculoskeletal complaints in Hordaland](Nor). Tidsskr Nor Laegeforen. 2003;123:2855–9.