

5th Amino Acid Assessment Workshop

The Hordaland Homocysteine Study: A Community-Based Study of Homocysteine, Its Determinants, and Associations with Disease¹

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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 $\mu\text{mol/L}$, 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.

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

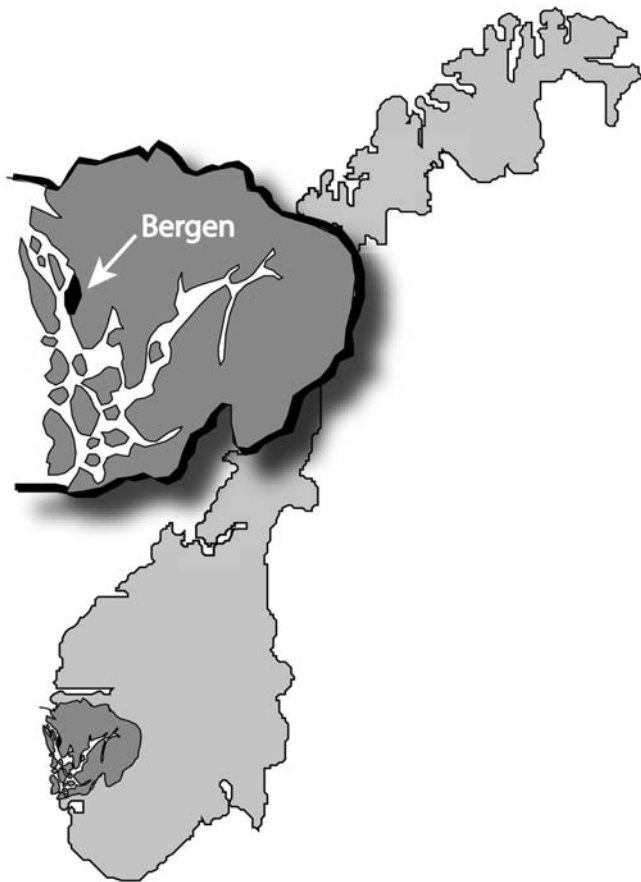


FIGURE 1 The county of Hordaland is located on the western coast of Norway. From Ueland et al. (1) and reprinted with permission of AOCs. Originally published in *Lipids* 36, Suppl (2001), S33.

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

The Hordaland Homocysteine Study I

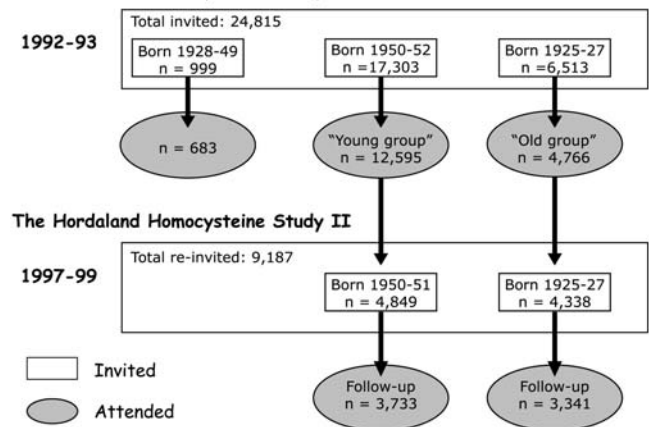


FIGURE 2 Recruitment into the Hordaland Homocysteine Study. The cohort was established in 1992–1993 and included 18,044 community-dwelling individuals born in 1925–1952. The “younger group” was recruited from the entire county of Hordaland, the older group was recruited from the city of Bergen and 3 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

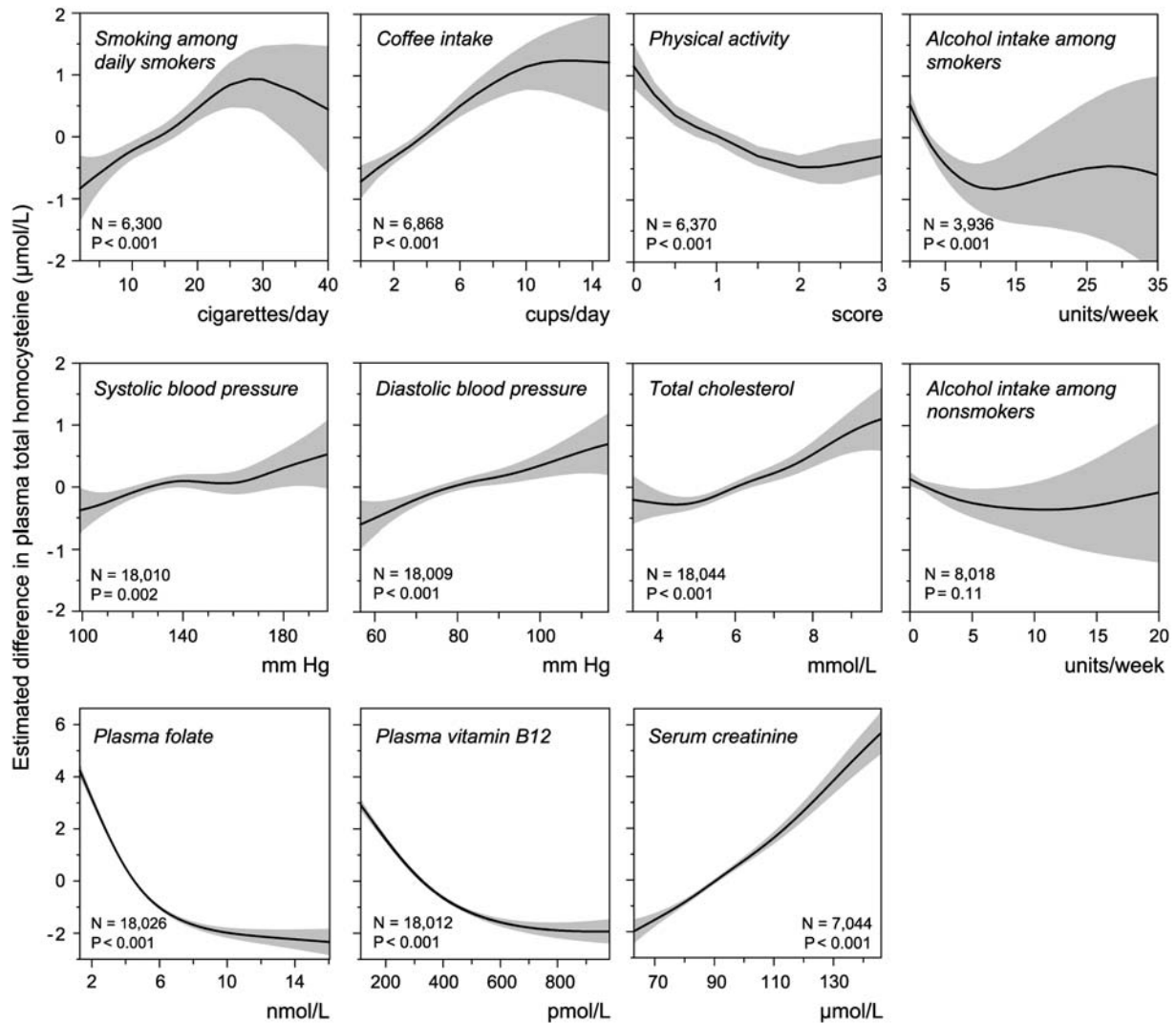


FIGURE 3 Factors associated with total homocysteine in the Hordaland Homocysteine Study. Dose– or concentration–response relationships between various factors and tHcy obtained by generalized additive logistic regression adjusted for age and gender. Shaded areas represent 95% confidence intervals. The *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 Simes 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 $\mu\text{mol/L}$ 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 $\geq 40 \mu\text{mol/L}$ (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).

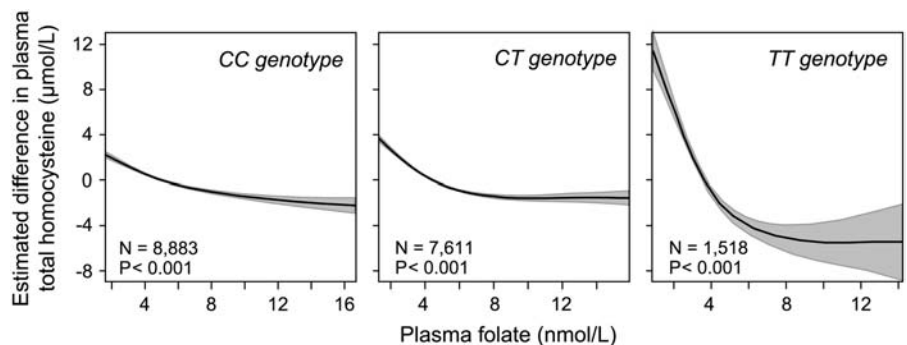
Other factors that influence tHcy levels in the general population include diet, in particular folate intake, blood levels of folate, vitamin B-12, and betaine, renal function, and the *MTHFR* 677C > T polymorphism (24,53–56). Some of these associations are depicted in Figure 3 (lowest panel) and Figure 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.



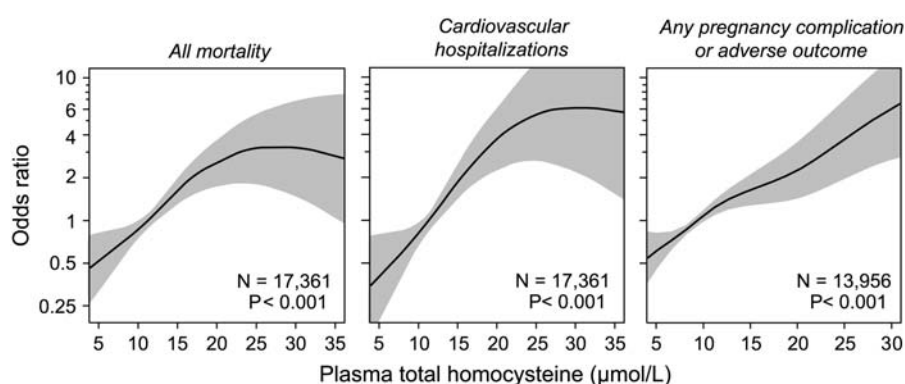


FIGURE 5 Association between plasma total homocysteine and various clinical outcomes. The concentration–response relation is obtained by generalized additive regression adjusted for age and gender, except for any complication or adverse outcomes of pregnancy (adjusted for maternal age and parity). Shaded 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

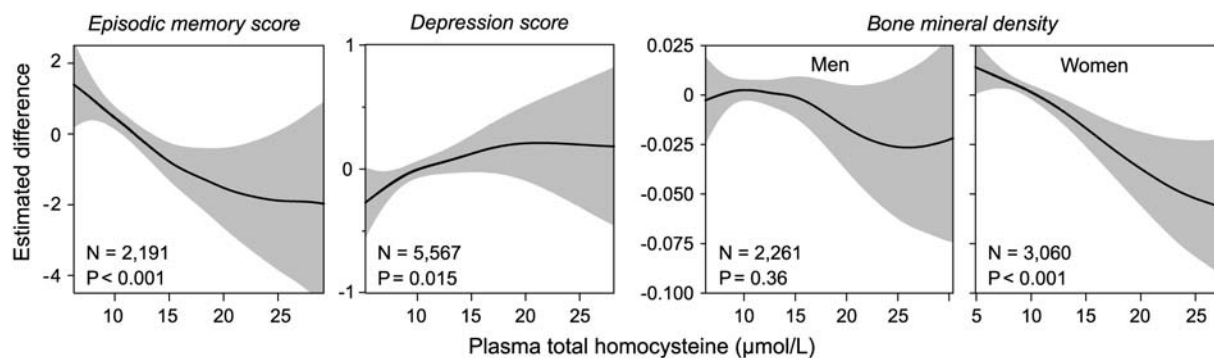


FIGURE 6 Association between plasma total homocysteine and various scores reflecting episodic memory, depressive mood, or bone mineral density. The concentration–response relationships are obtained by generalized additive regression. Depression score (HADS-D) and episodic memory score (Kendrick Object 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.

$<9 \mu\text{mol/L}$ had 3.8% mortality, whereas patients with $\geq 20 \mu\text{mol/L}$ 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 $\sim 13,000$ 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 $>15 \mu\text{mol/L}$; they had 2-fold higher risk of having depression compared with subjects with tHcy $<9 \mu\text{mol/L}$. 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 $\mu\text{mol/L}$ compared with tHcy <9 $\mu\text{mol/L}$ 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 $\mu\text{mol/L}$. 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.

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