The Hordaland Homocysteine Studies

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ABSTRACT: The Hordaland Homocysteine Study is a population-based screening of total plasma homocysteine (tHcy) in ~18,000 men and women aged 40–67 yr that took place in 1992–1993 in the county of Hordaland in Western Norway. In this cohort, tHcy was associated with several physiologic and life-style factors, including age and gender, blood pressure, serum cholesterol, smoking, alcohol and coffee consumption, physical activity, diet, and vitamin status. All associations with established cardiovascular risk factors were in the direction expected to confer increased risk. In a subset of 5,883 women aged 40–42 yr, tHcy was associated with previous pregnancy outcomes, including preeclampsia, placental abruption, and neural tube defects. This article reviews the published results from the Hordaland Homocysteine Study in the light of relevant literature. The Hordaland Homocysteine cohort will be used for future investigations of the stability of tHcy and vitamin status over time, and to investigate associations with mortality and morbidity including cancer incidence.

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The Hordaland Homocysteine Study is an investigation of 18,043 men and women, aged 40–67 y, living in Hordaland County in Western Norway (Fig. 1). The study was a collaboration between the University of Bergen and the National Health Screening Service. Eligible subjects were selected from the National Population Register, identified by site of residence and age on December 31, 1992, and plasma total homocysteine (tHcy) was determined in all subjects in 1992–1993. The aim of the study was to obtain cross-sectional data on the relationship between tHcy and life style and risk factors related to chronic diseases, in particular, cardiovascular disease (CVD). Furthermore, the long-term prospect of this study was to relate the tHcy concentration to future all-cause and cardiovascular mortality and morbidity. Finally, we used the collected data to assess relationships between tHcy level in 1992–1993 and previous pregnancy outcomes in the female participants of the Hordaland Study.

Recruitment and Data Collection

A total of 24,815 subjects from three different age groups were invited to participate; the overall acceptance rate was 72.7%. The younger and the largest group (n = 12,594) included all subjects in the county aged 40–42 yr. The older group (n = 4,766) covered all subjects aged 65–67 yr in the city of Bergen. A third group (n = 683), aged 43–64 yr, was a 2% random sample of residents in Bergen. Men and women in the younger and older age group represent the four main groups.

Data on type of work, physical activity, smoking habits, medical history of CVD, hypertension, diabetes mellitus, and food and vitamin intake were obtained by questionnaire. Blood samples from nonfasting subjects were collected into evacuated EDTA tubes and placed in a refrigerator (4–5°C) for 15–30 min; the plasma fraction was isolated within 1–3 h and stored at –20°C until analysis. tHcy was determined in plasma with high-performance liquid chromatography (HPLC) and fluorescence detection (1).

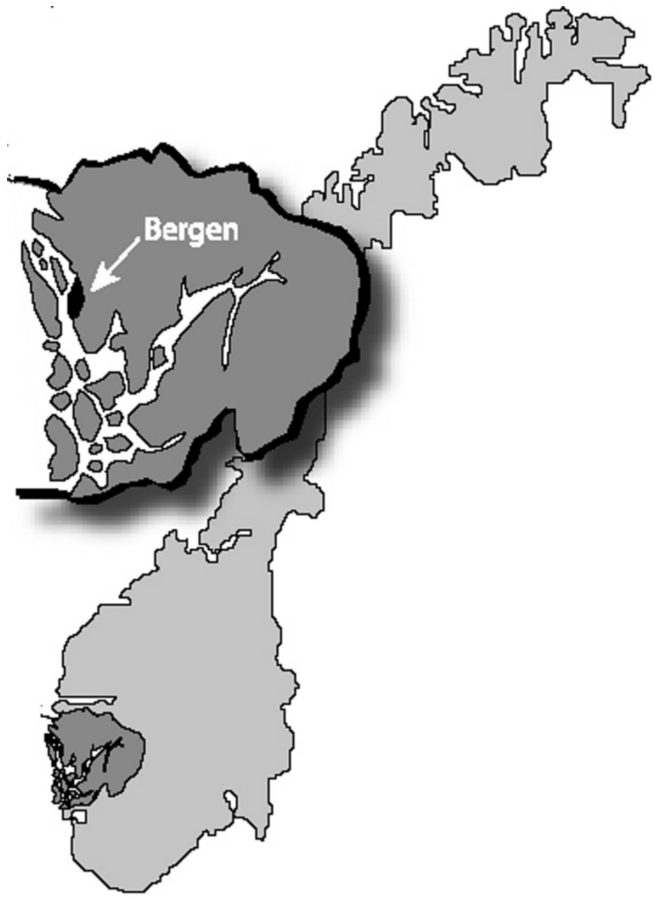

FIG. 1. The county of Hordaland is located on the western coast of Norway.

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Abbreviations: CVD, cardiovascular disease; HPLC, high-performance liquid chromatography; MTHFR, methylenetetrahydrofolate reductase; tCys, total cysteine; tHcy, total homocysteine.

Age, Gender, Blood Pressure and Cholesterol

Plasma tHcy showed a skew distribution, with a tail toward higher levels in all four main age and sex groups investigated. The arithmetic means were 11.4 μM in 5,918 healthy men and 9.6 μM in 6,348 women aged 40–42 yr, demonstrating that tHcy is ~2 μM higher in men than in women. In the oldest age group (65–67 yr), the corresponding values were 13.0 and 11.6 μM. Thus, there was an increase in tHcy by age, which was more pronounced in women than in men (2).

There are consistent data based on large populations (3,4), demonstrating age- and sex-related differences in tHcy. Higher tHcy in men than in women has been explained by difference in muscle mass and hormonal effects, whereas the age-related increase is probably due to deterioration of renal function and impaired vitamin status with age (4,5).

The Hordaland study demonstrates a positive relation between tHcy and diastolic and systolic blood pressure. tHcy also showed a positive relation to serum cholesterol. These relations were essentially confined to the younger age group, and the difference in tHcy between the extreme groups was <1 μM after multivariate adjustments (2). A weak relation between tHcy and serum cholesterol has been demonstrated in some studies (6–8). Conceivably, thyroid status is a strong determinant of both tHcy and serum cholesterol (9), and may explain the correlation between these indices. The weak association between tHcy and blood pressure is not a consistent finding (10–12).

Life-Style Factors

Smoking and alcohol. The first large study to address these issues was the Hordaland Homocysteine Study (13). There was a strong graded relationship between the number of cigarettes and tHcy levels, independent of age and sex (2), and folate intake (14). Notably, smoking was associated with an increased mean tHcy and a shift of the whole tHcy distribution curve to higher levels, similar to that observed in populations with low folate intake (14). The effect from smoking was more pronounced in women than in men. In women aged 40–42 yr, the estimated tHcy increase corresponded to 2% per cigarette/d. Comparing never smokers with heavy smokers (≥20/d), plasma tHcy levels were 8.7 and 10.7 μM in 40- to 42-yr-old women, and 10.5 and 11.7 μM in 40- to 42-yr-old men. There was no significant difference in tHcy between former smokers and subjects who had never smoked. Among smokers, the increase in tHcy was independent of the number of years of smoking (14).

A positive relation between smoking and tHcy has been demonstrated in most (12,15–17) but not all (18) smaller studies. Smokers in general consume a less healthy diet than nonsmokers, and elevated tHcy may be related to the effect of smoking on homeostasis of B-vitamins involved in homocysteine metabolism, including folate, vitamin B_6, and vitamin B_{12} (5).

In the Hordaland cohort, alcohol consumption showed a weak U-shaped relation to tHcy with a negative association with consumption up to 14 units per week. This effect was markedly stronger in smokers (19).

The first studies on alcohol effect on tHcy were done in abusers and chronic alcoholics, and in these subjects, a marked increase in tHcy was observed (13). A positive relation between tHcy and blood alcohol concentration in alcohol abusers has recently been reported (20). The effect of moderate alcohol consumption was investigated in the large population-based Caerphilly cohort (21), and in this study, alcohol intake was negatively associated with tHcy and showed a positive relation to folate intake. The authors suggest that the tHcy reduction is mediated by folate (21). Smaller studies have demonstrated increased tHcy or no effect from moderate alcohol consumption (13). A recent intervention trial demonstrated that consumption of liquor and red wine, but not beer, increases the tHcy level (22). The beverage specificity may be related to the large amount of folate and vitamin B_6 in beer and negligible amounts in red wine and spirits (22).

Physical activity. There was a negative relation between physical activity in leisure time and plasma tHcy in the Hordaland cohort. After multivariate adjustment, individuals reporting heavy exercise had tHcy ~0.5 μM lower than individuals who characterized themselves as sedentary (2). Other observational studies noted no (11,23) or only a minor (12) effect from physical activity. One experimental study demonstrated a moderate increase in tHcy after acute exercise, which paralleled hemoconcentration (24), whereas another study demonstrated no tHcy response (25).

Coffee. Daily coffee consumption was registered for 89% of the Hordaland study participants, and among these, 95% used filtered coffee. There was an unexpected and strong positive association between plasma tHcy and consumption of various types of coffee, except decaffeinated coffee. This relation was almost as strong as that observed between tHcy and smoking, and was also found in nonsmokers and at both high and low folate intake. Heavy coffee consumption increases mean tHcy by decreasing the proportion with low and intermediate tHcy (<17 μM), and in this respect can be distinguished from folate deficiency and cigarette smoking (26).

The strong effect of heavy coffee consumption on plasma tHcy has been confirmed in several recent studies (27,28). Moderate coffee consumption in the Atherosclerosis Risk In Communities (ARIC) cohort was not associated with elevated tHcy (29), but recent intervention trials demonstrated that 1 L/d of unfiltered (30) as well as filtered coffee (31) increased tHcy by ~1.5 M. The coffee effect was observed within 2 wk and was reversible (31). Serum levels of folate, vitamin B_6, or vitamin B_{12} did not change during the coffee consumption period (31). In the Framingham offspring cohort, the tHcy rising effect was observed from coffee and other caffeine-containing beverages (e.g., cola) but not from decaffeinated coffee (28). The elevation of tHcy by coffee is probably mediated by caffeine, which may influence the cardiovascular system or kidney function, or possibly interfere with vitamin B_6 function (5).

Diet and vitamins. In the Hordaland study, we constructed and verified folate and cobalamin intake scores, based on con-

sumption of various dietary items and vitamin supplements (14). Folate derived from food showed a weaker negative relation to plasma tHcy than folate taken as supplements. We could distinguish between the reduction of high tHcy to normal levels, which is usually conferred by folate derived from food, and the reduction from normal to subnormal levels, which is attributable to intake of folic acid supplements (14).

A large and consistent literature describes the effect of B-vitamins involved in homocysteine metabolism on plasma concentration of tHcy. In folate- or cobalamin-deficient subjects, tHcy is markedly reduced by treatment with the deficient vitamin (32). In subjects with no overt vitamin B deficiency, folate supplementation induces an average reduction of tHcy by ~25% (33), and there is essentially no additional effect from vitamin B_6 or cobalamin (33,34). Vitamin B_6 supplementation alone has no or only a modest effect on fasting tHcy (35). In several large population-based studies, tHcy shows a strong, inverse relation with serum folate concentration or folate intake, a weaker relation to serum cobalamin concentration, and often no relation to estimated intake of cobalamin. Both serum level and intake of vitamin B_6 show a significant relation to tHcy (13,28,36). Thus, folate status in particular but also cobalamin status are established determinants of fasting tHcy, whereas the observed vitamin B_6 effect may represent confounding from folate, and vitamin B_6 seems important to control postmethionine load tHcy.

Combined effects and tail effects. A life-style profile that reflects the combined effect of three major modifiable tHcy determinants, i.e., folate intake, smoking, and coffee consumption, is strongly correlated with tHcy (14). Subjects with a contrasting lifestyle with respect to these factors have a difference of 3–5 μM in tHcy, a difference that is larger than the effect attributable to each factor alone. This supports the notion of different mechanisms underlying the tHcy elevating effects of smoking, low folate intake, and heavy coffee consumption. Furthermore, tHcy is essentially normally distributed in a population characterized by a high folate intake, low coffee consumption, and nonsmoking (14).

The major tHcy determinants (age, gender, coffee consumption, smoking, and no intake of vitamin supplements) show different relationships with the extremes of the tHcy distribution curve (13). Male gender, old age, and heavy coffee consumption decreased the likelihood of having low tHcy (<7 μM), but had essentially no effect on the likelihood of having moderately (15–30 μM) or markedly elevated (30–100 μM) tHcy. Thus, these factors had their main effect on the lower part of the tHcy distribution curve. In contrast, smoking and no intake of vitamin supplements decreased the likelihood of low tHcy and increased the likelihood of moderately and markedly elevated tHcy, and thereby were associated with a displacement/shift of the whole tHcy distribution curve to a higher level (Fig. 2). These distribution effects suggest different mechanisms whereby age, gender, coffee consumption, smoking, and no intake of vitamin supplements affect the tHcy concentration, but also raise the possibility that the tHcy elevation related to coffee consumption and increasing age has less adverse effects than the high levels associated with smoking and impaired vitamin status (13).

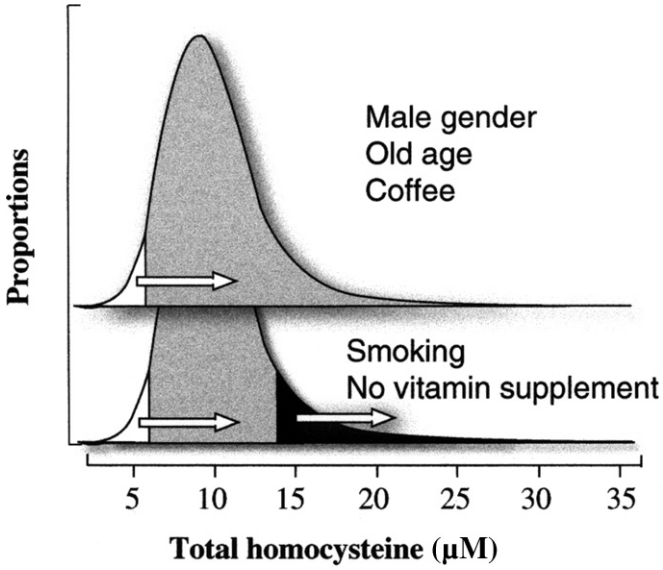

FIG. 2. Scheme of tail effects of total homocysteine (tHcy) determinants. The determinants gender, age, and coffee consumption, presented in the upper panel, have a selective effect on the lower part of the tHcy distribution curve. Smoking and no use of vitamin supplements (lower panel) cause a shift of the whole distribution curve to higher levels.

Intermediate Hyperhomocysteinemia and the 677C→T Methylenetetrahydrofolate Polymorphism

Among the 18,043 subjects investigated in the Hordaland Homocysteine Study, 67 (0.4%) had tHcy ≥40 μM (37). Compared with controls, these subjects had lower plasma folate and cobalamin levels, lower intake of vitamin supplements, consumed more coffee, and were more frequently (60%) smokers. When seven subjects with cobalamin deficiency were excluded, 92% of these hyperhomocysteinemic subjects (compared with 10.4% of controls) were homozygous for the 677C→T methylenetetrahydrofolate reductase (MTHFR) polymorphism. These findings demonstrate a strong positive interaction between MTHFR genotype and life-style determinants of tHcy (37).

The 677C→T MTHFR polymorphism has been studied extensively during the last 5 yr because it is a genetic determinant of tHcy. MTHFR catalyzes the irreversible formation of 5-methyletrahydrofolate which serves as a methyl donor in the remethylation of Hcy to methionine (38). The T-allele codes for a thermolabile enzyme variant with low catalytic activity and reduced affinity for 5-methyltetrahydrofolate and the MTHFR co-factor, FAD. This explains the finding of elevated tHcy in subjects with the combination of the TT genotype and impaired folate status, and also the recent observation that low plasma riboflavin (vitamin B_2) increases tHcy in subjects with the T-allele (39). However, there is effect modification by the MTHFR polymorphism of several factors predisposing to hyperhomocysteinemia, which cannot readily be

explained by the enzymic properties of the thermolabile MTHFR variant. Thus, low serum cobalamin, renal failure, and drugs such as L-dopa and antifolate agents cause higher tHcy in subjects with the TT-genotype than those with the CC genotype (38). It therefore seems that the T-allele is associated with increased propensity toward hyperhomocysteinemia, which is in accordance with the high prevalence of the TT genotype and negative life-style factors in hyperhomocysteinemic subjects in the Hordaland cohort.

Total Cysteine vs. the Cardiovascular Risk Profile

The relationship between the cardiovascular risk profile (which included life-style factors, age, gender, blood pressure, and cholesterol) and total concentration of another plasma aminothiol, cysteine, was investigated in the Hordaland cohort (40). Total cysteine (tCys) and tHcy showed a distinct and differential relation to components of the risk profile. Age, cholesterol, diastolic blood pressure, and coffee were positively related to both tCys and tHcy; body mass index showed a strong positive relation only to tCys, whereas smoking, folate and vitamin intake, heart rate, and physical activity (which were associated with tHcy) showed no relation to tCys (40).

There are a few studies (17,41–43) demonstrating significantly higher levels of plasma tCys in vascular patients than in healthy controls, suggesting that tCys is associated with cardiovascular risk. Furthermore, cysteine and homocysteine are interactive components of the plasma redox thiol status (44). Therefore, knowledge of tCys determinants is important to understand the possible pathogenic role of cysteine and to assess confounding of the observed disease-tCys relationships.

Pregnancy Outcomes

Adverse pregnancy outcomes were investigated in 5,883 women from the age group 40–42 yr in the Hordaland cohort (45). Records of 14,492 pregnancies in the period from 1967 to 1996 were retrieved from the Medical Birth Registry of Norway, and outcomes were related to the tHcy level measured in 1992–1993.

The levels of tHcy in 1992–1993 showed a strong, concentration-dependent association with preeclampsia, prematurity, and low birth weight, whereas a moderate association was observed with stillbirth. An increased frequency of placental abruption was observed at tHcy > 15 μM. Plasma tHcy was also related to malformations such as neural tube defects and clubfoot, but not to orofacial cleft (45).

Of the pregnancies investigated, ~80% occurred more than 10 yr before the tHcy measurements. The associations with preeclampsia, prematurity, low birth weight, and stillbirth were strongest in the time interval closest to the tHcy determinations. Thus, the long time interval weakens the association between plasma tHcy and pregnancy outcomes.

The relationship among folate status, plasma tHcy, and pregnancy outcome has been investigated in several smaller studies (46–48). Most have a case-control design and demonstrate that hyperhomocysteinemia is associated with habitual miscarriage, preeclampsia, placenta abruption, thromboembolic events, neural tube defects, and perhaps with fetal death *in utero* and intrauterine growth retardation (46–48).

Prospective Analysis of Mortality

The ability of tHcy to predict mortality was investigated in a population of 587 patients with angiographically verified coronary artery disease recruited from the Haukeland University hospital. The median follow-up time was 4.6 yr. There was a strong, graded relation between tHcy and both cardiovascular and overall mortality. After 4 yr, 3.8% of the patients with tHcy < 9 μM had died, compared with 24.7% of those with tHcy ≥ 15 μM (49). Notably, mortality rate was highest in patients with other established CVD risk factors, in particular diabetes mellitus, low ejection fraction, and elevated fibrinogen (Fig. 3). These data have inspired investigations of the association between tHcy and future CVD events and overall mortality and morbidity in the Hordaland cohort, and two Norwegian ongoing secondary intervention studies with tHcy-lowering B-vitamins in coronary patients

By the end of year 2000, there were ~35 prospective studies on the association between tHcy and CVD (50–61). About half (9 of 18) of the population-based studies and most (14 of 17) studies in clinical cohorts or in elderly demonstrated a significant positive relation. Thus, tHcy seems to be a particularly strong predictor of cardiovascular events or death in subjects with a preexisting illness, such as coronary heart disease, diabetes, or renal failure (62).

Conclusion and Perspectives

The first part of the Hordaland Homocysteine Study provided cross-sectional data on the relation between plasma tHcy and several established cardiovascular risk factors and life style in a large general population (n = 18,043) of men and women. The study demonstrated for the first time a positive relation between tHcy and coffee consumption, and affirmed the as-

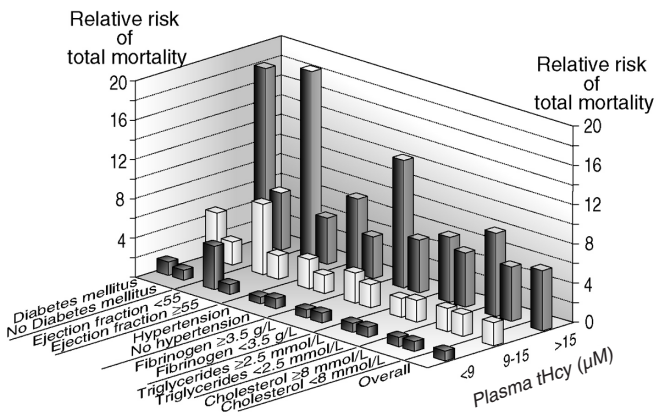

FIG. 3. Total mortality according to plasma total homocysteine (tHcy) and other established risk factors for cardiovascular disease. Data from Reference 49.

sociations between tHcy and age, sex, smoking, and blood pressure. The magnitude of this cohort allows the investigation of a diversity of clinical conditions related to tHcy in the same population, as demonstrated by the study on pregnancy outcomes. Ongoing and future investigations include studying the relationship between baseline tHcy and all-cause and cause-specific mortality, cardiovascular morbidity, and cancer incidence. Finally, on-going reinvestigation in 1998–1999 of ~6000 subjects from the age groups 40–42 and 65–67 yr in 1992–1993 allows assessment of the stability of tHcy and folate status over time, the relation to single nucleotide polymorphisms, and the association of two measurements with mortality and morbidity due to common diseases.

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