Total Plasma Homocysteine and Cardiovascular Risk Profile

The Hordaland Homocysteine Study

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Objective.—To estimate the relations between established cardiovascular risk factors and total homocysteine (tHcy) in plasma.

Design.—Health examination survey by the Norwegian Health Screening Service in 1992 and 1993.

Setting.—General community, Hordaland County of Western Norway.

Participants.—A total of 7591 men and 8585 women, 40 to 67 years of age, with no history of hypertension, diabetes, coronary heart disease, or cerebrovascular disease were included.

Main Outcome Measure.—Plasma tHcy level.

Results.—The level of plasma tHcy was higher in men than in women and increased with age. In subjects 40 to 42 years old, geometric means were 10.8 μmol/L for 5918 men and 9.1 μmol/L for 6348 women. At age 65 to 67 years, the corresponding tHcy values were 12.3 μmol/L (1386 men) and 11.0 μmol/L (1932 women). Plasma tHcy level increased markedly with the daily number of cigarettes smoked in all age groups. Its relation to smoking was particularly strong in women. The combined effect of age, sex, and smoking was striking. Heavy-smoking men aged 65 to 67 years had a mean tHcy level 4.8 μmol/L higher than never-smoking women aged 40 to 42 years. Plasma tHcy level also was positively related to total cholesterol level, blood pressure, and heart rate and inversely related to physical activity. The relations were not substantially changed by multivariate adjustment, including intake of vitamin supplements, fruits, and vegetables.

Conclusions.—Elevated plasma tHcy level was associated with major components of the cardiovascular risk profile, ie, male sex, old age, smoking, high blood pressure, elevated cholesterol level, and lack of exercise. These findings should influence future studies on the etiology and pathogenesis of cardiovascular disease.

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PATIENTS with homocystinuria, a rare inborn error of metabolism, have extremely high levels of total homocysteine (tHcy) in plasma. They also have a high incidence of cardiovascular disease in early adolescence and even in childhood. Homocystinuria results from several enzymic defects in homocysteine (Hcy) metabolism, but premature cardiovascular disease develops irrespective of the site of metabolic deletion,[1] suggesting that some form of Hcy is responsible for the vascular damage.[2]

Plasma tHcy refers to the sum of protein-bound, free-oxidized, and reduced species of Hcy in plasma[3] and is usually about 5 to 15 μmol/L in healthy subjects.[4] Moderate hyperhomocysteinemia (15 to 30 μmol/L)[5] is related to genetic or acquired factors.[4] Genetic causes are heterozygous deficiency of cystathionine β-synthase or methylenetetrahydrofolate reductase or a thermolabile variant of the latter enzyme,[5] whereas impaired cobalamin or folate status is among the acquired causes.[6,7]

Since the first report in 1976,[8] more than 20 retrospective[5,9-11] and two prospective[12,13] studies of more than 3300 patients have demonstrated a relation between moderate hyperhomocysteinemia and premature vascular disease in the coronary, cerebral, and peripheral arteries. Most studies conclude that plasma Hcy is an independent risk factor for cardiovascular disease,[5,9,14-16] but an association between Hcy levels and established cardiovascular risk factors, such as serum cholesterol level,[17,18] blood pressure,[18,19] or cigarette smoking,[13,20] has occasionally been demonstrated. Knowledge of such associations is needed to identify potential confounders in studies of Hcy and disease and may contribute toward understanding of the pathogenesis of cardiovascular disease.

Recently, we initiated a population-based, prospective study on plasma tHcy and cardiovascular disease. In the current report, we have used the baseline data to estimate the relations between plasma tHcy level and major components of the cardiovascular risk profile. Our study is the first to address these issues in a large number of subjects.

SUBJECTS AND METHODS

Study Population

The study, which is part of a national cardiovascular risk survey,[21] was conducted in the Hordaland County in Western Norway from April 1992 to April 1993 by the National Health Screening

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Table 1.—Population Size, Attendance Rates, and Numbers Included in the Study

Characteristic	Invited No.	Attendance Rate, No. (%)	Study Subjects, No. (%)*
Sex			
Male	12 488	8573 (68.6)	7591 (60.8)
Female	12 327	9470 (76.8)	8585 (69.6)
Age, y			
40-42	17 303	12 594 (72.8)	12 266 (70.9)
43-64	999	683 (68.4)	592 (59.3)
65-67	6513	4766 (73.2)	3318 (50.9)
Total Series	**24 815**	**18 043** (72.7)	**16 176** (65.2)

*Includes all who attended without reporting a history of diabetes mellitus, angina, former acute myocardial infarction, stroke, or treatment for hypertension. One subject with homocystinuria was excluded.

Service (NHSS) in cooperation with the University of Bergen and local health services. The total population of Hordaland is approximately 420 000, of whom about 50% live in the city of Bergen.

The eligible subjects were selected from the National Population Registry, identified by site of residence and their age on December 31, 1992. A total of 24 815 subjects from three different age groups were invited to participate in the study. The younger age group included all subjects in the county who were 40 to 42 years of age. The older age group covered all subjects aged 65 to 67 years in Bergen and three neighboring suburban municipalities. A third group (43 to 64 years) was a 2% random sample of residents in Bergen.

The attendance rate for the whole group was 72.7%. All participating subjects gave their written, informed consent, and the study protocol was approved by the Regional Ethical Committee of Western Norway.

A total of 1866 participants reporting a previous diagnosis of coronary heart disease, cerebrovascular disease, hypertension, or diabetes were excluded from the analyses to avoid potential influence of the disease itself on tHcy levels directly or indirectly from treatment or from change in lifestyle. One patient with homocystinuria was also excluded. Thus, 16 176 individuals were included in the current study (Table 1).

Data Collection

The screening procedures performed by the NHSS have been described previously.[21] Briefly, data were collected through questionnaires, examinations, and blood tests. The subjects received the invitation letter and a one-page questionnaire. This was completed at home by the participants and collected and checked for logical errors by a nurse on the day of examination. The questionnaire provided information about type of work, physical activity, smoking habits, medical history of cardiovascular disease, hypertension and diabetes mellitus, and family history of coronary heart disease. A second questionnaire about recent food intake was filled in by a nurse during the examination. The attending subjects also received a third questionnaire on the examination day, covering more details about lifestyle, medical history, and dietary habits. This was later completed at home and mailed to the NHSS by 86% of the participants.

Smoking Habits.—Current and former cigarette smokers were asked to report the average number of cigarettes smoked per day and the duration of smoking. Based on this information, cigarette smokers were grouped in five categories, ie, never smokers, former smokers, light smokers (one to nine cigarettes per day), moderate smokers (10 to 19 cigarettes per day), and heavy smokers (≥20 cigarettes per day).

Physical Activity.—The subjects were asked to mark one of the following categories that best fitted their average degree of activity in leisure time for the last year: (1) sedentary or no activity; (2) walking, cycling, or other type of moderate physical activity for at least 4 hours a week (moderate activity); (3) exercise, gardening with physical exertion, or similar degree of physical activity for at least 4 hours a week (active exercise); or (4) regular heavy training or competitive sport several times a week (heavy training).

The grading of leisure time physical activity obtained by the questionnaire was validated by the finding of a strong inverse relation between triglycerides and reported activity level. Low level of triglycerides is a recognized response to increased physical activity in leisure time.[22-24]

Intake of Vitamin Supplements, Fruits, and Vegetables.—A vitamin supplement score (n=11 940) was created according to use during the year and frequency of intake during the week and categorized into five groups. The lowest category included subjects who never used vitamins (34%), while the highest category represented those who took vitamins 6 to 7 days a week during the whole year (14%). Among those taking vitamins, approximately 80% reported use of vitamin B and/or multivitamin combinations, which usually contain folic acid (100 μg per tablet).

A fruit-vegetable score (n=13 378) was based on the sum of frequencies of intake of fruits and vegetables (four categories). The highest category represented subjects consuming both fruits and vegetables at least 6 days a week (38%), while the lowest category represented those with intake of fruits and vegetables once a week or less (4.3%).

In a subpopulation (n=329), we found a significant correlation between plasma folate and the vitamin supplement score (r=0.31) and the fruit-vegetable score (r=0.12). No relation was observed between these scores and plasma cobalamin. Another score based on intake of vegetables and fruits, which are rich in folates, showed a stronger relation to plasma folate (r=0.21) but a weaker relation to plasma tHcy (r=0.10) than the fruit-vegetable score (r=−0.14), and it was therefore not included in the analyses.

Examinations

Trained nurses and technical staff from the NHSS performed the examinations and collected the blood samples. After the participants had registered and height and weight were measured, they were allowed to sit for 10 minutes while the nurse checked the first questionnaire and obtained information for the second questionnaire. Then, three blood pressure measurements using Dinamap 845 XT equipment (Criticon, Tampa, Fla) were performed. Values from the second measurement were used in this study.

Blood Sample Collection and Biochemical Analysis

The participants were nonfasting, and blood samples were obtained immediately after the examination with the person in the sitting position. Blood samples used for the preparation of serum were collected into an evacuated tube containing sodium sulfite titration gel (Becton Dickinson Co, Meylan, France) and centrifuged within 2 hours. The serum tubes were transported to the Department of Clinical Chemistry, Ullevål Hospital, Oslo, where determination of total cholesterol and triglycerides was performed within 7 days after collection of the sample.

Blood samples used for the preparation of plasma were collected into an evacuated tube containing ethylenediaminetetraacetic acid, placed in a refrigerator (4°C to 5°C) within 15 to 30 minutes, and centrifuged usually within 1 hour (maximum, within 3 hours). The plasma fraction was then transferred to plastic vials. Compared with immediate sample handling, the described proce-

Table 2.—Total Plasma Homocysteine (tHcy) Level by Age and Sex*

	Age 40-42 y		Age 43-64 y		Age 65-67 y	
		tHcy, μmol/L		tHcy, μmol/L		tHcy, μmol/L
Sex	No.	Mean (95% CI)	No.	Mean (95% CI)	No.	Mean (95% CI)
Male	5918		287		1386	
Geometric mean		10.84 (10.76-10.91)		11.22 (10.87-11.58)		12.27 (12.11-12.44)
Arithmetic mean		11.34 (11.23-11.45)		11.70 (11.21-12.18)		12.93 (12.60-13.25)
Female	6348		305		1932	
Geometric mean		9.14 (9.06-9.22)		9.89 (9.53-10.25)		11.04 (10.89-11.19)
Arithmetic mean		9.60 (9.50-9.69)		10.47 (9.96-10.97)		11.58 (11.38-11.78)

*CI indicates confidence interval.

dure may cause an increment in mean plasma tHcy level of 0.5 μmol/L.[25] The unfrozen plasma samples, kept at room temperature, usually arrived at our laboratory the following day, but occasionally they arrived up to 4 days after collection. The samples were stored at −20°C until the tHcy analysis was performed. The duration of storage was between a few days and up to 6 months.

Total plasma Hcy, cysteine, and cysteinylglycine levels were determined using a modification[25] of a fully automated assay based on precolumn derivatization with monobromobimane followed by reversed-phase high-performance liquid chromatography.[25,26] The precision (between-day coefficient of variation) of the assay is less than 3%, and results obtained correlate well with other established methods for determination of tHcy level.[4] The stability of analytical procedures was controlled for by inserting quality-control samples for every 18th sample. Replicate measurements were performed for about every 15th sample. This included all samples with very high (≥40.0 μmol/L) or low (≤4.0 μmol/L) levels of plasma tHcy and samples with total cysteine and/or cysteinylglycine levels outside a 95% confidence interval based on results from about 2000 samples that had previously been determined in our laboratory. An intraclass coefficient (correlation between replicate measurements) was obtained (r=0.99).

Statistical Methods

The distribution of plasma tHcy was skewed with a long tail toward high values. The levels ranged from 3.6 to 137 μmol/L with overall mean and median values of 10.8 μmol/L and 10.1 μmol/L, respectively. Analysis of variance and regression models for a continuous variable assume that the dependent variable is normally distributed. To better satisfy this requirement, all analyses were done on log (base 10) tHcy, and then the results were transformed back to the original scale. Thus, geometric means were used, unless otherwise stated. The overall geometric mean was 10.3 μmol/L, which is closer to the median than to the arithmetic mean. The 95% confidence interval of the geometric mean was found by taking the antilog of the 95% confidence interval of log tHcy.

Mean tHcy levels were compared using two-group t tests or analysis of variance models. To assess the simultaneous relationship among the various predictors of tHcy and to provide effect estimates adjusted for other factors, multiple linear regression models were used. The logarithm of plasma tHcy was the dependent variable, whereas the independent variables were represented in the models as indicator variables denoting membership to one of four categories for heart rate, cholesterol, triglycerides, exercise, blood pressure, and intake of fruits and vegetables, as well as one of five categories for cigarette smoking and intake of vitamin supplements. Thus, each regression coefficient estimated the difference in log tHcy between the reference category and the other categories for each risk factor. This difference was transformed back to the original scale by taking its antilogarithm.

The ability of different risk factors to predict hyperhomocysteinemia (≥15.0 μmol/L) was studied by logistic regression. The odds ratio estimated the risk that an individual with a specific risk profile is hyperhomocysteinemic relative to subjects with a baseline risk profile.

The analyses were performed with the statistical packages BMDP[27] and S-Plus.[28] All tests were two-tailed, and a P value less than .05 was considered significant.

RESULTS

Age and Sex

The characteristics of the study population are given in Table 1. Plasma tHcy level was higher in men than in women and increased progressively with age in both sexes (Table 2 and Figures 1 and 2). The difference in geometric mean values between the younger and older age group was higher in women (1.9 μmol/L) than in men (1.4 μmol/L) (Table 2). In all main sex and age subgroups, there was a skew distribution with a long tail toward higher tHcy values (Figure 1).

Cigarette Smoking

Compared with nonsmokers, current smokers had a distinctly higher plasma tHcy level that increased almost linearly with the daily number of cigarettes smoked (Table 3 and Figure 3). This increase in current smokers was not related to the number of years smoked. The effect of cigarette smoking was stronger in women than in men and in the older age group compared with the younger age group (Table 3). The combined effect of cigarette smoking, age, and sex was strong (Figure 3), with a difference of 4.8 μmol/L in mean tHcy level between younger never-smoking women and older heavy-smoking men.

There was no significant difference in plasma tHcy level between former smokers and never smokers apart from the higher levels among female former smokers aged 65 to 67 years (Table 3 and Figure 3). However, in former smokers, the age-adjusted tHcy level was positively related to both the number of years smoked and the number of cigarettes smoked daily. Thus, in former smokers, tHcy level increased with the number of cigarette-years, with a difference in tHcy level between the highest and lowest quintile of cigarette years ranging from 0.3 to 0.9 μmol/L in the four main sex and age subgroups (data not shown).

Physical Activity

There was an inverse relation between mean tHcy level and amount of exercise in leisure time. In the group aged 40 to 42 years, the difference in tHcy levels between subjects who reported heavy training and those who reported sedentary activity was 0.76 μmol/L in men and 0.94 μmol/L in women (Table 3 and Figure 3). In the older age group, only five persons reported heavy training, and they were combined with the active exercise group. In this combined group, men had 1.08 μmol/L and women had

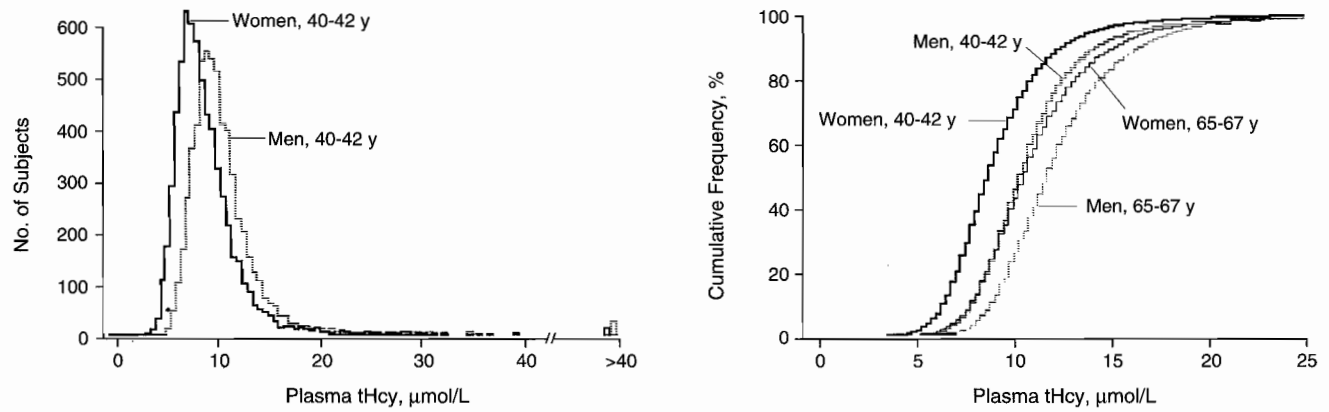

Figure 1.—Distribution of total plasma homocysteine (tHcy) according to sex and age. The left panel shows the distribution of tHcy in 5918 men and 6348 women aged 40 to 42 years, and the right panel compares the cumulative frequency curves for these two groups with the curves for tHcy in 1386 men and 1932 women aged 65 to 67 years.

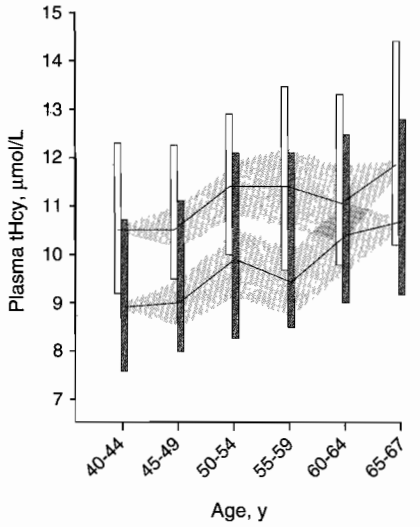

Figure 2.—Total plasma homocysteine (tHcy) levels in 7591 men and 8585 women according to different age groups. The solid lines are median values, and the shaded area indicates the 95% confidence intervals, while the boxes show the 25th to 75th percentile intervals and are open for men and darkly shaded for women.

0.65 µmol/L lower tHcy levels than the sedentary group (Figure 3).

Blood Pressure and Heart Rate

Plasma tHcy level showed a positive linear association with diastolic blood pressure (Table 3 and Figure 3) and systolic blood pressure (data not shown). This relation was essentially confined to the younger age group. In contrast, tHcy level was positively related to heart rate in both age groups (Table 3 and Figure 3).

Cholesterol and Triglycerides

There was a positive linear association between plasma tHcy levels and serum cholesterol levels. This relation was strong in subjects aged 40 to 42 years, weaker but significant in older women (aged 65 to 67 years), but absent in older men (Table 3 and Figure 3). A weak positive relation between serum triglycerides and plasma tHcy level was observed in younger men and older women (Table 3 and Figure 3).

Multivariate Analyses and Analyses of Subgroups

The results of the regression analyses, given as estimated change in mean plasma tHcy concentration and odds ratio for hyperhomocysteinemia, are listed in Table 4.

The first model shows a summary of the univariate results presented in the previous sections, adjusted for age and/or sex. For all variables, there was a progressive increase or decrease in tHcy level with each category away from the reference category. The largest differences in tHcy levels were observed between the older and younger age group (2.20 µmol/L), between heavy smokers and never smokers (1.91 µmol/L), and between men and women (1.82 µmol/L). Smaller, but significant differences in tHcy level were found for the other variables tested.

To assess the possible influence of smoking on the relations between tHcy level and the other factors, the sex- and/or age-adjusted analyses were repeated in never smokers. The association between heart rate and tHcy level was weaker in the never smokers, whereas the remaining associations were almost the same as for the total population.

In a multiple regression analysis with all factors included, triglycerides no longer contributed in the prediction of tHcy level. The association with physical activity, diastolic blood pressure, heart rate, and total cholesterol level were weakened, while the effect of age, sex, and cigarette smoking remained virtually unchanged.

The vitamin supplement and the fruit-vegetable scores were both highly significantly related to plasma tHcy level. The estimated adjusted differences between low- and high-intake groups were 1.35 µmol/L and 0.79 µmol/L, respectively, but adjustment for these scores had a moderate impact on the coefficients of the factors considered in Table 4. In the final model, sex, age, cigarette smoking, and the vitamin supplement score were the strongest determinants of tHcy level.

Between plasma tHcy level and body mass index (BMI), there was only a weak U-shaped relation (P=.04), which disappeared in the multivariate analyses. However, there was an effect modification of BMI since subjects with low BMI had the strongest inverse association between tHcy level and exercise. In contrast, a positive relation was seen in subjects with very high BMIs (>90th percentile). This positive relation in the very obese was confined to the younger subjects and was most pronounced in men (data not shown).

The odds ratios for hyperhomocysteinemia (≥15 µmol/L) and the estimated changes in mean plasma tHcy levels provided essentially the same information (Table 4). However, categories with increasing exercise were characterized by a shift of the upper tail of the tHcy distribution to the left. This reduced skewness was associated with only a small reduction in mean level and explains the relatively strong effect of heavy exercise on the odds ratio compared with the coefficient in the multiple linear regression model.

COMMENT

In this population-based study of 16 176 men and women aged 40 to 67 years, plasma tHcy level was strongly associ-

Table 3.—Total Plasma Homocysteine (tHcy) by Sex and Cardiovascular Risk Factors in Subjects Aged 40 to 42 Years

	Male			Female		
		tHcy, μmol/L*			tHcy, μmol/L*	
Variable	No.	Mean (95% CI)	P	No.	Mean (95% CI)	P
Smoking habits, cigarettes/d						
Never smokers	1907	10.45 (10.33-10.57)	<.001‡§	2497	8.67 (8.58-8.76)	<.001‡§
Former smokers	1585	10.57 (10.44-10.70)		1410	8.66 (8.54-8.78)	
Light smokers, 1-9	377	10.96 (10.67-11.26)		634	9.45 (9.24-9.66)	
Moderate smokers, 10-19	1298	11.28 (11.10-11.46)		1413	9.98 (9.82-10.14)	
Heavy smokers, ≥20	709	11.66 (11.40-11.93)		388	10.67 (10.30-11.05)	
Physical activity in leisure time†						
No activity	1033	11.13 (10.93-11.33)	<.001‡§	1066	9.49 (9.31-9.68)	<.001‡§
Moderate	3128	10.84 (10.74-10.95)		4539	9.08 (9.00-9.15)	
Active exercise	1608	10.68 (10.55-10.81)		691	9.04 (8.84-9.24)	
Heavy training	147	10.37 (9.94-10.82)		51	8.55 (7.98-9.15)	
Diastolic blood pressure, mm Hg						
<70	692	10.27 (10.08-10.48)	<.001‡§	1492	8.92 (8.79-9.05)	<.001‡§
70-84	3226	10.79 (10.69-10.89)		3611	9.16 (9.08-9.25)	
85-99	1723	11.12 (10.97-11.27)		1103	9.35 (9.18-9.52)	
≥100	254	11.23 (10.81-11.67)		140	9.15 (8.75-9.58)	
Heart rate, beats/min						
<60	821	10.43 (10.25-10.62)	<.001‡§	312	8.82 (8.55-9.11)	<.001‡§
60-79	3537	10.79 (10.69-10.88)		3610	9.05 (8.96-9.13)	
80-99	1352	11.14 (10.98-11.30)		2044	9.28 (9.16-9.41)	
≥100	185	11.61 (11.07-12.18)		380	9.47 (9.18-9.76)	
Cholesterol, mmol/L (mg/dL)						
<4.00 (<155)	187	10.33 (9.91-10.76)	<.001‡§	343	9.03 (8.73-9.34)	<.001‡§
4.00-5.99 (155-231)	3558	10.71 (10.62-10.81)		4529	9.04 (8.97-9.12)	
6.00-7.99 (232-308)	2031	11.02 (10.89-11.15)		1396	9.43 (9.27-9.58)	
≥8.00 (≥309)	142	12.20 (11.52-12.93)		80	9.92 (9.31-10.58)	
Triglycerides, mmol/L (mg/dL)						
<1.00 (<88)	1009	10.62 (10.44-10.81)	.02‡ .005§	3092	9.05 (8.95-9.14)	.06‡§
1.00-1.99 (88-176)	2727	10.85 (10.74-10.96)		2630	9.24 (9.14-9.34)	
2.00-2.99 (177-264)	1305	10.83 (10.66-10.99)		440	9.15 (8.90-9.40)	
≥3.00 (≥265)	877	11.06 (10.87-11.26)		186	9.17 (8.70-9.66)	
Total	**5918**	10.84 (10.76-10.91)		**6348**	9.14 (9.06-9.22)	

*Geometric mean of tHcy. CI indicates confidence interval.
†The grading of physical activity in leisure time is defined in the "Methods" section.
‡*P* value for homogeneity.
§*P* value for linear trend.

ated with several demographic and clinical variables that are known to be traditional harbingers of cardiovascular disease. The most notable finding was the strong relation between cigarette smoking and tHcy level. This contrasts to most earlier investigations on plasma tHcy,[9] although such a relation has been suggested in a few recent studies.[13,20,29-32] We found that in current smokers, tHcy level increased almost proportionally to the number of cigarettes smoked per day. The dose-response relationship was strongest in the oldest age group and in women. Overall, multivariate regression analyses showed that the increase in tHcy level per cigarette smoked per day was 1% in women and 0.6% in men. The concentration was only marginally higher in former smokers than in never smokers, and this suggests a transient effect of smoking on tHcy level.

The reason why plasma tHcy level is increased in smokers is not known. Smoking is accompanied by changes in plasma thiol redox status,[29] possibly due to a higher formation of reactive oxygen species.[33] Furthermore, reduced intake of nutrients and vitamins[34,35] and lower levels of plasma folate,[36] red blood cell folate,[37] vitamin B_{12},[38,39] and plasma pyridoxal 5'-phosphate[40] have been demonstrated in smokers. In the current study, adjustment for vitamin supplementation and intake of fruits and vegetables had only a marginal effect on the relation between smoking and plasma tHcy level. However, in smokers as well as in nonsmokers, tHcy level was inversely related to vitamin consumption. This points to extra vitamin intake as a possible means to reduce plasma tHcy level in smokers.

The Hordaland study demonstrates for the first time that plasma tHcy level is inversely related to physical activity. The most pronounced difference was found in the older age group between the physically inactive and active subjects. Moderate and active exercise were associated with almost identical mean tHcy levels. Heavy physical activity conferred a further reduction in mean tHcy level and a marked reduction in the odds ratio for hyperhomocysteinemia. With increasing activity levels, a reduction in skewness of tHcy distribution was observed. This suggests that exercise, especially heavy physical activity, exerts its most favorable effect in subjects with hyperhomocysteinemia.

Several studies have shown that there is a dose-dependent reduction in risk for coronary heart disease with physical activity,[22,41] and a greater benefit has been demonstrated in the older age groups.[42-44] Since this effect of exercise cannot be fully explained by changes in other established risk factors,[22] decreased plasma tHcy level may contribute to the beneficial effect of physical activity on coronary risk.

A positive correlation between tHcy level and total or low-density lipoprotein cholesterol level has been demonstrated in a few previous reports.[13,17,18,30,45] We found a positive relation between tHcy level and total cholesterol level, which was particularly strong in the younger age group. The relation was moderately reduced after adjustment for the other risk factors and intake of fruit, vegetables, and vitamin supplements. A biochemical explanation for the association between plasma tHcy level and serum cholesterol level has not been identified.

Plasma tHcy as a function of heart rate has not been investigated previously, whereas an association with blood pressure has been found only in hypertensive and diabetic patients.[18,46,47] In the current study, plasma tHcy level correlated to blood pressure in the younger subjects and to both heart rate and blood pressure in older subjects. The relation

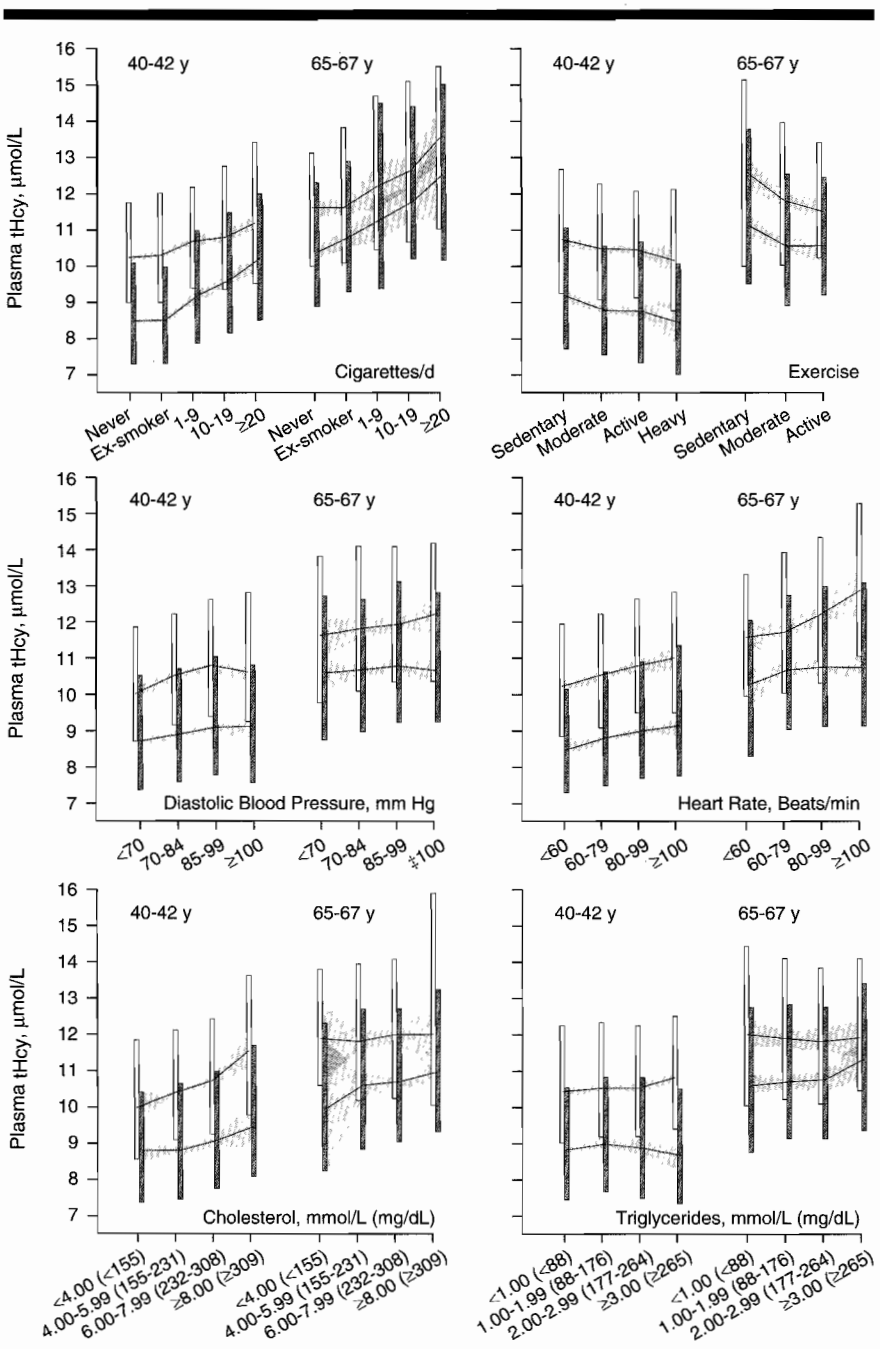

Figure 3.—Total plasma homocysteine (tHcy) levels according to the levels of cardiovascular risk factors in young and old men and women. The group aged 40 to 42 years comprised 5918 men and 6348 women, and the group aged 65 to 67 years included 1386 men and 1932 women. The solid lines are median values, and the shaded area indicates the 95% confidence intervals, while the boxes show the 25th to 75th percentile intervals and are open for men and darkly shaded for women. In the active exercise group of subjects aged 65 to 67 years indicates that the heavy and active exercise groups were combined.

to heart rate was weaker in never smokers and may partly be due to increased pulse rate in smokers.[48] The mechanism behind the relation to blood pressure is unknown. Impaired renal function, an established determinant of plasma tHcy level,[9] is unlikely since the association was strongest in the youngest age group. Likewise, others have found that the difference in plasma tHcy level between hypertensive patients and normotensive patients persisted after adjustment for creatinine.[18]

The current study demonstrates that plasma tHcy level is markedly higher in men than in women and that it increases significantly with age. This confirms results from several smaller studies.[19,31,49-54] Sex hormones may play a role for these sex and age differences. Higher Hcy levels after menopause have been reported in some studies[55,56] but not all studies,[31,52] while reduced levels have been observed during pregnancy, in estrogen replacement therapy in postmenopausal women, and during treatment with the partial estrogen agonist tamoxifen.[49,57-61] Thus, a change in hormonal status may explain the steeper age-related increase in plasma tHcy levels in women. However, even at age 65 to 67 years, men had higher levels than women, suggesting additional mechanisms.

Low levels of folate, cobalamin, and vitamin B_6 explain both sex[7] and age differences in some studies[62] but not all studies.[54] Vitamin levels were not determined in our study, but adjustment for intake of vitamin supplements, fruits, and vegetables strengthened the relation between tHcy level and age, whereas the association between tHcy level and sex was slightly reduced.

In two recent studies, the relation of tHcy level with sex[30,54] and age[30] disappeared after adjustment for serum creatinine level. Creatine-creatinine synthesis, a function of muscle mass, is the major source of Hcy formation[1] and may explain the higher tHcy level in men. In addition, tHcy level is increased during renal failure.[9] Higher tHcy levels in the elderly may therefore be related to the decline in renal function with age.[54]

In the Hordaland population, we observed changes in plasma tHcy level between different cardiovascular risk factor levels, which may be of clinical relevance. Smoking, age, and sex were each related to 1.5- to 2.0-µmol/L (15% to 20%) change in mean plasma tHcy level. In combination, these three variables accounted for a 4.8-µmol/L higher tHcy level in heavy-smoking older men compared with younger women who had never smoked. Physical activity, diastolic blood pressure, heart rate, or total cholesterol level was associated with changes of 0.5 to 1.0 µmol/L. Most retrospective studies have found that mean plasma tHcy level is 2 to 3 µmol/L (20% to 30%) higher in coronary patients than in healthy control subjects,[5] and a recent meta-analysis estimated that a 5-µmol/L increase in plasma tHcy level is associated with an odds ratio for coronary artery disease of 1.6 to 1.8 and an odds ratio for cerebrovascular disease of 1.5.[11] These data concur with the prospective Tromsø study[13] that demonstrated a 40% increase in risk per 4-µmol/L increase in plasma tHcy level. In another prospective study (Physicians' Health Study[12]), patients with myocardial infarction had only a 6% higher mean plasma tHcy level than control subjects, but the relative risk was 3.3 in subjects with levels greater than

Table 4.—Estimated Change in Total Plasma Homocysteine (tHcy) From Multiple Linear Regression Models and Odds Ratio (OR) for Hyperhomocysteinemia (≥15.0 μmol/L) According to Different Levels of Cardiovascular Risk Factors*

	Estimated Change in tHcy, μmol/L†						OR (95% CI)
	Adjusted for Sex and Age				Adjusted for All Risk Factors in This Table		
			Never Smokers			Additional Adjusted for Intake of Fruits, Vegetables, and Vitamin Supplements	
Risk Factor	(n=16 062)		(n=6059)		(n=16 062)	(n=11 496)	(n=11 496)
Female (vs male)	−1.82	P<.001	−1.93	P<.001	−1.57 P<.001	−1.43 P<.001	0.70 (0.59-0.82)
Age, y (vs 40-42)							
43-64	0.74	P<.001	0.75	P<.001	0.56 P<.001	0.65 P<.001	1.21 (0.83-1.76)
65-67	2.20		2.40		1.89	2.22	2.91 (2.44-3.47)
Smoking status (vs never smoker)							
Former smoker	0.11	P<.001	...		0.12 P<.001	0.13 P<.001	1.33 (1.09-1.62)
Light smoker	0.93				0.92	1.04	1.81 (1.39-2.37)
Moderate smoker	1.42				1.33	1.37	2.44 (2.00-2.96)
Heavy smoker	1.91				1.73	1.69	2.99 (2.34-3.82)
Physical activity in leisure (vs no activity)							
Moderate	−0.60	P<.001	−0.48	P<.001	−0.35 P<.001	−0.36 P<.001	0.69 (0.58-0.82)
Active exercise	−0.65		−0.45		−0.28	−0.23	0.77 (0.61-0.97)
Heavy training	−1.16		−1.19		−0.51	−0.43	0.45 (0.18-1.12)
Diastolic blood pressure, mm Hg (vs <70)							
70-84	0.39	P<.001	0.50	P<.001	0.32 P<.001	0.43 P<.001	1.13 (0.91-1.40)
85-99	0.68		0.81		0.54	0.60	1.32 (1.01-1.72)
≥100	0.80		0.86		0.62	0.55	1.14 (0.66-1.99)
Heart rate, beats/min (vs <60)							
60-79	0.41	P<.001	0.38	P<.01	0.08 P<.01	0.08 P<.01	1.05 (0.80-1.39)
80-99	0.76		0.49		0.17	0.25	1.20 (0.90-1.62)
≥100	1.21		0.72		0.48	0.62	1.77 (1.21-2.59)
Total cholesterol, mmol/L (mg/dL) [vs <4.00 (<155)]							
4.00-5.99 (155-231)	0.16	P<.001	0.22	P<.001	0.00 P<.001	−0.16 P<.001	0.82 (0.54-1.24)
6.00-7.99 (232-308)	0.58		0.56		0.27	0.04	0.89 (0.58-1.36)
≥8.00 (≥309)	1.36		1.05		0.94	0.82	1.39 (0.84-2.28)
Triglycerides, mmol/L (mg/dL) [vs <1.00 (<88)]							
1.00-1.99 (88-176)	0.27	P<.001	0.27	P<.01	0.07 P=.28	0.08 P=.34	0.88 (0.73-1.04)
2.00-2.99 (177-264)	0.24		0.14		−0.08	−0.08	0.91 (0.72-1.14)
≥3.00 (≥265)	0.48		0.50		0.01	0.00	0.92 (0.71-1.21)

*CI indicates confidence interval.
†Indicates the estimated change in geometric mean tHcy concentration associated with each specified level compared with the reference level of the risk factor. All levels of a risk factor were tested jointly (test for homogeneity).

15.5 μmol/L. The latter study, which suggests a threshold effect, emphasizes that predictors of hyperhomocysteinemia may reveal important relations not disclosed by comparing the mean levels.

A crucial question is whether plasma Hcy is directly involved in the pathogenesis of vascular disease or just a marker for increased risk. Our study cannot answer this question, and it cannot explain the mechanisms of the observed relations. However, in addition to the previous clinical and epidemiologic studies,[16] there are two lines of evidence in favor of Hcy as a causal factor. First, in patients with homocystinuria, the risk of a fatal thromboembolic event is substantially reduced after Hcy-lowering therapy.[1] Second, in experimental studies, Hcy has been shown to interact with several factors connected with vascular disease, including platelets, endothelial cell function, low-density lipoproteins, the coagulation system, lipoprotein(a), and nitric oxide.[10] The clinical significance of these factors in relation to Hcy is unknown, and intervention studies with Hcy-lowering therapy are necessary to clarify the importance of plasma tHcy level in vascular disease.

In conclusion, our data show that elevated tHcy level is related to old age, male sex, cigarette smoking, high blood pressure, elevated cholesterol level, and lack of exercise, which all contribute to an unfavorable cardiovascular risk profile. This intriguing series of associations should incite intervention studies on the effect of Hcy-lowering therapy and influence design and analysis of future studies on plasma tHcy level and cardiovascular disease.

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