# Plasma Total Homocysteine, B Vitamins, and Risk of Coronary Atherosclerosis

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*Abstract* Epidemiological research has shown that elevated plasma total homocysteine (tHcy) is a risk factor for atherosclerotic disease. In the present case-control study, we investigated whether fasting or postmethionine-loading tHcy was a stronger predictor of risk of severe coronary atherosclerosis. Furthermore, we studied levels of B vitamins, which are involved in homocysteine metabolism. Subjects were recruited from men and women, aged 25 to 65 years, who underwent coronary angiography between June 1992 and June 1994 in a hospital in Rotterdam, The Netherlands. Cases (n=131) were defined as those with ≥90% occlusion in one and ≥40% occlusion in a second coronary artery, while control subjects (n=88) had ≤50% occlusion in only one coronary vessel. In addition, a population-based control group free from clinical cardiovascular disease (n=101) was studied. Coronary patients were studied at least 2.5 months after angiography or other acute illness, such as myocardial infarction. After adjusting for age and sex differences between the groups, cases had 9% ($P$=.01) higher geometric mean fasting and 7% ($P$=.04) higher geometric mean postload tHcy than the combined control groups. Despite higher levels of tHcy for cases, their geometric mean levels of red cell folate and pyridoxal 5′-phosphate were higher than for control subjects, whereas plasma vitamin $B_{12}$ was only slightly lower in cases. The frequency distribution of tHcy values in cases was slightly shifted toward the right, across the entire range, compared with the distribution in the combined control group. This was somewhat more obvious for fasting than postload tHcy levels. The odds ratio (OR) for severe coronary atherosclerosis (case status) for each 1 SD increase in fasting tHcy (5 μmol/L) was 1.3 (95% confidence interval [CI], 1.0-1.6), similar to the OR for each 1 SD increase (12 μmol/L) in postmethionine-loading tHcy (1.3 [95 CI, 1.0-1.7]), after adjustment for sex, age, and other potential confounders. Furthermore, there was a significant linear trend of increasing fasting tHcy with increasing number of occluded arteries ($P$=.01), correcting for sex, age, and other potential confounders. Our data show a positive association between plasma tHcy and risk of severe coronary atherosclerosis, of similar strength for fasting and postload tHcy levels. The data suggest that the association exists over a wide range of tHcy levels, without a clear cutoff point below which there is no increased risk. (***Arterioscler Thromb Vasc Biol.* 1997;17:989-995.**)

***Key Words*** • plasma total homocysteine • vitamins • risk factor • coronary atherosclerosis

Homocysteine is a sulfur amino acid, which is formed from the essential amino acid methionine. Homocysteine is either transsulfurated to cysteine via two vitamin $B_6$–dependent reactions or is remethylated to methionine. Notably, in most cells and tissues, the remethylation pathway depends both on vitamin $B_{12}$ and folate, and low intakes of these vitamins are common causes of elevated plasma tHcy.[1-3] It has been suggested that the fasting level may be determined by homocysteine remethylation, while increased postload tHcy may reflect abnormalities in the transsulfuration pathway.[4]

An extensive number of epidemiological studies has shown that elevated plasma total homocysteine (tHcy) is an independent risk factor for atherosclerotic disease in the coronary, cerebrovascular, and peripheral vessels.[5,6] Positive associations with coronary atherosclerosis have been shown for both fasting tHcy and tHcy levels in response to a methionine load.[7-14] However, most studies were small or investigated either fasting or postload tHcy.

The mechanisms for the atherogenic properties of the amino acid homocysteine are unknown, and hypotheses have mainly been generated by experimental and in vitro studies, mostly in animals. Mechanisms include direct effects on the vascular endothelium, subsequently affecting its antithrombotic properties. Furthermore, endothelial cell damage may cause increased uptake of modified LDL cholesterol in the vascular wall.[1,15] A recently proposed atherogenic mechanism involves inhibition of growth of endothelial cells and stimulation of proliferation of smooth muscle cells by homocysteine, finally leading to thickening of arterial walls.[16]

In the present investigation, we compared both fasting and postload plasma tHcy, as well as the difference between the two (ie, increase after methionine loading), in groups of subjects with and without angiographically documented severe coronary occlusion and a group of apparently healthy subjects with no history of cardiovascular disease. We evaluated whether there was a graded risk of coronary atherosclerosis within the normal range of plasma tHcy, as opposed to a threshold effect, restricted to those with abnormally high levels. Blood levels of PLP, vitamin $B_{12}$, and folate were compared for cases and control subjects, and

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**Selected Abbreviations and Acronyms**

BMI = body mass index
CI = confidence interval
OR = odds ratio
PLP = pyridoxal 5′-phosphate
tHcy = total homocysteine

relationships between plasma tHcy and levels of each of the vitamins were examined.

## Methods

### Study Population

A case-control study was conducted from June 1992 to June 1994. Cases and one control group were selected from patients aged 25 to 65 years who underwent coronary angiography in the Zuiderziekenhuis Hospital in Rotterdam, the Netherlands. Subjects with either severe coronary occlusions (referred to as cases) or without substantial coronary occlusions (referred to as coronary control subjects) were included. A second control group was drawn from the general population and comprised subjects with no history of cardiovascular disease (referred to as population-based control subjects). Exclusion criteria for all groups were diabetes; renal, hepatic, or thyroid disease; cancer; gastrointestinal disease; alcohol or drug abuse; and psychiatric illness.

At angiography, projections were made of the major coronary vessels by using standard catheterization techniques. A team of cardiologists reviewed the projections and prepared angiography reports. A trained research nurse selected potential cases and coronary control subjects. Cases were defined as those having ≥90% occlusion in one and ≥40% occlusion in one additional coronary artery. Notably, 77.1% of the cases had ≥70% occlusion in a second vessel. Coronary control subjects were defined as those having ≤50% occlusion in only one coronary artery. The majority (79.5%), however, had no substantial coronary narrowing in all three arteries, whereas only 5.7% of them had 50% stenosis in a single coronary vessel. Thus, there was a marked contrast between cases and coronary control subjects, reducing the possibility of disease misclassification.

The conditions that led to angiography were mainly angina pectoris, whereas in some coronary control subjects a known valve defect was the reason. Sixty-seven cases (51.1%) and 6 coronary control subjects (6.8%) had a history of myocardial infarction before angiography. In the coronary control subjects, the myocardial infarctions were due to coronary spasms or other nonatherosclerotic causes. Since coronary atherosclerosis was the end point of interest, these control subjects were not excluded from analyses. Some patients had undergone previous angiographic exams, ie, 23.6% of cases and 3.4% of coronary control subjects. The median for the interval between the initial diagnosis of coronary heart disease and the angiographic exam used for selection into the study was 4.8 months (mean: 35.3, range: 0 to 221.2) in cases and 3.0 months (mean: 14.1, range: 0 to 90.6) in coronary control subjects. The time between coronary angiography and day of the methionine-loading test was at least 2.5 months in all subjects.

During the study period, a total of 2659 patients underwent coronary angiography. Of these, 2292 were not included, mainly because of age >65 years (n=1122, 49.0%), coronary occlusion outside ranges of case and control definition (n=486, 21.2%), or the presence of one or more other exclusion criteria. Of the 369 subjects that were invited, 353 (95.6%) could be reached, and of those 222 (62.9%) were willing to participate (131 cases, 91 coronary control subjects). Three of the 91 coronary control subjects that had originally participated were excluded from analysis, as their angiography reports showed too much coronary narrowing at second evaluation.

We obtained a population-based control group from a register of about 10 000 men previously sampled from the catchment area of cases for participation in a cholesterol-lowering trial (which was not conducted). Among men with no prior history of cardiovascular disease or diabetes, a random sample of 152 was invited for participation. Fifteen could not be reached, 14 did not meet the inclusion criteria, and 47 were not interested, leaving 76 (61.3%) study subjects for participation. One participant was excluded from analysis because he reported diabetes at the interview. Spouses of 45 male participants were invited to participate, of whom 12 were not eligible, 7 were not willing, leaving 26 (78.8%) women for participation. Thus, a total of 101 population-based control subjects was studied.

All participants gave their written informed consent. The study protocol was approved by the medical ethics committee.

### Blood Sampling and Examination

At the day of the examinations, venous blood samples were obtained from all subjects between 8:30 and 9:30 AM, after a 10- to 12-hour fast. l-Methionine (0.1 g/kg body weight) mixed with orange juice was given orally, together with a standardized low-protein breakfast. After breakfast, subjects were interviewed about current and past smoking habits, alcohol consumption, and use of medication. Subjects received a standardized low-protein lunch and were asked not to consume any protein-containing foods, such as milk, cheese, or meat. Six hours after methionine administration, a second blood sample was drawn for estimation of plasma tHcy in response to methionine provocation. A blood sample after methionine loading was not obtained from one subject. Duplicate blood pressure readings were taken before and after the methionine-loading test with the subject seated after 5 minutes' rest. Height and weight (without shoes and heavy clothing) were measured in the morning.

For measurement of whole-blood folate, 200 μL of EDTA blood was mixed with 4 mL (1:20) of freshly prepared 1% (wt/vol) ascorbic acid solution. The rest of the EDTA blood, to be used for measurement of tHcy, creatinine, PLP, and cobalamin in plasma was placed on ice and in the dark immediately and centrifuged at 4°C within 1 hour. Serum was obtained for measurement of total and HDL cholesterol and triglycerides. Serum and plasma samples were stored at −80°C for a maximum of 6 months before analysis.

### Biochemical Analyses

The first 60 subjects (15 cases, 20 coronary control subjects, 25 population-based control subjects) of our study were included in a European case-control study, Homocysteinemia and Vascular Disease, which had biochemical analyses performed centrally. After completion of this European study, we continued sending samples to these central laboratories. Plasma tHcy, which refers to the sum of protein-bound, free oxidized, and reduced species of homocysteine in plasma, was determined by a modification of the method by Fiskerstrand et al.[17] The assay was performed at the Department of Clinical Biology, Division of Pharmacology, University of Bergen, Norway, and has a coefficient of variation of 3%. Estimations were performed in duplicate. All other determinations were performed at the laboratory of Mimelab-AB, Söråker, Sweden. For estimation of plasma PLP, enzymatic photometry with high-performance liquid chromatography separation was used, whereas folate in whole blood and plasma vitamin $B_{12}$ (as cobalamin) were determined by radioimmunoassay. We expressed folate concentration per hematocrit, referred to as erythrocyte folate. Creatinine, total cholesterol, and HDL cholesterol (after precipitation of LDL and very-low-density lipoprotein cholesterol) were determined with enzymatic photometry. LDL cholesterol was calculated with Friedewald's formula (except for seven subjects with too high levels of triglycerides). At both laboratories, personnel was unaware of the status (case or control) of the specimens.

### Data Analysis

Smoking status, hypertension, and hypercholesterolemia were based on the situation at the time of the investigation (ie, the day of methionine loading). Current smoking was defined as

the use of any tobacco. In addition, duration of heavy smoking (one or more packs per day) was calculated. Subjects were diagnosed as hypertensive at a systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥95 mm Hg or when they were using antihypertensive medication. Hypercholesterolemia was defined as serum cholesterol ≥6.5 mmol/L or use of cholesterol-lowering drugs.

Differences in cardiovascular risk factor levels between cases and control subjects were tested with Student's *t* test for continuous variables and Pearson's $\chi^2$ test for frequency measures. With exception of age and sex, we show age- and sex-adjusted means and frequencies. To evaluate potential confounding, we studied associations of the cardiovascular risk factors with plasma tHcy levels among the combined control groups by calculating Spearman's correlation coefficients.

By subtracting fasting plasma tHcy levels from postload tHcy levels, we calculated the postload increase in tHcy for each subject. Means, SDs and geometric means of tHcy, creatinine, and vitamins were calculated for all three study groups. Percentage differences in geometric means between cases and control subjects (separate and combined control groups) were calculated, adjusting for age and sex by means of linear regression analysis with the variable of interest (ie, tHcy, vitamins, or creatinine) as the dependent variable and case-control status, age, and sex as the independent variables.

By means of logistic regression analysis, the ORs for severe coronary atherosclerosis (case status) were calculated for those with elevated fasting, postload, or postload increase tHcy levels, defined as levels above the 75th percentile of the combined control groups. ORs were considered to estimate relative risks. Subjects with levels ≤75th percentile were considered as the reference group. To evaluate a possible graded association of plasma tHcy with coronary atherosclerosis, we computed the ORs per 1 SD increase of plasma tHcy. Multivariate logistic regression analysis was used to simultaneously adjust ORs for age, sex, and other confounding factors. Also, we calculated ORs for elevated fasting tHcy, based on previously established cutoff points (14.0 μmol/L and 15.8 μmol/L).[3,18]

To study whether tHcy related to the number of significantly occluded coronary arteries (0, 1, 2, or 3), we regrouped all 219 angiography patients into four groups, in a similar way as previously done in epidemiological research on the association between tHcy and risk of coronary atherosclerosis.[10,12]

Finally, we calculated Spearman's correlation coefficients of the vitamins with plasma tHcy levels. All reported probability values are two tailed.

## Results

### Characteristics of Study Groups

Table 1 shows the main characteristics of the cases and the two control groups at the time of examination. The percentage of males and mean age were higher in cases than in both control groups. The majority of subjects in all groups was aged 40 to 60 years. Mean total cholesterol levels were similar among the groups, but cases had a higher mean total/HDL cholesterol level. The proportion of subjects with hypertension was highest among cases. Mean serum level of triglycerides was highest in cases, whereas alcohol consumption was higher in control subjects. At the time of the examination, a lower percentage of cases was currently smoking, but mean years of heavy smoking was highest in the cases. Creatinine levels were slightly higher in cases than control subjects.

### Plasma tHcy Levels of Study Groups

Mean and geometric mean levels of plasma tHcy were higher in cases than in both control groups, for both fasting and postload levels (Table 2). Since the postload tHcy level is a combination of the fasting level and the increase after methionine loading, we also investigated the increase after loading (ie, postload minus fasting level). This value was also highest among the cases. Distributions of tHcy levels were very similar among coronary and population-based control subjects; therefore, the groups were combined in all subsequent analyses. Cases had significantly higher geometric mean levels of fasting and postload tHcy and nonsignificantly higher postload increase levels of tHcy than the combined control groups after adjusting for age and sex (Table 2). As the Figure shows, the higher mean levels of fasting and postload tHcy in cases were a consequence of a shift toward the right of the cases' frequency distribution. For fasting tHcy, this was slightly more consistent throughout the entire distribution, whereas for postload tHcy there was an additional shift at the far right end of the distribution. For men separately, similar distributions were observed (data not shown).

**TABLE 1. Characteristics of Cases With Severe Coronary Atherosclerosis and Two Groups of Control Subjects**

| | Cases (n=131) | Coronary Control Subjects (n=88) | Population Control Subjects (n=101) |
|---|---|---|---|
| Age, y | 52.5±7.5 | 48.2±8.0 | 49.9±6.9 |
| Sex, % male | 84.7 | 59.1 | 74.3 |
| BMI, kg/m² | 26.6±3.0 | 26.3±3.5 | 26.0±3.6 |
| Total cholesterol, mmol/L | 6.8±1.4 | 6.8±1.7 | 6.7±1.7 |
| Total:HDL cholesterol | 6.8±2.0* | 6.2±2.4 | 6.0±2.7 |
| Hypercholesterolemia, % | 67.2* | 62.5 | 49.5 |
| Triglycerides, mmol/L | 2.0±1.3* | 1.7±1.0 | 1.4±0.9 |
| Systolic BP, mm Hg | 134±13 | 133±16 | 135±14 |
| Diastolic BP, mm Hg | 81±8 | 79±9 | 82±8 |
| Hypertension, % | 83.2* | 53.4 | 16.8 |
| Currently smoking, % | 26.0* | 36.4 | 36.6 |
| Pack-years | 29.8±27.1* | 25.2±20.3 | 21.9±21.4 |
| Alcohol consumption, glasses per day | 0.8±1.3* | 1.1±1.7 | 1.3±1.6 |
| Creatinine, μmol/L | 75.8±11.5 | 73.9±11.2 | 73.5±11.7 |

BP indicates blood pressure. For variables other than age and sex, we show age- and sex-adjusted values (mean±SD or frequencies are shown).

*$P<.05$ cases vs combined control groups. Tested with Student's *t* test for continuous variables and Pearson's $\chi^2$ test for frequencies.

### Evaluation of Confounding by Age, Sex, and Coronary Risk Factors

Age was not associated with fasting or postload tHcy in the combined control groups, which may be caused by the small age range in this study population. Men (n=127) had a 12% higher ($P=.008$) geometric mean fasting plasma tHcy level than women (n=62). Geometric mean postload tHcy and postload increase of tHcy were 5% ($P=.31$) and 12% ($P=.03$) higher in women than in men. This finding was due to a marked response to the methionine-loading test in a subfraction of the women, as has been reported previously by others.[19,20] BMI was positively associated with postload tHcy ($r=.15$, $P=.04$) and postload increase in tHcy ($r=.18$, $P=.01$). Furthermore, fasting tHcy correlated inversely with alcohol consumption ($r=-.15$, $P=.04$). Creatinine

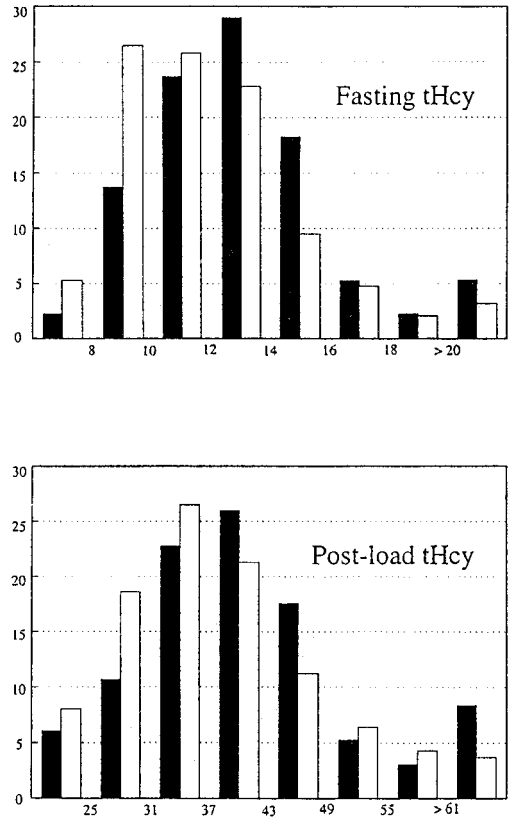
**TABLE 2. Concentrations of Total Homocysteine in Cases With Severe Coronary Atherosclerosis and Two Groups of Control Subjects**

| | Cases (n=131) | | Coronary Control Subjects (n=88) | | Population Control Subjects (n=101) | | Percent Case-Control Difference* |
|---|---|---|---|---|---|---|---|
| | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | |
| Fasting tHcy, μmol/L | 13.5±6.6 | 12.7 | 12.1±3.5 | 11.6 | 12.5±5.7 | 11.9 | 9 (0.01) |
| Postload tHcy, μmol/L | 41.1±12.4 | 39.4 | 37.8±11.2 | 36.5 | 39.0±12.8 | 37.3 | 7 (0.04) |
| Difference of fasting and postload, μmol/L | 27.6±9.7 | 26.1 | 25.8±9.6 | 24.4 | 26.5±9.1 | 25.1 | 6 (0.15 |

Plasma postload tHcy was not measured for one population-based control subject. Values are age- and sex-adjusted mean±SD.
*Age- and sex-adjusted percentage difference in geometric means between cases and combined control groups.

correlated positively with fasting tHcy ($r$=.29, $P$=.0001) and postload tHcy ($r$=.16, $P$=.02). Smoking, blood pressure, total cholesterol, HDL cholesterol, or triglycerides did not correlate with tHcy. Nor was use of antihypertensives and lipid-lowering drugs related to tHcy levels.

Thus, sex was the only variable that was statistically significantly related to tHcy levels and that statistically significantly differed by case-control status. However, by matter of convention, in the subsequent risk analyses, we also controlled for age and traditional risk factors for coronary heart disease (smoking, hypertension, hypercholesterolemia). Furthermore, we controlled for the factors that showed a relationship with tHcy, ie, creatinine, alcohol consumption, and BMI.




Frequency distribution of fasting and postload plasma total homocysteine levels (tHcy) among 131 cases with severe coronary atherosclerosis (solid bars) and 189 control subjects (open bars).

## Plasma tHcy and Risk of Coronary Atherosclerosis

Table 3 shows ORs for severe coronary atherosclerosis for subjects with elevated tHcy relative to subjects with levels at or below the cutoff points. Controlling for age, sex, and other potential confounders, the ORs for severe coronary atherosclerosis for subjects with elevated fasting and postload tHcy levels were 1.3 (95% CI, 0.8-2.3) and 1.5 (95% CI, 0.9-2.5), respectively, whereas the OR for elevated postload increase tHcy levels was 1.8 (95% CI, 1.1-3.2).

When using previously established cutoff points for fasting hyperhomocysteinemia, the age- and sex-adjusted ORs were 1.6 (95% CI, 0.8-3.1) for levels higher than 15.8 μmol/L (cutoff point of Stampfer et al[18]) and 1.6 (95% CI, 0.9-2.7) for levels higher than 14.0 μmol/L (cutoff point of Selhub et al[3]). After additional adjustment for other possible confounders, these ORs were 1.7 (95% CI, 0.8-3.6) and 1.5 (95% CI, 0.9-2.7), respectively.

The ORs for an increase of 1 SD in plasma tHcy, adjusting for the full set of possible confounders, were 1.3 (95% CI, 1.0-1.6) for fasting levels, 1.3 (95% CI, 1.0-1.7) for postload levels, and 1.2 (95% CI, 1.0-1.6) for postload increase levels.

## Degree of Coronary Atherosclerosis

For fasting tHcy there was a linear trend of increasing plasma tHcy levels with increasing number of occluded coronary arteries also when adjusting for age, sex, and potential confounders ($P$=.01). Although postload tHcy levels were higher in groups with 1, 2, and 3 occluded coronaries, compared with those with no occluded coronaries, there was no obvious linear trend (Table 4).

## Vitamins

Compared with coronary control subjects, cases had slightly lower plasma levels of PLP and vitamin $B_{12}$ but higher erythrocyte folate levels (Table 5). The higher folate levels among cases, in comparison with the coronary control subjects, may have been a result of more frequently changed dietary habits after start of the cardiological treatment (39.7% in cases versus 22.0% in coronary control subjects, $P$<.005). Most dietary regimens were meant to lower fat intake, but an increased vegetable or fruit intake may have occurred as well.

Population-based control subjects had lowest mean levels of plasma PLP, plasma vitamin $B_{12}$, and erythrocyte folate. When pooling the control groups and adjusting for age and sex differences, cases had higher plasma PLP, slightly lower plasma vitamin $B_{12}$, and higher geometric mean levels of erythrocyte folate than control subjects (Table 5). One case, two coronary control subjects,

**TABLE 3. ORs of Severe Coronary Atherosclerosis for Subjects With Elevated Plasma Total Homocysteine and per 1 SD Increase in Plasma Total Homocysteine**

| | Fasting Levels | Postload Levels | Increase After Load |
|---|---|---|---|
| Elevated tHcy, μmol/L | >13.5 | >43.5 | >29.8 |
| Cases/controls, n (%) | 47/47 (35.9/24.9) | 41/47 (31.3/24.9) | 42/47 (32.1/24.9) |
| Age- and sex-adjusted OR (95% CI) | 1.4 (0.9-2.4) | 1.4 (0.8-2.3) | 1.6 (1.0-2.7) |
| Multivariate* adjusted OR (95% CI) | 1.3 (0.8-2.3) | 1.5 (0.9-2.5) | 1.8 (1.1-3.2) |
| Per 1 SD increase in tHcy, μmol/L | Per 5 | Per 12 | Per 10 |
| Age- and sex-adjusted OR (95% CI) | 1.3 (1.0-1.6) | 1.3 (1.0-1.6) | 1.2 (0.9-1.5) |
| β±SE† | 0.0478±0.0253 | 0.0199±0.00992 | 0.0180±0.0127 |
| Multivariate* adjusted OR (95% CI) | 1.3 (1.0-1.6) | 1.3 (1.0-1.7) | 1.2 (1.0-1.6) |
| β±SE | 0.0488±0.0253 | 0.0226±0.0103 | 0.0214±0.0134 |

*Multivariate logistic regression, adjusting for age and sex, smoking habits, hypertension, hypercholesterolemia, creatinine, alcohol consumption, and BMI.
†Given per unit increase (μmol/L) in tHcy levels.

and six population-based control subjects were using multivitamins or B vitamin supplements on a daily basis.

Levels of all vitamins correlated inversely with plasma fasting and postload tHcy, in a very similar way for cases and control subjects. In the group of combined control subjects, the correlation coefficients of fasting plasma tHcy with the vitamins were −.22 ($P$=.003) for erythrocyte folate, −.26 ($P$=.0004) for plasma vitamin $B_{12}$, and −.28 ($P$=.0001) for plasma PLP. However, the associations between the vitamins and postload tHcy were to a large extent explained by the strong intercorrelation of fasting and postload tHcy, and only plasma PLP correlated inversely with the increase in plasma tHcy after a methionine load ($r$=−.15, $P$=.04).

## Discussion

Our study findings support the hypothesis that elevated plasma tHcy is a risk factor for severe coronary atherosclerosis, independent of other risk factors for coronary artery disease. The relation was apparent over a wide range of tHcy levels and of similar strength for fasting and postload tHcy. The relative risk per 1 SD increase in tHcy was slightly smaller for the postload increase in plasma tHcy (ie, postload minus fasting tHcy), possibly caused by effect attenuation as a result of double-exposure measurement error (ie, measurement of fasting and postload levels of tHcy). Overall, the increased relative risk associated with elevated postload increase in plasma tHcy suggests that the association observed for postmethionine-loading tHcy is to a large extent independent of fasting tHcy.

Several other retrospective case-control studies on the association of plasma tHcy with angiographically defined coronary atherosclerosis have demonstrated positive associations, with fasting[8-14] or postload tHcy levels.[7] Our study is the first one with relatively large numbers of cases and control subjects to demonstrate that both fasting and postload tHcy levels are positively related to risk of coronary atherosclerosis, over a wide range of values. Our data concur with a retrospective case-control study of peripheral vascular disease[20] showing that frequency distributions of both fasting and postload tHcy were displaced to the right in cases relative to control subjects. A clear advantage of using angiographically defined coronary atherosclerosis as a disease end point is the possibility to grade the vascular disease. Others[10,12] have shown that fasting tHcy increased with increasing number of occluded coronary vessels. In our study, we had the same observation, which reinforces the hypothesis that elevated tHcy plays a role in the atherosclerotic process, although a clear cause-effect relationship cannot be derived from this type of study. However, it is uncertain why the positive linear trend that was observed for fasting tHcy was not apparent for postload tHcy.

With few exceptions,[21,22] most other retrospective epidemiological studies of coronary heart disease showed a positive relation with plasma tHcy.[23-27] However, data from prospective studies have shown weaker associations. In the Physicians' Health Study, with a 5-year follow-up, plasma tHcy levels above the 95th percentile of the control distribution were associated with a 3.4-fold increased risk of myocardial infarction.[18] However, the relative risk was 1.7 with 7.5 years of follow-up, and this

**TABLE 4. Plasma Total Homocysteine Among Subjects With Varying Severity of Coronary Atherosclerosis**

| | Group 0 (n=83) | | Group 1 (n=10) | | Group 2 (n=69) | | Group 3 (n=57) | |
|---|---|---|---|---|---|---|---|---|
| | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean |
| Fasting tHcy , μmol/L | 12.1±3.6 | 11.7 | 12.6±2.3 | 12.4 | 13.2±5.4 | 12.6* | 13.6±8.0 | 12.7 |
| Postload tHcy, μmol/L | 37.4±10.9 | 36.0 | 44.6±10.3 | 43.6* | 40.6±11.5 | 39.1* | 41.6±13.9 | 39.7* |

Values are age- and sex-adjusted mean±SD. Group 0 consisted of patients with <50% occlusion in any of the three major coronary arteries; group 1, subjects with at least 50% narrowing in only one coronary artery; group 2, subjects with at least 50% occlusion in two coronary arteries; and group 3, subjects with at least 50% occlusion in all three coronary vessels.
*$P$<.05 for age- and sex-adjusted differences in geometric means between each of the groups and group 0.

**TABLE 5. Concentrations of Erythrocyte Folate, Serum Cobalamin, and Plasma Pyridoxal 5'-Phosphate in Cases With Severe Coronary Atherosclerosis and Two Groups of Control Subjects**

| | Cases (n=131) | | Coronary Control Subjects (n=88) | | Population Control Subjects (n=101) | | Percent Case-Control Difference* |
|---|---|---|---|---|---|---|---|
| | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | Mean±SD | Geometric Mean | |
| Folate, nmol/L | 853±271 | 809 | 814±277 | 768 | 766±263 | 718 | 10 (0.02) |
| Vitamin $B_{12}$, pmol/L | 261±97 | 245 | 284±102 | 269 | 248±97 | 229 | -1 (0.84) |
| PLP, nmol/L | 29.0±11.4 | 26.9 | 29.1±10.8 | 27.0 | 24.7±8.2 | 23.3 | 8 (0.08) |

PLP indicates pyridoxal 5'-phosphate. Erythrocyte folate levels were missing for two cases and two coronary control subjects, whereas plasma PLP was missing for one population-based control subject. Values are age- and sex-adjusted mean±SD.

*Age- and sex-adjusted percentage difference in geometric means between cases and combined control groups.

was no longer statistically significant.[28] Two other prospective studies clearly failed to show an association,[29,30] whereas another[31] showed no association with incident coronary heart disease and an increased risk of coronary heart disease mortality only during the first 1.5 years of follow-up. Two other studies[32,33] found positive associations, but these were generally smaller than those observed in retrospective studies, especially when subjects with preexisting cardiovascular disease were excluded from analyses.[33] Reasons for weaker associations in prospective studies may include deterioration of tHcy in blood samples or variation in subjects over time, leading to effect attenuation. Of course, another possibility may be that elevated tHcy is not a cause but merely a consequence of cardiovascular disease.

Although our data are compatible with a positive association between plasma tHcy and risk of coronary atherosclerosis, most findings did not reach statistical significance, possibly due to relatively small numbers, especially when using cutoff points for elevated tHcy. However, the estimated multivariately adjusted OR of 1.7 for tHcy levels higher than 15.8 μmol/L was the same as observed for myocardial infarction by Chasan-Taber et al[28] in their analysis based on a follow-up of 7.5 years. Like other studies,[14,27,31] ours found that elevated tHcy is a graded risk factor, but our ORs per unit increase of plasma tHcy were smaller. In a recent meta-analysis,[6] summary ORs for coronary artery disease per 5 μmol/L increment in fasting plasma tHcy were estimated as 1.6 (95% CI, 1.4-1.7) for men and 1.8 (95% CI, 1.3-1.9) for women, based on data of 17 epidemiological studies. We think it is unlikely that large error for tHcy measurement explains the smaller ORs in our study, since protocols for drawing, handling, and shipment of blood samples were carefully followed and coefficients of variation were small for the tHcy determination. It is more likely that the case-control contrast for tHcy was reduced due to more frequent changes of dietary habits (and thus possible improvement of vitamin status) among cases than coronary control subjects. Contributing to this reduced contrast in tHcy may have been the lower vitamin status of population-based control subjects, probably in part accounted for by the fact that both cases and coronary control subjects had adopted healthier dietary habits, in response to cardiological treatment. Use of vitamin supplements did not explain the higher vitamin status in cases relative to control subjects. Also, there was no correlation between tHcy or vitamin levels of spouses among population-based control subjects, indicating that this way of sampling population-based female control subjects has not affected the results.

We found that levels of PLP, vitamin $B_{12}$, and erythrocyte folate were inversely correlated with fasting tHcy, whereas only PLP was inversely related to increase in tHcy after methionine loading. These findings support the hypothesis that fasting level may be determined by homocysteine remethylation, while increased postload tHcy may reflect abnormalities in the transsulfuration pathway.[1,4] However, we found plasma PLP and erythrocyte folate levels not to be lower among cases compared with combined control subjects, despite higher tHcy levels in cases. Similar results were seen in two other studies.[13,14] This observation raises the possibility that factors other than inadequate vitamin status were the cause of elevated tHcy levels in cases. One explanation may be that there was a higher prevalence of subjects with homozygosity for a mutation that renders the enzyme methylenetetrahydrofolate reductase thermolabile (leading to elevated tHcy levels) among cases relative to control subjects, as observed in a recent study.[34] This condition may lead to elevated erythrocyte folate levels[35] and may in fact be another explanation for higher erythrocyte folate levels in cases than in control subjects.

In conclusion, our data suggest that elevated plasma tHcy, fasting and after a methionine load (as well as the increase after methionine loading), is an independent risk factor for severe coronary atherosclerosis. The association exists over a wide range of tHcy levels. The observation that levels of PLP, vitamin $B_{12}$, and folate were not lower among cases than among control subjects in this population, was unexpected, since these levels are important determinants of plasma tHcy levels. This finding may indicate that other determinants of tHcy, ie, genetic influences, were more important in this population. Thus, further studies are necessary to complement the growing evidence of the important role of elevated tHcy in cardiovascular disease etiology and to further elucidate the role of genetic, nutritional, and lifestyle factors. In our opinion, reduction of tHcy levels in the general population, and not only in those with clearly abnormal tHcy levels, may protect against cardiovascular disease, but this has yet to be proven in clinical trials.

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