ORIGINAL INVESTIGATION

Homocysteine and Short-term Risk of Myocardial Infarction and Stroke in the Elderly

The Rotterdam Study

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Background: Elevated homocysteine level increases vascular disease risk. Most data are based on subjects younger than 60 years; data for the elderly are more limited. We examined the relationship of homocysteine level to incident myocardial infarction and stroke among older subjects in a nested case-control study.

Methods: Subjects were participants in the Rotterdam Study, a cohort study among 7983 subjects residing in the Ommoord district of Rotterdam, the Netherlands. Baseline examinations were performed from March 1, 1990, to July 31, 1993. The analysis is restricted to myocardial infarction and stroke that occurred before December 31, 1994. One hundred four patients with a myocardial infarction and 120 with a stroke were identified with complete data. Control subjects consisted of a sample of 533 subjects drawn from the study base, free of myocardial infarction and stroke. Nonfasting total homocysteine levels were measured.

Results: Results were adjusted for age and sex. The risk of stroke and myocardial infarction increased directly with total homocysteine. The linear coefficient suggested a risk increase by 6% to 7% for every 1-µmol/L increase in total homocysteine. The risk by quintiles of total homocysteine level was significantly increased only in the group with levels above 18.6 µmol/L (upper quintile): odds ratios were 2.43 (95% confidence interval, 1.11-5.35) for myocardial infarction and 2.53 (95% confidence interval, 1.19-5.35) for stroke. Associations were more pronounced among those with hypertension.

Conclusions: The present study, based on a relatively short follow-up period, provides evidence that among elderly subjects an elevated homocysteine level is associated with an increased risk of cardiovascular disease.

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ELEVATED LEVELS of homocysteine have been associated with an increased risk of vascular disease, including myocardial infarction, stroke, and peripheral artery disease. These findings have been obtained in hospital-based studies, as well as cross-sectional and prospective population-based studies.[1] The extant studies have been based on populations that mostly included middle-aged subjects. Therefore, it is not known whether homocysteine level remains an important risk factor for vascular disease with advancing age.

Studies on other cardiovascular disease risk factors, such as cholesterol level, smoking, or elevated blood pressure, suggest that risk estimates found in middle-aged subjects are significantly different from those obtained in older persons.[2,3] This may also apply to homocysteine. For instance, in the Physicians Health Study, the relative risk associated with elevated homocysteine level was higher in men younger than 60 years than in men 60 years and older.[4,5] The Hordaland Homocysteine Study investigators reported that the magnitude of the relationship of homocysteine level to several cardiovascular risk factors was lower in the 65- to 67-year-olds than in the 40- to 42-year-olds.[6] Since these studies included few subjects older than 70 years, they could not adequately assess the risk of vascular disease associated with homocysteine in older persons. We examined the association of total homocysteine (tHcy) levels to occurrence of myocardial infarction and stroke in a nested case-control study in which 44% of subjects were older than 70 years. Subjects were participants in the Rotterdam Study, a population-based prospective follow-up study of disease and disability among older persons.

RESULTS

General characteristics of the study population are presented in **Table 1**. Compared with controls, cases of myocardial infarction were more often men, were older, had higher systolic blood pressure and total cholesterol levels, had lower HDL cholesterol levels, and

SUBJECTS AND METHODS

STUDY BASE

The Rotterdam Study is a single-center study described in detail elsewhere.[7] Eligible participants included all residents 55 years old and older living in the Rotterdam suburb of Ommoord, the Netherlands. Of the 10 275 eligible participants, 7983 participated (77.7%) at baseline. Baseline data were collected between March 1, 1990, and July 31, 1993, at an extensive home interview and 2 subsequent visits at the research center. A follow-up examination took place between September 9, 1993, and December 31, 1994, which was attended by 6315 participants (88.4% of those still alive). For this study, we included incident events that occurred after the subject's baseline examination and before January 1, 1995. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus University, Rotterdam, and written informed consent was obtained from all participants at baseline.

CEREBROVASCULAR AND CARDIOVASCULAR RISK INDICATORS

Prevalent disease was assessed by the questions "Did you ever suffer from a myocardial infarction that led to hospitalization?" and "Did you ever suffer from a stroke diagnosed by a physician?" Smoking status was assessed by a standard questionnaire and classified as current, former, and never. At the research center, height and weight were measured and body mass index was calculated. Sitting blood pressure was measured at the right upper arm with a random-zero sphygmomanometer. The average of 2 measurements obtained on 1 occasion, separated by a count of the pulse rate, was used in the present analysis. Hypertension was defined as a systolic blood pressure of 160 mm Hg or more, a diastolic blood pressure of 95 mm Hg or more, or current use of antihypertensive drugs for the indication of hypertension. Diabetes mellitus was considered present when subjects currently used oral blood glucose–lowering drugs or insulin.

A nonfasting venipuncture was performed with the use of a 21-gauge butterfly needle with tube. A detailed description of the blood sampling technique has been given elsewhere.[8] In short, samples were taken with minimal stasis, put on ice directly after sampling, processed within 30 minutes, snap frozen and stored in liquid nitrogen (–80°C), and later stored at –20°C for prolonged storage. Serum total cholesterol level was determined by means of an automated enzymatic procedure.[9] Similarly, high-density lipoprotein (HDL) cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium. Total nonfasting homocysteine was measured as a fluorescent derivative by high-performance liquid chromatography according to the method of Araki and Sako[10] as modified by Ubbink and coworkers.[11] A number of quality control samples were incorporated into runs. The estimation of the tHcy concentration of these samples had to be with 2 SDs of the level of the control serum. The within-run coefficient of variation ranged from 2.3% to 4.0%, and the day-to-day coefficient of variation, from 3.2% to 4.0% for elevated and normal tHcy concentrations, respectively.

OCCURRENCE OF CEREBROVASCULAR AND CARDIOVASCULAR DISEASE

In the Rotterdam Study, information on occurrence of fatal and nonfatal events is obtained from approximately 20 general practitioners (GPs) working in the study district of Ommoord. These GPs cover approximately 85.2% of the Rotterdam Study cohort, and their practices are computerized. The GPs involved report all possible cases of stroke and myocardial infarction to the Rotterdam research center on a weekly basis. Events are presented on a computer file in coded information according to the International Classification of Primary Care.[12] Information on vital statistics is obtained from the GPs as well as at regular intervals from the municipal authorities in Rotterdam. Follow-up information from GPs with practices outside the Ommoord area, covering 15% of the cohort, was obtained retrospectively through checking the participant's GP file and by interviewing the GP. These data were first collected in 1996. When an event or death is reported, additional information is obtained by interviewing the GP and scrutinizing information from hospital discharge records in case of admission or referral. Events were classified after all available information was considered.

A myocardial infarction was considered to have occurred when (1) the event had led to a hospitalization and the hospital discharge record showed a diagnosis of a new myocardial infarction, based on signs and symptoms, electrocardiographic recordings, and repeated laboratory investigations during hospital stay (definite myocardial infarction); or (2) in case of no hospitalization, when a subject died within 1 hour after onset of symptoms (sudden death), or in case of unwitnessed death and the GP reported a cardiac source as the most likely cause of death (probable myocardial infarction).

As 25% to 30% of subjects who suffer an acute stroke in the Netherlands are not hospitalized,[13] all suspected cerebrovascular events reported by the GPs were submitted for review to the EUROSTROKE case-review board, consisting of 4 Dutch neurologists.[14] Based on all information, including symptoms and signs obtained by interviewing the GP or, in case of hospital referral, hospital data, the neurologists classified the events as definite, probable, and

Continued on next page

more frequently had a history of myocardial infarction. Increasing age, current smoking, higher systolic and diastolic blood pressure, hypertension, diabetes mellitus, history of myocardial infarction, and history of stroke were associated with stroke (Table 1). Increasing age, male sex, current smoking, and history of myocardial infarction were significantly and positively associated with tHcy level. Other cardiovascular risk factors were not significantly associated with elevated tHcy level (**Table 2**). Among the strokes, there were 12 hemorrhagic strokes and 61 cerebral infarctions, whereas the remainder of the strokes could not be further classified. Of the cerebral infarctions, 17 were considered lacunar strokes; 26, partial anterior circulation infarctions; 10, total anterior cerebral infarctions; and 13, posterior circulation infarctions.

A logistic regression model with tHcy level as a continuous variable showed that an increase of 1 µmol/L in tHcy level was associated with an age- and sex-adjusted 6% increase in risk of myocardial infarction (OR, 1.06;

possible stroke. Events were classified by 2 neurologists. In case of disagreement, a third neurologist (P.J.K.) was consulted, whose opinion was decisive for the final classification. The present analysis is restricted to definite and probable events. An incident stroke was considered to have occurred when (1) the event had led to a hospitalization and the hospital discharge record indicated a diagnosis of a new stroke; the clinical diagnosis was based on signs and symptoms and neuroimaging investigations during hospital stay (definite stroke); (2) in case of no hospitalization, signs and symptoms associated with the event obtained from the GP records and interview were highly suggestive of a stroke according to the neurologists (probable stroke); or (3) in case of out-of-hospital death, when the GP reported that the cause of death was a cerebrovascular accident and a cardiac cause was judged by the GP to be highly unlikely (probable stroke). Cerebral infarction was further classified according to internationally accepted criteria.[15,16]

SELECTION OF CASES AND SAMPLING OF CONTROLS

For reasons of availability and completeness of information on cardiovascular events, we restricted the present study to follow-up events registered by the approximately 20 GPs with computerized practices in the Ommoord area (coverage of nearly 85% of the cohort). This resulted in a cohort of 6749 subjects from which cases were drawn. The mean duration of follow-up was 2.7 years. Participants enlisted with these general practices were on average 5 years older than those from GPs covering the other 15% of the cohort. Sex, systolic and diastolic blood pressure, total and HDL cholesterol levels, presence of diabetes mellitus, and history of stroke or myocardial infarction did not significantly differ between the 2 groups. We had stored plasma samples from 128 of the 157 subjects with a definite or probable stroke and from 115 of the 140 subjects with a definite or probable myocardial infarction. Of those, tHcy level could be determined in 120 stroke cases and 104 cases of myocardial infarction.

Controls were drawn from a random selection of 630 subjects who participated in both the baseline and follow-up examinations. This restriction was imposed on the sample to meet the requirements of an earlier substudy examining the relationship of homocysteine level to cognitive decline (M.L.B., L.J.L., J.L., A.H., S. Kalmijn, MD, and M. Breteler, MD, PhD; unpublished data; July 1997). A subject was eligible as a control in this analysis when he or she (1) was registered with 1 of the 20 GPs in the Ommoord area and (2) remained free of a myocardial infarction or a stroke during follow-up. The total number of control subjects was 533. Compared with other subjects of the baseline Rotterdam Study cohort enlisted with practices in the Ommoord area and eligible as control subjects (n = 5919), these 533 control subjects were on average 2.7 years younger, had a 3.3–mm Hg lower mean systolic blood pressure, and had a 2.6–mm Hg lower mean diastolic blood pressure. Sex, serum lipid levels, body mass index, current smoking, and history of previous myocardial infarction or stroke did not differ between the 2 groups.

DATA ANALYSIS

First, a linear regression model was used to evaluate the association of tHcy level with potential confounding factors such as age, sex, smoking, body mass index, systolic and diastolic blood pressure, hypertension, total and HDL cholesterol levels, diabetes mellitus, and history of myocardial infarction and stroke. Second, using a logistic model, we studied the association between incident cardiovascular disease and tHcy level expressed as a continuous value. An influential outlier with a tHcy value of 202 µmol/L was excluded from this analysis. A logarithmic transformation of the tHcy values did not change the results with respect to direction and significance, and their results are therefore not presented. Third, we assessed the association of tHcy level to cardiovascular disease by categorizing the sample into quintiles of tHcy level. We estimated the odds ratio (OR) (95% confidence interval [CI]) for incident disease with the lowest quintile as the reference. The 20th, 40th, 60th, and 80th percentile cutoff points were 12.0, 13.8, 15.9, and 18.6 µmol/L, respectively.

The analyses were performed for myocardial infarction and stroke. Also, separate analyses were performed for type of stroke and type of cerebral infarction. Several analysis models are presented: 1 model with adjustments for age and sex only; 1 model with additional adjustment for confounders (current smoking, previous myocardial infarction); and 1 model that included age (continuous), sex, current smoking, systolic blood pressure (continuous), hypertension, total cholesterol level (continuous), HDL cholesterol level (continuous), diabetes mellitus, previous myocardial infarction, and stroke.

In addition to age (55-74 years, 75 years and older), we also evaluated whether sex, smoking, and hypertension modified the association between tHcy level and incident cardiovascular disease as was suggested in earlier studies. To examine effect modification, we dichotomized the sample at the 80th percentile cutoff of tHcy level (≥18.6 µmol/L) and entered a multiplicative interaction term into the logistic model. All data were analyzed with STATA statistical software (Version 4.0; STATA Collaboration, College Station, Tex). Analyses in which subjects with a history of myocardial infarction or stroke were excluded showed associations similar in direction and magnitude to those for all events. Therefore, we only present the latter.

95% CI, 1.02-1.11). Adjustment for confounders (current smoking, previous myocardial infarction) did not alter the findings (OR, 1.06; 95% CI, 1.02-1.11). Similarly, when all cardiovascular factors were accounted for in the analyses, the OR was 1.05 (95% CI, 1.01-1.12).

An increase of 1 µmol/L in tHcy level was associated with an age- and sex-adjusted 7% increase in risk of stroke (OR, 1.07; 95% CI, 1.03-1.11). Adjustment for confounders (current smoking, previous myocardial infarction) did not alter the findings (OR, 1.07; 95% CI, 1.03-1.12). Similarly, when all cardiovascular factors were accounted for in the analyses, the OR was 1.07 (95% CI, 1.02-1.12).

The association between tHcy level and hemorrhagic stroke (12 cases) was as strong as that for cerebral infarction (66 cases). The age- and sex-adjusted ORs per 1-µmol/L increase in tHcy level were 1.09 (95% CI, 1.00-1.18) and 1.07 (95% CI, 1.02-1.12), respectively. With respect to type of cerebral infarction, the strongest

Table 1. General Characteristics of the Study Population by Case Status*

Characteristic	Myocardial Infarction (n = 104)	Stroke (n = 120)	Controls (n = 533)
Age, y	71.8 ± 8.0†	76.5 ± 8.4†	67.9 ± 7.2
Female, %	33.6†	62.5	62.2
Smoking, %	25.2	27.5†	24.2
Body mass index, kg/m^2	26.2 ± 3.3	26.0 ± 3.9	26.5 ± 3.7
Blood pressure, mm Hg			
Systolic	141.3 ± 20.8†	150.4 ± 23.5†	134.8 ± 20.1
Diastolic	71.7 ± 12.7	75.0 ± 14.5†	71.2 ± 10.5
Hypertension, %	31.2	49.5†	27.1
Total cholesterol, mmol/L (mg/dL)	6.90 ± 1.22† (267 ± 47)	6.41 ± 1.25 (248 ± 48)	6.68 ± 1.24 (258 ± 48)
HDL cholesterol, mmol/L (mg/dL)	1.14 ± 0.26† (44 ± 10)	1.33 ± 0.41 (51 ± 16)	1.35 ± 0.41 (52 ± 16)
Diabetes mellitus, %	6.9	12.9†	2.1
Previous myocardial infarction, %	24.0†	13.7†	6.5
Previous stroke, %	6.7	16.2†	3.5
Homocysteine, µmol/L‡	17.3 ± 5.5†	18.4 ± 8.1†	15.2 ± 4.3

*Values are unadjusted proportions or means ± SDs. HDL indicates high-density lipoprotein.

†Significantly different from controls (P<.05), evaluated by logistic regression analysis with adjustment for age, sex, or both for characteristics other than age and sex.

‡One subject with a homocysteine level of 202 µmol/L was excluded.

Table 2. Associations Between Cardiovascular Risk Factors and Total Homocysteine*

Characteristic	β Coefficient	95% CI
Age (per year)	0.19	0.15 to 0.24†
Female (women vs men)	–1.42	–2.19 to –0.65†
Smoking (yes vs no)	1.29	0.43 to 2.15†
Body mass index (per 10 kg/m^2)	0.40	–1.0 to 0.95
Blood pressure (per 10 mm Hg)		
Systolic	0.05	–0.22 to 0.13
Diastolic	0.03	–0.27 to 0.35
Hypertension (yes vs no)	0.59	–0.23 to 1.41
Total cholesterol (mmol/L)	0.12	–0.18 to 0.42
HDL cholesterol (mmol/L)	0.36	–0.58 to 1.31
Diabetes mellitus (yes vs no)	–0.98	–2.79 to 0.83
Previous myocardial infarction (yes vs no)	1.74	0.52 to 2.97†
Previous stroke (yes vs no)	1.32	–0.23 to 2.88

*The β coefficients reflect the mean change in total homocysteine level with the change in characteristic by 1 "unit"; eg, in smokers, the mean total homocysteine level is 1.29 µmol/L (95% CI, 0.43 to 2.15) higher than in nonsmokers, and an increase of 10 mm Hg in systolic blood pressure is associated with an increase in total homocysteine level of 0.05 µmol/L (95% CI, –0.22 to 0.13). Results are adjusted for age and sex, by linear regression analysis. One subject with a homocysteine level of 202 µmol/L was excluded. CI indicates confidence interval; HDL, high-density lipoprotein.

†Statistically significant association (P<.05).

association was seen for lacunar stroke: the age- and sex-adjusted OR per 1-µmol/L increase in tHcy level was 1.10 (95% CI, 1.04-1.17). The ORs for partial anterior circulation infarctions, total anterior cerebral infarctions, and posterior circulation infarctions were 1.08 (95% CI, 1.02-1.15), 1.04 (95% CI, 0.91-1.18), and 0.89 (95% CI, 0.75-1.06), respectively.

The risk of cardiovascular disease increased markedly beyond the upper quintile (18.6 µmol/L) (**Table 3**). Relative to the lowest quintile (<12.0 µmol/L), the age- and sex-adjusted risk of myocardial infarction was 2.43 (95% CI, 1.11-5.35) and the risk of stroke was 2.53 (95% CI, 1.19-5.35). For myocardial infarction and stroke combined, the OR was 2.50 (95% CI, 1.39-4.48). Adjustment for confounders (current smoking, previous myocardial infarction) slightly attenuated the associations (OR, 2.21; 95% CI, 1.21-4.03). Similarly, when all cardiovascular factors were accounted for in the analyses, the OR was 1.85 (95% CI, 0.96-3.55).

The multiplicative interaction terms for evaluating whether the association between tHcy level and cardiovascular disease differed by age, sex, smoking behavior, or hypertension were significant for hypertension ($P = .01$), but not for age ($P = .99$), sex ($P = .17$), and smoking ($P = .16$). Results are presented in **Table 4**.

COMMENT

In this population-based follow-up study among elderly subjects, the short-term risk of stroke and myocardial infarction increased directly with the level of tHcy. When tHcy was analyzed as a continuous variable, the linear coefficient suggested that the risk increased by 6% to 7% for every 1-µmol/L increase in tHcy level. However, the risk by quintiles indicated that the risk for cardiovascular disease was significantly increased only in the group with levels greater than 18.6 µmol/L (upper quintile). Associations did not differ with age (55-74 years, ≥75years) but were more pronounced among women, noncurrent smokers, and those with hypertension.

Several aspects of our nested case-control study that might affect the validity of the findings should be addressed. First, the exposure (tHcy) was measured without knowledge of the case-control status of the participant. Also, the outcome events were based on documented medical information, which limits the extent of misclassification of the diagnosis. However, if such misclassification is still present, the observed associations would represent an underestimation of the true associations, since misclassification is likely to be nondifferential. Similarly, inclusion of subjects with silent myocardial infarctions or silent strokes in the control group, which we could not adjust for, might have led to attenuation of the associations with homocysteine. Second, unlike the cases, our control subjects visited the research center at baseline and follow-up and may represent a somewhat healthier sample of the Rotterdam Study participants. In fact, we have shown that the control subjects were younger and had lower mean blood pressure levels. The issue is whether the selection procedure resulted in control subjects with lower tHcy levels, which might have biased the case-control comparison toward a positive finding. We believe that such a bias is not present in this study, because, apart from age, no differences in determinants of homocysteine level were found between the selected control subjects and those who were potentially control subjects. Furthermore, the direction and magnitude of the associations only marginally changed when confounding cardiovascular risk factors were taken into account. Also,

Table 3. Association Between Total Homocysteine Level and Myocardial Infarction and Stroke by Quintile of Homocysteine Distribution*

	Myocardial Infarction by Total Homocysteine, µmol/L				Stroke by Total Homocysteine, µmol/L			
	12.0-13.7	13.8-15.5	15.6-18.5	≥18.6	12.0-13.7	13.8-15.5	15.6-18.5	≥18.6
N	20	19	23	31	21	16	25	45
Model 1	1.86 (0.86-4.15)	1.28 (0.57-2.89)	1.53 (0.68-3.43)	2.43 (1.11-5.35)	1.51 (0.68-3.32)	0.92 (0.41-2.91)	1.33 (0.61-2.91)	2.53 (1.19-5.35)
Model 2	1.87 (0.84-4.17)	1.22 (0.54-2.77)	1.53 (0.68-3.44)	2.46 (1.11-5.42)	1.42 (0.64-3.15)	0.85 (0.37-1.97)	1.33 (0.61-2.91)	2.30 (1.08-4.90)
Model 3	1.75 (0.78-3.93)	1.22 (0.54-2.77)	1.39 (0.62-3.15)	2.04 (0.91-4.57)	1.50 (0.66-3.40)	0.88 (0.37-2.08)	1.38 (0.62-3.09)	2.33 (1.07-5.08)
Model 4	1.95 (0.80-4.74)	1.08 (0.43-2.67)	1.63 (0.66-3.99)	2.10 (0.88-5.03)	1.61 (0.67-3.89)	0.89 (0.35-2.22)	1.38 (0.58-3.27)	1.90 (0.80-4.48)

**Results are odds ratios with 95% confidence intervals in parentheses, obtained by logistic regression analyses. Category with homocysteine levels <12.0 µmol/L is used as reference. N indicates number of events. Model 1 is adjusted for age and sex; model 2, adjusted for age, sex, and smoking; model 3, adjusted for age, sex, smoking, and previous myocardial infarction; and model 4, adjusted for age, sex, smoking, hypertension, total cholesterol level, high-density lipoprotein cholesterol level, diabetes mellitus, previous myocardial infarction, and previous stroke.*

Table 4. Association Between Total Homocysteine Level and Myocardial Infarction and Stroke by Several Potential Modifiers*

	Myocardial Infarction			Stroke			
Modifiers	Exposed	Nonexposed	OR (95% CI)	Exposed	Nonexposed	OR (95% CI)	Combined, OR (95% CI)
Age, y							
55-74	17/56	48/381	1.85 (0.96-3.55)	12/56	42/381	1.53 (0.75-3.14)	1.70 (1.01-2.88)
≥75	14/22	25/74	1.60 (0.69-3.70)	33/22	33/74	3.21 (1.52-6.76)	2.42 (1.27-4.61)
Sex							
Male	21/40	48/161	1.58 (0.83-2.97)	18/40	27/161	1.84 (0.88-3.87)	1.69 (0.99-2.92)
Female	10/38	25/294	2.11 (0.89-4.97)	27/38	48/294	2.48 (1.27-4.85)	2.33 (1.29-4.22)
Smoking							
No	24/52	53/352	2.21 (1.21-4.05)	29/52	55/352	2.16 (1.18-3.98)	2.23 (1.38-3.62)
Current	7/26	19/103	0.99 (0.37-2.81)	14/26	18/103	1.77 (0.69-4.53)	1.44 (0.67-3.10)
Hypertension							
No	17/61	49/322	1.06 (0.54-2.09)	16/61	38/322	1.31 (0.64-2.69)	1.14 (0.67-1.97)
Yes	13/17	17/126	4.80 (1.93-11.9)	19/17	34/126	2.65 (1.14-6.15)	3.67 (1.79-7.51)

**Odds ratios (ORs) and 95% confidence intervals (CIs) are age and sex adjusted. Exposed indicates number of cases/controls among the exposed (homocysteine level, ≥18.6 µmol/L); nonexposed, number of cases/controls among the nonexposed (homocysteine level, ≤18.5 µmol/L).*

the associations of homocysteine to incident disease were consistent within age strata. Finally, it has been suggested that nonfasting homocysteine measurements may lead to more misclassification of exposure status than fasting homocysteine measurements.[17,18] Misclassification in exposure status in general leads to attenuation of the association, and thus, if present in our study, the results most likely represent an underestimation of the true association.

Compared with previous studies, our levels of total homocysteine are notably higher both in cases and controls. An increase in homocysteine level may be a consequence of incorrect handling of the blood samples, in particular when the sample is kept at room temperature for more than 4 hours.[19] In our study, however, samples were put on ice directly and were generally processed within 60 minutes and stored at –80°C. A higher homocysteine level may result from a higher mean age of the participants in our study compared with that in other studies. A significant positive association of homocysteine levels with age has been noted in most studies able to examine this association.[5,20-22] Our estimates with age showed that a difference of 10 years may lead to a mean increase in tHcy level of 1.9 µmol/L. Yet, our analyses of risk were based on internal comparisons, so the shifted homocysteine distribution is not likely to have biased our results. In addition, high levels of homocysteine may result from poor vitamin status, ie, low levels of vitamin B_{12}, vitamin B_6, and folic acid, and from high creatinine levels. Unfortunately, information on vitamin status and creatinine level was not available in this studied sample, and thus we are unable to explore whether the high tHcy levels resulted from poor vitamin status. Finally, the use of different standards for tHcy measurement across studies may explain in part the differences in absolute tHcy levels.

In this study, the main determinants of elevated tHcy levels were increasing age, male sex, current smoking, and history of myocardial infarction. These findings are in agreement with reports from other studies.[6,17-19]

Most of the prospective evidence that links elevated homocysteine levels to future cardiovascular disease risk comes from studies performed in middle-aged subjects.[4,5,23,24] In the Physicians Health Study, subjects with homocysteine levels above the 95th percentile of controls had a 3.1-fold increased risk for myocardial infarction compared with those with homocysteine levels below the 90th percentile of controls.[4] In the Tromso study, a 30% increased risk of coronary heart disease was found per 4-µmol/L increase in tHcy level.[21] In a report from the British Regional Heart Study, a graded positive association was shown between the level of tHcy and risk of

stroke.[20] However, in the Physicians Health Study, a nonsignificant 1.4-fold increase in risk of stroke was found, when those in the top quintile of homocysteine levels were compared with all others.[5] In a Finnish prospective population-based study, an association between homocysteine level and risk of coronary heart disease and stroke was not found.[25]

The present study shows that, among elderly subjects, an elevated homocysteine level is associated with an increased risk of both myocardial infarction and stroke. These results and those from other studies on markers of atherosclerosis, including an earlier report from our group,[26] support the hypothesis that elevated homocysteine levels are associated with an increased risk of cardiovascular disease. An increased risk of extracranial carotid artery stenosis of 25% and higher was reported in elderly subjects with homocysteine levels above the median in the Framingham cohort.[27] In middle-aged subjects participating in the population-based Atherosclerosis Risk in Communities study, those with homocysteine levels in the top quintile had an increased risk for thickening of the carotid artery intima-media wall, compared with those in the bottom quintile.[28] Our results from a logistic model in which homocysteine was included as a continuous variable provided a significant estimate of a linear association between homocysteine and future disease, but results from analyses in quintiles did not support a linear relationship. Rather, the increased risk was confined to the upper quintile. However, the CIs of the second, third, fourth, and fifth quintiles do overlap considerably, indicating that a linear graded association cannot be excluded.

The present study showed that the magnitudes of the associations of tHcy level to hemorrhagic stroke and cerebral infarction did not significantly differ from one another. Also, the strongest associations were seen with lacunar infarction compared with other types of cerebral infarction. Yet, these results are limited in that they are based on a small number of cases. Confirmation by other studies is therefore warranted.

We found that, among hypertensive subjects, the association of homocysteine level to myocardial infarction and to stroke was markedly stronger than in normotensive subjects. For myocardial infarction, effect modification by blood pressure could not be demonstrated in the Physicians Health Study,[29] whereas effect modification was not reported in the results of the Tromso study or the Finnish study. For stroke our findings are in agreement with those from the British Regional Heart Study, in which, among hypertensive men, the risk of stroke for subjects in the upper quintile of the tHcy distribution was 3.7-fold (95% CI, 1.0- to 13.1-fold) higher than that for those in the lowest quintile, whereas for normotensive men a relative risk of 1.8 (95% CI, 0.6-5.5) was found. Yet, in the Physicians Health Study, the reverse was found: a higher relative risk among normotensive subjects.[19] This effect modification has also been reported in the Atherosclerosis Risk in Communities study in a report on tHcy and intima-media thickness.[25] Elevated tHcy level is thought to induce endothelial dysfunction possibly via cell loss, a reduced survival of blood platelets, and increased formation of atherosclerotic lesions.[30] An increased turnover of blood platelets has been associated with elevated tHcy levels.[31,32] Results from animal studies indicate that the deleterious effect of elevated homocysteine level on the development of atherosclerosis occurs earlier and is more pronounced in hypertension than in normotension.[33]

When a potential causal association between risk factors and disease is studied, it can be argued whether one should make adjustment for all cardiovascular risk factors measured. For causative analyses, in principle, adjustments should only be done for variables that may confound the association in the current data set, ie, factors that associate with both homocysteine level and event and are not considered to be in the causal pathway (model 3, Table 3). On the other hand, when the interest is whether the association is independent of other cardiovascular risk factors, one may want to adjust for a larger number of cardiovascular risk factors (model 4, Table 3). The results of the latter model are important for the use of homocysteine measurements relative to other cardiovascular risk factors in risk profiling of individual patients.

The main results of the present study may have important implications for prevention of cerebrovascular and coronary heart disease in older subjects. Elevated levels of tHcy may result from genetic defects or nutrient deficiencies.[18,34] At greater age, the contribution of genetic defects to the elevation of homocysteine level appears to be limited compared with other factors.[35] Several studies have pointed toward the possibility of lowering homocysteine through dietary intervention with vitamin B_{12}, folate, and vitamin B_6.[36,37]

In conclusion, the present study, based on a relatively short follow-up period, provides evidence that among older subjects an elevated homocysteine level indicates an increased risk of cardiovascular disease.

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