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Serum Homocysteine and Risk of Coronary Heart Disease and Cerebrovascular Disease in Elderly Men

A 10-Year Follow-Up

Coen D.A. Stehouwer, Matty P. Weijnenberg, Michiel van den Berg, Cornelis Jakobs, Edith J.M. Feskens, Daan Kromhout

Abstract—Hyperhomocysteinemia is an independent risk factor for atherosclerotic disease in the middle-aged. We investigated whether a high serum homocysteine level is a risk factor for vascular disease in 878 elderly men (mean age at baseline, 71.5 years; range, 64 to 84 years) in a population-based, representative cohort followed up for 10 years in Zutphen, the Netherlands. Thirty-one percent had nonfasting homocysteine levels $\geq 17 \mu\text{mol/L}$. After adjustment for other major risk factors, high homocysteine levels at baseline (the third compared with the first tertile) were associated with an increased baseline prevalence of myocardial infarction (odds ratio [OR], 1.81; 95% confidence interval [CI], 1.07 to 3.08; P for trend, 0.03) and with a marginally significant increase in the risk of dying of coronary heart disease (relative risk [RR], 1.58; 95% CI, 0.93 to 2.69; P for trend, 0.09) but not with an increased risk of first-ever myocardial infarction. In addition, high homocysteine levels at baseline were associated with an increased baseline prevalence of stroke (OR, 4.61; 95% CI, 1.79 to 11.89; P for trend, 0.002) and with an increased risk of dying of cerebrovascular disease in subjects without hypertension (RR, 6.18; 95% CI, 2.28 to 16.76) but not in those with hypertension. High homocysteine levels were associated with an increased risk of first-ever stroke among normotensive subjects that was not statistically significant (RR, 1.77 [95% CI, 0.83 to 3.75; P for trend, 0.14]). In a general population of elderly men, a high homocysteine level is common and is strongly associated with the prevalence of coronary heart disease and cerebrovascular disease. It is a strong predictive factor for fatal cerebrovascular disease in men without hypertension but less so for coronary heart disease. (*Arterioscler Thromb Vasc Biol.* 1998;18:1895-1901.)

Key Words: homocysteine ■ atherosclerosis ■ vascular disease ■ elderly

More than 20 cross-sectional¹⁻³ and 3 prospective⁴⁻⁶ studies in young and middle-aged subjects have shown that high levels of homocysteine are associated with an increased risk of myocardial infarction and stroke. These associations were weak⁷ or absent^{8,9} in 2 other prospective studies and in a longer follow-up¹⁰ of the Physicians' Health Study,⁴ however, and most prospective studies observed weaker associations with increasing age.^{4,5,7,10}

High homocysteine levels are particularly common among the elderly,¹¹ but there are no population-based data on homocysteine and risk of myocardial infarction or stroke in the elderly. We therefore investigated these issues in the Zutphen Elderly Study, a population-based prospective investigation in elderly men.

Methods

Patients

The Zutphen Elderly Study is a longitudinal investigation of risk factors for chronic diseases in elderly men. It is an extension of the

Dutch contribution to the Seven Countries Study. The study started in 1960 with a cohort of 878 men then 40 to 59 years old who had lived for at least 5 years in Zutphen, in the eastern Netherlands.¹² In 1985, 555 men of the original cohort were still alive and were invited for new investigations, together with an additional random sample of 711 men of the same age group living in Zutphen and not part of the original cohort. Of those invited, 74% (939/1266) entered the study: 62 had moved or could not be reached, 109 could not be examined because of serious illness, and 156 refused. Complete information was available for 878 men 64 to 84 years old.

Baseline Assessment

The baseline examination took place between March and June 1985.¹² In brief, the body mass index was calculated as weight (kg)/height (m)². Systolic and diastolic (Korotkoff phase V) blood pressures were measured in duplicate with a random-zero sphygmomanometer, with the men supine. Hypertension was defined as a systolic blood pressure of ≥ 160 mm Hg, a diastolic blood pressure of ≥ 95 mm Hg, and/or use of antihypertensive drugs. The diagnosis of myocardial infarction required 2 or more of the following 3 criteria: severe chest pain lasting for >20 minutes that did not disappear in rest, characteristic changes on electrocardiography, and

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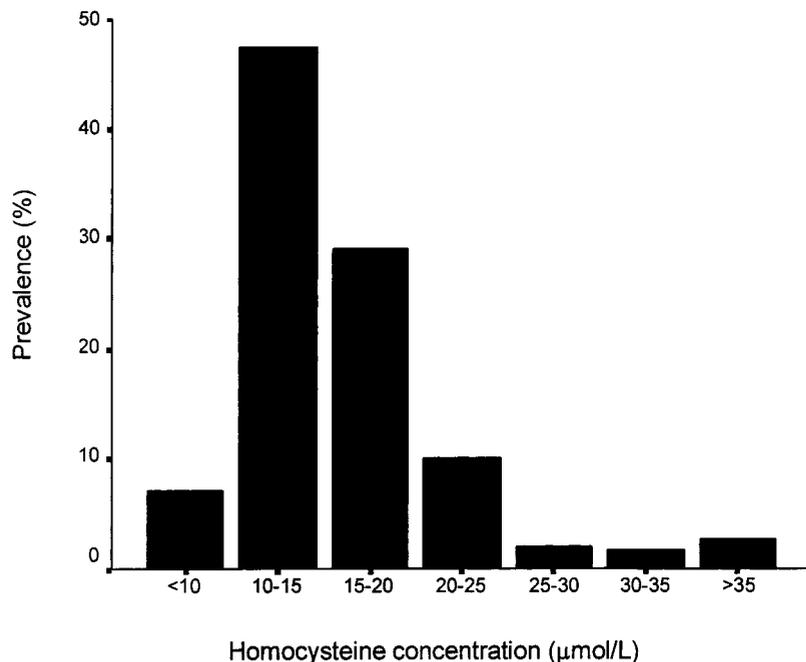


Figure 1. Distribution of homocysteine levels in 878 elderly men.

specific enzyme elevations. Information on stroke was collected with a standardized questionnaire. All diagnoses were verified with hospital discharge data and written information from the subjects' general practitioners and, in the case of stroke, their neurologists.

Venous blood samples were obtained in the nonfasting state. Samples were centrifuged after ≈ 60 minutes, which is sufficient to prevent increases in serum homocysteine resulting from ex vivo generation of homocysteine by erythrocytes.¹³ Serum was stored at -20°C and assayed in 1995. Total (free plus protein-bound) homocysteine levels are stable in serum or plasma stored for 10 years or more.^{8,14} Serum total homocysteine was measured as previously described in detail.¹⁵ The intra-assay and interassay coefficients of variation are 2.1% and 5.1%. Because the available amount of serum was limited, we performed duplicate assays in only 64 of 878 samples (mean difference, 6.0%). Serum homocysteine levels are consequently given as whole numbers. Serum total and HDL cholesterol were determined with standard methods.¹² Serum creatinine was determined with a modified Jaffé method.

Follow-Up

Information on vital status on December 31, 1994, was obtained. Information on the causes of death was obtained from Statistics Netherlands for deaths that occurred between the baseline assessment and June 1990 and from the subjects' general practitioners for deaths that occurred thereafter. The causes of death were coded according to the 9th revision of the International Classification of Diseases (ICD) by a single physician. Because the underlying cause of death in the elderly is often difficult to determine, both the primary and the secondary causes of death were considered in the analyses. Death from coronary heart disease and cerebrovascular disease was defined by ICD codes 410 to 414 and 430 to 438. Information on the occurrence of myocardial infarction and stroke between the baseline assessment and December 31, 1994 was obtained in 1990 (at an assessment similar to the baseline assessment; see above), in 1993, and in 1995 (using the Dutch translation of the Rose Questionnaire for myocardial infarction and a standardized questionnaire for stroke). In all cases, myocardial infarction and stroke were defined as described above (see Baseline Assessment). For nonresponders, information on major chronic diseases was obtained from a standardized nonresponse questionnaire filled in by the subjects themselves or their closest relative or caregiver. "First-ever" myocardial infarction or stroke was defined as fatal or nonfatal myocardial infarction or stroke in the absence of a history of

myocardial infarction or stroke at baseline. All diagnoses were verified with hospital discharge data and written information from the subjects' general practitioners and were coded by 2 physicians.

Statistical Methods

SAS statistical programs were used for the analyses (SAS Institute Inc, 1989, version 6.08). All tests were 2-sided. Values of $P < 0.05$ were considered statistically significant. The subjects were categorized according to tertiles of homocysteine level. Differences in their baseline characteristics were then evaluated by use of ANOVA for normally distributed variables, the Kruskal-Wallis test for variables with a skewed distribution, and an overall χ^2 test for categorical variables. Event rates were calculated as the number of cases divided by the sum of time periods of observation. Logistic regression analysis was used to investigate the associations with the prevalence of myocardial infarction and stroke. Cox's proportional hazard (survival) analysis was used to investigate the associations with mortality outcomes and with the incidence of first-ever myocardial infarction and stroke. The highest tertile was compared with the 2 lower tertiles if necessary to avoid (near-) empty cells and thus the inability to estimate the relative risk (RR) reliably. Interaction terms were investigated at the 0.10 level. Three persons had moved and were lost to follow-up. The date on which they moved was used as their (censored) end-point date. Unless stated otherwise, adjusted analyses are those in which the effects of major risk factors are taken into account, ie, age, body mass index, systolic blood pressure, total and HDL cholesterol, diabetes mellitus, and cigarette smoking habits.

We chose to categorize the subjects according to tertiles of homocysteine levels, because the alternative approach, ie, survival analysis with homocysteine as a continuous variable, assumes that the relationship between homocysteine level and the risk of vascular disease is linear. It is not clear that this assumption is correct,^{1-10,16-18} but, for comparison, we nevertheless repeated the above analyses with homocysteine as a continuous variable.

Results

Baseline

The mean (\pm SD) homocysteine level at baseline was 15.8 (± 8.2) $\mu\text{mol/L}$. Figure 1 shows the distribution of homocysteine levels. The prevalence of homocysteine levels ≥ 16 , 17,

TABLE 1. The Zutphen Elderly Study: Baseline (1985) Characteristics According to Tertiles of Serum Homocysteine in 878 Men

Characteristic	Homocysteine Tertile			P
	First (n=287)	Second (n=323)	Third (n=268)	
Homocysteine, $\mu\text{mol/L}$; range	10.5 (1.4) 6–12	14.3 (1.1) 13–16	23.5 (11.3) 17–97	
Age, y	70.0 (4.6)	71.5 (5.3)	73.0 (5.7)	<0.0001
BMI, kg/m^2	25.8 (3.3)	25.3 (2.7)	25.3 (3.6)	0.08
BP, mm Hg				
Systolic	149.1 (21.4)	151.6 (21.7)	152.7 (21.2)	0.1
Diastolic	85.2 (11.2)	86.3 (10.8)	84.9 (12.5)	0.3
Hypertension, %	39	43	46	0.2
Serum cholesterol, mmol/L	6.13 (1.00)	6.13 (1.10)	6.02 (1.21)	0.4
Serum HDL cholesterol, mmol/L	1.15 (0.31)	1.11 (0.27)	1.09 (0.30)	0.05
Serum creatinine, $\mu\text{mol/L}^*$	96.9 (13.5)	105.2 (19.7)	113.1 (28.9)	<0.0001
Current cigarette smokers, %†	27	30	35	0.02
History of diabetes mellitus, %	9	6	5	0.1
History of myocardial infarction, %	9.8	13.0	17.5	0.03
History of stroke, %	2.1	3.7	10.1	0.001

BMI indicates body mass index; BP, blood pressure. Data are mean (SD) or percentage, unless otherwise indicated.

*†Information available for *849 and †877 men.

and 18 $\mu\text{mol/L}$ was 37.8%, 30.5%, and 25.1%. Table 1 shows that higher homocysteine levels were associated with increasing age, lower levels of HDL cholesterol, higher serum creatinine levels, current smoking, and a history of myocardial infarction and stroke. The associations with myocardial infarction and stroke remained after adjustment for major risk factors (see Statistical Methods). For myocardial infarction, the adjusted odds ratios (ORs) for the second and third tertiles compared with the first were 1.28 (95% confidence interval [CI], 0.76 to 2.15) and 1.81 (95% CI, 1.07 to 3.08; *P* for trend, 0.03). For stroke, these ORs were 1.74 (95% CI, 0.63 to 4.83) and 4.61 (95% CI, 1.79 to 11.89; *P* for trend, 0.002).

Follow-Up

For coronary heart disease, the mortality rate per 100 person-years was 1.45 (98/878). It was highest in subjects with homocysteine levels in the highest tertile (Table 2; Figure 2A). This risk was reduced after adjustment (*P* for trend, 0.09) and decreased further when we included a history of myocardial infarction at baseline as a potential confounder in the analysis (adjusted RRs for the second and third tertiles compared with the first, 1.11 [95% CI, 0.66 to 1.87] and 1.42 [95% CI, 0.83 to 2.41]; *P* for trend, 0.20). (Note that such an analysis may result in overadjustment if a history of myocardial infarction is an intermediate in the causal pathway linking a high homocysteine level to a subsequent fatal myocardial infarction.) The incidence rate per 100 person-years of first-ever myocardial infarction was 1.76 (115/761) and was not clearly related to homocysteine levels (adjusted RR for the third compared with the first tertile, 1.17 [95% CI, 0.72 to 1.89]; *P* for trend, 0.51). There was no evidence for

interactions between homocysteine and other risk factors, including hypertension (see below).

For cerebrovascular disease, the mortality rate per 100 person-years was 0.86 (58/878). It was highest in subjects with homocysteine levels in the highest tertile (Table 2). This association was significant only in subjects without hypertension (Table 2 and Figure 2B and 2C; *P* for interaction, 0.0003), a finding that remained when we included a history of stroke at baseline as a potential confounder in the analysis (adjusted RR for the highest tertile compared with both lower tertiles combined, 3.92 [95% CI, 1.38 to 11.14]; *P*=0.01) or when we excluded subjects with stroke at baseline (RR, 4.42; *P*=0.01). The incidence rate per 100 person-years of first-ever stroke was 1.49 (98/833) and was not clearly related to homocysteine levels (adjusted RR for the third compared with the first tertile, 1.27 [95% CI, 0.78 to 2.07]; *P* for trend, 0.34). The risk was somewhat higher in normotensive subjects (RR, 1.77 [95% CI, 0.83 to 3.75]; *P* for trend, 0.14) than in hypertensive subjects (RR, 0.99 [95% CI, 0.52 to 1.91]; *P* for trend, 0.98; *P* for interaction, 0.15).

Adjustment for serum creatinine did not materially alter the above risk estimates (data not shown). Multivariate survival analyses with homocysteine as a continuous variable gave results similar to those shown above.

The adjusted RR of mortality from coronary heart disease per 1 $\mu\text{mol/L}$ increase in homocysteine level was 1.014 (95% CI, 0.997 to 1.030; *P*=0.11) and 1.013 (95% CI, 0.995 to 1.031; *P*=0.15) after additional adjustment for history of myocardial infarction. For first-ever myocardial infarction, the adjusted RR was 1.010 (95% CI, 0.993 to 1.028; *P*=0.25). There was no evidence for interactions between homocysteine and other risk factors, including hypertension.

TABLE 2. Relative Risks and 95% CIs for Mortality From Coronary Heart Disease and Cerebrovascular Disease in 878 Men During 10-Year Follow-Up According to Tertiles of Serum Homocysteine

Mortality	Homocysteine Tertile			P for Trend
	First	Second	Third	
Coronary heart disease				
Crude	1*	1.38	1.89	0.01
		0.83–2.29	1.14–3.15	
Adjusted†	1*	1.23	1.58	0.09
		0.73–2.06	0.93–2.69	
Cerebrovascular disease				
Crude	1*	0.78	1.87	0.04
		0.40–1.55	1.02–3.43	
Adjusted†	1*	0.67	1.26	0.48
		0.34–1.35	0.67–2.36	
Normotensive‡ adjusted†	1*	1*	6.18	0.0003
			2.28–16.76	
Hypertensive‡ adjusted†	1*	1*	0.65	0.28
			0.30–1.41	

*Reference group.

†Adjusted for age, body mass index, systolic blood pressure, total and HDL cholesterol, diabetes mellitus, and cigarette smoking habits.

‡Among normotensive subjects, 6 cases per 2984 person-years in the first two tertiles vs 16/984 in the third tertile; among hypertensive subjects, 27/1952 vs 9/833.

For mortality from cerebrovascular disease, the adjusted RRs were 1.007 (95% CI, 0.984 to 1.032; $P=0.55$) in the entire group, 0.937 (95% CI, 0.865 to 1.014; $P=0.11$) in the hypertensive subjects, and 1.024 (95% CI, 1.002 to 1.047; $P=0.03$) in the normotensive subjects (P for interaction, 0.03). After additional adjustment for a history of stroke, the RR in the normotensive subjects was 1.023 (95% CI, 0.997 to 1.051; $P=0.09$). For first-ever stroke, the adjusted RRs were 1.001 (95% CI, 0.979 to 1.023; $P=0.92$) in the entire group, 0.997 (95% CI, 0.961 to 1.033; $P=0.85$) in the hypertensive subjects, and 1.005 (95% CI, 0.978 to 1.033; $P=0.73$) in the normotensive subjects (P for interaction, 0.65).

Discussion

This is the first prospective, population-based study of the role of high homocysteine levels as a risk factor for coronary heart disease and cerebrovascular disease in the elderly. The subjects' mean age at baseline was 71.5 years (range, 64 to 84 years), which is substantially higher than the mean ages in previous prospective studies (46 to 60 years).^{4–9}

Suggested cutoff values for high homocysteine levels have ranged from 11.4 to 15.8 $\mu\text{mol/L}$.^{2,4–7,11,16} In our study, 33% of the subjects had levels of ≥ 17 $\mu\text{mol/L}$. Thus, our study confirms that high homocysteine levels are extremely common among the free-living elderly.¹¹

We found that a high serum homocysteine level was strongly associated with the baseline prevalence of myocardial infarction and stroke. During 10-year follow-up, it was associated with a modest, borderline significant increase in the risk of dying of coronary heart disease and, among normotensive subjects, with a large increase in the risk of

fatal cerebrovascular disease and a nonsignificant increase (RR, 1.77) in the risk of first-ever stroke. Remarkably, it was not associated with the incidence of first-ever myocardial infarction.

The associations between homocysteine and coronary heart and cerebrovascular disease are not consistent. On the one hand, many cross-sectional^{1–3} and 3 prospective studies have shown an increased risk of myocardial infarction^{4,5} and stroke⁶ with high homocysteine levels. In addition, high plasma homocysteine levels were a strong predictor of all-cause and cardiovascular mortality in patients with coronary artery disease.¹⁷ On the other hand, 2 other prospective studies^{8,9} and an extended follow-up¹⁰ of an earlier study⁴ were negative with regard to myocardial infarction, as were 2 studies on stroke.^{7,8} There is no straightforward explanation for these discrepancies. One possibility is that homocysteine is related to other cardiovascular risk factors, ie, that homocysteine is not an independent cause of vascular disease, and that studies showing an association of hyperhomocysteinemia and cardiovascular risk have not been fully adjusted for possible confounders. However, plausible biological mechanisms have been demonstrated by which high homocysteine levels may lead to vascular disease: homocysteine is thought to induce endothelial dysfunction with respect to the regulation of vasomotor tone and hemostatic balance^{19–21} and to stimulate vascular smooth muscle cell proliferation,²² both important events in the pathogenesis of atherothrombotic disease. Moreover, severe hyperhomocysteinemia in young people is strongly associated with arteriosclerosis and arterial and venous thrombosis at a young age.²³ These findings constitute important evidence in favor of a causal association between homocysteine and vascular disease.

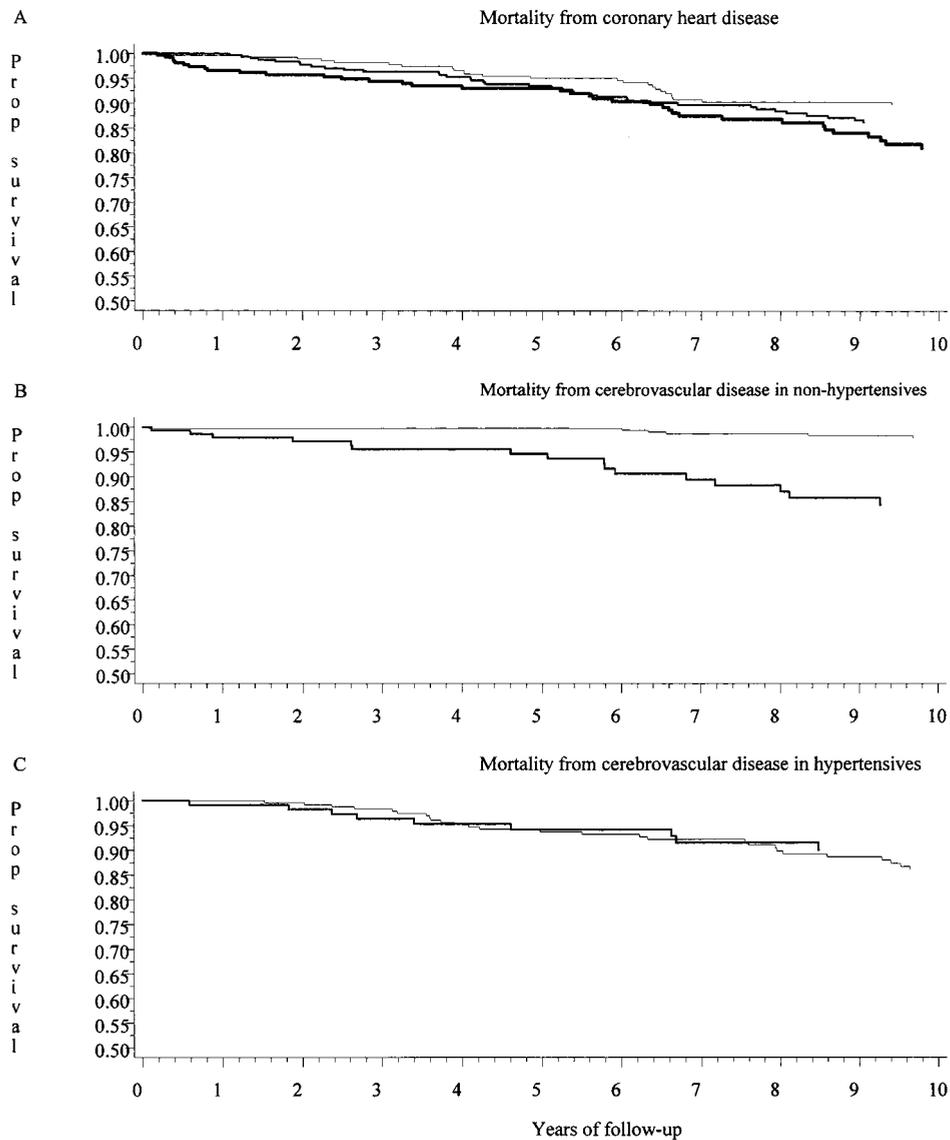


Figure 2. A, Mortality from coronary heart disease according to tertiles of homocysteine levels. Gray line indicates first tertile (6 to 12 μmol/L); black line, second tertile (13 to 16 μmol/L); heavy black line, third tertile (17 to 97 μmol/L); and Prop, proportion. B and C, Mortality from cerebrovascular disease in normotensive (B) and hypertensive (C) subjects. Gray line indicates first and second tertile; black line, third tertile.

Another possibility is that the association between homocysteine level and vascular disease depends on some threshold¹⁷ and that the negative results of at least some studies⁸ may be explained by relatively low homocysteine levels. Whether or not such a threshold exists and if so, at what level, however, remains uncertain.^{4-9,17} Although our data do not suggest a clear threshold, the present study was not large enough to investigate this with any agree of confidence.

An alternative hypothesis is that the association between mild hyperhomocysteinemia and both atherosclerotic and thrombotic disease in adults is modulated by other factors, such as ethnicity and other cardiovascular risk factors and that this can explain, at least in part, the inconsistent results among various studies. Several intriguing reports appear to support this idea.

First, the association between homocysteine level and extent of atherosclerotic disease as assessed by angiography

or ultrasound is strong in some studies^{16,24,25} but weak or absent in others.^{17,18,26} The association between homocysteine levels and incidence of angina pectoris, the pathogenesis of which is determined more by atherosclerosis than by thrombosis, was also weak.²⁷ If the strength of the associations between homocysteine level and atherogenesis and thrombogenesis varied among populations, this might account for some of the variability in the association of homocysteine with vascular disease. For example, in a situation in which the association of homocysteine level and thrombogenesis is strong and that with atherosclerosis weak, one may find that the association between homocysteine level and myocardial infarction is limited to the first few years of follow-up. At least some data^{4,10,27} support this notion. In this regard, it is interesting that the risk of mortality from coronary heart disease among subjects with high homocysteine levels in the present study did appear to be greatest in the first few years

of follow-up (Figure 2A). We did not further analyze this, both because such an analysis would be post hoc and because our study lacked sufficient power. Nevertheless, future larger studies need to investigate this issue.

A second important factor may be ethnicity. A study that used a low serum folate level as a proxy for a high homocysteine level found an increased risk of stroke among black but not among white subjects.²⁸

Third, hyperhomocysteinemia may interact with other risk factors. In some populations, the association between hyperhomocysteinemia and vascular disease was especially strong among smokers² and in the presence of hypertension^{2,6} or non-insulin-dependent diabetes mellitus.³ Other studies found that the association of high homocysteine levels with myocardial infarction¹⁰ and stroke⁷ was stronger among normotensive than among hypertensive subjects. We found that among elderly men, the risk of stroke was lowest in normotensive subjects with low homocysteine levels, whereas it was equally increased among subjects with hypertension, high homocysteine levels, or both. In contrast, we observed no interaction between blood pressure and homocysteine levels with regard to coronary heart disease. Age may be another risk factor that modifies the association between homocysteine level and cardiovascular risk. Our data and previous studies^{4,5} raise the possibility that high homocysteine levels are linked predominantly to myocardial infarction at a relatively young age and to recurrent infarction but not to first-ever infarction at an advanced age.

Taken together, these findings support the concept that age and other cardiovascular risk factors, at least in some populations, may modulate the association between homocysteine level and risk of vascular disease. However, the biological basis of these effects is poorly understood and requires further investigation. In particular, the discrepant results with respect to the interaction between homocysteine levels and blood pressure in relation to cerebrovascular disease^{2,6,7,16,18} are not easily explained. We stress that our subgroup analysis was prompted by an earlier study⁷ and therefore planned in advance. Our findings thus suggest that, in elderly men, the effects of hypertension and hyperhomocysteinemia on risk of cerebrovascular disease do not reinforce each other.

An important limitation of our study was that it included only men. Therefore, the generalizability of our findings with respect to elderly women remains uncertain. In addition, we had no data on fibrinogen levels, an important cardiovascular risk factor or indicator that has been linked to homocysteine levels in one study.^{24,29} Only one^{17,30} of the previous prospective studies^{4-9,27} has included fibrinogen levels in the analysis, and this issue therefore merits further investigation. The interpretation of our data, moreover, was hampered by the lack of knowledge regarding the time course, the dose-response characteristics, and the modulation by other risk factors of the atherothrombotic effects of homocysteine. Our data nevertheless strongly suggest that the vascular risks associated with hyperhomocysteinemia in the elderly differ from those in younger subjects. A final limitation is that our study lacked information on possible determinants of homocysteine levels, notably vitamin status.¹¹ The latter, however,

clearly does not detract from our findings on homocysteine level and risk of vascular disease.

In the elderly, high homocysteine levels are to a large extent related to an inadequate folate, vitamin B₁₂, and vitamin B₆ status.¹¹ Because high homocysteine levels can be reduced by simple treatment with folic acid and vitamin B₆ even in the absence of deficiencies of these vitamins,¹⁹ studies are now needed of the effect of treatment with these vitamins and with vitamin B₁₂ on cardiovascular disease not only among the middle-aged, but also in the elderly.

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