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Hyperhomocysteinemia Is Associated With an Increased Risk of Cardiovascular Disease, Especially in Non–Insulin-Dependent Diabetes Mellitus
A Population-Based Study

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Abstract—A high serum total homocysteine (tHcy) level is an independent risk factor for cardiovascular disease. Because it is not known whether the strength of the association between hyperhomocysteinemia and cardiovascular disease is similar for peripheral arterial, coronary artery, and cerebrovascular disease, we compared the three separate risk estimates in an age-, sex-, and glucose tolerance–stratified random sample (n=631) from a 50- to 75-year-old general white population. Furthermore, we investigated the combined effect of hyperhomocysteinemia and diabetes mellitus with regard to cardiovascular disease. The prevalence of fasting hyperhomocysteinemia (>14.0 μmol/L) was 25.8%. After adjustment for age, sex, hypertension, hypercholesterolemia, diabetes, and smoking, the odds ratios (ORs; 95% confidence intervals) per 5 -μmol/L increment in tHcy were 1.44 (1.10 to 1.87) for peripheral arterial, 1.25 (1.03 to 1.51) for coronary artery, 1.24 (0.97 to 1.58) for cerebrovascular, and 1.39 (1.15 to 1.68) for any cardiovascular disease. After stratification by glucose tolerance category and adjustment for the classic risk factors and serum creatinine, the ORs per 5 -μmol/L increment in tHcy for any cardiovascular disease were 1.38 (1.03 to 1.85) in normal glucose tolerance, 1.55 (1.01 to 2.38) in impaired glucose tolerance, and 2.33 (1.11 to 4.90) in non–insulin-dependent diabetes mellitus (*P*=.07 for interaction). We conclude that the magnitude of the association between hyperhomocysteinemia and cardiovascular disease is similar for peripheral arterial, coronary artery, and cerebrovascular disease in a 50- to 75-year-old general population. High serum tHcy may be a stronger (1.6-fold) risk factor for cardiovascular disease in subjects with non–insulin-dependent diabetes mellitus than in nondiabetic subjects. (*Arterioscler Thromb Vasc Biol*. 1998;18:133–138.)

Key Words: homocysteine ■ non–insulin-dependent diabetes mellitus ■ cardiovascular disease ■ epidemiology

R etrospective and prospective studies have demonstrated that hyperhomocysteinemia is a risk factor for cardiovascular disease that is independent of classic risk factors such as smoking, hypercholesterolemia, diabetes mellitus, and hypertension.[1–4] In a recent meta-analysis,[1] the association between hyperhomocysteinemia and peripheral arterial disease (summary OR, 6.8) was considerably stronger than with coronary artery and cerebrovascular disease (ORs, 1.8 and 1.5). The summary estimate of the association between hyperhomocysteinemia and peripheral arterial disease, however, was inferred from one population-based study,[5] which consisted of only men, and two hospital-based studies.[6,7] Therefore, to further investigate this issue, we compared the risk estimates of peripheral arterial, coronary artery, and cerebrovascular disease in a random sample of a 50- to 75-year-old general white population.

A recent large study showed that the risk of cardiovascular disease was especially high among subjects with hyperhomocysteinemia who also smoked or had hypertension, ie, there was evidence of interaction with these risk factors.[2] However, this study excluded diabetic subjects. Our study was specifically designed to examine glucose tolerance as a cardiovascular risk factor,[8] and therefore we investigated the combined effect of hyperhomocysteinemia and diabetes mellitus with regard to relative risk of cardiovascular disease.

Finally, there is increasing evidence that hyperhomocysteinemia is common in the elderly population.[9,10] A large part of the prevalence of hyperhomocysteinemia in the elderly population is attributable to a low intake of the B vitamins, folate, vitamin B_6, and vitamin B_{12}.[10] Therefore, it has been suggested that lowering serum tHcy levels by increasing the intake of folate, probably the most important dietary determinant of serum tHcy levels, may be an effective means of decreasing cardiovascular risk.[11] To estimate the potential maximum benefit of such a strategy, we estimated the propor-

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Selected Abbreviations and Acronyms

ABPI = ankle brachial pressure index
BMI = body mass index
CI = confidence interval
ECG = electrocardiogram
HbA_{1c} = glycosylated hemoglobin
IGT = impaired glucose tolerance
IQR = interquartile range
NGT = normal glucose tolerance
NIDDM = non–insulin-dependent diabetes mellitus
OR = odds ratio
PAR = population-attributable risk
tHcy = total homocysteine
TIA = transient ischemic attack

tion of preventable cardiovascular disease caused by hyperhomocysteinemia.

Methods

Design and Study Population

The Hoorn Study is a cross-sectional survey of glucose tolerance and other cardiovascular risk factors in a 50- to 75-year-old general white population conducted from 1989 to 1992. A random sample of all men and women aged 50 to 75 years was drawn from the municipal population registry office of Hoorn (The Netherlands); 2484 subjects participated (response rate, 71%). An extensive cardiovascular investigation (detailed below) was performed in an age-, sex-, and glucose tolerance–stratified random subsample (n=631; response rate, 89.1%).[8] The Hoorn Study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit. Informed consent was obtained from all participants.

Cardiovascular Disease

Cardiovascular disease was defined as coronary artery, cerebrovascular, or peripheral arterial disease. Coronary artery disease was defined as a history of myocardial infarction, coronary artery bypass grafting, or Minnesota codes 1–1 or 1–2 on the ECG (n=625).[12] Cerebrovascular disease was defined as a history of TIA or stroke, or a carotid artery stenosis of >80%. (A carotid artery stenosis in excess of 80% is associated with a high risk of stroke within 2 years: more than 25% for symptomatic and 10% for asymptomatic carotid stenosis.[13,14]) Peripheral arterial disease was defined as a peripheral arterial reconstruction or limb amputation, or an ABPI <0.50. (A low ABPI is related to both more extensive peripheral arterial disease and a higher risk of cardiovascular mortality.[15–18]) The cardiovascular history was obtained by means of a self-administered questionnaire and, if positive, accepted only when confirmed by written information from the participant's general practitioner. Ultrasonographic examination of both common, internal, and external carotid arteries (n=628) was performed by means of a color-coded Duplex scanner as previously described in detail.[19] We classified subjects into two categories on the basis of the maximal percentage of stenosis of the more diseased of the two carotid arteries: 0% to 80% or 81% to 100%.[20] The ABPI was obtained by means of Doppler-assisted systolic blood pressure measurements taken from the brachial and the three crural arteries on both sides as previously described in more detail.[8] The lowest ABPI of either limb was used for statistical analysis.

Measurement of Serum Total Homocysteine

Fasting blood samples were centrifuged within 1 hour after collection. Serum was stored at −20°C for 4 to 6 years. There is good evidence that serum tHcy levels are stable for 10 years or more.[9,21] Serum total (free plus protein bound) homocysteine level was measured by using tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as the thiol-specific fluorochromophore, fol-

lowed by high-performance liquid chromatography with fluorescence detection.[22] The intra-assay and interassay coefficients are 2.1% and 5.1%.

Other Cardiovascular Risk Factors

We measured levels of fasting serum total cholesterol, HDL cholesterol, triglycerides (enzymatic techniques, Boehringer Mannheim), and creatinine (modified Jaffé method). HbA_{1c} was determined by ion-exchange high-performance liquid chromatography. Hypercholesterolemia was defined as total cholesterol ≥6.5 mmol/L or the current use of cholesterol-lowering medication. Hypertension was defined as a blood pressure ≥160 mm Hg systolic, ≥95 mm Hg diastolic, or the current use of antihypertensive medication. IGT and NIDDM were defined according to the World health Organization criteria[23] applied to the mean of two oral glucose tolerance tests. Subjects were classified as either nonsmokers or ever smokers. BMI and waist-hip ratio were calculated as described elsewhere.[8] All laboratory and vascular measurements and codings of the ECGs were carried out in a blinded fashion with respect to history of cardiovascular disease and glucose tolerance status.

Statistical Analysis

Variables are presented as mean±SD, number (percentage of the total), or in case of skewed distribution, median and IQR. Associations of cardiovascular risk factors with serum tHcy level (logarithmically transformed) were studied by calculating Pearson correlation coefficients. All reported probability values are two-tailed. We assessed sex-specific prevalences of hyperhomocysteinemia for different cutoff values (>12, 13, 14, 15, and 16 μmol/L), standardized for age and glucose tolerance as described previously in detail.[8] Briefly, the frequency of hyperhomocysteinemia was determined in 24 strata (age [3], sex [2], and glucose tolerance [4]) of the subsample. To assess the prevalence of hyperhomocysteinemia in the original population-based sample (standard, n=2484), the prevalence of hyperhomocysteinemia was back-calculated from the magnitude of each age, sex, and glucose tolerance category stratum.

We performed logistic regression analyses to study the association of serum tHcy with peripheral arterial, coronary artery, and cerebrovascular disease separately and combined (ie, total cardiovascular disease). We calculated ORs and 95% CIs per 5-μmol/L (about 1 SD) increment of serum tHcy (assuming a linear logistic relationship between homocysteine and risk of cardiovascular disease) and by tertiles with the lowest tertile as a reference category. We used multiple logistic regression analysis to control for age, sex, hypertension, hypercholesterolemia, smoking, and diabetes mellitus. We also tested models that also included serum creatinine, total cholesterol, triglycerides, HDL and LDL cholesterol, systolic blood pressure, BMI, or waist-hip ratio. To evaluate a possible modifying role of other risk factors, we repeated the previous analyses in strata of sex, glucose tolerance categories, smoking, hypertension, and hypercholesterolemia.

We calculated the PAR, ie, the percentage of excess cardiovascular disease in the population attributable to elevated serum total homocysteine levels, as $[P_e(RR-1)\times100]/[P_e(RR-1)+1]$, where RR is the relative risk estimated as the OR, and P_e is the proportion of the population liable to benefit from a reduction of serum tHcy levels. The potential benefit of a distribution shift of 5 μmol/L was calculated because we assumed that this is within attainable limits.[1] To calculate the PAR, we conservatively assumed that reduced serum tHcy level would benefit only individuals with levels higher than 12 μmol/L, although the epidemiological evidence more strongly supports a graded than a threshold association between serum tHcy and cardiovascular disease. The cutoff of 12 μmol/L is based on homocysteine levels of vitamin B_{12}- and folate-replete subjects[10,24,25] and thus on nutritional status, not on an estimate of the association with cardiovascular disease. This calculation of the PAR assumes that there is no important risk gradient up to a serum tHcy level of 12 μmol/L. To investigate whether this assumption is reasonable, we also calculated the ORs for cardiovascular disease for several ranges of homocysteine concentrations with 9 to 12 μmol/L serum tHcy as the reference category. We chose boundaries as small as possible to evaluate the dose–response relationship between homocysteine and cardiovascular disease as accurately as possible.

All analyses were performed with SPSS for Windows 6.1.

TABLE 1. General Characteristics of Subjects

N	631
Men, %	48
Age, y	64.3 (7.2)
BMI, kg/m^2	27.3 (4.0)
Waist-hip ratio	0.92 (0.09)
Ever smoker, %	66
Blood pressure, mm Hg	
Systolic	139 (19)
Diastolic	83 (10)
Hypertension, %	39.1
Impaired glucose tolerance, %	26.9
Diabetes mellitus, %	27.4
Fasting glucose, mmol/L	6.7 (2.6)
HbA$_{1c}$, % of hemoglobin	5.9 (1.3)
Total cholesterol, mmol/L	6.6 (1.2)
HDL cholesterol, mmol/L	1.3 (0.4)
LDL cholesterol, mmol/L	4.5 (1.1)
Triglycerides, mmol/L	1.6 (1.1-2.2)*
Creatinine, μmol/L	92 (19)
Total homocysteine, μmol/L	11.4 (9.3-14.1)*
Cardiovascular disease	67 (10.6)†
Peripheral arterial disease	17 (2.7)†
Coronary artery disease	40 (6.3)†
Cerebrovascular disease	19 (3.0)†

Cardiovascular disease indicates peripheral arterial disease, coronary artery disease, and/or cerebrovascular disease; peripheral arterial disease indicates history of vascular surgery or major amputation (n=10) and/or ankle brachial pressure index <0.50 (n=9); coronary artery disease indicates history of coronary artery bypass grafting (n=8), myocardial infarction (n=24), and/or Minnesota codes 1-1 or 1-2 on the ECG (n=19); and cerebrovascular disease indicates history of TIA/stroke (n=12) and/or carotid artery stenosis >80% (n=8). Data are presented as mean (SD), *median (interquartile range) or †number (percentage).

Results

Table 1 shows the main characteristics of the study population. The median serum tHcy level was 12.2 (IQR, 10.0 to 15.3) μmol/L in men and 10.7 (IQR, 9.0 to 13.3) μmol/L in women. Fig 1 shows the standardized sex-specific prevalences of hyperhomocysteinemia according to different cutoff values. The medians (IQR) for serum tHcy were 11.2 (9.2 to 14.4) μmol/L in NGT, 12.2 (9.7 to 14.5) μmol/L in IGT, and 11.2 (9.2 to 13.6) μmol/L in NIDDM. Serum tHcy levels correlated with age ($r=.17$; $P<.0001$), serum creatinine ($r=.41$; $P<.0001$), systolic blood pressure ($r=.10$; $P=.01$), waist-hip ratio ($r=.12$, $P=.004$), and inversely with HDL cholesterol ($r=-.09$; $P=.03$) but not with BMI ($r=-.02$; $P=.6$), fasting glucose ($r=-.07$; $P=.08$), HbA$_{1c}$ ($r=-.02$; $P=.7$), or duration of NIDDM ($r=-.06$, $P=.6$).

The mean±SD HbA$_{1c}$ was 5.3±0.5% in NGT, 5.6±0.5% in IGT, and 7.2±1.8% in NIDDM. Of all NIDDM subjects, 96 (55.5%) were newly diagnosed. Ten (5.8%) were treated with diet alone and 67 (38.7%) with glucose-lowering agents; 15 (8.7%) with insulin, 51 (29.5%) with sulfonylureas, and 3 (1.7%) with metformin (2 of whom also used sulfonylureas). The median (IQR) duration of NIDDM of those subjects

treated with diet or glucose-lowering agents was 6.1 (2.5 to 11.2) years. The prevalence of cardiovascular disease was 7.3% in NGT, 11.2% in IGT, and 15.6% in NIDDM.

A 5-μmol/L increment of serum tHcy was associated with an increased risk of cardiovascular disease, which was of similar magnitude in each of the vascular territories examined (Table 2). Additional adjustment for serum creatinine did not materially change the ORs, nor did inclusion of total cholesterol, triglycerides, HDL and LDL cholesterol, systolic blood pressure, BMI, or waist-hip ratio in the model. There was no evidence for a threshold if risks were calculated by tertiles of serum tHcy (data not shown). Risk of total cardiovascular disease increased with increasing serum tHcy levels (Fig 2).

We evaluated possible effect modification and did not observe substantial differences among the strata of the following risk factors: male sex, hypertension, hypercholesterolemia, and smoking (data not shown). However, after stratification by glucose tolerance category, exclusion of one outlier, and adjustment for age, sex, hypertension, hypercholesterolemia, smoking, and serum creatinine, the ORs (95% CI) per 5-μmol/L increment in serum tHcy of cardiovascular disease were 1.38 (1.03 to 1.85) in NGT, 1.55 (1.01 to 2.38) in IGT, and 2.33 (1.11 to 4.90) in NIDDM ($P=.07$ for interaction; Fig 3). These results indicate that high serum tHcy is a stronger (1.6-fold) risk factor for cardiovascular disease in NIDDM than in subjects with normal or impaired glucose tolerance.

The standardized prevalence of cardiovascular disease was 8.0%. From the incremental PAR percent values for serum tHcy levels >12 μmol/L, we calculated that the proportion of preventable cardiovascular disease caused by a 5-μmol/L decrease was 10.6% for a 50- to 75-year-old general white population.

Discussion

There are four main findings in this study. First, the magnitude of the association between hyperhomocysteinemia and cardiovascular disease was similar with respect to peripheral, coronary, and cerebral arterial disease. Second, hyperhomocysteinemia appeared to be a stronger (1.6-fold) risk factor for cardiovascular disease in subjects with NIDDM than in subjects with normal or impaired glucose tolerance. Third, the prevalence of hyperhomocysteinemia was high in this 50- to 75-year-old general population. Finally, we estimated a potential reduction of approximately 10% of the total burden of cardiovascular disease by a distribution shift of 5 μmol/L serum tHcy level.

In a recent meta-analysis,[1] the summary ORs per 5-μmol/L increment of fasting serum tHcy were 1.7 for coronary artery disease, 1.5 for cerebrovascular disease, and 6.8 for peripheral arterial disease. Compared with the ORs we found, these ORs were somewhat higher for coronary and cerebrovascular disease but much higher for peripheral disease. For coronary and peripheral arterial disease, it is unlikely that we underestimated the relative risk because of misclassification of disease because the diagnostic criteria we used are quite specific.[8,14] In contrast, the diagnostic category "TIA/stroke," which was part of our definition of cerebrovascular disease, included self-reported TIAs, a diagnosis that is liable to nondifferential misclassification. Thus, we may have underestimated the OR for cerebro-
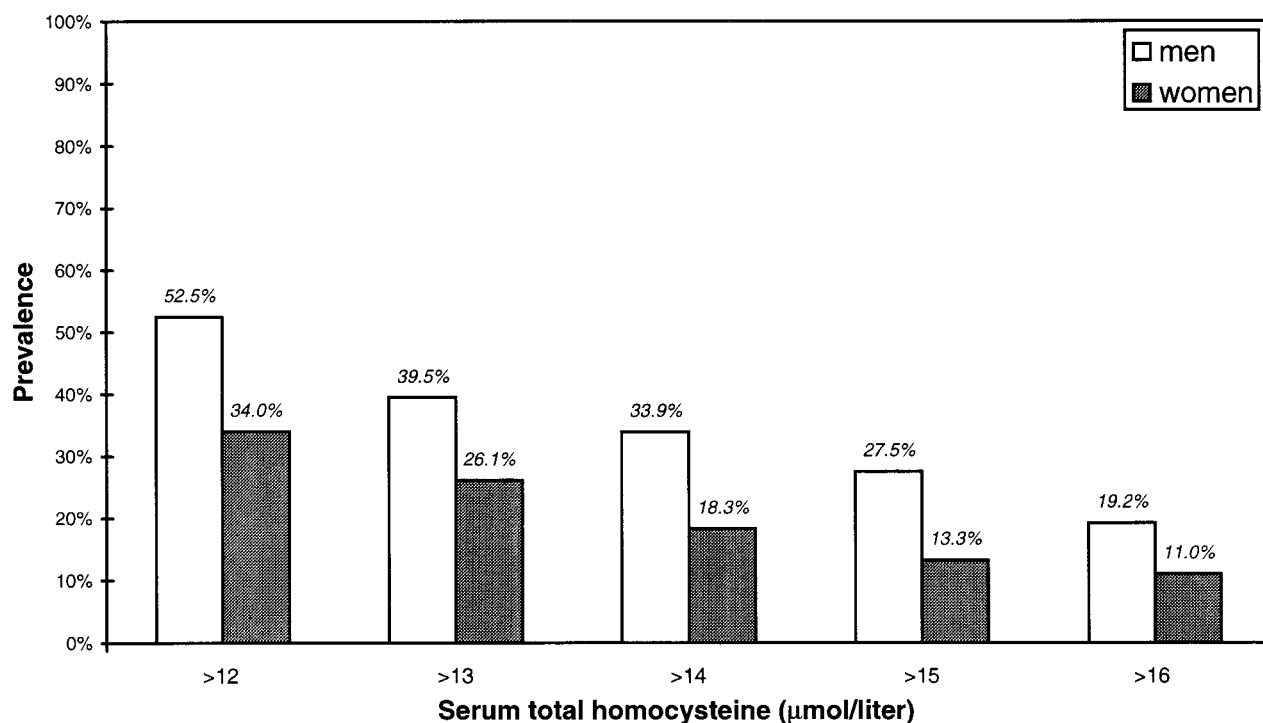
Figure 1. Sex-specific prevalence of hyperhomocysteinemia in Hoorn, The Netherlands, between 1989 and 1992. Prevalences are presented for men and women separately for different cutoff values of serum tHcy.

vascular disease to some extent. Nevertheless, the results of the present study clearly do not support the hypothesis that hyperhomocysteinemia is a stronger risk factor for peripheral arterial than for coronary and cerebrovascular disease, at least among 50- to 75-year-olds. A recent study in younger subjects (mean age, 45 years) reached a similar conclusion.[2]

Because the previously mentioned meta-analysis[1] was based mostly on studies that comprised to a large extent persons younger than 55 years, another explanation for the weaker association we found might be that the relative risk of hyperhomocysteinemia with regard to cardiovascular disease is weaker among older persons. However, in a recent study[26] that comprised subjects aged 25 to 65 years, an OR of 1.3 per 5-μmol/L increment of tHcy for severe coronary artery disease

was found, which is of a magnitude similar to the OR in the present study.

Little is known about the impact of NIDDM on serum tHcy levels. As in previous studies,[27,28] we found no important difference in fasting serum tHcy level between diabetic and nondiabetic subjects. Although Araki et al[29] and Munshi et al[27] have demonstrated that diabetic subjects who also had macrovascular disease had a higher fasting and post–methionine load tHcy level, respectively, than nondiabetic control subjects who were free of cardiovascular disease, it is not clear from their studies that the higher tHcy levels were due to the diabetic state per se. In addition, we found no relationship between serum tHcy and fasting glucose, HbA$_{1c}$, or duration of NIDDM. Although more than 55% of the diabetic subjects

TABLE 2.   Odds Ratios (95% CIs) of Cardiovascular Disease per 5-μmol/L Increment of Serum Total Homocysteine

	Subjects, n	Crude OR	Age- and Sex-Adjusted OR	Multivariate* Adjusted OR
Cardiovascular disease	67†	1.39‡	1.34§	1.39‡
		(1.16-1.67)	(1.12-1.60)	(1.15-1.68)
Peripheral arterial disease	17	1.38§	1.38§	1.44§
		(1.13-1.68)	(1.12-1.69)	(1.10-1.87)
Coronary artery disease	40	1.26§	1.23‖	1.25‖
		(1.06-1.51)	(1.02-1.47)	(1.03-1.51)
Cerebrovascular disease	19	1.24‖	1.24	1.24
		(1.01-1.54)	(0.98-1.56)	(0.97-1.58)

*Adjusted for age, sex, hypertension (yes/no), ever smoking (yes/no), hypercholesterolemia (yes/no), and NIDDM (yes/no).

†Subjects were counted once with regard to cardiovascular disease end point.
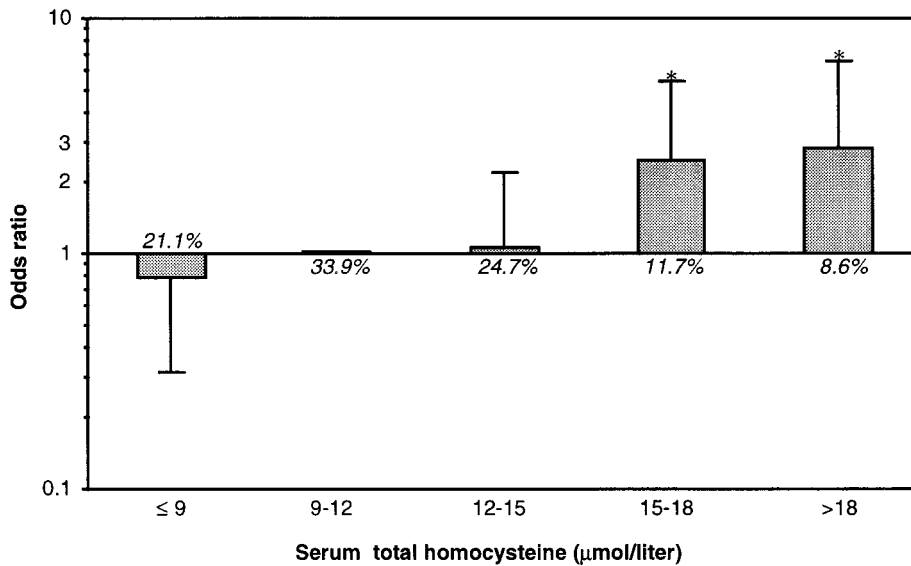
‡$P<.001$; §$P<.01$; ‖$P<.05$.

Figure 2. OR for cardiovascular disease according to serum tHcy level adjusted for age and sex. The reference category was serum tHcy values of 9 to 12 μmol/L. Percentages of the subsample for each serum tHcy range are presented. The error bars represent the lower or upper half of the 95% CI. *$P<.05$, significantly different from the reference category (A logarithmic scale was used because the OR is a multiplicative measure of association; equal differences on the logarithmic scale correspond to equal ratios between OR).

were newly diagnosed, we cannot rule out that changes of dietary habits of the 45% of patients with diabetes who were aware of their disease may have improved their B-vitamin status and thereby lowered their tHcy level. There is no indication that insulin or sulfonylureas alter tHcy metabolism.[29] In contrast, metformin may induce vitamin B_{12} malabsorption and thereby increase the serum tHcy level. However, we did not find an important effect on serum tHcy levels in subjects with NIDDM.[30] Taken together, there is no clear evidence that the diabetic state influences tHcy levels, but more detailed studies of this issue are needed.

The design of the study, with oversampling of diabetic subjects, provided an opportunity to investigate the combined role of diabetes and hyperhomocysteinemia with regard to cardiovascular disease. Because the oversampling was performed before identification of cardiovascular disease, there was no introduction of bias. Hyperhomocysteinemia appeared to be a stronger risk factor for cardiovascular disease in patients with NIDDM than in subjects with normal or impaired glucose tolerance. The biological mechanism for the interaction between diabetes and hyperhomocysteinemia with regard to cardiovascular disease is not known. However, both smoking and hypertension interact with hyperhomocysteinemia.[2] Taken together, these data suggest that hyperhomocysteinemia can enhance atherogenic and/or thrombogenic pathways common to classic risk factors such as smoking, hypertension, and diabetes mellitus. Because NIDDM is associated with a high risk of cardiovascular disease, interaction with hyperhomocysteinemia may have important implications with regard to risk management. The substantial difference we found therefore merits further examination in a larger number of subjects than were available in this study. In contrast to the findings of a recent study,[2] we found no interactions between serum tHcy and other classic risk factors, but our study had limited power to do so.

Because the ORs for the three arterial territories did not differ significantly, we calculated a summary OR for cardiovascular disease by pooling all subjects with coronary, peripheral, and/or cerebrovascular disease. We estimated a proportion of preventable cardiovascular disease of 10% for a distribution shift of 5 μmol/L serum tHcy level. A similar result was obtained in a recent meta-analysis[1]: 10% of the proportion of death caused by coronary heart disease was estimated to be attributable to hyperhomocysteinemia. The present study illustrates that although the OR of hyperhomocysteinemia for cardiovascular disease is relatively modest, hyperhomocysteinemia is an important risk factor because the frequency is high in the general population. An increased risk of cardiovascular disease has been observed if homocysteine levels exceed 14 μmol/L.[2–4] The prevalences of hyperhomocysteinemia (>14.0 μmol/L) we found was 34% for men and 18% for women, which are somewhat higher than the 25% and 20% observed in a 67- to 74-year-old population of the Framingham Study (based on plasma tHcy).[10] Part of this difference could be related to the fact that levels of serum compared with plasma tHcy levels are slightly higher.[31]

A limitation of the present study is the absence of assessment of serum folate, which would have provided important information about the relation between serum tHcy and folate in
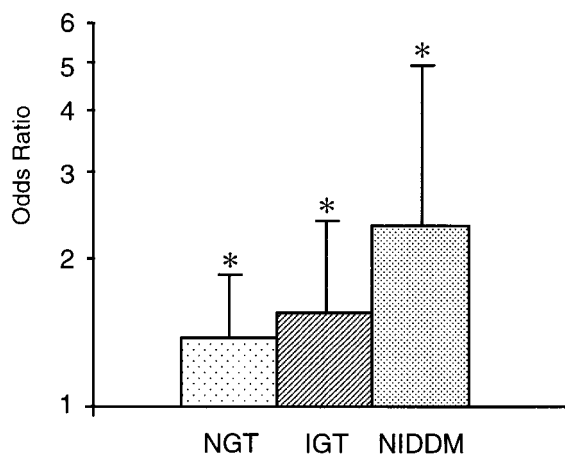

Figure 3. OR for cardiovascular disease after stratification by glucose tolerance category. The error bars represent the upper half of the 95% CI. ORs are calculated per 5-μmol/L increment of serum tHcy, adjusted for age, sex, hypertension, ever smoking, hypercholesterolemia, and serum creatinine. *$P<.05$; $P=.07$ for interaction.

the general population. However, additional measurements of serum folate would not have altered the conclusions of the present study with regard to the association between serum tHcy and cardiovascular disease, because the associations between hyperhomocysteinemia and cardiovascular disease exist regardless of the underlying cause of hyperhomocysteinemia.

Obviously, this cross-sectional study cannot resolve the temporal relationship between homocysteine concentration and cardiovascular disease. However, there is evidence that the relation between tHcy and cardiovascular disease is causal because prospective studies have shown a positive association between hyperhomocysteinemia and cardiovascular disease.[3,4,32,33]

In conclusion, hyperhomocysteinemia is positively associated with cardiovascular disease in a 50- to 75-year-old general population, independent of classic risk factors. The magnitude of the relative risk of hyperhomocysteinemia is similar with regard to peripheral arterial, coronary arterial, and cerebrovascular disease. With regard to cardiovascular disease, hyperhomocysteinemia appeared to be a stronger risk factor in patients with NIDDM than in subjects with normal or impaired glucose tolerance.

Acknowledgments

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