Plasma Total Cysteine as a Risk Factor for Vascular Disease

The European Concerted Action Project

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Background—Elevated plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease. Although cysteine is structurally similar and metabolically linked to tHcy, its relation to the risk of cardiovascular disease has received little attention. We studied the relation between plasma total cysteine (tCys) levels and the risk of vascular disease in the coronary, cerebral, and peripheral vessels.

Methods and Results—This case-control study included 750 patients with vascular disease and 800 age- and sex-matched control subjects recruited from 19 centers in 9 European countries. Conventional risk factors for cardiovascular disease were recorded. In addition, plasma levels of tCys, tHcy, folate, B_6, B_{12}, and creatinine were measured. Overall, a U-shaped relationship was observed between tCys and risk of vascular disease. With the middle range of 250 to 275 μmol/L tCys used as the reference category, the adjusted risk of vascular disease at low ($\leq$225 μmol/L) tCys levels was 2.1 (95% CI 1.2 to 3.6), and the risk at high ($>$300 μmol/L) tCys levels was 1.6 (95% CI 1.1 to 2.3). Different shapes of the dose-response relationship were seen for the 3 vascular disease categories. The relation with peripheral vascular and cerebrovascular disease was U-shaped, whereas a weak positive relation was observed with coronary heart disease.

Conclusions—Our data show a significant U-shaped relationship between tCys and cardiovascular disease after adjustment for tHcy, creatinine, and other cardiovascular disease risk factors. **(*Circulation*. 2001;103:2544-2549.)**

Key Words: cardiovascular diseases ■ amino acids ■ risk factors

Both prospective and case-control studies have shown that an elevated plasma total homocysteine (tHcy) level is an independent risk factor for occlusive vascular disease.[1–5] A variety of mechanisms have been suggested for the vascular lesions associated with hyperhomocysteinemia,[6] and the redox property of the sulfhydryl group of homocysteine, leading to the formation of reactive oxygen species,[7,8] is believed to play a pivotal role.

Cysteine is another sulfhydryl-containing amino acid with structural and chemical properties similar to those of homocysteine.[9] Autoxidation of cysteine in vitro promotes several processes considered to be involved in atherogenesis and thrombogenesis.[8–12] Cysteine has a cytotoxic effect in vitro against several cell types.[13] Cysteine supports superoxide-mediated modification of LDL, which may facilitate foam cell formation.[8,14] Finally, cysteine forms an adduct with nitric oxide[15] and may thereby impair endothelial function.

The concentration of total cysteine (tCys) in serum/plasma from healthy subjects is $\approx$250 μmol/L, which is 20-fold higher than the plasma tHcy level.[16] Cysteine, homocysteine, and other amino thiols exist in plasma in reduced, oxidized, and protein-bound forms, interacting with each other through redox and disulfide exchange reactions.[16] We have previously shown that high levels of tHcy cause complex changes in tCys and the overall aminothiol status in plasma.[17] Therefore, hyperhomocysteinemia should not be considered an isolated factor in relation to cardiovascular disease, because the associated changes in other plasma aminothiols may modulate or even mediate atherogenesis and thrombogenesis.

Few studies have analyzed the relation between cysteine and vascular occlusive disease.[18–21] These studies showed significantly higher levels of plasma tCys in vascular patients than in healthy control subjects.[18–21]

Recently, we investigated the relation between plasma tCys and lifestyle and cardiovascular disease risk factors among 16 176 healthy participants in the Hordaland Homocysteine Study.[22] The strongest determinants of tCys were age, body mass index (BMI), sex, diastolic blood pressure, serum total cholesterol, and coffee consumption. There was no relation to folate and vitamin intake, smoking, or physical activity, which are established determinants of plasma tHcy.[22]

The European Concerted Action project, which recruited 750 patients with vascular disease and 800 control subjects from 9 European countries, confirmed that an elevated

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TABLE 1. Estimated Change in Plasma tCys Concentration by Cardiovascular Risk Factor and Other Potential Determinants

	Adjusted for Sex and Age		Multiple Adjustment*	
Variable	Change, μmol/L	*P*	Change, μmol/L	*P*
Age (per year)	1.17	<0.001	0.86	<0.001
Sex (male vs female)	12.66	<0.001	6.48	0.011
Smoking (ever vs never)	−3.39	<0.001	−3.10	<0.001
BMI (per kg/m^2)	1.78	<0.001	1.70	<0.001
Diastolic blood pressure (per 10 mm Hg)	1.27	0.30	−0.90	0.46
Cholesterol (per mmol/L)	3.56	<0.001	3.66	<0.001
Apolipoprotein A-1 (per g/L)	2.43	0.46	4.31	0.17
Apolipoprotein B (per g/L)	15.47	<0.001	8.64	0.012
HDL (per mmol/L)	−0.47	0.86	1.41	0.59
LDL (per mmol/L)	3.14	<0.001	−3.36	0.15
Triglycerides (per mmol/L)	5.31	<0.001	2.62	0.083
Creatinine (per μmol/L)	0.43	<0.001	0.38	<0.001
Plasma B_{12} (per nmol/L)	1.59	0.84	2.70	0.72
Plasma B_6 (per nmol/L)	−0.05	0.56	−0.01	0.93
Plasma folate (per nmol/L)	0.69	<0.001	0.65	<0.001
tHcy (per μmol/L)	1.22	<0.001	1.07	<0.001

*Adjusted for sex, age, diastolic blood pressure, BMI, cholesterol, smoking, and creatinine.

plasma tHcy level is an independent risk factor for cardiovascular disease.[2] The magnitude of risk was similar to that of smoking or hyperlipidemia. Moreover, tHcy interacted with smoking and hypertension and thereby conferred a marked risk enhancement.[2] In the present work, we investigated the relation between levels of plasma tCys and occlusive disease in the coronary, cerebral, and peripheral vessels by using data from this large case-control study.

Methods

Study Population

A total of 750 patients with coronary heart disease, cerebrovascular disease, or peripheral vascular disease (cases) and 800 control subjects (controls) were included in this study. Both cases and controls were <60 years of age, of both sexes, and were recruited from 19 centers in 9 European countries.

The exclusion and inclusion criteria have been reported in detail elsewhere.[2,23] In brief, exclusion criteria included nonatherosclerotic vascular disease, cardiomyopathy, diabetes mellitus, pregnancy, systemic illness during the previous 3 months, and psychiatric illness. Patients with conditions thought to influence tHcy concentrations, such as renal or thyroid disease, anticonvulsant therapy, and recent (<3 months) exposure to nitrous oxide, were also excluded.

Cases had defined clinical and investigational evidence of vascular disease, and 69% were recruited within 1 year of diagnosis. Controls were free of overt disease, and ≈50% of these subjects were from community samples, ≈33% were from employee health insurance registers, and ≈17% were hospital employees. Two percent of control subjects were hospital patients.[2]

Study Variables

Briefly, data collected included information about age, sex, smoking habits, blood pressure, weight, height, and drug and vitamin usage.

For both systolic and diastolic blood pressure, the mean of 4 values was used (2 obtained before and 2 after the administration of methionine). Blood measurements included tHcy, tCys, folate, vitamin B_{12}, vitamin B_6, lipid concentrations, and creatinine. Variables examined in this study and their collection are extensively reported elsewhere.[2]

Smokers were defined as those currently smoking any tobacco (at the time of diagnosis for cases and at the time of the methionine-loading test for controls). Subjects were divided into 5 categories: never smokers, former smokers, light smokers (1 to 9 cigarettes/d), moderate smokers (10 to 19 cigarettes/d), and heavy smokers (≥20 cigarettes/d).

Creatinine levels were grouped into 5 categories: ≤50, 50 to 70, 70 to 100, 100 to 120, and >120 μmol/L.

Fasting tCys values were used for the analyses and were categorized as follows: <225, 225 to 250, 250 to 275, 275 to 300, and >300. The lowest risk for vascular disease was observed in the middle category of 250 to 275 μmol/L. Therefore, this category served as the reference group. Throughout the text, intervals defining categories of tCys, tHcy, and creatinine are half-open, including the exact lower value and excluding the upper.

Biochemical Analyses

Plasma tCys and tHcy were measured by a previously described method involving reduction with sodium borohydride, derivatization with monobromobimane, high-performance liquid chromatography (HPLC) separation, and fluorescence detection.[24,25]

Measurements of serum lipids, folate, vitamin B_{12}, vitamin B_6 (determined as pyridoxal 5′-phosphate), and creatinine were performed at Mime-AB, as described.[2] Vitamin B_{12} and folate concentrations were measured with a radioimmunoassay technique, and pyridoxal 5′-phosphate was measured by enzymatic photometry with HPLC separation.[2]

Statistical Methods

Linear regression analyses were performed to assess the relationship between tCys, conventional cardiovascular disease risk factors, and other possible confounders. These analyses were performed only in controls to avoid a possible effect of treatment or change in lifestyle on these relationships.

The relationship between disease and tCys was studied by use of conditional logistic regression performed for all vascular disease

patients combined and for the 3 vascular subcategories separately. The analyses were stratified by age group (<40, 40 to 49, and ≥50 years old), sex, and center and included adjustment for diastolic blood pressure, serum cholesterol, triglycerides, BMI, smoking, creatinine, and fasting tHcy. Odds ratios are given with 95% CI.

The analyses were also performed for different combinations of low (≤250 μmol/L), medium (250 to 300 μmol/L), and high (>300 μmol/L) tCys and low (≤12 μmol/L) and high (>12 μmol/L) tHcy levels. Subjects with a combination of a medium level of tCys and a low level of tHcy served as the reference group.

The statistical analyses were performed with SAS statistical software (release 6.12 for Windows). Generalized additive logistic regression was used for constructing the dose-response graphs with S-plus software (version 4.0 for Windows).

Results

tCys Level According to Case-Control Status and Sex

The study population consisted of 750 vascular disease cases (544 men, 206 women) and 800 controls (570 men, 230 women). Mean±SD plasma tCys concentration was 275.3±35.3 μmol/L. The tCys levels were higher in cases than in controls (279.9±37.2 versus 271.0±32.9 μmol/L, $P<0.0001$) and were higher in men than in women (279.9±34.7 versus 263.6±33.6 μmol/L, $P<0.0001$).

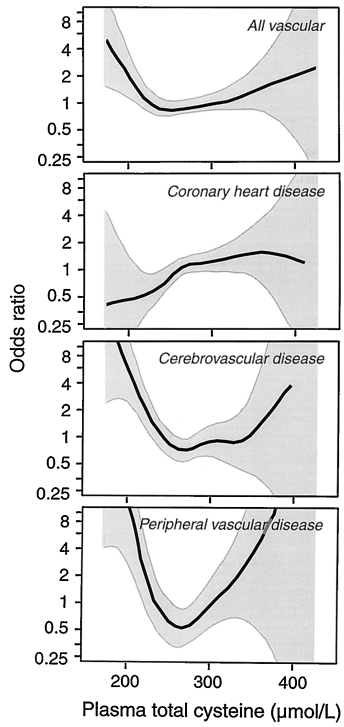
Determinants of tCys

We investigated the associations of tCys with conventional cardiovascular disease risk factors and other possible confounders in controls, as shown in Table 1. Plasma tCys was positively associated with age, sex, BMI, cholesterol, apolipoprotein B, tHcy, creatinine, and triglyceride levels. tCys was associated with plasma folate but had no association with vitamin B_{12} or B_6. Plasma tCys showed a significant negative relation to smoking.

tCys Levels and Risk of Cardiovascular Disease

We investigated the relationship between tCys and the risk of all vascular disease and also separately for the risk of vascular disease in the coronary, cerebral, and peripheral arteries. This was carried out by generalized additive logistic regression yielding continuous dose-response curves between tCys and risk (Figure) and by calculation of odds ratios (ORs) for 5 categorical levels of tCys (Table 2). In the latter analyses, plasma tCys levels of 250 to 275 μmol/L served as the reference category. All analyses were adjusted for age, sex, center, smoking, BMI, diastolic blood pressure, serum cholesterol, creatinine, triglycerides, and tHcy.

Plasma tCys showed a U-shaped relation with overall vascular disease, as shown in the Figure and Table 2. Analyses for each disease category separately showed different dose-response relationships for coronary heart disease, cerebrovascular disease, and peripheral vascular disease. The association of tCys with peripheral and cerebrovascular disease was U-shaped, with the highest risk at the extreme tCys levels. For coronary heart disease, there was a gradual increase in risk from low to high tCys, which was significant before ($P=0.05$) but not after ($P=0.09$) adjustment for tHcy (Figure, Table 2).



Dose-response relation between tCys levels and odds ratio for all vascular disease, coronary heart disease, cerebrovascular disease, and peripheral vascular disease. Values are adjusted for age, sex, center, creatinine, smoking, diastolic blood pressure, tHcy, BMI, and cholesterol by use of generalized additive logistic regression. Solid line indicates estimated dose-response curve; shaded area, 95% CI.

Dose-response graphs between tCys and the risk of disease were performed at separate tHcy tertiles (results not shown). For coronary heart disease, there was no relation with tCys at low tHcy levels, but a positive relation existed at medium and at high tHcy concentrations. For cerebrovascular and peripheral vascular disease, low tCys was associated with increased risk at all tHcy levels.

Analyses were repeated in men and in women separately, and essentially similar results were obtained (data not shown).

Cardiovascular Risk at Various Combinations of tCys and tHcy

We investigated a combination variable of tCys and tHcy in relation to the vascular risk.

The lowest adjusted risk was observed in subjects having a combination of medium tCys (250 to 300 μmol/L) and low tHcy (≤12 μmol/L); we used this group as reference. The relationship between the tCys-tHcy combinations and the risk of disease differed between the 3 vascular disease categories. Notably, the combination of low tCys and low tHcy had an increased risk for cerebrovascular disease (OR 2.2, 95% CI 1.3 to 3.9) and peripheral vascular disease (OR 1.7, 95% CI 0.9 to 3.5) but not for coronary heart disease (OR 0.6, 95% CI 0.3 to 0.9). The highest risk for all disease categories was observed for the combination of low tCys and high tHcy. The odds ratios and 95% CIs for coronary heart disease, cerebrovascular disease, and peripheral vascular disease in this group were 2.3 (1.0 to 5.3),

TABLE 2. ORs (95% CI) for All Vascular Disease, Coronary Heart Disease, Cerebral Vascular Disease, and Peripheral Vascular Disease by Plasma tCys Level*

Plasma tCys, μmol/L	Controls, n	All Vascular Disease		Coronary Heart Disease		Cerebral Vascular Disease		Peripheral Vascular Disease	
		n†	OR (95% CI)	n†	OR (95% CI)	n†	OR (95% CI)	n†	OR (95% CI)
≤225	52	52	2.1 (1.2–3.6)	13	0.7 (0.3–1.6)	24	8.3 (3.5–19.3)	15	3.1 (1.1–8.4)
225–250	163	109	1.2 (0.8–1.8)	40	0.8 (0.5–1.2)	46	2.6 (1.4–4.9)	23	2.3 (1.0–5.1)
250–275 (reference)	246	170	1	95	1	42	1	33	1
275–300	178	206	1.6 (1.2–2.3)	111	1.3 (0.9–2.0)	55	2.4 (1.3–4.4)	40	1.6 (0.8–3.3)
>300	161	213	1.6 (1.1–2.3)	124	1.3 (0.9–2.0)	44	1.8 (0.9–3.6)	45	1.5 (0.7–3.5)
P for heterogeneity			0.0074		0.16		0.0001		0.18
P for linear trend			0.97		0.09		0.025		0.36

*Adjusted for sex, age, center, diastolic blood pressure, cholesterol, smoking, creatinine, BMI, tHcy, and triglycerides.
†Number of cases.

5.7 (2.2 to 14.6), and 4.3 (1.2 to 14.8), respectively. Increased risk was also observed for the group characterized by high tCys and high tHcy for all vascular disease categories.

Vitamin and Creatinine Levels According to the Combination Categories

In an attempt to understand the basis of the different tCys-tHcy profiles, we calculated levels of creatinine and folate in each of the 6 tCys-tHcy combination groups in controls (Table 3). The highest creatinine concentrations were seen in subjects with high tCys and high tHcy and the lowest concentrations of folate, but also vitamins B_6 and B_{12} (results not shown), in subjects with low tCys and high tHcy levels. The contrasts were similar but more pronounced when the analyses were done in cases.

Discussion

In this multicenter case-control study, we analyzed the relationship between plasma tCys and the risk of cardiovascular disease in the coronary, cerebral, and peripheral arteries. Our results showed significant relationships between tCys and vascular disease. Different dose-response relationships for the 3 types of vascular disease were observed, however. The relationship with peripheral and cerebrovascular disease was U-shaped, with lowest risk in subjects with intermediate levels (250 to 275 μmol/L) of tCys, whereas a weak positive relation was observed with coronary heart disease. The association between tCys and cerebrovascular and peripheral vascular disease persisted after adjustment for conventional cardiovascular disease risk factors, tHcy, creatinine, and other potential confounders (Figure). The relationship between tCys and coronary heart disease, however, was statistically insignificant after adjustment for tHcy.

TABLE 3. Levels of Creatinine and Plasma Folate in Controls in the Different tCys-tHcy Combinations*

tCys†	tHcy‡	n	Controls	
			Creatinine, μmol/L	Plasma Folate, nmol/L
L	L	198	65.5±11.9	10.5±5.5
M	L	336	68.8±12.3	11.7±5.8
H	L	105	73.7±11.3	14.5±8.7
P, ANOVA			<0.0001	<0.0001
L	H	17	65.3±10.2	7.3±4.1
M	H	88	70.7±11.8	7.0±3.2
H	H	56	77.3±13.5	9.0±4.3
P, ANOVA			0.0004	0.007

*Values are mean±SD.
†L indicates low tCys, ≤250 μmol/L; M, medium tCys, 250–300 μmol/L; H, high tCys, >300 μmol/L.
‡L indicates low tHcy, ≤12 μmol/L; H, high tHcy, >12 μmol/L.

This case-control study was originally designed to evaluate the relation between tHcy and the risk of vascular disease.[2] The availability of data on plasma levels of tCys allowed us to evaluate the relation between tCys and the risk of vascular disease in this large multicenter study. The major strength of our study is the large number of cases that allowed us to study the tCys-disease relationship separately for coronary heart disease, cerebrovascular disease, and peripheral vascular disease. This also allowed us to make more precise estimates of the strength and shape of the tCys-disease relationships.

Because the blood samples in cases were drawn after the disease episode, however, we cannot rule out the possibility that tCys levels might be influenced by the disease itself. There is also the possibility that medication or change in lifestyle and dietary habits might have influenced the levels of tCys in cases. We have previously demonstrated that tCys is positively related to cholesterol, diastolic blood pressure, and BMI,[22] which is essentially confirmed in the present study. Conceivably, cholesterol-reducing or antihypertensive therapy or weight reduction in cases may weaken rather than increase the difference in tCys between cases and controls.

The relation between tCys and cardiovascular disease was evaluated for different combinations of tCys and tHcy concentrations. Subjects with low tCys levels and high tHcy had the highest ORs for disease in all 3 vascular disease categories. The elevation in tHcy in this group may be due to low vitamin B status.[26,27] In line with this, we observed a lower mean concentration of folate in this group (Table 3). Subjects

with high tCys and high tHcy levels also had a significant increase in the risk of disease in the 3 vascular disease categories. The associated increase in serum creatinine (Table 3) suggests that elevation of both tCys and tHcy is due to an impaired renal function.[28]

Renal failure has consistently been shown to cause increased levels of both tHcy and tCys,[29] and it is associated with high cardiovascular mortality and morbidity.[30] Our finding of increased risk of cardiovascular disease and elevated serum creatinine in subjects with the combination of high tCys and tHcy (Table 3) are in accordance with these published data. One may speculate whether elevated tCys affects the development of vascular lesions in patients with renal failure.

Subjects with low tCys and low tHcy had an odds ratio >1 for having cerebrovascular and peripheral vascular disease but not coronary heart disease. The metabolic processes responsible for this aminothiol profile and the possible mechanisms behind increased risk are not readily apparent. Low tCys might be a marker of low glutathione, however, which recently was associated with increased risk of coronary heart disease.[31] Cysteine is the limiting amino acid for the biosynthesis of glutathione,[32] and this explained a possible link between these 2 thiol compounds.

The relation between tCys and cardiovascular disease was also evaluated at low, medium, and high tHcy levels by use of dose-response curves. For coronary heart disease, there was no relation at low tHcy levels, but a positive relation existed at medium and high tHcy concentrations. This indicates that the effects of high tCys on risk of coronary heart disease are dependent on the associated levels of tHcy. For cerebrovascular and peripheral vascular disease, low tCys was associated with increased risk at all tHcy levels, which indicates that low tCys levels are associated with cerebrovascular and peripheral vascular disease independently of tHcy.

Previous studies[18–20,33] have demonstrated increased tCys levels in patients with myocardial infarction,[18] cerebral infarction,[19] or peripheral vascular disease.[20] In addition, a recent study showed that plasma tCys is independently associated with cardiovascular diseases as well as with atherosclerotic lesions in hyperlipidemic patients.[33] These studies, however, did not adjust for tHcy and other determinants of tCys.

Our results are partly in agreement with published studies on tCys and cardiovascular risk. We found a weak relation with coronary heart disease that was attenuated after adjustment for tHcy. In addition, a new finding of the present study is the association of low tCys levels with cerebrovascular and peripheral vascular disease, independent of tHcy level.

In conclusion, using data from a large European case-control study, we demonstrate that tCys is associated with vascular disease in the coronary, cerebral, and peripheral arteries. The relationship was strong and U-shaped for cerebrovascular and peripheral vascular disease, whereas for coronary heart disease, the relationship was weak and positive. Large prospective studies are needed to confirm our results and to elucidate the possible interaction between cysteine and homocysteine in the pathogenesis of cardiovascular disease.

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