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Predictors of Change in Plasma Total Cysteine: Longitudinal Findings from the Hordaland Homocysteine Study

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Background: **Total cysteine (tCys) in plasma has recently been linked to cardiovascular risk and is also associated with cardiovascular risk factors, including body mass index (BMI) and cholesterol. Changes and predictors of change in tCys concentrations over a mean follow-up time of 6.0 (5.2–7.2) years were assessed in this study.**

Methods: **Baseline data from the Hordaland Homocysteine Study recorded in 1992–1993 included tCys, total homocysteine (tHcy), and various lifestyle and cardiovascular risk factors. In 1998–1999, the same measurements were repeated in 3732 individuals born in 1950–1951 and 3339 individuals born in 1925–1927. Most of the statistical analyses were done separately in the four age and sex groups.**

Results: **The overall mean values of tCys were higher at follow-up [mean (SD), 296 (41) μmol/L] than at baseline [278 (36.5) μmol/L]; $P < 0.0001$. The mean percentage of increase in tCys in the different age and sex groups ranged from 4.9% to 8.5%. There was a significant correlation between the tCys values measured on the two occasions (Spearman correlation coefficient, 0.55–0.59 in the different age and sex groups; $P < 0.0001$). The change in tCys correlated with changes in BMI, cholesterol, and diastolic blood pressure in the younger age group, whereas only changes in BMI predicted changes in tCys in the older age group.**

Conclusions: **tCys increased in the 6 years between the two measurements. Factors related to the baseline tCys values, including BMI and the change in BMI, predicted the tCys changes over time.**

Increased plasma total homocysteine (tHcy)[3] is regarded as an independent risk factor for occlusive disease in the coronary, cerebral, and peripheral arteries *(1–3)*.

Homocysteine is produced from the essential amino acid methionine and is remethylated to methionine. This reaction is catalyzed by methionine synthase, which requires vitamin B_{12} as a cofactor and methyltetrahydrofolate as a cosubstrate. An alternative route of homocysteine disposal is degradation to cysteine through the transsulfuration pathway by two sequential vitamin B_6-dependent reactions *(4)*.

Cysteine is structurally similar and metabolically linked to homocysteine. However, the relationship between plasma total cysteine (tCys) and the risk of cardiovascular disease (CVD) has received less attention. Some studies have shown a relationship between tCys and vascular occlusive disease *(5–9)*. In these studies, significantly higher tCys concentrations were found in vascular patients than in healthy controls. Data from the European Concerted Action Project showed a significant U-shaped relationship between tCys and all cardiovascular disease categories after adjustment for tHcy, creatinine, and other cardiovascular disease risk factors *(10)*.

We recently investigated the relationship between plasma tCys and lifestyle and CVD risk factors among 16 176 healthy individuals in the Hordaland Homocysteine Study. The strongest determinants of tCys were age, sex, body mass index (BMI), diastolic blood pressure, serum total cholesterol, and coffee consumption *(11)*.

Plasma tCys and lifestyle and CVD risk factors that were measured in 1993 in the Hordaland Homocysteine Study were measured again in 1998 in a subset of 7071 individuals. In the present study, we assessed the stability of tCys and the determinants of change in plasma tCys concentrations from 1993 to 1998 in this subset.

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[3] Nonstandard abbreviations: tHcy, total homocysteine; tCys, total cysteine; CVD, cardiovascular disease; and BMI, body mass index.

Participants and Methods

STUDY POPULATION

In 1992–1993, the baseline data for Hordaland Homocysteine Study I were collected from three different age groups: individuals 40–42 years of age (all individuals living in Hordaland were invited); individuals 43–64 years of age (a 2% random sample of the residents in the city of Bergen were invited); and individuals 65–67 years of age (all individuals living in Bergen and three neighboring suburban municipalities were invited). The whole study population was 18 043 individuals, of whom 96.2% belonged to the youngest and oldest age groups. Details on the data collection have been given previously *(12)*.

In 1997–1998, ~9500 men and women who had participated in Hordaland Homocysteine Study I were invited to a new health screening. They included all living participants from the oldest age group (born in 1925–1927) and the youngest age group (born in 1950–1951) who were living in Bergen or three neighboring suburban municipalities. The attendance rate for the whole group was 74.4%, constituting a total of 7071 participants.

Baseline and follow-up data were collected through blood tests, questionnaires, and examinations. The questionnaires provided information about age, lifestyle, and dietary habits. Coffee consumption was divided into four groups according to the number of cups consumed per day: 0, 1–4, 5–8, and ≥9. Smoking was classified into four categories: never smokers, light smokers (1–9 cigarettes/day), moderate smokers (10–19 cigarettes/day), and heavy smokers (≥20 cigarettes/day).

The examinations included measurements of height, weight, and blood pressure. For diastolic blood pressure, three measurements were performed with the Dinamap 845 XT (Criticon). The mean of the second and third measurements was used in this study. Blood samples were taken from the participants in the nonfasting state. Plasma concentrations of tCys and tHcy were determined by HPLC and fluorescence detection *(13, 14)*. Serum total cholesterol and triglycerides were measured by enzymatic methods. In 1998, HDL-cholesterol, serum glucose, creatinine, plasma folate, and plasma vitamin B_{12} were also measured. The folate and B_{12} were measured from samples stored at −20 °C.

The samples were assayed within 2 years of sampling, and tCys, tHcy *(15)*, serum cobalamin *(16)*, and blood lipids were stable under these conditions. Plasma folate decreased moderately (by ~30%) during the first year and remained constant thereafter *(16)*.

STATISTICAL METHODS

Differences between values for tCys, BMI, cholesterol, diastolic blood pressure, and triglycerides measured at baseline and at follow-up were evaluated statistically by the paired *t*-test.

Spearman correlation coefficients were computed between baseline and follow-up measurements of tCys, tHcy, cholesterol, diastolic blood pressure, and triglycerides to show the variability in the measurements of these variables between baseline and follow-up. In addition, Spearman correlations between CVD risk factors and plasma tCys measured at follow-up were also computed.

Multiple linear regression models were used to assess the simultaneous relationships among the various predictors of tCys measured separately at baseline and at follow-up. Plasma tCys was the response variable, whereas the predictor variables age, sex, BMI, triglycerides, diastolic blood pressure, total cholesterol, and coffee consumption were represented in the models as indicator variables. For each factor, the difference in mean tCys concentrations between the reference category and the other categories was estimated by the regression coefficient. Plasma tCys concentrations across categories of each risk factor were tested jointly for homogeneity of the means and for linear trend.

Multiple linear regression was used to assess the relationship between change in plasma tCys and each of the predictor variables separately. Changes in tCys between baseline and follow-up was used as the response variable, and the baseline risk factor or the change in the risk factor was used as the predictor variable. The standardized regression coefficients were the estimates that would be obtained if all variables in the model were standardized to zero mean and unit variance before we performed the regression computations. Each coefficient indicated the number of SD changes in the dependent variable associated with a 1 SD change in the independent variable, holding constant all other variables *(17)*. Standardized coefficients were computed to allow for comparison among the different predictors.

The statistical analyses were performed with SAS statistical software (release 8.2 for Windows). In addition, S-PLUS software (Ver. 6.0 for Windows) was used to construct the graphs for the distribution of absolute change in tCys between baseline and follow-up.

Results

The overall mean (SD) values of tCys were higher at follow-up [296.0 (41.0) μmol/L] compared with baseline [278.0 (36.5) μmol/L]. The overall mean tCys change was 17.9 (29.7) μmol/L, and the mean percentage increase in tCys in the different age and sex groups was 4.9–8.5% (Table 1). There was a highly significant correlation between baseline and follow-up tCys concentrations (Spearman correlation coefficient, 0.55–0.59 for the different age and sex groups; $P < 0.0001$).

The distributions of absolute change in tCys between baseline and follow-up and the changes in tCys for baseline values at or below the 10th percentile and at or above the 90th percentile are shown in Fig. 1. Most of the participants with low tCys at baseline had an increase in tCys, whereas those with high tCys at baseline had a decrease in tCys.

Table 1. Mean baseline and follow-up plasma tCys by age and sex.

Age groups	Sex	n	Mean tCys (95% CI),[a] μmol/L		Mean absolute tCys change (95% CI), μmol/L	Mean % of tCys change
			Baseline	Follow-up		
All		7071	278.0 (277.2–278.9)	296.0 (295.0–297.0)	17.9 (17.2–18.7)	6.91
Born in 1950–1951	M	1664	270.5 (269.1–271.9)	282.4 (280.9–283.9)	11.8 (10.5–13.2)	4.85
	F	2068	250.4 (249.1–251.6)	266.2 (264.9–267.6)	15.9 (14.6–17.1)	6.87
P			<0.0001	<0.001	0.22	
Born in 1925–1927	M	1473	298.1 (296.4–299.8)	318.1 (316.3–320.0)	20.0 (18.4–21.7)	7.24
	F	1866	298.0 (296.5–299.6)	321.7 (320.0–323.4)	23.6 (22.2–25.1)	8.45
P			0.94	0.006	0.28	

[a] CI, confidence interval.

BASELINE AND FOLLOW-UP tCys BY AGE AND SEX

In all age and sex groups, mean tCys concentrations were higher at follow-up that at baseline (Table 1). In the younger age group (born in 1950–1951), men had higher mean tCys than did women at both baseline and follow-up. In the older age group (born in 1925–1927), there was no difference between men and women in tCys measured at baseline. However, follow-up data showed a marginally higher mean tCys in women than in men. The analyses were repeated separately for participants that were free from CVD at baseline, and essentially similar results were obtained (results not shown).

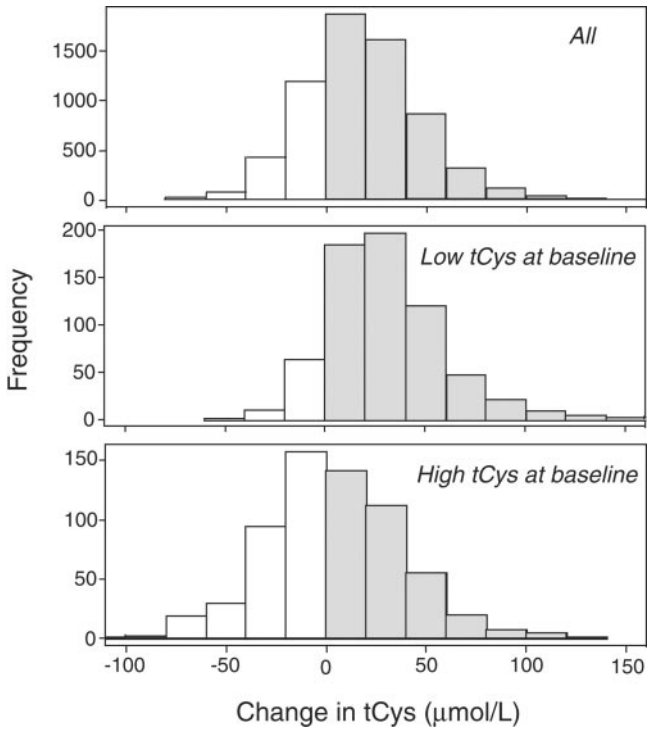

Fig. 1. Distribution of absolute change in plasma tCys between baseline and follow-up in all participants (*top*) and in the subgroups with low (*middle*) or high tCys (*bottom*) at baseline.

Low tCys, at or below the 10th percentile at baseline (n = 705); *High tCys*, at or above the 90th percentile at baseline (n = 708). *Shaded columns* indicate increases in tCys values; *open columns* indicate decreases.

We evaluated the increase in tCys per year in the cross-sectional data. The increase was evaluated in the 1992–1993 data and the 1998–1999 data separately. In the 1992 data, the increase in tCys per year was 1.1 μmol/L in men and 1.9 μmol/L in women. In the 1998 data, the increase in tCys per year was 1.5 μmol/L in men and 2.3 μmol/L in women.

The increase in tCys per year was also investigated in the longitudinal data (from 1992–1993 to 1998–1999). In the younger men, the increase in tCys was 1.9 μmol/L, and in the younger women, the tCys increase was 2.6 μmol/L. For the older age group, the longitudinal increase in tCys was 3.3 μmol/L per year in men and 3.9 μmol/L per year in women.

BASELINE AND FOLLOW-UP VALUES FOR CARDIOVASCULAR RISK FACTORS BY AGE AND SEX

The mean values for cholesterol, triglycerides, BMI, and diastolic blood pressure, measured at baseline and at follow-up, are presented in Table 2. The results are presented separately for the younger and older men and women.

In the younger age group, mean values for cholesterol, triglycerides, and BMI were significantly higher at follow-up than at baseline. Mean diastolic blood pressure was significantly lower at follow-up compared with baseline values. The same trends were seen for men and women. When we compared variables at follow-up and at baseline, the older age group had lower cholesterol, lower diastolic blood pressure, and higher BMI at follow-up. Triglycerides at follow-up were higher in females and lower in males, but the latter finding was not statistically significant.

The mean values for creatinine, HDL-cholesterol, plasma folate, and plasma B_{12} at follow-up are also presented in Table 2. In both age groups, women had higher HDL-cholesterol, plasma folate, and plasma B_{12} compared with men, whereas men had higher creatinine than women. Serum creatinine and HDL-cholesterol and plasma folate and B_{12} were higher in the older than the younger age group.

Table 2. Mean values and changes for variables at baseline and follow-up by age and sex.

	Age groups							
	Younger				Older			
	Men		Women		Men		Women	
	n	Mean	n	Mean	n	Mean	n	Mean
BMI, kg/m^2	1662		2066		1470		1860	
Baseline		25.1		23.8		25.7		25.8
Follow-up		26.1		24.9		26.0		26.3
Change		1.0[a]		1.1[a]		0.3[a]		0.4[a]
Cholesterol, mmol/L	1664		2065		1473		1863	
Baseline		5.74		5.39		6.30		7.06
Follow-up		5.84		5.66		5.86		6.59
Change		0.09[a]		0.27[a]		−0.41[a]		−0.47[a]
Triglycerides, mg/L	1664		2065		1473		1863	
Baseline		1943.5		1193.6		1884.8		1740.6
Follow-up		2069.9		1444.5		1853.2		1800.0
Change		126.4[a]		250.9[a]		−31.6		59.4[b]
Diastolic blood pressure, mmHg	1662		2067		1472		1859	
Baseline		79.88		75.63		86.08		82.91
Follow-up		78.27		71.84		79.79		75.77
Change		−1.61[a]		−3.80[a]		−6.29		−7.16[a]
HDL-cholesterol, mmol/L	1664		2065		1473		1863	
Follow-up		1.16		1.45		1.22		1.47
Creatinine, μmol/L	1664		2064		1471		1863	
Follow-up		95.0		81.6		101.7		86.1
Plasma folate, nmol/L	1656		2055		1458		1853	
Follow-up		7.2		8.5		7.5		9.5
Plasma-vitamin B_{12}, pmol/L	1658		2059		1466		1859	
Follow-up		370		380		382		405

[a,b] Paired *t*-test: [a] $P < 0.0001$; [b] $P = 0.002$.

STABILITY OVER TIME

The stability of tCys, tHcy, cholesterol, diastolic blood pressure, and triglyceride measurements over time was estimated by computing Spearman correlations between baseline and follow-up measurement for each of these variables (Table 3). Highly significant correlations were seen for each of these variables. The correlations were also calculated in the different age and sex groups separately, and similar results were obtained. tHcy showed a stronger and more consistent correlation than tCys overall and in the different age and sex groups (Table 3).

Table 3. Spearman correlations between baseline and follow-up measurements.[a]

Age group	Sex	tCys	tHcy	Cholesterol	Diastolic blood pressure	Triglycerides
All		0.72	0.75	0.66	0.61	0.62
Born 1950–1951	M	0.57	0.71	0.68	0.59	0.56
	F	0.55	0.69	0.69	0.66	0.58
Born 1925–1927	M	0.57	0.71	0.56	0.54	0.57
	F	0.59	0.67	0.51	0.56	0.64

[a] All $P < 0.0001$.

SIMPLE CORRELATIONS BETWEEN tCys AND CARDIOVASCULAR RISK FACTORS MEASURED AT FOLLOW-UP

Correlations between CVD risk factors and plasma tCys measured at follow-up were calculated. Plasma tCys was strongly correlated with age ($r = 0.54$), BMI ($r = 0.31$), cholesterol concentration ($r = 0.25$), and creatinine; ($r = 0.32$). In addition, tCys was weakly associated with triglycerides, diastolic blood pressure, and coffee intake and negatively associated with HDL-cholesterol. There was no significant association between tCys and smoking or tCys and polymorphism in the gene encoding 5,10-methylenetetrahydrofolate reductase (*MTHFR* 677C→T). Similar associations were seen for the different age and sex groups. Nevertheless, coffee intake and cholesterol were strongly associated with tCys in the younger age group but not in the older age group.

RISK FACTORS MEASURED AT BASELINE AND AT FOLLOW-UP AS DETERMINANTS OF tCys AT FOLLOW-UP

Multiple regression analysis was used to identify the predictors of plasma tCys measured at follow-up (Table 4). For all variables, the difference in mean tCys concen-

Table 4. Estimated change in plasma tCys measured at follow-up by risk factors measured at baseline and at follow-up relative to the reference group in each risk factor.[a]

Risk factor	n	At baseline	n	After 6 years[b]
Age, years				
40–42[c]	3732		3732	
65–67	3339	40.36 (38.52–42.21)	3339	41.78 (40.11–43.45)
P-trend		<0.0001		<0.0001
BMI, kg/m^2				
<20[c]	318		263	
20–24.99	3492	5.12 (1.27–8.96)	2925	5.15 (1.14–9.17)
25–29.99	2658	15.72 (11.73–19.72)	2997	16.03 (11.96–20.11)
≥30	587	26.47 (21.75–31.18)	873	28.33 (23.81–32.84)
P-trend		<0.0001		<0.0001
Cholesterol, mmol/L				
<4.00[c]	170		128	
4.00–5.99	3385	5.34 (0.09–10.59)	3633	6.70 (0.78–12.62)
6.00–7.99	3002	9.86 (4.48–15.23)	3008	12.25 (6.26–18.23)
≥8.00	508	14.16 (8.06–20.26)	296	16.52 (9.51–23.54)
P-trend		<0.0001		<0.0001
Triglycerides, mg/L				
<880[c]	1403		830	
880–1760	3264	2.66 (0.48–4.84)	3548	0.52 (−2.00 to 3.05)
1770–2640	1458	4.20 (1.53–6.87)	1664	1.80 (−1.09 to 4.69)
≥2650	940	5.09 (2.01–8.18)	1023	0.82 (−2.43 to 4.08)
P-trend		0.0002		0.28
Diastolic blood pressure, mmHg				
<70[c]	1142		2118	
70–84	3610	1.88 (−0.39 to 4.14)	3485	1.31 (−0.51 to 3.13)
85–99	1896	2.31 (−0.30 to 4.91)	1258	0.79 (−1.58 to 3.17)
≥100	407	6.20 (2.23–10.18)	199	1.09 (−3.73 to 5.92)
P-trend		0.0076		0.29
Coffee, cups/day				
0[c]	463		629	
1–4	3980	7.61 (5.07–10.16)	4208	7.80 (5.54–10.07)
5–8	1807	10.00 (7.21–12.79)	1572	10.78 (8.16–13.40)
≥9	228	14.11 (9.10–19.11)	284	9.70 (5.26–14.14)
P-trend		<0.0001		<0.0001

[a] 95% confidence intervals in parentheses.
[b] Adjusted for all risk factors in this table and for sex and creatinine.
[c] Reference group.

trations among the categories of each risk factor and the reference category were estimated. The risk factors assessed at baseline had a significant association with tCys across different categories of age, sex, BMI, cholesterol, triglycerides, coffee consumption, and diastolic blood pressure.

The same analyses were performed with the categories of the cardiovascular and lifestyle factors measured at follow-up. Similar results were obtained for most of the variables (Table 4), but there was no significant association between tCys across categories of sex, triglycerides, and diastolic blood pressure.

CHANGES ACCORDING TO BASELINE VALUES AND CHANGES IN RISK FACTORS

The relationship between baseline risk factors and the change in tCys between baseline and follow-up were studied by multiple linear regression (Table 5). All analyses were adjusted for sex and age. The standardized regression coefficients are presented to allow comparison among the different predictors. The association with change in tCys was significant with age, BMI, and triglycerides measured at baseline. When the analyses were performed separately in younger and older men and women, the significant associations between baseline BMI and the change in tCys were confined to women. Furthermore, baseline triglycerides had a significant association with the change in tCys in the younger men and in all women, but not in the older men.

The relationship between changes in the risk factors (from baseline to follow-up) and the change in tCys are also presented in Table 5. Overall, a strong positive association was seen with change in BMI and total cholesterol. A weaker association was seen with the change in

Table 5. Standardized linear regression coefficients for tCys change per SD of the predictor variable.[a]

	Regression coefficients				
		Younger		Older	
	All	Men	Women	Men	Women
Baseline risk factor[b]					
Age	0.14[c]				
BMI, kg/m^2	0.04[c]	0.03	0.07[c]	0.003	0.06[c]
Weight, kg	0.05[c]	0.05	0.06[c]	0.007	0.07[c]
Total cholesterol, mmol/L	0.01	−0.01	0.02	−0.03	0.03
Diastolic blood pressure, mmHg	0.01	0.02	0.01	−0.02	0.03
Triglycerides, mg/L	0.08[c]	0.11[c]	0.12[c]	0.05	0.05[c]
Coffee, cups/day	0.0002	−0.003	0.03	−0.04	0.004
Change in risk factor[d]					
BMI, kg/m^2	0.09[c]	0.13[c]	0.09[c]	0.09[c]	0.07[c]
Weight, kg	0.07	0.09[c]	0.08[c]	0.01	0.07[c]
Total cholesterol, mmol/L	0.07[c]	0.11[c]	0.14[c]	0.03	0.02
Diastolic blood pressure, mmHg	0.03[c]	0.07[c]	0.09[c]	0.02	−0.04
Triglycerides, mg/L	−0.04[c]	−0.08[c]	−0.03	−0.04	−0.03
Coffee, cups/day	0.02	0.04	0.004	0.06[c]	−0.01

[a] Change in tCys (in SD units) per 1 SD change in the predictor variable.
[b] Adjusted for age and sex.
[c] $P < 0.05$.
[d] Adjusted for age, sex, and creatinine.

diastolic blood pressure, and a weak negative association was seen with the change in triglycerides. The analyses were adjusted for sex, age, and creatinine measured at follow-up. The addition of creatinine to the model only slightly weakened the results. Analysis of subgroups demonstrated that in the older age group, only the association with BMI was significant, whereas the other variables, including change in cholesterol, were not associated with tCys changes.

The change in tCys was evaluated in different categories of change in BMI from baseline to follow-up (Table 6). The results showed that, on average, tCys decreased in individuals whose BMI decreased from baseline to follow-up, whereas tCys increased in individuals who had an increase in BMI higher than one unit at follow-up compared with baseline. There was no significant change in tCys when BMI was not changed or was slightly increased (less than one unit of change).

Discussion

Using data on 7071 participants from the Hordaland Homocysteine Study, we assessed the changes in plasma tCys and the corresponding changes in the determinants of tCys from 1992–1993 to 1998. The results show that tCys was significantly higher at follow-up compared with baseline in both the younger and older age groups. The determinants of change were different for the older and younger groups. In the younger group, changes in BMI, cholesterol, and diastolic blood pressure were the strongest predictors of change in tCys, whereas only a change in BMI determined the change in tCys in the older age group (Table 5).

The major strength of our study is the large number of participants, which allowed us to assess the changes in several lifestyle and CVD risk factors and the changes in tCys over a period of 6 years. The lack of measurements of creatinine, folate, and vitamin B_{12} at baseline is a weakness in this study because the changes in these factors could not be evaluated. Participants with low tCys at baseline had an increase in plasma tCys, whereas those with high tCys at baseline had a decrease in tCys (Fig. 1). This might be attributable to changes in tCys determinants within these individuals, but this observation probably reflects the phenomenon of regression toward the mean *(18)*.

We evaluated the increase in tCys on a per year of age

Table 6. Change in tCys (μmol/L) with change in BMI.

			Younger				Older			
BMI change, kg/m^2	n	All	n	Men	n	Women	n	Men	n	Women
<0	2132	−3.69[a]	345	−4.30[a]	479	−3.99[a]	600	−3.47[a]	708	−3.33[a]
0–1	2106	−0.33	538	−2.43	587	−1.49	479	2.07	502	1.00
>1	2817	3.60[a]	778	5.10[a]	999	4.09[a]	391	1.99	649	2.63[a]

[a] $P < 0.05$.

basis. For the younger age group, there was general agreement between the cross-sectional and longitudinal findings, with an increase of 1.1–2.6 μmol/L per year in men and women. In the older age group, however, there was an increase in tCys in the longitudinal data beyond that expected from the cross-sectional data. We speculate that the increase in tCys in the older age group may be attributable to a decline in renal function, a condition that is associated with an increase in tCys and is common in the elderly. In both the younger and older age groups, however, a slight calendar time effect toward increasing tCys in the population cannot be ruled out.

Correlations between baseline and follow-up (self-correlations) were calculated to assess the stability of tCys and other metabolites between baseline and follow-up. tCys showed more stability and stronger correlations than did cholesterol, diastolic blood pressure, and triglycerides (Table 3). Both tCys and tHcy were stable over the 6-year follow-up time, as demonstrated by the strong self-correlations of each of these amino thiols. However, tCys showed a slightly weaker self-correlation than did tHcy, particularly when the correlations were assessed for the different age and sex groups separately. There are no studies on the stability of tCys over time. However, a few studies have evaluated the short- and long-term variability of tHcy. Clarke et al. *(19)* showed that an individual's plasma tHcy is relatively constant over a 1-year period, with little seasonal variation. Garg et al. *(20)* found that an individual's plasma tHcy is relatively constant over at least 1 month but that the variability increases over 30 months, a result confirmed by Clarke et al. *(21)*, who found that the variability in tHcy increases with longer intervals between measurements.

The determinants of tCys were previously studied in this population, using the baseline data *(11)*. In the present study, the determinants of tCys were evaluated again, using the follow-up data. The determinants of tCys were mainly the same for follow-up as for baseline values. Age, sex, BMI, cholesterol, and coffee consumption were among the strongest predictors of tCys at follow-up. Diastolic blood pressure, which was a strong determinant of tCys at baseline, had no relationship with tCys at follow-up. The decrease in diastolic blood pressure from baseline to follow-up, however, may indicate effects from medication and/or changes in lifestyle, which in turn may explain this lack of association between tCys and diastolic blood pressure at follow-up.

Creatinine, which was measured at follow-up but not at baseline, was a strong determinant of tCys. Likewise, Brattström et al. *(22)* found that creatinine was a strong determinant of tCys. Plasma folate and B_{12} were also measured in the present study, and the results showed weak associations between tCys and both folate and B_{12}. We have previously found associations between tCys and creatinine and tCys and folate but not B_{12} in the European Concerted Action Project *(10)*.

BMI and cholesterol are strong predictors of tCys at baseline *(11)* and at follow-up (Table 3). In the present study, the change in each of these risk factors was a strong predictor of change in plasma tCys in the younger age group. In the older age group, however, mean cholesterol concentrations were lower at follow-up compared with baseline values, possibly because of changes in lifestyle or the use of cholesterol-lowering medications. The difference in patterns observed between the older and the younger groups was not attributable to the higher mortality in participants with high cholesterol at baseline (data not shown). Other factors, e.g., a decline in renal function, could be responsible for the increase in tCys in this older age group *(23)*. This is reflected in the strong association between tCys and creatinine (Table 4) and in the high creatinine values in the older age groups compared with the younger groups (Table 2). Creatinine was measured only in the follow-up data; therefore, changes in plasma creatinine between baseline and follow-up could not be determined.

BMI was the strongest determinant of tCys in both men and women and in both age groups. The increase in tCys was confined to those with a greater than one unit increase in BMI between baseline and follow-up (Table 6). The mechanism(s) behind the association of plasma tCys with serum cholesterol and BMI is unknown. The possibility that tCys concentrations are related to body cell mass *(24)* is unlikely because the increase in BMI in our study participants probably reflects an increase in body fat rather than muscle.

In conclusion, this is the first study on changes in tCys and its determinants with time. The results demonstrated an increase in tCys during a 6-year period with a strong correlation between the two measurements. The changes in cholesterol and diastolic blood pressure, which were determinants of baseline tCys values, were also predictors of the change in tCys with time. The change in BMI, however, emerged as the strongest determinant of change in tCys in all age and sex groups.

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