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Changes in lifestyle and plasma total homocysteine: the Hordaland Homocysteine Study[1–3]

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ABSTRACT

Background: Elevated plasma concentrations of total homocysteine (tHcy) are a risk factor for cardiovascular disease. tHcy is a marker of folate and cobalamin deficiencies and is also related to several lifestyle factors.

Objective: We examined whether changes in lifestyle influence tHcy over time.

Design: A population-based, prospective study was conducted in 7031 subjects from western Norway who constituted 2 age groups (41–42 and 65–67 y) at baseline (1992–1993). The subjects were reinvestigated in 1997–1999 ($\bar{x}$ follow-up: 6 y).

Results: During follow-up, median tHcy concentrations decreased 0.10 (25th and 75th percentiles: −1.24, 1.00) μmol/L in the younger subjects and increased 0.39 (25th and 75th percentiles: −0.99, 1.79) μmol/L in the older subjects. Changes in plasma vitamin status and vitamin supplement use were the strongest determinants of changes in tHcy over time. Each unit increase in plasma folate (nmol/L) and vitamin B-12 (pmol/L) was associated with reductions in tHcy concentrations of 0.2 and 0.1 μmol/L, respectively. Among the younger and older age groups, those who started to take vitamin supplements during follow-up had significant reductions in tHcy concentrations of 0.42 (95% CI: −0.65, −0.20) and 0.41 (−0.78, −0.03) μmol/L, respectively. In the younger subjects who quit smoking, tHcy concentrations decreased 0.54 (−0.91, −0.16) μmol/L. Weight changes were inversely related to tHcy. Both baseline history of cardiovascular disease or hypertension and cardiovascular events during follow-up were significantly associated with changes in tHcy.

Conclusions: Changes in lifestyle factors over time influence tHcy concentrations. These changes are modest when compared with the strong associations between tHcy and lifestyle factors in cross-sectional studies. *Am J Clin Nutr* 2004;79:812–9.

KEY WORDS Body weight, cardiovascular disease, coffee, folate, homocysteine, smoking, vitamin B-12, vitamin supplements

INTRODUCTION

Elevated plasma concentrations of total homocysteine (tHcy) are a marker of folate and cobalamin deficiencies and a risk factor for cardiovascular disease (CVD) (1–3). In addition, elevated tHcy concentrations are associated with adverse pregnancy outcomes (4), cancer, and cognitive dysfunction in the elderly (1, 3).

tHcy concentrations increase with age. Other causes of elevated tHcy include low intakes or deficiencies of B vitamins, genetic defects, polymorphisms of enzymes involved in homocysteine metabolism, impaired renal function, and lifestyle factors, such as smoking, heavy coffee consumption, and lack of exercise (1, 3, 5, 6).

There are few longitudinal studies on the variability of tHcy over time (7–10). These studies indicate that the within-person variability of tHcy is relatively small over ≥1 mo (7) or even 1 y (8, 10). Van den Berg et al (9) showed substantial intraindividual variability in tHcy over a period of 1–4 mo, which was attributed to changes in serum folate concentration. Because tHcy concentrations are related to several health behaviors (diet, smoking, and exercise), an altered lifestyle could conceivably cause changes in plasma tHcy concentration. Thus far, no population-based studies of the effect of lifestyle changes over several years on tHcy concentrations have been published.

The Hordaland Homocysteine Study (11) was the first large-scale, population-based study of lifestyle factors and tHcy, and Nygård et al (12–14) showed that smoking, high coffee consumption, lack of exercise, and low dietary folate intake are associated with elevated tHcy concentrations. The primary objective of the present study was to examine whether changes in lifestyle during a period of 6 y predicted variability in tHcy concentrations during this period in the Hordaland cohort.

SUBJECTS AND METHODS

Study population

The Hordaland Homocysteine Study (11) was a collaborative study between the University of Bergen, local health services, and the National Health Screening Service. The cohort was established in 1992–1993 in western Norway and consisted of >18 000 subjects who were born in 1925–1952 (11, 12). In 1997–1999, all cohort members who were born in 1925–1927 or

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1950–1951 and who were living in Bergen or 3 neighboring suburban municipalities were invited to participate in a second survey. The mean follow-up period was 6 y (range: 5.2–7.2 y). The response rate was 76.7%, and 7047 subjects were reexamined. Subjects who had a tHcy concentration $>$ 40 μmol/L at baseline (n = 18) were offered treatment with cobalamin or folic acid, and those subjects were excluded from the present study. All participating subjects gave their written informed consent. The study protocol was approved by the Regional Committee for Medical Research Ethics of Western Norway.

Data collection

At baseline (1992–1993), participants underwent the standard cardiovascular examinations of the National Health Screening Service (including determination of height and weight) (15), and nonfasting plasma samples were collected into tubes containing EDTA for tHcy, folate, and vitamin B-12 analyses. Several self-administered questionnaires focusing on CVD risk factors, lifestyle factors, and dietary habits were used. Details on the baseline data collection were reported previously (12). The follow-up examination included essentially the same variables that were included at baseline.

In the present study, changes in tHcy concentration between the 2 surveys were assessed in relation to lifestyle changes. Plasma tHcy, which includes both the free and protein-bound fractions of homocysteine, was measured on both occasions by using a fully automated HPLC assay (16, 17). The samples were stored at −20 °C (samples collected in 1992–1993) or −80 °C (samples collected in 1997–1999) until the tHcy analyses were performed. The duration of storage ranged from a few days to 6 mo for baseline samples and 18 mo for follow-up samples.

Folate and vitamin B-12 concentrations were measured by using microbiological assays on microtitre plates with a chloramphenicol-resistant strain of *Lactobacillus casei* and a colistin sulfate–resistant strain of *Lactobacillus leichmannii*, respectively (18, 19). Before baseline folate and vitamin B-12 measurements were performed, the plasma samples were stored at −20 °C for ≤10 y and were frozen and thawed 1–2 times during that time period. As reported by Ocké et al (20), folate concentrations decrease during storage, whereas vitamin B-12 concentrations do not change. In a sample subset (n = 329) of the Hordaland cohort, plasma folate was measured in 1995 (21) and reanalyzed for the present study in 2000. The mean folate concentration decreased ≈20% from 1995 to 2000. However, the correlation between folate and tHcy was the same at both times (data not shown). In the present study, baseline folate concentrations were multiplied by a coefficient (1.2978) that was calculated as follows: plasma folate concentrations measured in a sample subset (n = 316; subjects who had a tHcy concentration $>$ 40 μmol/L were excluded) in 1995 divided by plasma concentrations reanalyzed in 2000. Follow-up vitamin measurements were performed in plasma stored at −80 °C for ≤12 mo.

In the baseline survey, the frequency (almost daily, 4–5 times/wk, 2–3 times/wk, once a week, 1–3 times/mo, and seldom or never) and the seasonal variability (whole year, only during the winter half of the year, less often, and never) of vitamin supplement use were recorded. A question about the type of vitamin (multivitamin, vitamin B, vitamin C, vitamin A or D, and vitamin E) consumed was also asked. In the follow-up survey, similar categories were used for vitamin supplement use. Those who reported taking multivitamin supplements or any type of B vitamin supplements during the winter half of the year or the whole year and at least once a week were defined as vitamin supplement users in the present study. Information from all questions that concerned multivitamin or B vitamin intake was used to compute a new variable counting the number of days per year when vitamin supplements were taken.

Those who reported daily smoking of cigarettes, cigars, cigarillos, or a pipe were considered as smokers in both surveys. In addition, the number of cigarettes smoked per day was recorded.

Information about coffee consumption included the type and amount of coffee. If a person indicated consumption of more than one type of coffee, all types were registered, whereas the consumption of decaffeinated coffee was considered as nonconsumption of coffee. In the baseline survey, subjects were classified into 4 groups according to the number of cups consumed per day: 0 or $<$1, 1–4, 5–8, and ≥9. In the follow-up survey, the number of cups consumed per day was recorded. For comparison, in the follow-up survey, the numbers of cups consumed were categorized into 4 groups according to the criteria defined for the baseline survey. By replacement of the category number with a value of 0, 2.5, 6.5, or 9 cups/d, respectively, daily coffee consumption was estimated for each subject both at baseline and follow-up.

In the follow-up survey, the questionnaire included a question about nutritional habits. This question was as follows: Have you tried to eat more healthy food during the past 12 mo?

At baseline, self-reported information on previous instances of myocardial infarction, angina pectoris, stroke, thrombosis or phlebitis, and treatment for hypertension was recorded. In addition, computerized records containing discharge diagnoses for all hospitalizations occurring between the baseline screening and May 1998 at the 6 hospitals serving Hordaland County were searched for CVD cases. A self-reported questionnaire at follow-up was used to obtain possible CVD cases outside the county hospitals, cases that occurred between May 1998 and the follow-up survey, and information about the use of antihypertensive treatment. On the basis of information from these 3 different sources, the subjects were categorized as having ever or never had CVD or hypertension (defined as receiving antihypertensive treatment).

Statistical analyses

Absolute (μmol/L) and percentage changes in tHcy concentration from baseline to follow-up examination were calculated for each subject. Median tHcy concentrations are presented with 25th and 75th percentile values. t tests for paired data and McNemar tests for paired proportions were applied to assess changes in lifestyle and the history of CVD or hypertension over time. Mean changes with 95% CIs are presented when the influence of changes in various lifestyle factors and the history of CVD or hypertension on changes in tHcy concentration was examined. Two-factor analysis of variance with an interaction term was used to test whether the age groups and sexes differed in the response variable. To investigate a possible age-by-sex interaction, a logistic regression model with an interaction term (chi-square test) was used for binary variables (baseline and follow-up). For binary changes over time, a logistic regression model (a generalized estimating equations method) for repeated measurements was applied with the use of PROC GENMOD of SAS statistical software 8.2 for WINDOWS (SAS Institute Inc,

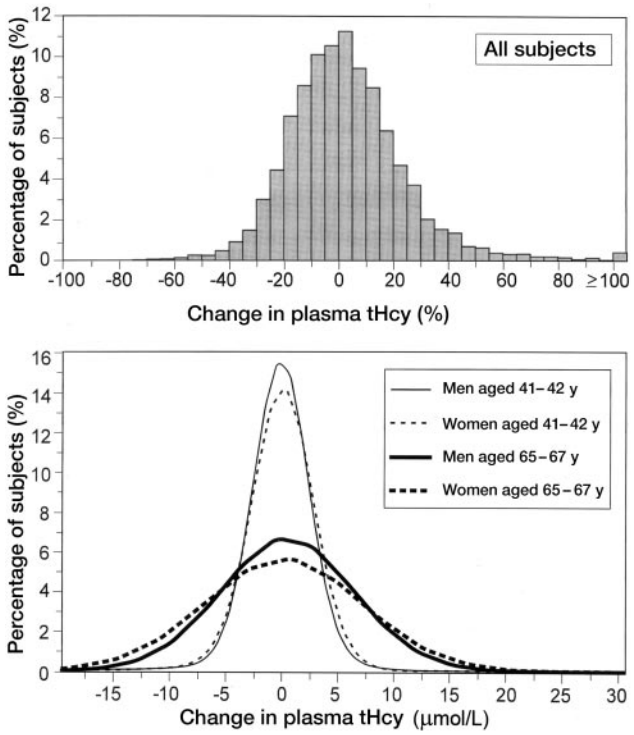

FIGURE 1. Distributions of percentage and absolute changes in plasma total homocysteine (tHcy) concentration between baseline (1992–1993) and follow-up (1997–1999) in subjects who participated in the Hordaland Homocysteine Study (Norway). The total study population was 7031 subjects, and the mean follow-up time was 6 y. In the lower panel, the ages that are given are those at baseline. Also in the lower panel, the lowest and highest 0.1 percentiles of change in plasma tHcy concentration are not presented.

Cary, NC). Between-group differences in changes in tHcy concentration according to variations in lifestyle factors were tested with the use of linear regression analysis in which the negative factor status (ie, no vitamin supplement use, smoking, or coffee consumption) both at baseline and at follow-up was considered as a reference. Linear regression analyses (adjusted for age and sex) were also used to estimate the associations of changes in plasma folate and vitamin B-12 concentrations, smoking, coffee consumption, and body weight with simultaneous changes in tHcy concentration. Additional analyses were done in which vitamin supplement use replaced plasma folate and vitamin B-12 concentrations as an indicator of vitamin status in a linear regression model. Because of missing information, only 56% of the participants were included in these analyses. P values < 0.05 were considered significant. Most statistical analyses were performed by using SPSS 11.0 for WINDOWS (SPSS Inc, Chicago).

RESULTS

Distributions of percentage and absolute (μmol/L) changes in plasma tHcy concentration between baseline and follow-up are shown in **Figure 1**. Most follow-up tHcy concentrations (87.9%) were within ±30% of baseline concentrations. Baseline and follow-up tHcy concentrations were significantly correlated (Spearman's correlation coefficients varied from 0.67 to 0.71 in the 4 age- and sex-specific groups; $P < 0.001$ in all groups). Changes in tHcy concentration were more pronounced in the older subjects than in the younger subjects and did not differ between the men and the women (Figure 1, lower panel).

Median baseline and follow-up tHcy concentrations and changes in tHcy concentration from baseline to follow-up in the 4 age- and sex-specific groups are presented in **Table 1**. On average, during the mean follow-up period of 6 y, median plasma tHcy concentrations decreased 0.10 μmol/L (range: −22.1–30.6 μmol/L) in the younger subjects and increased 0.39 μmol/L (range: −26.5–126.7 μmol/L) in the older subjects. Both age and sex were associated with tHcy concentrations at baseline and

TABLE 1
Plasma total homocysteine (tHcy) concentrations at baseline (1992–1993) and follow-up (1997–1999) and changes between baseline and follow-up in subjects who participated in the Hordaland Homocysteine Study (Norway)[1]

	Age of 41–42 y at baseline		Age of 65–67 y at baseline		P[2]		
	Men (n = 1658)	Women (n = 2058)	Men (n = 1461)	Women (n = 1854)	Age	Sex	Interaction
tHcy							
Baseline (μmol/L)	10.3 (9.0, 12.0)[3]	8.8 (7.6, 10.5)	11.9 (10.1, 13.9)	10.6 (9.1, 12.5)	<0.001	<0.001	0.05
Follow-up (μmol/L)	10.2 (8.8, 11.9)	8.6 (7.3, 10.3)	12.2 (10.4, 14.5)	10.9 (9.1, 13.2)	<0.001	<0.001	0.25
Change over time							
(μmol/L)	−0.07 (−1.27, 1.06)	−0.11 (−1.24, 0.95)	0.48 (−0.93, 1.90)	0.31 (−1.05, 1.72)	<0.001	0.19	0.64
(%)	−0.8[4]	−1.3	4.1	3.1	<0.001	0.55	0.82
tHcy ≥ 15 μmol/L (%)							
Baseline	6.6	4.4	16.7	9.9	<0.001	<0.001	0.36
Follow-up	7.5	3.7	22.2	13.3	<0.001	<0.001	0.51
Change over time	0.9	−0.7	5.5	3.4	<0.001	<0.001	0.84
tHcy ≥ 20 μmol/L (%)							
Baseline	1.9	1.4	2.5	2.0	0.057	0.09	0.75
Follow-up	2.0	1.1	5.3	3.3	<0.001	0.001	0.66
Change over time	0.1	−0.3	2.8	1.3	<0.001	0.002	0.76

[1] The mean follow-up period was 6.0 y (range: 5.2–7.2 y).
[2] Two-factor ANOVA with an interaction term was used for continuous variables, and a logistic regression model with an interaction term (chi-square test) was used for binary data: a logistic regression model for repeated measurements with an interaction term (chi-square test) was used for binary changes over time.
[3] Median; 25th and 75th percentiles in parentheses (all such values).
[4] Median (all such values).

follow-up, whereas only age was significantly related to changes in tHcy concentration over time. A borderline significant interaction between age and sex was found for baseline tHcy concentrations.

During follow-up, the proportions of subjects who had a tHcy concentration $\geq$ 15 or 20 μmol/L increased significantly in the older subgroups but not in the younger subgroups. Age and sex were significantly associated with the prevalence of hyperhomocysteinemia (tHcy concentration $\geq$ 15 μmol/L) both at baseline and at follow-up and with changes in the prevalence over time. When the cutoff for hyperhomocysteinemia was set at $\geq$20 μmol/L, age and sex were no longer significantly associated with baseline hyperhomocysteinemia.

At baseline and follow-up, the use of vitamin B or multivitamin supplements was moderately correlated with plasma folate concentrations [$r = 0.25$ ($P < 0.001$) and $r = 0.22$ ($P < 0.001$), respectively] and weakly correlated with plasma vitamin B-12 concentrations [$r = 0.09$ ($P < 0.001$) and $r = 0.04$ ($P = 0.002$), respectively].

Values for plasma folate and vitamin B-12, multivitamin or B vitamin supplement use, smoking, coffee consumption, body weight, and history of CVD or hypertension at baseline and follow-up and changes between baseline and follow-up in the 4 age- and sex-specific groups are shown in **Table 2**. During the follow-up period, mean plasma folate concentrations increased significantly in all groups, whereas mean plasma vitamin B-12 concentrations increased significantly only among the younger women and the older men. In general, the prevalence of vitamin supplement use and the mean intake (in d/y) decreased significantly in the older group and increased in the younger group. However, in the younger group, only the change in the prevalence of vitamin supplement use among men was significant. Independent of age group and sex, the prevalence of smokers and the mean number of cigarettes smoked per day among smokers decreased significantly. The prevalence of coffee consumers increased significantly in all 4 subgroups, whereas the mean number of cups of coffee consumed per day did not change among the women and changed marginally among the men. Body weight increased in all groups (significant increases in all groups except the older men). The prevalence of CVD or hypertension increased significantly in all 4 subgroups.

We found a significant age-by-sex interaction for plasma folate concentration and body weight both at baseline and follow-up. Significant interactions between age and sex were found for baseline plasma vitamin B-12 concentrations, daily smoking at baseline, vitamin supplement use among supplement users at follow-up, and coffee consumption (cups/d) at follow-up. We found significant age-by-sex interactions for changes in plasma vitamin B-12 concentration, daily smoking, and coffee consumption (cups/d).

The study population was divided into categories according to individual changes in the various lifestyle factors (no or yes), including vitamin supplement use, daily smoking, and coffee consumption. Significant interactions were found between age and the status of both vitamin supplement use and daily smoking but not between age and the status of coffee consumption. Subgroup analyses for vitamin supplement use and daily smoking are presented in **Table 3**. Changes in tHcy over time within each of the 4 categories of vitamin supplement use or daily smoking were assessed as paired comparisons between baseline and follow-up tHcy concentrations. We also compared mean changes in tHcy from baseline to follow-up between categories.

Among the younger subjects, those who had started to take vitamin supplements or quit smoking by the follow-up survey had significant within-category decreases in tHcy concentration over time. When compared with the younger subjects who did not take vitamin supplements or smoke at baseline and at follow-up, those who stopped smoking had significant decreases in tHcy concentration. The younger subjects who had ceased taking vitamin supplements or who had started smoking did not have significant changes in tHcy concentration. The younger subjects who had no change in these 2 lifestyle variables tended to have decreased tHcy concentrations, but the only significant decrease occurred in those who smoked at both time points.

Among the older subjects, tHcy concentrations decreased only among those who started to take vitamin supplements; this decrease was significant over time and in comparison with the change in tHcy concentration among the subjects who did not take vitamin supplements at baseline or follow-up. The older subjects in all the other groups of vitamin supplement use had significant within-category increases in tHcy concentration. Regarding smoking habits, the within-category changes in tHcy concentration over time were significant in all groups of older subjects except those who had started smoking by the follow-up survey. However, the latter group had the highest mean increase in tHcy concentration, and this increase was significant when compared with the change in tHcy concentration in nonsmokers at both time points. In addition, the group who smoked at both time points had a significant increase in tHcy concentration relative to the change in tHcy concentration observed in the reference group.

With respect to coffee consumption, there was no significant interaction by age, and therefore both age groups were combined. The subjects who stopped drinking coffee had a nonsignificant mean decrease in tHcy concentration [-0.28 μmol/L (95% CI: -0.79, 0.23 μmol/L); $n = 141$], and those who started to drink coffee had a significant increase [0.38 μmol/L (95% CI: 0.01, 0.75 μmol/L); $n = 292$]. None of the between-category changes were significant. The subjects who reported at the follow-up survey that they had tried to eat more healthy food during the past year had a marginal mean decrease in tHcy concentration, whereas those who had not tried to eat more healthy food had a mean increase in tHcy concentration [-0.03 μmol/L (95% CI: -0.13, 0.07 μmol/L) compared with 0.28 μmol/L (95% CI: 0.15, 0.41 μmol/L), respectively; $P < 0.001$].

Changes in tHcy concentration according to changes in vitamin status, lifestyle factors, and body weight are presented in **Table 4**. Because the results by age and sex were in the same direction and did not differ significantly, only combined data are given. Because the variables in the table have different units, standardized regression coefficients are presented to allow for a direct comparison between the variables in their relative power to explain changes in tHcy concentration over time. The strongest association was found for changes in plasma folate concentration, which were inversely and significantly associated with changes in tHcy concentration. Similarly, changes in plasma vitamin B-12 concentration and body weight were inversely and significantly associated with changes in tHcy concentration. No significant association between changes in daily smoking or coffee consumption and changes in tHcy concentration was found.

TABLE 2
Values for plasma folate and vitamin B-12, multivitamin or B vitamin supplement use, smoking, coffee consumption, body weight, and history of cardiovascular disease (CVD) or hypertension at baseline (1992–1993) and follow-up (1997–1999) and changes between baseline and follow-up in subjects who participated in the Hordaland Homocysteine Study (Norway)[1]

	Age of 41–42 y at baseline		Age of 65–67 y at baseline		*P*[2]		
	Men	Women	Men	Women	Age	Sex	Interaction
Plasma folate (nmol/L)							
n	1650	2047	1449	1848			
Baseline[3]	6.7 ± 0.07	6.9 ± 0.07	6.8 ± 0.09	7.8 ± 0.10	<0.001	<0.001	<0.001
Follow-up	7.2 ± 0.09	8.5 ± 0.13	7.5 ± 0.14	9.5 ± 0.20	<0.001	<0.001	0.009
Change over time	0.5 ± 0.10	1.6 ± 0.13	0.6 ± 0.15	1.7 ± 0.20	0.55	<0.001	0.74
Plasma vitamin B-12 (pmol/L)							
n	1650	2052	1457	1851			
Baseline	369.8 ± 2.89	336.7 ± 2.91	360.0 ± 6.53	390.9 ± 9.38	<0.001	0.85	<0.001
Follow-up	369.3 ± 3.22	380.2 ± 3.20	379.7 ± 7.87	402.0 ± 6.79	0.003	0.002	0.30
Change over time	−0.5 ± 2.93	43.6 ± 3.08	19.7 ± 7.47	11.1 ± 9.05	0.32	0.004	<0.001
Vitamin supplement users (%)							
n	984	1356	788	1082			
Baseline	23.8 ± 0.01	38.4 ± 0.01	31.5 ± 0.02	49.1 ± 0.02	<0.001	<0.001	0.72
Follow-up	29.9 ± 0.01	40.8 ± 0.01	26.9 ± 0.02	32.2 ± 0.01	<0.001	<0.001	0.10
Change over time	6.1 ± 0.02	2.4 ± 0.02	−4.6 ± 0.02	−16.9 ± 0.02	0.14	<0.001	0.49
Vitamin supplement use among users (d/y)							
n	404	788	347	645			
Baseline	130.7 ± 7.13	155.2 ± 5.16	196.7 ± 8.32	223.9 ± 5.69	<0.001	<0.001	0.84
Follow-up	142.8 ± 7.06	154.7 ± 5.27	165.3 ± 8.75	148.5 ± 6.42	0.24	0.73	0.04
Change over time	12.1 ± 10.01	−0.5 ± 7.61	−31.4 ± 13.02	−75.4 ± 8.92	<0.001	0.004	0.12
Daily smokers (%)							
n	1653	2047	1445	1844			
Baseline	37.3 ± 0.01	37.0 ± 0.01	23.3 ± 0.01	17.5 ± 0.01	<0.001	<0.001	0.002
Follow-up	34.1 ± 0.01	34.3 ± 0.01	16.6 ± 0.01	14.3 ± 0.01	<0.001	0.15	0.12
Change over time	−3.2 ± 0.02	−2.7 ± 0.01	−6.7 ± 0.01	−3.2 ± 0.01	<0.001	0.010	0.011
Daily smoking among smokers (cigarettes/d)							
n	670	814	315	341			
Baseline	13.5 ± 0.30	12.2 ± 0.24	12.4 ± 0.40	9.3 ± 0.29	<0.001	<0.001	0.011
Follow-up	11.4 ± 0.31	10.2 ± 0.23	7.3 ± 0.43	6.7 ± 0.29	<0.001	0.013	0.47
Change over time	−2.1 ± 0.32	−2.0 ± 0.22	−5.0 ± 0.50	−2.6 ± 0.31	<0.001	<0.001	0.001
Coffee consumers (%)							
n	1623	1996	1430	1803			
Baseline	89.5 ± 0.01	87.7 ± 0.01	92.0 ± 0.01	91.0 ± 0.01	0.001	0.06	0.76
Follow-up	92.1 ± 0.01	89.8 ± 0.01	93.7 ± 0.01	93.0 ± 0.01	0.001	0.030	0.39
Change over time	2.6 ± 0.01	2.1 ± 0.01	1.7 ± 0.01	2.0 ± 0.01	<0.001	0.024	0.52
Coffee consumption (cups/d)							
n	1530	1835	1373	1713			
Baseline	4.6 ± 0.06	4.0 ± 0.05	3.5 ± 0.05	3.1 ± 0.04	<0.001	<0.001	0.12
Follow-up	4.7 ± 0.06	4.0 ± 0.05	3.3 ± 0.05	3.1 ± 0.04	<0.001	<0.001	<0.001
Change over time	0.2 ± 0.06	0 ± 0.05	−0.2 ± 0.05	0 ± 0.04	0.001	0.84	0.001
Body weight (kg)							
n	1655	2056	1458	1847			
Baseline	80.8 ± 0.26	65.4 ± 0.23	79.2 ± 0.28	67.5 ± 0.25	0.27	<0.001	<0.001
Follow-up	83.9 ± 0.29	68.3 ± 0.26	79.4 ± 0.29	67.8 ± 0.28	<0.001	<0.001	<0.001
Change over time	3.1 ± 0.11	2.9 ± 0.10	0.2 ± 0.11	0.3 ± 0.11	<0.001	0.85	0.40
History of CVD or hypertension (%)							
n	1381	1716	1307	1577			
Baseline	4.3 ± 0.01	5.0 ± 0.01	37.9 ± 0.01	35.9 ± 0.01	<0.001	0.78	0.24
Follow-up	10.4 ± 0.01	10.4 ± 0.01	55.2 ± 0.04	49.7 ± 0.01	<0.001	0.11	0.11
Change over time	6.1 ± 0.01	5.4 ± 0.01	17.3 ± 0.04	13.8 ± 0.02	<0.001	0.44	0.16

[1] For plasma folate and vitamin B-12, vitamin supplement use among users, daily smoking among smokers, coffee consumption, and body weight, values are $\bar{x}$ ± SEM. For vitamin supplement users, daily smokers, coffee consumers, and history of CVD or hypertension, values are % ± SE.

[2] Two-factor ANOVA with an interaction term was used for continuous variables, and a logistic regression model with an interaction term (chi-square test) was used for binary data; a logistic regression model for repeated measurements with an interaction term (chi-square test) was used for binary changes over time.

[3] Because folate concentrations decrease during storage, baseline plasma folate concentrations were multiplied by 1.2978 (*see* Subjects and Methods).

TABLE 3
Changes in plasma total homocysteine (tHcy) concentration according to changes in 2 lifestyle factors (vitamin supplement use and daily smoking) during 6 y of follow-up (1992–1999) in subjects who participated in the Hordaland Homocysteine Study (Norway)

		Factor status				
	Age at baseline	Baseline	Follow-up	*n*	Change in tHcy[1]	*P*[2]
	y				*μmol/L*	
Vitamin supplement use[3]	41–42	No	No	1148	−0.15 (−0.32, 0.03)	Reference
		Yes	No	345	0.06 (−0.18, 0.30)	0.24
		No	Yes	437	−0.42 (−0.65, −0.20)	0.09
		Yes	Yes	410	−0.10 (−0.31, 0.11)	0.75
	65–67	No	No	878	0.59 (0.40, 0.79)	Reference
		Yes	No	432	0.83 (0.48, 1.18)	0.18
		No	Yes	213	−0.41 (−0.78, −0.03)	<0.001
		Yes	Yes	347	0.43 (0.09, 0.76)	0.61
Daily smoking[4]	41–42	No	No	2184	−0.07 (−0.16, 0.03)	Reference
		Yes	No	250	−0.54 (−0.91, −0.16)	0.003
		No	Yes	141	−0.03 (−0.41, 0.34)	0.87
		Yes	Yes	1125	−0.34 (−0.51, −0.17)	0.003
	65–67	No	No	2593	0.46 (0.31, 0.60)	Reference
		Yes	No	193	0.80 (0.34, 1.26)	0.23
		No	Yes	37	2.02 (−0.32, 4.37)	0.015
		Yes	Yes	466	1.08 (0.50, 1.66)	0.005

[1] $\bar{x}$; 95% CI in parentheses.
[2] Compared with subjects who did not take vitamin supplements or smoke at baseline or follow-up (linear regression analyses adjusted for age and sex).
[3] *P* for interaction between age and vitamin supplement use in 4 categories = 0.047.
[4] *P* for interaction between age and smoking in 4 categories < 0.001.

The effect on changes in tHcy was weaker when vitamin supplement use (days per week in which multivitamin supplements or any type of B vitamin supplements were consumed) was included in the model than when plasma vitamin concentrations were included instead. The standardized multiple linear regression coefficient (based on 3959 subjects with available data) was −0.110 ($P < 0.001$).

The subjects with preexisting CVD or hypertension at baseline had a significantly larger mean increase in tHcy concentration between the 2 surveys than did those who were free of CVD or hypertension both at baseline and at follow-up [0.34 μmol/L (95% CI: 0.01, 0.67 μmol/L) compared with 0.02 μmol/L (95% CI: −0.06, 0.10 μmol/L); $P = 0.035$]. Similarly, previously healthy subjects who developed CVD or hypertension during follow-up had a significantly higher mean increase in tHcy concentration (0.40 μmol/L; 95% CI: 0.08, 0.72 μmol/L; $P = 0.003$) than did those who were free of CVD or hypertension. The subjects who had preexisting CVD or hypertension at baseline and who had an additional diagnosis of CVD or hypertension during follow-up had the largest mean increase in tHcy concentration [0.82 μmol/L (95% CI: 0.39, 1.25 μmol/L); $P < 0.001$ for the comparison with the subjects who were free of CVD or hypertension both at baseline and at follow-up].

TABLE 4
Standardized multiple linear regression coefficients as predictors of changes in plasma total homocysteine (tHcy) concentration in subjects who participated in the Hordaland Homocysteine Study (Norway)

Predictor variable[1]	β[2]	*P*
	μmol/L	
Plasma folate (nmol/L)	−0.217	<0.001
Plasma vitamin B-12 (pmol/L)	−0.094	<0.001
Daily smoking (cigarettes/d)	0.017	0.16
Coffee consumption (cups/d)	0.010	0.42
Body weight (kg)	−0.058	<0.001

[1] Computed as follow-up (1997–1999) values minus baseline (1992–1993) values. Estimates were based on 6545 subjects with complete data on these variables. All 5 variables were included in a single analysis.
[2] Adjusted for age and sex.

DISCUSSION

The present study shows that intraindividual changes in tHcy concentration over 6 y are relatively modest. In the subjects aged 41–42 y at baseline, median tHcy concentrations decreased ≈0.1 μmol/L, whereas in those aged 65–67 y at baseline, median tHcy concentrations increased ≈0.4 μmol/L between baseline and follow-up. A decrease in tHcy concentration from baseline to follow-up was associated with the onset of vitamin supplement use and the cessation of smoking.

The strengths of our study are that it was population based, had a prospective design, included a large number of participants ($n = 7031$), and had a high follow-up response rate (72.1%). The limitation of the study is its lack of information about the exact time when different lifestyle changes occurred, which may have weakened the observed associations.

In cross-sectional studies, tHcy concentrations have been consistently shown to increase with age (3, 5, 22–24). Jacques et al (22) examined tHcy concentrations in ≈9000 US citizens aged 12 to >80 y. They found that tHcy concentrations increased linearly up to 25 y of age and then leveled off at ≈40 y of age, after which a second increase of ≈0.1 $\mu mol \cdot L^{-1} \cdot y^{-1}$ was observed. This pattern was similar in non-Hispanic whites, non-Hispanic blacks, and Mexican Americans and in both sexes. In the present study, tHcy concentrations increased 0.4 μmol/L during the 6 y of follow-up in the older group but decreased

≈0.1 μmol/L in the younger group. Inconsistencies between these 2 studies may be partly explained by a difference in study design, ie, the study by Jacques et al (22) was cross-sectional whereas our study was longitudinal. A reduction in tHcy concentrations in the younger group was observed not only among those who started to take vitamin supplements or quit smoking, but also among those who did not change these behaviors. After the baseline survey, in which tHcy concentrations were measured in >18 000 Hordaland County inhabitants, both the public and primary care physicians became more aware of homocysteine as a potential modifiable CVD risk factor. Norway has a relatively high rate of clinical tHcy testing, which may be linked to recommendations of tHcy-lowering therapy and lifestyle changes, and this may partly explain why the mean tHcy concentration did not increase in the younger subjects.

Combinations of folic acid, vitamin B-6, and vitamin B-12 supplements have a tHcy-lowering effect, and this effect is greatest among subjects with high blood tHcy concentrations or low blood folate concentrations before treatment (25, 26). Supplementation with folic acid at doses ≥0.5 mg reduces blood tHcy concentrations ≈25%, which corresponds to an absolute reduction of ≈3–4 μmol/L in a population with a mean tHcy concentration of 12 μmol/L (25). In the present study, the magnitude of the changes in tHcy among the subjects who had started to take vitamins between the 2 surveys was lower than that reported in clinical trials on vitamin supplementation (25, 26). This is not unexpected because the dose and exact timing of supplement use among our study participants was unknown; the subjects may have taken supplements at irregular intervals, and most Norwegian multivitamin and B vitamin supplements did not contain folic acid until after 1997, after which they contained 0.1–0.2 mg. Despite the low folic acid dose, the use of vitamin supplements was significantly related to changes in tHcy concentration in the present study. In the older group, who had an overall median increase of ≈0.4 μmol/L, the only subjects in whom tHcy concentrations decreased were those who did not take vitamin supplements at baseline but did at follow-up. Likewise, among the younger subjects, in whom tHcy concentrations generally decreased, one of the few subgroups that showed an increase in tHcy concentration was the group that stopped taking vitamin supplements between the 2 surveys (increase of ≈0.1 μmol/L). In general, each additional day per week of vitamin supplementation decreased tHcy concentrations by ≈0.1 μmol/L. Each unit of increase in plasma folate (nmol/L) and vitamin B-12 (pmol/L) was associated with a reduction in tHcy concentration of 0.2 and 0.1 μmol/L, respectively.

Body weight was also a significant factor influencing tHcy in the present study. An inverse association between gain in body weight and change in tHcy concentration was seen, even after adjustment for vitamin status, smoking, and coffee consumption. The mechanism by which weight affects tHcy is certainly difficult to disentangle, because various factors with partly opposite effects may be involved. These include alterations in vitamin B status by diet or physical activity (27, 28), insulin effects (29, 30), and decreased creatinine synthesis and homocysteine formation by reduction in muscle mass (24, 31–33).

Several studies showed that smoking is associated with elevated tHcy concentrations and that there is a strong dose-response relation between the number of cigarettes smoked and tHcy concentrations (12, 23, 34–36). We found that among the younger subjects who quit smoking, tHcy concentrations decreased 0.5 μmol/L. However, in the older subjects who quit smoking, tHcy concentrations increased 0.8 μmol/L. In this age group, the cessation of smoking may be related to deteriorating health status, which is known to be a determinant of tHcy (37). Thus, the unexpected increment in tHcy concentrations between the younger and older subjects who quit smoking may be due to a relatively high prevalence of CVD or hypertension (60.6% in the men and 45.8% in the women). On the other hand, the older subjects who started smoking or continued smoking during the follow-up period had significantly higher increases in tHcy concentration than did nonsmokers at both time points; this is in agreement with the known adverse effect of smoking on tHcy.

The association between tHcy and coffee consumption was first shown in the Hordaland homocysteine cohort (13) and was later confirmed in both observational (23, 35) and intervention studies (38–40). The effect is reversible, because abstention from coffee causes a reduction in tHcy concentrations (38–40). In the present study, the subjects who stopped drinking coffee had a decrease in tHcy concentration of 0.3 μmol/L, whereas much larger decreases in tHcy concentration of 1.1–1.5 μmol/L were detected in intervention studies (38–40). The long follow-up time and the lack of information about when the subjects stopped drinking coffee could explain the lower reduction in tHcy concentration in our study. The subjects in our study who stopped drinking coffee might also have already had low coffee consumption. In addition, changes in such a lifestyle habit may be secondary to incident disease, which may disturb true associations between lifestyle and tHcy concentrations.

Both preexisting CVD or hypertension at baseline and new diagnoses of CVD or hypertension during follow-up were related to a modest elevation of tHcy concentrations, which confirms the widely accepted associations between CVD and tHcy (1, 3, 41, 42). However, whether elevated tHcy concentrations are a cause rather than a consequence of CVD and subclinical nephrosclerosis is still unclear (43). Homocysteine may not be a CVD risk factor in healthy subjects but may provoke the acute event only in patients with underlying vascular dysfunction (44, 45).

In conclusion, in this population-based, 6-y follow-up study, we found that median tHcy concentrations increased in older adults and decreased slightly in middle-aged adults. The strongest lifestyle-associated determinants of changes in tHcy concentration over time were improved plasma folate and vitamin B-12 status and the onset of B vitamin or multivitamin supplement use. Changes in weight and other lifestyle factors, such as smoking and coffee consumption, had weaker associations with changes in tHcy concentration. Although the overall changes in tHcy concentration were modest in comparison with the effects of health behaviors observed in cross-sectional studies, our study suggests that changes in lifestyle affect tHcy concentrations. Changing to a lifestyle characterized by vitamin intake and nonsmoking leads to a significant reduction in tHcy concentration over a 6-y period.

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EN was responsible for the statistical data analysis and for writing the first draft of the manuscript. GST, SEV, HR, and PMU participated in the study design and the organization of data collection. SEV and RMN contributed to the statistical data analysis. All authors were involved in the interpretation of the results and contributed to the study design and the writing of the paper. None of the authors had any financial conflicts of interest.

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