Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study[1–3]

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ABSTRACT We report on the location and skewness of the distribution of plasma total homocysteine (tHcy) according to lifestyle indexes in 11 941 apparently healthy participants of the Hordaland Homocysteine Study. Most subjects were in two age groups: 9165 subjects were aged 40–42 y and 2351 subjects were aged 65–67 y. The remaining 425 subjects were of intermediate ages. In multivariate analysis, sex, age, folate intake, smoking status, and coffee consumption were the strongest determinants of tHcy concentration. The combined effect of the three modifiable factors was larger than the effect from each factor alone. A lifestyle profile characterized by low folate intakes, smoking, and coffee consumption was associated with a high median tHcy concentration and a pronounced skewness toward high tHcy values. In subjects characterized by a contrasting lifestyle profile [high folate intakes, nonsmoking status, and low coffee consumption (< 1 cup/d)], tHcy values were almost normally distributed and the median concentration was 3.0–4.8 μmol/L lower. Among all 40–42-y-old subjects, the 95% reference ranges based on geometric mean tHcy concentrations were 5.1–16.5 μmol/L for women and 6.2–18.7 μmol/L for men. The corresponding ranges for subjects characterized by high folate intakes, nonsmoking status, and low or moderate coffee consumption (< 5 cups/d) were 4.8–12.8 μmol/L and 6.2–14.7 μmol/L . These findings are relevant for establishing adequate reference ranges for tHcy and emphasize folate intake, smoking status, and coffee consumption as major acquired determinants of tHcy concentration in this general population. *Am J Clin Nutr* 1998;67:263–70.

KEY WORDS Plasma total homocysteine, distribution, reference range, lifestyle, smoking, coffee consumption, vitamin supplements, Hordaland Homocysteine Study, folate intake

INTRODUCTION

The plasma total homocysteine (tHcy) concentration is ≈5–15 μmol/L in healthy subjects, but the distribution has a long tail toward higher tHcy values (1). Plasma tHcy concentrations increase with age, are higher in men than in women (2, 3), and are influenced by renal function (4). Folate and cobalamin status are important modifiable determinants of plasma tHcy in the general population (5), and negative relations between plasma tHcy and these vitamins are observed even within their established normal and subnormal concentration ranges (6, 7). The combination of impaired folate status and homozygosity for the common C677T mutation in the methylenetetrahydrofolate reductase gene (8) was recently identified as a frequent cause of high tHcy concentrations in the general population (9, 10).

In laboratory diagnostic tests, plasma tHcy concentration is emerging as a sensitive marker of cobalamin and folate deficiencies (11), and it is a strong risk factor for cardiovascular disease (12). It is also related to birth defects in pregnant women (13–15) and to cognitive impairment in the elderly (16). The diagnostic utility of tHcy in various clinical conditions makes it mandatory to establish references ranges that are relevant for the various populations under investigation. It has been advocated that reference intervals be determined in a population with adequate vitamin status (17–19). In line with this, tHcy concentrations have been reported after 3 wk of vitamin injections (18), predicted from a linear regression model by using data from vitamin supplementation trials (17), and reported only for subjects without definite or suspected deficiency of cobalamin or folate (19).

The baseline data from the Hordaland Homocysteine Study, a population-based investigation of 18 043 subjects, revealed several lifestyle factors that affect tHcy, including cigarette smoking, exercise, and coffee consumption (3, 20). In the present publication, we report on the interactions among the strongest lifestyle determinants of tHcy concentrations and their relation to the tHcy distribution.

SUBJECTS AND METHODS

Study population

The baseline data of the Hordaland Homocysteine Study cohort have been used in the present report. This cohort of 18 043 men and women aged 40–67 y was established from April 1992 to April 1993 (3). We excluded one person with homocystinuria;

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1866 participants who in the questionnaires reported a previous diagnosis of ischemic heart disease, cerebrovascular disease, hypertension, or diabetes mellitus; and 4235 subjects not answering questions on use of vitamin supplements. Thus, 11 941 subjects were included in the present investigation. Most subjects were in two main age groups: 9165 subjects were aged 40–42 y and 2351 subjects were aged 65–67 y. The remaining 425 subjects were of intermediate ages (**Table 1**). Plasma concentrations of folate and cobalamin were measured in a subsample of 329 subjects aged 40–67 y.

Data collection

Data were collected through questionnaires, clinical examinations, and blood tests (3). Information on dietary habits was obtained from the questionnaires and included information on the frequency of intake of 41 food items and of vitamin supplements. Subjects who used supplements were also asked whether they took one or more of the following types of vitamins: multivitamins, B vitamins, vitamin C, vitamin E, and vitamins A and D combined. We did not specifically ask for information on use of supplements containing folic acid or cobalamin.

In an earlier study, we found no difference in plasma tHcy between nonsmokers and former smokers (3). Smoking habits were therefore classified in four categories: nonsmoking, current light smoking (1–9 cigarettes/d), moderate smoking (10–19 cigarettes/d), and heavy smoking ($\geq$ 20 cigarettes/d). Coffee consumption was recorded in five categories according to number of cups consumed/d: 0, $<$ 1, 1–4, 5–8, and $\geq$ 9. A total of 95% of coffee drinkers drank filtered coffee (20).

Assessment of nutrient intake

On the basis of the information from the questionnaires, we created three scores: a vitamin supplement score, a folate score, and a cobalamin score. The vitamin supplement score was created according to frequency of intake as well as seasonal variations in intake of any type of vitamin supplement. The lowest of five categories included subjects who never used vitamins and the highest category represented those who took vitamins 6–7 d/wk for an entire year.

The folate score was based on two sources of folate intake and was calculated as the sum of a supplement component and a dietary component. By weighting the vitamin supplement score according to the type of vitamin used, the score was modified to specifically estimate variation in intake of folic acid from supplements. In 1992–1993, only $\approx$50% of multivitamin or B vitamin tablet preparations in Norway contained folic acid (100 μg per tablet). Liquid multivitamins or B vitamins, which were rarely used, or other types of vitamins did not contain folic acid. This modified score constituted the supplement component of the folate score. The dietary component was created as the unweighted sum of frequency of use of dietary items that in a multiple linear regression analysis best explained the variation in plasma folate in the subsample of 329 subjects. These items were fruit, vegetables, oranges, fruit juices, eggs, and meat.

Similarly, a cobalamin score was composed from dietary items that best explained the variation in plasma cobalamin. Intake of vitamin supplements was not related to cobalamin concentration. The cobalamin score included the frequency of intake of skim and semiskim milk, fresh and frozen fish, meat, and entrails. The score showed a correlation with plasma cobalamin of r = 0.40 and with plasma tHcy of r = –0.12, and the difference in mean tHcy between subjects in the highest and lowest deciles for the cobalamin score was 0.8–2.3 μmol/L in the four main age and sex groups.

The number of subjects with complete data to compute the folate score, cobalamin score, or both were 9571, 7948, and 7477, respectively. Thus, because the cobalamin score substantially decreased the number of subjects with complete data and also had a minimal influence on the various multivariate relations to tHcy, it was not included in the main analyses of this paper.

Blood sampling and biochemical determinations

Nonfasting venous blood samples were obtained with EDTA as the anticoagulant. Blood for preparation of plasma was placed in a refrigerator (4–5 °C) within 15–30 min and centrifuged for 10 min at 1010 $\times$ g at room temperature within 1–3 h. The unfrozen plasma samples were transported at room temperature to our laboratory, where they usually arrived the following day. The samples were then stored at –20 °C until analyzed. The plasma tHcy concentration, which includes both the unbound and bound fractions of Hcy, was measured by using HPLC with fluorescence detection (21, 22). The precision (between-day CV) of the assay was $<$ 3%. Plasma folate was assayed with the Quantaphase folate radioassay (Bio-Rad Laboratories, Hercules, CA). Cobalamin in plasma was measured with a microparticle

TABLE 1
Percentage distribution of vitamin supplement consumption and plasma total homocysteine (tHcy) concentration according to sex and age

Category of vitamin supplement score[1]	Total study population[2]	Age 40–42 y				Age 65–67 y			
		Men		Women		Men		Women	
		Number	tHcy	Number	tHcy	Number	tHcy	Number	tHcy
	n (%)	*n (%)*	*μmol/L*	*n (%)*	*μmol/L*	*n (%)*	*μmol/L*	*n (%)*	*μmol/L*
0	4066 (34.1)	1909 (44.8)	11.2	1225 (25.0)	9.6	450 (47.8)	12.5	345 (24.5)	11.6
1	1904 (15.9)	795 (18.7)	11.0	841 (17.1)	9.5	90 (9.6)	12.6	113 (8.0)	11.4
2	2674 (22.4)	853 (20.0)	10.5	1338 (27.3)	9.3	121 (12.9)	12.8	261 (18.5)	11.4
3	1685 (14.1)	372 (8.7)	10.1	825 (16.8)	8.6	119 (12.6)	11.9	310 (22.0)	11.0
4	1612 (13.5)	331 (7.8)	10.0	676 (13.8)	8.3	161 (17.1)	11.0	381 (27.0)	10.2
Total	11941	4260	10.8	4905	9.1	941	12.2	1410	11.0

[1]This variable is defined in the Methods; category 0 included subjects who never used vitamin supplements and category 4 included subjects who used supplements 6–7 d/wk during an entire year.

[2]The total study population included 425 subjects aged 43–64 y who are not included in this table.

enzyme–intrinsic factor assay run on an IMx system (Abbott Laboratories, Abbott Park, IL).

Statistical analyses

The frequency distributions of tHcy or cobalamin concentrations were positively skewed. To better satisfy the assumption of a normally distributed dependent variable, multiple linear regression analyses and correlations were carried out with the logarithm (base 10) of these variables. Thus, geometric mean concentrations of tHcy and cobalamin are presented unless stated otherwise. Various percentiles of tHcy were estimated in analyses stratified according to the strongest predictors of mean tHcy concentration in multivariate analysis: sex, age, folate score, smoking habit, and coffee consumption. The dose response between the folate score and tHcy was estimated by using generalized additive regression (23) as implemented in S-PLUS (24). The values of tHcy used in this analysis were not logarithmically transformed. Percentiles, 95% CIs for the median, and the density function of the tHcy distribution were also estimated with S-PLUS, whereas all other analyses were performed with BMDP (25). Tests were two-tailed, and a P value < 0.05 was considered significant.

RESULTS

Population characteristics and vitamin intake

The vitamin supplement score contained information on the frequency and seasonal use of any type of vitamin supplement. Overall, 34.1% of subjects (45% of men and 25% of women) reported no use of supplements (group 0), whereas 13.5% of subjects (9.5% of men and 16.8% of women) reported daily use throughout the entire year (group 4) (Table 1). A total of 53.8% of the subjects who took vitamins used multivitamins, 28.8% took B vitamins, 34.4% took vitamin C, 13.7% took vitamin E, and 19.7% took combined vitamin A and D preparations.

Overall, intake of vitamin supplements was a strong determinant of plasma tHcy concentration (Table 1). In the four main sex and age groups, plasma tHcy concentrations in individuals who used supplements on a daily basis were 1.2–1.5 μmol/L lower than in nonusers. The relation between intake of vitamin supplements and tHcy was limited to users of multivitamins or B vitamin tablets, which in Norway are the only vitamin preparations that contain folic acid. The difference in the mean tHcy concentration between regular users and nonusers of multivitamins was 1.5 μmol/L in both sexes in the 40–42-y-old group. In the 65–67-y-old group, this difference was 2.0 μmol/L for women and 2.2 μmol/L for men. Intake of vitamin supplements was also strongly associated with lifestyle (**Table 2**). The associations were stronger in men than in women and stronger in the younger than in the older group.

Validation of the folate score

The folate score was validated against, and showed a strong relation to, plasma folate in a subsample of 329 subjects (**Table 3**). Notably, the supplement component showed a much stronger relation to plasma folate than the dietary component; in a model with age, sex, and both components, their partial correlations with folate were 0.39 and 0.10, respectively.

The folate score showed a much stronger association with plasma folate than with tHcy, whereas sex, age, and smoking were more strongly related to tHcy (Table 3). None of these variables were significantly related to plasma cobalamin ($r < 0.05$). With sex, age, and smoking in the model, additional adjustment for the folate score only weakly changed the relations of these variables to plasma tHcy. Notably, adjustment with plasma folate instead of the folate score gave similar results. With the exception of coffee, both crude and adjusted relations to tHcy were of similar magnitude in the subsample and main sample.

TABLE 2
Selected characteristics of men and women aged 40–42 y according to consumption of vitamin supplements

Characteristics	Category of vitamin supplement score[1]					P for trend
	0	1	2	3	4	
			%			
Men						
Current smokers	41.3	43.1	33.8	33.3	31.8	<0.001
Active exercise[2]	27.1	29.4	31.3	31.5	32.6	0.005
Consumption of ≥ 5 cups of coffee/d	52.7	51.9	45.0	44.5	40.2	<0.001
Daily consumption of fruit and vegetables	19.9	21.3	25.7	26.7	32.7	<0.001
High education[3]	32.4	36.8	44.4	46.2	45.0	<0.001
Women						
Current smokers	37.9	40.3	35.5	35.9	36.8	0.20
Active exercise[2]	10.4	12.1	10.9	14.8	14.9	0.001
Consumption of ≥ 5 cups of coffee/d	39.9	41.3	38.3	34.3	31.1	<0.001
Daily consumption of fruit and vegetables	39.7	43.5	41.3	51.1	50.7	<0.001
High education[3]	26.5	25.2	29.0	28.9	31.0	0.01

[1] The definition of this score and the number of subjects in each category is given in Table 1.
[2] Defined as exercise, gardening with physical exertion, or similar degree of physical activity for ≥ 4 h/wk, or regular heavy training or competitive sport.
[3] Defined as college or university graduate.

TABLE 3
Correlations of selected variables with plasma folate and plasma total homocysteine (tHcy) in a subsample of men and women aged 40–67 y and with tHcy in the total study population[1]

	Subsample (n = 329)				tHcy in total population (n = 11941)	
	Folate		tHcy			
Variables	Crude	Adjusted[2]	Crude	Adjusted[3]	Crude	Adjusted[4]
Sex (1 = M, 2 = F)	0.17[5]	0.16[5]	−0.25[5]	−0.21[5]	−0.24[5]	0.22[5]
Age (40–67 y)	−0.04	−0.06	0.18[5]	0.23[5]	0.20[5]	0.23[5]
Smoking habit (4 categories)	−0.16[5]	−0.09	0.23[5]	0 21[5]	0.17[5]	0.15[5]
Coffee consumption (5 categories)	−0.07	0.03	0.09	−0.01	0.16[5]	0.10[5]
Folate score	0.45[5]	0.41[5]	−0.21[5]	−0.17[5]	−0.20[5]	−0.17[5]

[1] Log_{10} concentrations of tHcy were used in the analyses. The folate score is defined in the Methods. In the subsample, 326 subjects had data on smoking habits, 324 on plasma folate, and 200 on the folate score.
[2] Adjusted for sex, age, and the folate score and included 197 subjects.
[3] Adjusted for sex, age, smoking habit, coffee consumption, plasma cobalamin, and plasma folate and included 321 subjects.
[4] Adjusted for sex, age, smoking habit, coffee consumption, and the folate score and included 9530 subjects.
[5] $P < 0.05$. All other correlations are $P \geq 0.12$.

Folate intake and tHcy

Overall, there was a marked dose-response relation between the folate score and plasma tHcy. The dose-response curve showed an initial steep slope at low folate scores followed by a slight decrease at higher values (**Figure 1**, lower panel). Comparison with the frequency distribution curve (Figure 1, middle panel) suggests that the marked variability at low folate scores was related to marginal folate status among the 15% of subjects eating fruit and vegetables < 4 d/wk and taking no folate supplements. The additional moderate decline in tHcy at high folate scores concurred with folic acid supplementation in subjects with daily intake of fruit and vegetables.

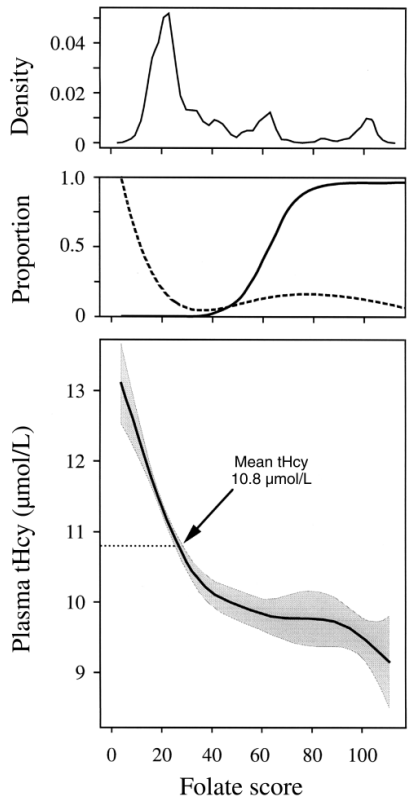

FIGURE 1. Relations to the folate score. The upper panel shows the probability density function of the folate score (the distribution of folate intake). The middle panel displays the proportions of subjects with intakes of fruit and vegetables ≤ 3 d/wk (dashed line) and intakes of multivitamin or B vitamins ≥ 2 d/wk (solid line) in an entire year. The bottom panel shows the dose-response relation between the folate score and plasma total homocysteine (tHcy); the solid line indicates the estimated dose-response curve and the outer margins of the shaded area indicate 95% CIs. The mean tHcy noted indicates the arithmetic mean of tHcy.

The mean folate score was significantly higher in women than in men, and higher in the older than in the younger group (both $P < 0.001$). In the 40–42-y-old group, the difference in tHcy between its highest and lowest decile was 1.7 μmol/L in men and 1.8 μmol/L in women. In the 65–67-y-old group, the corresponding differences were 2.4 and 2.7 μmol/L, respectively.

Combined effect of folate intake, coffee consumption, and smoking habit

The large number of tHcy measurements in the 40–42-y-old group allowed us to study the shape and location of the tHcy distribution in relation to each of the three lifestyle factors (**Table 4**). To achieve adequate numbers in subgroups, we defined high and low folate intake as the upper and lower quintile of the folate score, respectively. In women, we noted a dramatic variation in the 97.5% percentile from 11.4 μmol/L in those with a lifestyle profile characterized by no smoking, consumption of < 1 cup of coffee/d, and high folate intake, to 34.1 μmol/L in those with a contrasting lifestyle (smoking, consuming ≥ 5 cups of coffee/d, and low folate intake). Similar patterns were also seen at the other percentiles. At all levels of coffee consumption and smoking habits and for all percentiles, there was a clear shift toward lower tHcy values by folate intake. Similar relations were seen in men although they were less clear at or above the 95th percentile.

Notably, the combined effect of the folate score, smoking habit, and use of coffee was strong, and the difference in median tHcy between subjects with contrasting lifestyles was 3.2 μmol/L in men and 3.4 μmol/L in women in the younger group. Corresponding differences in the 65–67-y-old group were 3.0 μmol/L in men and 4.8 μmol/L in women.

Reference ranges of tHcy

Plasma tHcy values showed a skewed, right-tailed distribution in subjects with a lifestyle characterized by smoking, coffee drinking,

TABLE 4
Different percentiles of plasma total homocysteine (tHcy) in 40–42-y-old women and men according to vitamin intake, coffee consumption, and smoking habit[1]

Characteristic	tHcy percentiles									
	n	2.5	5	10	25	50	75	90	95	97.5
					μmol/L					
Women										
All subjects	4125	5.6	6.1	6.6	7.5	8.9	10.6	12.8	14.6	17.4
0 cigarettes/d, <1 cup coffee/d										
High folate score	123	4.7	5.0	5.4	6.3	7.2	8.4	9.8	10.2	11.4
Medium folate score	265	5.3	5.6	6.1	6.9	7.9	9.2	10.9	12.5	14.4
Low folate score	36	6.1	6.2	6.5	6.9	8.4	9.6	11.5	12.3	16.2
0 cigarettes/d, 1-4 cups coffee/d										
High folate score	377	5.3	5.5	6.1	6.8	8.0	9.2	11.1	12.1	12.9
Medium folate score	942	5.9	6.3	6.7	7.6	8.7	10.1	12.3	13.7	15.2
Low folate score	161	5.5	6.0	6.8	7.6	8.9	10.6	12.2	13.8	15.9
0 cigarettes/d, ≥5 cups coffee/d										
High folate score	151	5.7	6.0	6.3	7.2	8.2	9.5	10.9	12.1	13.5
Medium folate score	480	5.8	6.3	6.8	7.7	8.9	10.7	12.8	14.2	16.5
Low folate score	97	6.1	6.5	7.5	8.3	9.7	11.7	13.6	15.2	15.8
≥1 cigarette/d, ≤4 cups coffee/d										
High folate score	182	5.5	5.9	6.5	7.3	8.6	10.4	12.7	15.5	17.5
Medium folate score	402	5.9	6.6	7.0	7.9	9.5	11.4	14.0	16.9	26.6
Low folate score	105	6.1	6.7	6.9	7.8	9.5	11.8	14.8	17.3	18.7
≥1 cigarette/d, ≥5 cups coffee/d										
High folate score	152	5.8	6.0	6.6	7.7	9.0	11.0	13.0	13.6	14.4
Medium folate score	499	6.4	7.0	7.6	8.6	10.2	11.7	14.6	17.3	20.2
Low folate score	153	6.7	7.0	7.3	8.9	10.4	12.1	17.8	26.0	34.1
Men										
All subjects	3567	6.9	7.5	8.0	9.2	10.5	12.3	14.7	17.0	20.9
0 cigarettes/d, <1 cup coffee/d										
High folate score	56	6.3	6.6	6.9	8.0	9.0	10.3	11.4	12.8	13.1
Medium folate score	190	6.7	6.9	7.4	8.3	9.7	11.3	13.1	15.6	22.0
Low folate score	86	7.0	7.3	8.4	9.5	10.1	11.7	15.9	21.5	29.8
0 cigarettes/d, 1-4 cups coffee/d										
High folate score	154	6.3	6.9	7.5	8.4	9.8	11.1	12.7	13.5	14.7
Medium folate score	616	6.8	7.4	8.0	9.1	10.3	11.9	13.7	15.6	17.2
Low folate score	262	7.2	7.5	8.1	9.1	10.2	12.3	14.4	17.7	21.5
0 cigarettes/d, ≥5 cups coffee/d										
High folate score	105	6.8	7.2	7.9	8.6	10.4	11.1	12.5	12.9	15.1
Medium folate score	517	7.0	7.5	8.1	9.2	10.5	12.2	14.5	17.1	21.1
Low folate score	225	7.6	8.0	8.7	9.5	10.8	12.3	15.0	16.2	17.0
≥1 cigarette/d, ≤4 cups coffee/d										
High folate score	49	6.7	7.1	7.8	9.0	9.9	11.8	14.4	15.4	15.9
Medium folate score	269	7.2	7.6	8.0	9.0	10.5	12.2	14.4	17.2	31.0
Low folate score	135	7.0	7.7	8.2	9.2	10.1	12.1	14.2	17.1	21.6
≥1 cigarette/d, ≥5 cups coffee/d										
High folate score	71	7.3	7.5	7.5	9.5	10.4	13.0	14.6	16.5	17.6
Medium folate score	477	7.3	8.0	8.5	9.7	11.1	13.2	15.8	18.2	20.6
Low folate score	355	7.3	7.8	8.5	10.1	11.5	13.7	16.7	20.3	26.1

[1] High, medium, and low folate scores were defined as the upper, middle three, and lower quintile of this score, respectively. The score is defined in the Methods.

and low folate intake, compared with a left-shifted, almost symmetric distribution in subjects with opposite characteristics (**Figure 2**). This marked difference in tHcy distribution according to lifestyle is also illustrated in the calculated 95% reference ranges ($\bar{x}$ ± 1.96 SD) (**Table 5**). Compared with the overall value, the upper reference limit was 4–6 μmol/L lower in the subpopulation who did not smoke, drank < 5 cups of coffee/d, and had the highest folate intake. The upper limits were an additional 1 μmol/L lower if the analysis was further confined to individuals who drank < 1 cup of coffee/d (Table 4). There was good agreement between the geometric mean and direct percentile estimation (Table 5).

DISCUSSION

We found that folate intake, coffee consumption, and smoking are major lifestyle determinants of the plasma tHcy distribution

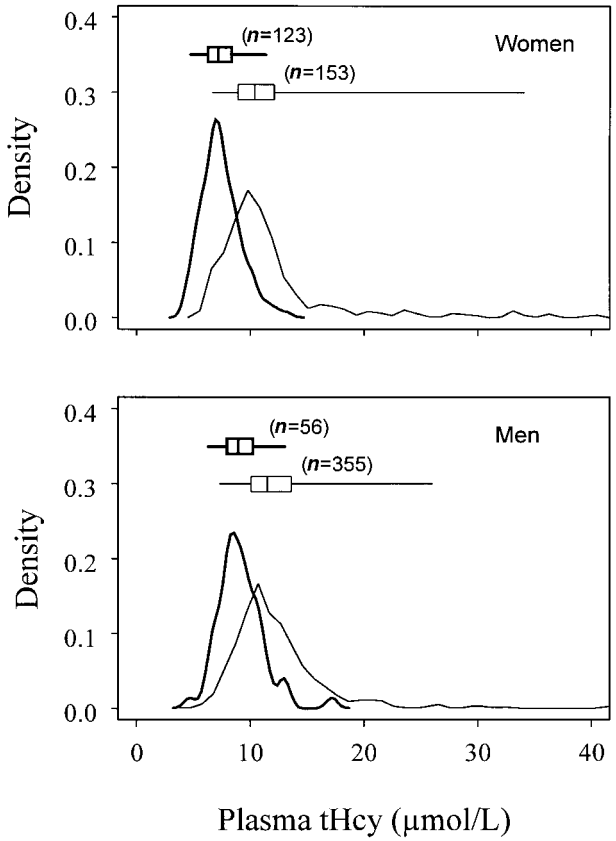

FIGURE 2. Distribution of plasma total homocysteine (tHcy) according to contrasting lifestyle groups in 40–42-y-old women (top) and men (bottom). Estimated density functions (continuous curves) are shown at the lower part of each figure and the same data are presented as box plots at the top of the figures. The boxes show the 25th to 75th percentile intervals, the lines inside the boxes are median values, and the ends of the whiskers indicate the 2.5th and 97.5th percentiles. The curve in boldface represents nonsmokers with high folate intakes and consumption of < 1 cup of coffee/d, whereas the thin line represents smokers drinking ≥ 5 cups of coffee/d and with low folate intakes. The various percentiles of tHcy for these groups are shown in Table 4.

in a large, apparently healthy population. In all four main age and sex groups of 40–42-y-old and 65–67-y-old men and women, plasma tHcy showed a skewed distribution with a tail toward high values. We found previously that coffee consumption and smoking are both associated with high mean tHcy concentrations, but have a differential effect on the tHcy distribution curve. Coffee consumption showed a particularly strong relation to tHcy at low and intermediate tHcy concentrations, whereas smoking also was associated with an in increase in the fraction with high values, thereby accentuating the skewness of the distribution curve (3, 20). In the present study, we showed that low folate intake was also associated with an increased proportion of subjects with high tHcy concentrations, and thus had an effect similar to that of smoking. Notably, both coffee consumption and smoking were related to tHcy at low as well as high folate intakes, and the combined effect of low folate intake, smoking, and coffee consumption greatly exceeded the effect from each factor alone. This is illustrated by the pronounced difference in tHcy (3.0–4.8 μmol/L) between subjects with contrasting lifestyle profiles with respect to smoking, coffee consumption, and folate intake. Our findings may indicate a different mechanism of action of these determinants.

We created scores to estimate variation in folate or cobalamin status that were strongly related to the corresponding plasma vitamin concentration in a subsample of 329 subjects. Notably, in the older group, we found a difference in mean tHcy between the highest and lowest decile of folate score (3.4 μmol/L) that was almost identical to the difference found in an elderly American population by Selhub et al [3.3 μmol/L (7)]. The difference in the mean tHcy concentration between the highest and lowest decile of cobalamin score was higher in our elderly subjects (2.5 μmol/L) than in elderly Americans (7). These data suggest that our vitamin scores were reliable measures of folate or cobalamin status.

There are no published data on folate intake in Norwegian populations. However, a study in 1989 from neighboring Sweden reported a median folate intake of 187 μg/d in women and 218 μg/d in men (26). These values may represent marginal intakes because a recent study showed that 200 μg/d was not sufficient for maintaining folate status or low plasma tHcy concentrations in most subjects (27, 28). We found 1.5–2.2-μmol/L lower tHcy concentrations in regular users compared with nonusers of multivitamin preparations, which in Norway contain ≤ 3.5 mg vitamin B-6 and 8 μg cobalamin with or without 100 μg folic acid. Intake of supplements that contain vitamin B-6 and cobalamin but not folic acid was unrelated to tHcy concentrations, which agrees with the findings of previous studies (10, 29–31).

As shown in earlier investigations (7, 32), there was a distinct dose-response relation between folate intake and plasma tHcy concentration. The largest variation in plasma tHcy was observed in subjects with low intakes of dietary folate, mostly determined by fruit and vegetable intake. Interestingly, low-dose folic acid supplements were associated with significantly lower tHcy concentrations even in subjects with the highest intake of dietary folate. These data therefore indicate that dietary folate intake was too low to ensure optimal Hcy metabolism in our population. A recent study also showed that folic acid in supplements is more effective than natural food folate in improving folate status (33).

A subgroup of the Hordaland population aged 40–42 y who did not smoke or consume coffee daily and who had the highest folate intakes had an almost normal tHcy distribution, with a low reference range of 4.7–11.4 μmol/L for women and 6.3–13.1 μmol/L for men (2.5–97.5 percentile, Table 4). Selection of individuals on the basis of these determinants may be both a convenient and adequate procedure for obtaining sex- and age-related reference ranges. Alternative strategies have been proposed, including selection of reference subjects without high tHcy or with adequate vitamin status (17–19). The reference ranges that resulted from use of these strategies were lower than those obtained in the present study, but a notable feature in common is an almost normal tHcy distribution and a marked shift toward lower tHcy concentrations compared with the values in the respective total populations (17–19).

Results from previous case-control and prospective studies show that a 5-μmol/L increment in tHcy may be associated with a ≥ 50% increase in risk of arteriosclerotic vascular disease (12). Recently, we found an equally strong dose-response relation between tHcy and mortality in patients with coronary artery disease (34). Thus, the differences in plasma tHcy that we observed according to contrasting lifestyle profiles may be of clinical relevance.

TABLE 5
Different measures of plasma total homocysteine (tHcy) with corresponding reference ranges[1]

Study group and method	Total subjects	Nonsmokers, <5 cups coffee/d, high folate score[2]
	μmol/L	
Men 40–42 y (*n* = 4260, 210)[3]		
Arithmetic	11.3 (2.9–19.7)	9.8 (5.4–14.1)
Geometric	10.8 (6.2–18.7)	9.5 (6.2–14.7)
Nonparametric	10.5 (6.9–20.9)	9.6 (6.2–14.5)
Women 40–42 y (*n* = 4905, 500)		
Arithmetic	9.6 (1.7–17.6)	8.1 (3.6–12.5)
Geometric	9.1 (5.1–16.5)	7.8 (4.8–12.8)
Nonparametric	8.9 (5.6–17.4)	7.8 (5.0–12.6)
Men 65–67 y (*n* = 941, 93)		
Arithmetic	12.9 (0–26.1)	10.9 (4.6–17.2)
Geometric	12.2 (6.9–21.6)	10.5 (6.4–17.3)
Nonparametric	11.9 (8.0–21.5)	10.2 (7.2–17.8)
Women 65–67 y (*n* = 1410, 214)		
Arithmetic	11.6 (2.4–20.8)	10.1 (4.4–15.7)
Geometric	11.0 (7.9–20.0)	9.8 (6.0–16.0)
Nonparametric	10.7 (6.7–21.0)	9.6 (6.4–16.7)

[1] $\bar{x}$ or median; reference range in parentheses. Arithmetic reference range is arithmetic mean ± 1.96 SD, geometric reference range is antilog (mean log-tHcy ± 1.96 SD), and nonparametric reference range is the 2.5–97.5th percentiles.

[2] High folate score is upper quintile of this score, which is defined in the Methods.

[3] *n* is for total subjects and for nonsmokers consuming < 5 cups of coffee/d and with high folate score, respectively.

In conclusion, we showed that lifestyle factors such as coffee consumption, smoking habit, and folate intake all contribute to the location and skewness of the tHcy distribution. These findings not only should have implications for the selection of reference populations and definition of reference ranges, but also should support established recommendations for a cardioprotective lifestyle.

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