Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women[1–3]

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ABSTRACT

Background: An elevated total homocysteine (tHcy) concentration is considered to be an independent risk factor for cardiovascular diseases and has also been associated with an increased risk of neural tube defects.

Objective: The objective of this study was to investigate folate intake, folate status, and the association between folate intake, other dietary and lifestyle factors, and tHcy concentrations in young and older women.

Design: tHcy concentrations were measured in 290 young women aged 25–30 y and in 288 older women aged 60–65 y. All participants completed questionnaires about factors including lifestyle, health, and use of vitamin supplements. Red blood cell folate was measured in 204 of the participants. A subgroup of 258 participants completed dietary records.

Results: Median tHcy was 7.6 μmol/L (range: 6.5–8.9) in the younger women and 9.4 μmol/L (7.7–11.1) in the older women. Folate intake from diet was 283 (224–348) and 268 (210–326) μg/d, respectively, in the 2 age groups. Folic acid intake from supplements ($P < 0.001$ for the younger women and $P = 0.026$ for the older women) and total folate intake ($P = 0.024$ and $P = 0.079$) were inversely associated with log tHcy in multiple linear regression analyses. Smoking status, coffee consumption, systolic blood pressure, and body mass index were positively associated and estrogen replacement therapy and tea consumption were inversely associated with log tHcy in some of the models.

Conclusions: According to the criteria used, between 1% and 36% of the women had suboptimal folate intake. Folic acid is a strong predictor of tHcy concentration; however, several dietary and other lifestyle factors seem to be important as well. *Am J Clin Nutr* 2000;72:1156–63.

KEY WORDS Homocysteine, women, folate intake, folic acid, lifestyle, vitamin supplements, observational study

INTRODUCTION

Homocysteine has attracted much interest during recent years because an elevated total homocysteine (tHcy) concentration is thought to be an independent risk factor for cardiovascular diseases (1). Various dietary and lifestyle factors can influence tHcy concentrations (2–5). A high folate intake from the diet or supplemental folic acid was associated with a decreased tHcy concentration in intervention (6, 7) and cross-sectional (8) studies. Intakes and blood concentrations of vitamin B-6 and blood concentrations of vitamin B-12 were found to be negatively associated with tHcy concentrations (4, 5). These associations are consistent with homocysteine metabolism. Homocysteine is formed in a series of reactions in which methionine serves as the source of methyl and homocysteine is remethylated mainly by a reaction that requires folate and vitamin B-12 (as a coenzyme), or it can be converted to cysteine in a series of vitamin B-6–dependent reactions (9).

Protein intake was also found to be inversely associated with tHcy concentration (3), whereas a positive association was shown for coffee consumption (3, 8, 10) and smoking status (8). Also, tHcy concentrations increase with age and are higher in males than in females (4, 11, 12). Associations between dietary or lifestyle factors and tHcy have been investigated mainly in elderly or middle-aged persons.

Elevated tHcy concentrations are associated with cognitive impairment in older persons (13) and in pregnant women with neural tube defects in the fetus (14). Therefore, a good folate status is important for both young women and elderly persons.

Folate status can be assessed by measuring plasma folate, red blood cell folate, or tHcy concentrations (15, 16), which are measurements normally found to be intercorrelated (4, 17). There are limitations in the use of all 3 measures. Plasma folate reflects the most recent folate intake (15) and red blood cell folate the long-term intake, but false high or low measurements are not uncommon (18). The concentration of tHcy is, as mentioned above, influenced by several factors other than folate intake.

The primary aim of this study was to investigate dietary folate intakes, use of folic acid supplements, red blood cell folate, and tHcy concentrations in a group of fertile women

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(aged 25–30 y) and a group of older women (aged 60–65 y). A further aim was to investigate the association of folate intake and other dietary and lifestyle factors with tHcy concentrations in younger and older women.

SUBJECTS AND METHODS

The data presented in this article resulted from analyses of baseline data collected from an ongoing study, the Danish Investigation of Iodine Intake and Thyroid Diseases (DanThyr), in the period March to June 1998. A total of 4649 persons, mostly women, in different age groups spanning the interval 18–65 y were examined in 2 cities in Denmark (Copenhagen and Aalborg). Participants were randomly selected from the civil registration system, in which all inhabitants in Denmark are registered. The study was approved by the Regional Ethical Committees of Copenhagen and Northern Jutland. All participants provided written, informed consent.

For the present study, women aged 25–30 y (young group) and 60–65 y (older group) were selected. All women in the diet subgroup and women taking part in DanThyr during the last 3 mo of the study gave extra blood for homocysteine analyses. tHcy was measured in 308 young and 291 elderly women (total $n = 599$). Plasma methylmalonate was measured if the tHcy concentration was >15 μmol/L, to exclude cobalamin deficiency. Four subjects (1 young and 3 older) were found to have a high probability of cobalamin deficiency according to the criteria of Nexø et al (19) (tHcy >15 μmol/L and methylmalonate > 0.45 μmol/L). These subjects were excluded from further analysis. Seventeen participants were pregnant and were excluded as well. Red blood cell folate was measured in 112 young and 79 elderly participants from one of the cities only (Aalborg).

All participants completed questionnaires that gave information about smoking habits (current smoker, former smoker, and number of cigarettes smoked daily), alcohol consumption (weekly intake of beer, wine, and liquor), level of physical activity in leisure time (level 1: mainly sedentary; level 2: 2–4 h light physical activity weekly; level 3: >4 h light or 2–4 h strenuous physical activity weekly; level 4: strenuous physical activity >4 h weekly), coffee and tea consumption, and use of medications. The subjects' heights and weights were measured while they wore no shoes. Blood pressure was determined with a mercury manometer while the participants were in a sitting position after being allowed 5 min rest. Participants were asked to bring all their vitamin supplements and medications with them, and brand names, doses, and how often the subjects used the supplements were recorded. Fewer than 5% of the subjects forgot to bring the supplements and medications and were interviewed about present use. If a subject did not remember the brand name of the multivitamin tablets used, the folic acid content was set at 100 μg, the vitamin B-6 content at 2 mg, and the vitamin B-12 content at 1 μg/daily dose, because these were the most common amounts used during the study period. Likewise, the amount of folic acid in supplements containing only folic acid was set at 200 μg/daily dose.

Diet subgroup

Women in the age groups 25–30 and 60–65 y who participated in DanThyr during the last 10 mo of the survey were asked to keep dietary records. Of the 417 participants who initially agreed to keep dietary records, 313 (75%) completed useful dietary records; of these, 14 were excluded because they were pregnant. The participants were asked to weigh and record all food and drink consumed during 4 consecutive days comprising 3 weekdays and 1 weekend day starting on Wednesday or Sunday. The participants were given oral and written information on how to keep the dietary records and digital weights were supplied. Thirty-one participants with cutoff values of reported energy intake divided by estimated basal metabolic rate < 1.06 (20) were excluded because of underreporting. To further validate the quality of the dietary records, 24-h urine samples were collected from 156 of the participants on the fourth day of dietary recording for the measurement of nitrogen excretion. Nitrogen excretion was measured only in complete 24-h urine samples validated with *p*-aminobenzoic acid (PABA). Eighty milligrams was taken orally 3 times—at 0800, 1200, and 1800—and urine samples with a PABA content below an earlier established cutoff point of 187 mg were rejected (21). The participants were asked to record on a form the time of the start and finish of the urine collection, the time at which they took the PABA tablets, the number of lost specimens, and the medications used during the urine-collection period. Thirty-six of the 24-h urine samples were rejected because of incomplete collection. Nitrogen excretion in urine was converted to protein excretion by adding 2 g nitrogen from extrarenal losses and multiplying by 6.25 (22). Mean protein excretion did not differ significantly from protein intake calculated from dietary records, indicating no systematic underreporting in these participants. Dietary intake was calculated with use of a computer database based on the Danish Food Database (Dankost 2000; Danish Catering Center, Copenhagen).

Specimens

Nonfasting blood samples were taken from an antecubital vein before 1400. Plasma samples for measurements of homocysteine and methylmalonic acid were collected in heparin-containing tubes to which sodium fluoride was added to a final concentration of 4 g/L blood according to the procedure described previously (23). Plasma was separated by centrifugation at 3000 × *g* for 10 min at 4°C within 2 h of collection and stored at −20°C until analyzed. Blood samples for red blood cell folate measurement were collected in glass tubes containing EDTA and ascorbic acid and analyzed within 24 h. Urine was stored cold and was received at the laboratory within 2 d after collection. Volumes were estimated by weight (specific gravity = 1 kg/L) and aliquots of 5 mL were stored at −20°C until analyzed.

Assays

tHcy and methylmalonic acid in plasma were measured by using stable-isotope dilution with solid-phase extraction of the sample (24). Red blood cell folate concentrations were measured with use of a radioassay (ICN Pharmaceuticals, New York). Urine samples were analyzed for PABA by HPLC as described by Jakobsen et al (21). Nitrogen in urine was analyzed by using the Kjeldahl method (Tecator; Perstorp Analytic, Bristol, United Kingdom).

Statistics

Results are expressed as medians with 25th to 75th percentiles or as geometric means with 95% CIs. The Mann-Whitney *U* test was used to compare variables between the 2 age groups and the chi-square test was used to compare dichotomous

TABLE 1
Total plasma homocysteine, red blood cell folate, and other characteristics of all study participants and the diet subgroup

	All participants		Diet subgroup	
	25–30 y (n = 290)	60–65 y (n = 288)	25–30 y (n = 145)	60–65 y (n = 113)
Homocysteine (μmol/L)	7.6 (6.5, 8.9)[1]	9.4 (7.7, 11.1)[2]	7.5 (6.6, 8.7)	9.3 (7.5, 10.5)[2]
Red blood cell folate (nmol/L)[3]	648 (471, 840)	936 (703, 1151)[2]	676 (491, 843)	959 (779, 1182)[2]
Use of folic acid–containing supplements (% of subjects)	36.2	44.2	37.1	46.0
Use of vitamin B-6–containing supplements (% of subjects)	41.0	56.9[2]	42.8	61.1
Use of vitamin B-12–containing supplements (% of subjects)	37.3	51.0[2]	40.0	53.1
Smokers (%)	34.1	35.1	22.8	34.5
No. of cigarettes smoked/d	15.0 (7.5, 20.0)	14.0 (8.0, 20.0)	15.0 (5.0, 20.0)	10.0 (8.0, 20.0)
BMI (kg/m^2)	22.9 (20.9, 24.9)	26.4 (23.6, 29.8)[2]	22.6 (20.9, 24.9)	25.8 (23.2, 29.0)[2]
Diastolic blood pressure (mm Hg)	80 (70, 81)	90 (80, 95)[2]	80 (70, 85)	85 (80, 95)[2]
Systolic blood pressure (mm Hg)	120 (110, 125)	150 (135, 165)[2]	120 (115, 125)	145 (135-165)[2]
Estrogen use (% of subjects)[4]	36.2	26.0[5]	34.5	31.9
Coffee intake (cups/d)	0.7 (0, 3.5)	3.5 (1.5, 5.5)[2]	0.28 (0, 3.5)	3.5 (1.5, 5.5)[2]
Tea intake (mugs/d)	0.7 (0.1, 1.5)	0.3 (0.0, 1.5)	0.9 (0.1, 2.2)	0.4 (0, 1.5)[6]

[1] Medians; 25th and 75th percentiles in parentheses.
[2,5,6] Significantly different from 25–30 y (Mann-Whitney U test and chi-square test): [2] $P < 0.001$, [5] $P < 0.01$, [6] $P < 0.05$.
[3] n = 117 of all participants in 25–30-y group, 87 of all participants in 60–65-y group, 73 of diet subgroup in 25–30-y group, 52 of diet subgroup in 60–65-y group.
[4] Contraceptives in the younger group and estrogen replacement therapy in the older group.

variables. Student's t test was used to compare variables given as geometric means and the P values were Bonferroni adjusted for the multiple comparisons.

The relation between tHcy concentration and folate (intake or red blood cell concentration) was determined by using linear regression (generalized linear models) in which log-transformed tHcy was the outcome variable. The models were constructed with variables that significantly correlated with log tHcy. Spearman correlations were used to examine correlations. Variables theoretically associated with tHcy, vitamin B-6 intake, and vitamin B-12 intake were included, even though a significant correlation with log tHcy was not found. Scatter plots of the variables were used to examine variable linearity. Possible confounding factors used in the linear models were supplemental folic acid, smoking status (smoking or not smoking), body mass index, physical activity level in leisure time, systolic blood pressure, estrogen use, alcohol intake, coffee intake, tea intake, supplemental vitamin B-6 intake, and supplemental vitamin B-12 intake. In the diet subgroup, total folate intake, total vitamin B-6 intake, total vitamin B-12 intake, fat intake (as a percentage of energy), and protein intake (g/d) were also included. Folic acid intake from supplements (all participants) was grouped into 3 categories: <100, 100–199, and ≥200 μg/d. Total folate intake in the diet subgroup was the sum of dietary folate intake and folic acid from supplements. Although the bioavailability of dietary folate is lower than is the bioavailability of folic acid (25), both were given the same value. Total folate intake was grouped into quintiles, as was red blood cell folate (red blood cell folate subgroup). The generalized linear models were used to estimate geometric means of tHcy adjusted for confounders with all covariates set to their respective sample means. Even though the intake curves for folate and other B vitamins, as well as for tHcy, were positively skewed, only tHcy was log transformed. The residuals of the final models were examined and all were normally distributed. P values <0.05 (two-sided) were considered statistically significant. All statistical tests were performed with SPSS (version 7.5.2; SPSS Inc, Chicago).

RESULTS

Folate status

Characteristics of the study participants are shown in **Table 1**. tHcy and red blood cell folate concentrations of all subjects in the 2 age groups and in the diet subgroup are also shown. Both tHcy and red blood cell folate were higher in the older group than in the younger group. Furthermore, more older than younger women took folic acid–containing supplements.

Relation between folic acid from supplements and log tHcy

Results of multiple linear regression analyses (generalized linear models) are shown in **Table 2**. In both age groups, folic acid intake from supplements was inversely associated with log tHcy. Furthermore, smoking status was positively associated with log tHcy in the younger group. Tea intake was weakly inversely associated with log tHcy in the older group. Estrogen use was negatively associated with log tHcy in the older group. Furthermore, in the younger group, systolic blood pressure was positively associated with log tHcy, and in the older group coffee intake was positively and physical activity level negatively associated with tHcy. Possible interactions of age group with coffee intake, folic acid intake, or smoking status and of smoking status with coffee intake, and of smoking status with folic acid intake on tHcy were examined in the full cohort, but none were found to be significant. In contrast, a significant interaction (P = 0.013) between age group and estrogen use on tHcy was found. If tea intake or tea intake and smoking status were not included in the model, coffee intake was strongly associated to tHcy (P = 0.006 and P = 0.001, respectively) in the older group.

In **Figure 1**, adjusted geometric means of tHcy in the 3 folic acid–supplemented groups are shown for the 2 age groups separately. In the younger group, tHcy was significantly lower in the middle intake (100–199 μg/d) and the highest intake (>200 μg/d) groups than in the lowest intake group (<100 μg/d). In the older group, tHcy was not significantly lower in the middle intake than

TABLE 2
Correlates of log-transformed total plasma homocysteine (tHcy) concentration from generalized linear models for all study participants[1]

Independent variable	25–30 y (n = 290)	P	60–65 y (n = 288)	P
Folic acid intake (μg/d)				
<100	0.084 (0.042, 0.126)[2]		0.060 (0.017, 0.104)	
100–199	0.018 (−0.034, 0.069)	<0.001	0.044 (−6.88 × 10^{-3}, 0.094)	0.026
>200	Reference		Reference	
Smoking status				
Yes	0.062 (0.030, 0.094)	<0.001	0.029 (−7.74 × 10^{-3}, 0.065)	0.123
No	Reference		Reference	
Tea intake (mugs/d)	−2.99 × 10^{-3} (−0.012, 6.41 × 10^{-3})	0.532	−0.011 (−0.022, 4.74 × 10^{-4})	0.041
Coffee intake (cups/d)	−3.42 × 10^{-3} (−8.43 × 10^{-3}, 1.60 × 10^{-3})	0.181	7.39 × 10^{-3} (4.51 × 10^{-4}, 0.014)	0.037
Estrogen use				
Yes	−0.021 (−0.051, 8.28 × 10^{-3})	0.158	−0.068 (−0.106, −0.029)	0.001
No	Reference		Reference	
Physical activity level				
1 (lowest)	Reference		Reference	
2	0.028 (−0.018, 0.075)		−0.081 (−0.144, −0.018)	
3	0.027 (−0.020, 0.074)	0.668	−0.062 (−0.128, 4.43 × 10^{-3})	0.020
4 (highest)	0.031 (−0.042, 0.104)		0.046 (−0.089, 0.181)	
Systolic blood pressure (mm Hg)	2.23 × 10^{-3} (9.69 × 10^{-4}, 3.49 × 10^{-3})	0.001	−3.75 × 10^{-4} (−1.18 × 10^{-3}, 4.27 × 10^{-4})	0.358
Intercept	0.537[3]		1.055[4]	

[1] The models were controlled for all of the variables in the table. BMI, diastolic blood pressure, alcohol intake, and supplemental vitamin B-6 and B-12 intakes were included in the analysis but not in the final model because they were not significantly related to log-transformed tHcy in any of the analyses. β coefficients are the log of tHcy per unit change in the independent variable.
[2] 95% CIs in parentheses.
[3] Adjusted R^2 = 0.13.
[4] Adjusted R^2 = 0.14.

in the lowest intake group, but was significantly lower in the highest intake group than in the lowest intake group.

Dietary intake data

Daily dietary intakes in the diet subgroup are shown in **Table 3**. Folate, vitamin B-6, and vitamin B-12 intakes did not differ significantly between the 2 age groups. Intakes of energy, carbohydrate, and protein (g/d) were lower in the older group than in the younger group, whereas intakes of fat and protein (percentage of energy) were higher in the older group.

Relation between total folate intake and log tHcy

Results of multiple linear regression for the diet subgroup are shown in **Table 4**. Total folate intake was inversely associated

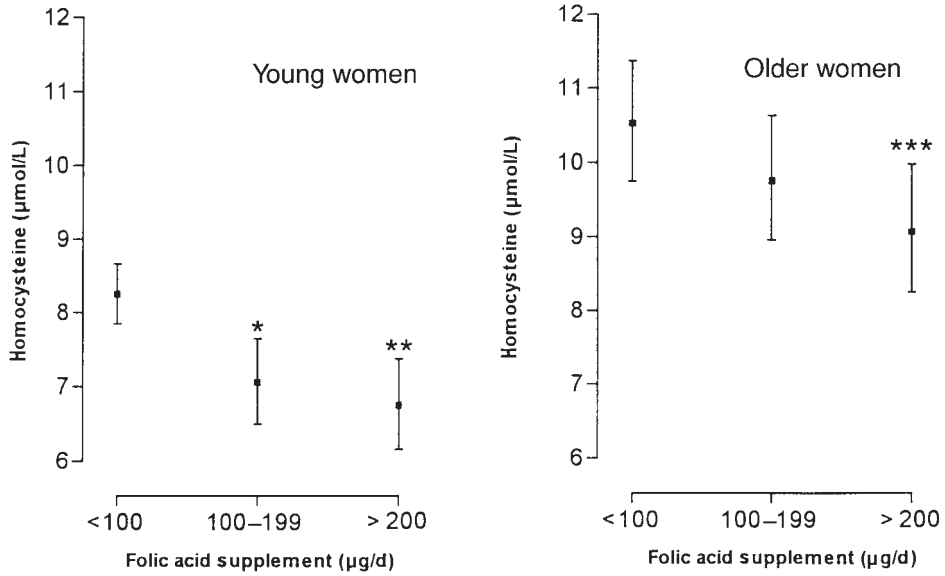

FIGURE 1. Adjusted geometric mean homocysteine concentrations in the young (25–30 y) and older (60–65 y) age groups in relation to folic acid intake from supplements. n = 199, 47, and 38 younger women and 152, 65, and 51 older women with folic acid intakes of <100, 100–199, and >200 μg/d, respectively. *,**,***Significantly different from lowest folic acid intake group (Student's t test): $^{*}P$ = 0.002, $^{**}P$ < 0.001, $^{***}P$ = 0.017.

TABLE 3
Daily dietary intake in the 2 age groups of the diet subgroup[1]

	25–30 y (n = 145)	60–65 y (n = 113)
Energy (MJ)	9.0 (7.9, 10.1)	7.9 (6.8, 8.8)[2]
Fat (% of energy)	32.3 (27.6, 35.7)	35.5 (29.8, 39.7)[2]
Carbohydrate (% of energy)	54.8 (49.8, 58.7)	49.7 (44.8, 54.6)[2]
Protein		
(% of energy)	13.7 (12.1, 15.9)	15.0 (12.8, 16.4)[3]
(g)	73.3 (62.5, 87.7)	64.8 (58.3, 80.3)[4]
Folate (μg)	283 (224, 348)	268 (210, 326)
Total folate intake (μg)[5]	316 (260, 415)	316 (238, 395)
Vitamin B-6 (mg)	1.4 (1.1, 1.7)	1.3 (1.1, 1.5)
Vitamin B-12 (μg)	5 (3, 6)	5 (3, 7)

[1] Medians; 25th and 75th percentiles in parentheses.
[2–4] Significantly different from 25–30 y (Mann-Whitney U test): [2] $P < 0.001$, [3] $P = 0.017$, [4] $P = 0.008$.
[5] Folic acid in supplements plus folate intake from the diet.

with tHcy in the younger group. Body mass index was significantly related to tHcy in the younger group and coffee intake was significantly related to tHcy in the elderly group.

Adjusted geometric means of tHcy in the total folate intake quintiles are shown in **Figure 2** for the 2 age groups separately. In both groups, tHcy concentrations were significantly lower in the highest folate intake quintile than in the lowest quintile.

In participants who did not take any folic acid–containing supplements, dietary folate intake was not significantly associated with tHcy concentration in the whole group (P = 0.074; n = 146) or in the 2 age groups, although a significant correlation between dietary folate and tHcy was found for the whole group (P = 0.009, Spearman $\rho = -0.21$). Furthermore, tHcy concentration was significantly lower in the highest dietary folate intake groups than in the lowest dietary folate intake group.

Red blood cell folate

Red blood cell folate was inversely associated with tHcy concentration in women aged 25–30 y and 60–65 y when controlled for smoking status and coffee intake (**Table 5**). Folic acid intake from supplements was significantly positively correlated with red blood cell folate in the younger (P = 0.008, Spearman ρ = 0.244; n = 177) and older ($P < 0.001$, Spearman ρ = 0.475; n = 87) women. Likewise, total folate intake was significantly positively correlated with red blood cell folate in the younger ($P < 0.001$, Spearman ρ = 0.447; n = 77) and the older ($P < 0.001$; Spearman ρ = 0.471; n = 59) women. In subjects who did not take folic acid supplements, dietary folate correlated significantly with red blood cell folate in the younger group (P = 0.002, Spearman ρ = 0.454; n = 46) but not in the older group (P = 0.644, Spearman ρ = 0.09; n = 29).

DISCUSSION

Median tHcy concentrations in the young and elderly women in this study were comparable with values found in healthy Danes in the same laboratory; however, 36.6% and 20.8%, respectively, were above the established 95% CIs for young and elderly (8.1 and 11.9 μmol/L, respectively; n = 182) women that was established after subjects with deviate concentrations of tHcy or methylmalonic acid were excluded, making an upper limit for optimal tHcy in persons without suspected vitamin deficiencies (26). In line with this, tHcy concentrations were predicted from a linear regression model by using data from vitamin supplementation trials (27) and, in the elderly, were reported after 3 wk of vitamin injections (28). However, no consensus of the definition of normal or elevated tHcy concentration has been reached, and it is not yet clear whether there is a threshold below which the tHcy concentration does not pose a risk of cardiovascular disease (27).

More older than younger women in our study took vitamin supplements containing folic acid. Most often, the folic acid was taken in multivitamin tablets. Other investigators also found that older women took vitamin supplements more often than did younger women (29).

Folate intake from the diet was ≈280 and 270 μg/d in the younger and older women, respectively. This is similar to the intake found in the most recent national dietary intake study in Denmark (30) and to the intake of 300 μg/d recommended in the Nordic nutrient recommendations (31). Likewise, the energy and

TABLE 4
Correlates of log-transformed total plasma homocysteine (tHcy) concentration from generalized linear models for the diet subgroup[1]

Independent variable	25–30 y (n = 145)	P	60–65 y (n = 113)	P
Total folate intake quintile[2]				
1 (lowest)	0.097 (0.040, 0.153)[3]	0.024	0.094 (0.026, 0.163)	0.079
2	0.040 (−0.015, 0.094)		0.067 (-4.1×10^{-3}, 0.139)	
3	0.036 (−0.017, 0.089)		0.056 (−0.011, 0.124)	
4	0.037 (−0.015, 0.089)		0.074 (7.41×10^{-3}, 0.140)	
5 (highest)	Reference		Reference	
Coffee intake (cups/d)	2.00×10^{-3} (-4.40×10^{-3}, 8.39×10^{-3})	0.538	0.020 (0.011, 0.029)	<0.001
BMI (kg/m^2)	9.30×10^{-3} (4.07×10^{-3}, 0.015)	0.001	-3.74×10^{-4} (-5.17×10^{-3}, 5.91×10^{-3})	0.894
Protein intake (g/d)	-8.00×10^{-4} (-1.75×10^{-3}, -1.52×10^{-4})	0.099	-1.01×10^{-3} (-2.21×10^{-3}, 1.82×10^{-4})	0.096
Intercept	0.683[4]		0.897[5]	

[1] The models were controlled for all of the variables in the table. Total vitamin B-12 intake, total vitamin B-6 intake, carbohydrate intake, fat intake, smoking status, tea intake, estrogen use, physical activity level, and systolic blood pressure were included in the analysis but not in the final model because they were not significantly related to log-transformed tHcy in any of the analyses. β coefficients are the log of tHcy per unit change in the independent variable.
[2] Exact figures are not shown because intake quintiles are different for the younger and older women. Total folate intake is the sum of folate from the diet and folic acid from supplements.
[3] 95% CIs in parentheses.
[4] Adjusted R^2 = 0.20.
[5] Adjusted R^2 = 0.21.

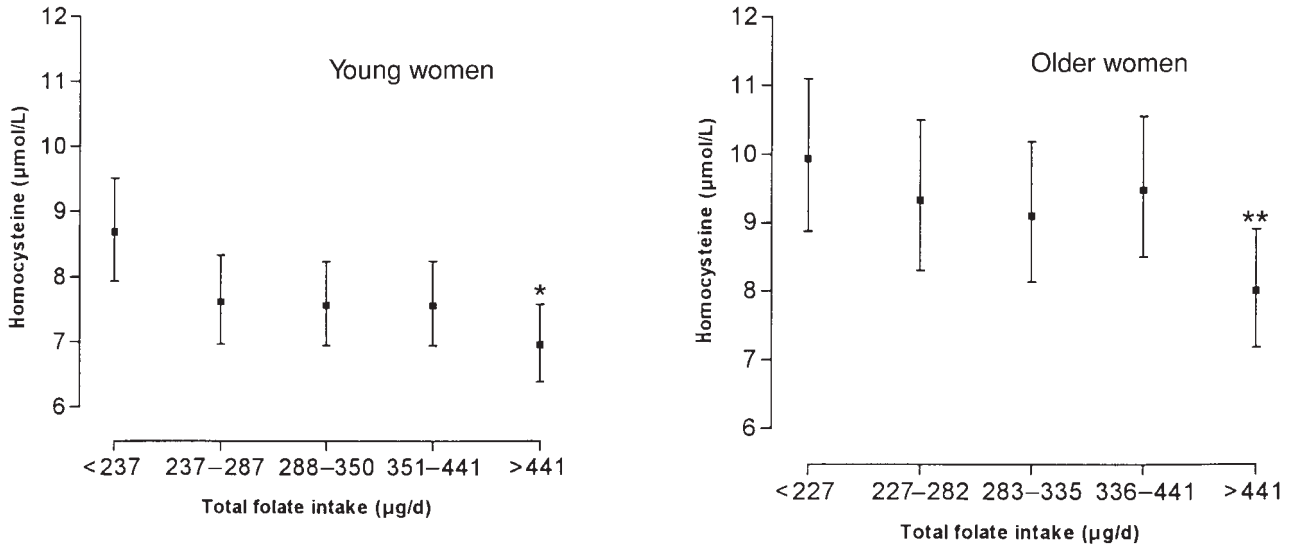

FIGURE 2. Adjusted geometric mean homocysteine concentrations in the young (25–30 y) and older (60–65 y) age groups in relation to total folate intake (intake from diet and supplements). *,**Significantly different from lowest intake quintile (Student's *t* test): $^{*}P < 0.005$, $^{**}P < 0.01$.

protein intakes in the younger group and the fat (percentage of energy) intake in the older group were comparable, whereas the fat intake in the younger group and energy and protein intakes in the older group were somewhat lower in this study than national figures (30). One should be careful when comparing folate intakes between countries in which different food databases are used.

Four days of dietary records are not enough to reflect habitual dietary intake of a single vitamin in an individual (32) and, therefore, the method could have led to misclassifications of subjects into folate intake groups. Because the half-life of folate in red blood cells is ≈100 d (33), the actual folate intake did not necessarily reflect the intake at the relevant time. However, use of a food-frequency questionnaire (ie, to record habitual food intake) was shown not to be associated with a stronger relation between dietary folate intake and red blood cell folate than a 3-d dietary record (34). Other possible limitations to our study were errors in the measurement of red blood cell folate concentration and uncertain information about dietary intake and supplements in some subjects. These limitations particularly weaken the associations between folate intake and red blood cell folate or tHcy. Fortification of foods with folic acid is not allowed in Denmark; hence, folic acid–fortified foods are not available.

The proportion of smokers (22.8%) was lower in the younger diet subgroup than in the remaining participants in this age group in DanThyr (32.3%). The low fat intake and the lower proportion of smokers suggests that the young diet subgroup was more health conscious than is the Danish population in general and, therefore, not representative.

Red blood cell folate was higher in the older women than in the younger women, as reported previously (35). Twelve of the samples from the younger group and one from the older group were below the cutoff of 400 nmol/L used in the laboratory that analyzed

TABLE 5
Correlates of log-transformed total plasma homocysteine (tHcy) concentration from generalized linear models for the red blood cell folate group[1]

Independent variable	25–30 y (n = 117)	P	60–65 y (n = 87)	P
Red blood cell folate quintile[2]				
1 (lowest)	0.115 (0.042, 0.188)[3]		0.173 (0.089, 0.257)	
2	0.076 (-6.82×10^{-3}, 0.145)		0.050 (−0.031, 0.131)	
3	−0.037 (−0.107, 0.034)	<0.001	0.040 (−0.044, 0.125)	0.001
4	-2.1×10^{-3} (−0.071, 0.067)		4.77×10^{-3} (−0.077, 0.087)	
5 (highest)	Reference		Reference	
Smoking status				
Yes	0.074 (0.022, 0.125)	0.006	−0.035 (−0.096, 0.026)	0.252
No	Reference		Reference	
Coffee intake (cups/d)	6.9×10^{-3} (-3.95×10^{-3}, 0.018)	0.211	0.012 (-7.01×10^{-4}, 0.024)	0.064
Intercept	0.828[4]		0.889[5]	

[1] The models were controlled for all of the variables in the table. BMI, estrogen use, physical activity level, diastolic and systolic blood pressure, and tea intake were included in the analysis but not in the final model because they were not significantly related to log-transformed tHcy in any of the analyses. β coefficients are the log of tHcy per unit change in the independent variable.

[2] Exact figures are not shown because intake quintiles are different for the younger and older women.

[3] 95% CIs in parentheses.

[4] Adjusted $R^2 = 0.22$.

[5] Adjusted $R^2 = 0.19$.

the samples. Others have suggested cutoffs of 253 nmol/L (36) or 320 nmol/L (37). Only 20 (17%) of the young women in this study had a red blood cell folate concentration >900 nmol/L, which has been associated with the lowest risk of neural tube defects (38).

The multiple regression analyses (generalized linear models) showed that various variables can influence tHcy concentration. In the full cohort we found that supplemental folic acid intake was a strong predictor of tHcy concentration. In both the younger and the older women the difference in geometric mean tHcy between the lowest and the highest folic acid intake groups was ≈1.5 μmol/L, similar to the difference found between users and nonusers of supplements containing folic acid (8).

Other researchers also found that age and smoking status predict tHcy concentration (4, 8, 11, 12). Likewise, it is a common finding that tHcy is lower in women who receive estrogen replacement therapy than in those who do not (39, 40). Tea intake was weakly, but significantly, inversely associated with tHcy in the older group. In univariate analyses, Nygård et al (41) found a strong inverse relation between tea consumption and tHcy concentration that disappeared after adjustment for coffee consumption and smoking status. Coffee consumption was found to be significantly positively associated with tHcy concentration in most (3, 8, 10) but not all (42) other studies. In the present study, coffee intake was strongly associated with tHcy concentration in the older women only if tea intake or both tea intake and smoking status were not included in the model. In the younger group, a relation between coffee intake and tHcy concentration was not found. The reason could be that there were fewer heavy coffee drinkers (>4.5 cups/d) among the younger women than among the older women (21% and 33%, respectively). We found that systolic blood pressure was positively associated with tHcy concentration in the younger group. This association was described in elderly subjects in a univariate analysis (5) but not in multiple linear regression analyses. We cannot explain this association.

The difference in tHcy concentration between the lowest and the highest total folate intake quintiles in the diet subgroup was ≈2 μmol/L, which was lower than the 3.3 μmol/L difference found by Selhub et al (4) in elderly American men and women and lower than the 3.4-μmol/L difference between the lowest and highest decile in folate score found by Nygård et al (8). Folate intake from diet did not significantly influence tHcy concentration in this study; however, a tendency was found ($P = 0.074$, $n = 146$), and tHcy concentration was significantly lower in the 2 highest dietary folate intake quintiles than in the lowest quintile. Other investigators have found that dietary folate can affect tHcy concentration significantly (43); in line with this, dietary folate was shown to decrease tHcy in an intervention study (6).

In the diet subgroup, smoking status was not significantly associated with tHcy concentration ($P = 0.357$ in the younger group and $P = 0.918$ in the older group). This lack of association was likely explained by a combination of 2 factors: the relatively low number of smokers among the younger women in this subgroup and the effect of smoking being overcome by other factors. When a model similar to the model used for all subjects was used in the diet subgroup, a weak but nonsignificant association between smoking status and tHcy concentration ($P = 0.077$ in the younger women and $P = 0.307$ in the older women) was found. Protein intake was weakly inversely associated with tHcy concentration. A strong inverse association between protein intake and tHcy concentration was described previously in elderly persons (3). The explanation for this association was speculative. One suggestion was that protein is a marker for vitamin B-12 intake and that vitamin B-12 accounts for the association, but this was disproved by the present study. Likewise, the mechanism behind the strong positive association in young women between body mass index and tHcy in our study was elusive. This association is not a common finding (3, 44), although body mass index was found to predict tHcy in one study (45).

Plasma concentrations of pyridoxal-5′-phosphate and cobalamin (2, 4) and dietary intake of vitamin B-6 (4) have been shown to be positively associated with tHcy; however, neither vitamin B-6 intake nor vitamin B-12 intake was associated with tHcy concentration in the present study. All participants in this study consumed more than the recommended amount of vitamin B-12 and almost all obtained the recommended amount of vitamin B-6. Thus, the reason that the intake of these vitamins did not influence tHcy could be that the intakes in general were sufficient. The results shown in Figure 1 suggest that there could be differences in the optimal folate intake between younger and older women, but this can be verified only in an intervention study.

In conclusion, folate status measured as red blood cell folate and plasma tHcy in a Danish population of young and older women showed that even in this select population, 11–36% of the younger women and 1–21% of the older women, depending on the criteria used, had suspected suboptimal folate intake. Several factors have been found to be associated with tHcy, and this study confirmed some of the commonly found predictors of tHcy (folate intake, coffee intake, smoking status, and age). Several other variables were not consistently associated with tHcy, stressing that results from observational studies should be interpreted with caution because some findings could be accidental. Furthermore, potential differences in the optimal folate intake between young and elderly women should be investigated.

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