See corresponding editorial on page 1446.

Dietary fat and plasma total homocysteine concentrations in 2 adult age groups: the Hordaland Homocysteine Study[1–3]

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ABSTRACT

Background: The intake of n−3 (formerly called omega-3) fatty acids (FAs) may be inversely associated with plasma total homocysteine (tHcy) concentrations, but the epidemiologic data are sparse.

Objective: We examined the association between dietary fat and tHcy in a Norwegian population.

Design: A cross-sectional, population-based study of 5917 subjects in 2 age groups (47–49 and 71–74 y old) was conducted with the use of food-frequency questionnaires and measurement of plasma tHcy concentrations.

Results: The intake of saturated FAs (SFAs) was positively and significantly (P for trend < 0.001) associated with tHcy concentrations; the difference in plasma tHcy concentrations between the highest and lowest quartiles of SFAs was 8.8%. The intake of marine very-long-chain n−3 FAs was inversely associated with tHcy concentrations; the difference in plasma tHcy concentrations between the lowest and the highest quartiles was −5.0% (P for trend $<$ 0.001). Intakes of total and monounsaturated fat also were positively associated with plasma tHcy concentrations (P for trend < 0.001 and < 0.005, respectively), whereas the intake of polyunsaturated fat was positively associated with tHcy concentrations only in the younger subjects (P for trend $= 0.03$). The associations were weakened by additional adjustment for B vitamin intake but remained significant for SFA intake ($P < 0.001$). When stratified for total B vitamin intake, the inverse association between tHcy concentrations and very-long-chain n−3 FAs was significant only in the highest quartile of B vitamin intake (P for trend $= 0.001$), regardless of supplement use.

Conclusions: High intakes of SFAs are associated with high plasma concentrations of tHcy. The inverse association between dietary intakes of very-long-chain n−3 FAs and plasma tHcy concentrations is apparent only at high B vitamin intakes. *Am J Clin Nutr* 2007; 85:1598–605.

KEY WORDS Diet, dietary fat, total homocysteine, n−3 fatty acids, saturated fat, fish, Hordaland Homocysteine Study

INTRODUCTION

Plasma total homocysteine (tHcy) is an independent risk factor for cardiovascular disease (1–3). Concentrations of tHcy increase with age and are higher in males than females (4). Certain lifestyle and dietary factors have been identified as predictors of tHcy concentrations in healthy subjects. Smoking and coffee consumption are associated with increasing concentrations, whereas dietary and plasma folate have been associated with lower concentrations (5–8).

Homocysteine is a nonprotein, sulfhydryl-containing amino acid in normal human plasma, and its only dietary precursor is the essential amino acid methionine. Homocysteine remethylation is catalyzed either by the ubiquitous methionine synthase, which requires cobalamin (vitamin B-12) as cofactor and folate (5-methyltetrahydrofolate) as cosubstrate, or by betaine-homocysteine methyltransferase using betaine as methyldonor (9). Alternatively, homocysteine is degraded to cysteine by the sequential action of 2 vitamin B-6–dependent enzymes. A fourth B vitamin, riboflavin, is essential in the formation of methyltetrahydrofolate. Thus, homocysteine exists at a point of convergence of several B vitamins, which explains their effects as tHcy-lowering agents in humans (3, 6).

The association between plasma tHcy and dietary fish and very-long-chain (VLC) n−3 fatty acids (FAs) has been studied; findings have been inconsistent (10–17). It has been hypothesized that a high intake of n−3 FAs may reduce tHcy but only in combination with a high B vitamin intake (18). There are even fewer studies of other types of dietary fat. It has been suggested that a low plasma tHcy concentration may be associated with the consumption of skimmed milk (19) and low intakes of saturated FAs (SFAs) (20).

The possible relation between fat intake and plasma tHcy may be explained by a biochemical link between homocysteine and lipid metabolism (21, 22). A major source of homocysteine in mammals is *S*-adenosylhomocysteine (23). Homocysteine is formed during the *S*-adenosylhomocysteine–dependent methylation of phosphatidylethanolamine to phosphatidylcholine,

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which is catalyzed by phosphatidylethanolamine methyltransferase (PEMT). Increased tHcy in animals fed a phosphatidylethanolamine-rich diet may be explained by enhancement of PEMT (22). In contrast, supplementation with phosphatidylethanolamine or choline has recently been shown to reduce plasma tHcy (24). There is experimental evidence that phosphatidylethanolamine synthesis via the PEMT pathway can be modified by dietary fat (25). Conceivably, the intake of different types of fat may have different effects on phosphatidylcholine synthesis and thereby on plasma tHcy concentration.

The association between the type of fat consumed and plasma tHcy concentrations has not been studied in a large population-based study. In the present population-based study of 5719 middle-aged and older adults, we address the question of whether dietary fat and especially VLC n−3 FAs, which are mainly present in fish and marine oils, are associated with tHcy concentrations. The study was conducted in Norway, where fish intake has traditionally been high.

SUBJECTS AND METHODS

Study population

The second round of the Hordaland Homocysteine Study (HHS II), from 1997 to 1999, conducted as part of the Hordaland Health Study (HUSK), was a collaboration of the National Health Screening Service, the University of Bergen (Bergen, Norway), the University of Oslo (Oslo, Norway), and local health services in the Bergen area. A dietary survey was included in HHS II for all persons in 4 selected communities in Hordaland County, Norway. A total of 9187 persons (4159 M, 5028 F) born in 1925–1927 and 1950–1951 were invited to join HHS II. Of this group, 7074 participated in a brief health examination, which included measurements of height and weight, and they provided a nonfasting blood sample. Information on smoking status was obtained from a questionnaire.

All participants gave written informed consent. The study protocol was approved by the Western Norway Regional Committee for Medical Research Ethics and by the Norwegian Data Inspectorate.

Assessment of dietary intake

Dietary data were collected by using a self-administered and optically readable food-frequency questionnaire (FFQ). The questionnaire was a modified version of an FFQ developed at the Department of Nutrition, University of Oslo (26). It included 169 food items grouped according to Norwegian meal patterns. The FFQ was designed to obtain information on usual food intake and vitamin supplements consumed during the past year. The frequency of consumption was given per day, week, or month. The portion sizes were given as household measures or as units such as slices or pieces. Questions regarding dietary supplement intake were included; the product names of the most-used supplements in Norway were used. Dietary intakes were calculated by using a database and a software system developed at the Department of Nutrition, University of Oslo (KOSTBEREGNINGSSYSTEM, version 3.2; University of Oslo, Oslo, Norway).

We obtained tHcy results from 7049 of the 7074 persons who attended the health examination. Of those 7049 persons, 6118 completed the FFQ. Participants with a very low [<3000 kJ for women (n = 105); <3300 kJ for men (n = 26)] or very high [>15 000 kJ for women (n = 24); 17 500 kJ for men (n = 46)] estimated daily energy intake were excluded, which left 5917 subjects (83.6% of those attending the health examination).

We assessed daily consumption of the main foods contributing to the intake of total fat, SFAs, and monounsaturated (MUFAs) and polyunsaturated (PUFAs) FAs, including n−3 PUFA. The selected foods were fatty and lean fish, milk products including whole (3.9% fat), reduced-fat (1.5% fat) and skimmed milk, cream products (cream, sour cream, and ice cream), margarine, butter and butter mixtures, vegetable oils, and fish-oil supplements (including cod liver oil and other fish-oil supplements). Moreover, we estimated intakes of total fat and the FA groups SFA, MUFA, PUFA, and n−6, n−3, and VLC n−3 PUFAs [ie, eicosapentaenoic, docosapentaenoic and docosahexaenoic acid (EPA, DPA, and DHA)].

Measurements of tHcy

Plasma tHcy was measured by using HPLC and fluorescence detection (27, 28). The between-day CV for the assay was about 3%. Measurements of plasma folate and vitamin B-12 were performed with microbiological assays as described previously (29, 30). Creatinine in serum was measured by the Jaffe method with bichromatic absorbance and reagent blank correction and by using reagents (Boehringer Mannheim, Mannheim, Germany) as adapted to a model 917 analyzer (Hitachi, Tokyo, Japan).

Statistical analysis

Potential differences in tHcy, BMI, current smoking, and dietary factors between the sex and age groups were tested by univariate analysis of variance. For the association between tHcy and dietary factors within the 2 age groups, we used multiple linear regression analysis (analysis of covariance) to estimate least-squares mean tHcy concentrations by categories of dietary factors. Values for tHcy were $\log_{10}$ transformed, and back-transformed means and 95% CIs are presented. Most dietary factors were categorized into quartiles within each separate age and sex group. For some of the foods for which a high proportion of the subjects had reported "no use," 3 nonequal categories were used to separate the users of these foods into 2 groups whose sizes were as equal as possible. These food items were whole milk (0.0, 0.1–1.0, or >1.0 mL/d), skimmed milk (0.0, 10–150, or >150 mL/d), butter and butter mixtures (0.0, 0.1–10.0, or >10.0 g/d), and vegetable oils (0.0, 0.1–1.0, or >1.0 g/d). We categorized fish-oil supplement use as "use" or "nonuse."

For the assessment of tHcy determinants, we considered the following variables to be potential confounders and adjusted for them in the multivariate models: sex (male or female), energy intake (kJ; continuous), daily smoking (yes or no), and coffee intake (mL/d; continuous). We added the following additional variables as potential confounders: intake of vegetables, fruit, and berries (g/d) for the association between foods and tHcy; and intake of folate (μg/d) and vitamins B-6 and B-12 and riboflavin (mg/d) for the association between fat types and tHcy. We present results with and without these latter adjustments.

We tried various ways of modeling confounders by replacing the continuous variables of vegetable, fruit, berries, folate, riboflavin, and vitamin B-6 and B-12 intakes with categorical variables. We also tried modeling other confounders, such as smoking, by replacing the dichotomous variable with a continuous smoking variable and with a categorical variable. Finally, we

TABLE 1
Characteristics of and dietary variables in subjects who participated in the Hordaland Homocysteine Study

	Men		Women		
	47–49 y old (n = 1298)	71–74 y old (n = 1314)	47–49 y old (n = 1734)	71–74 y old (n = 1571)	P^1
Characteristics					
tHcy (μmol/L)	10.8 ± 3.5[2]	13.0 ± 5.2	9.2 ± 3.3	11.5 ± 3.8	0.51
BMI (kg/m^2)	26.1 ± 3.3[3]	26.0 ± 3.2	24.9 ± 4.0[4]	26.2 ± 4.4	<0.001
Current daily smoker (%)	32[4]	16	34[4]	14	0.03[5]
Dietary intake					
Energy (kJ/d)	10350 ± 2584[3,4]	8519 ± 2347[3]	7850 ± 2075[4]	6672 ± 1984	<0.001
Coffee (mL/d)	555 ± 358[3,4]	402 ± 262[3]	455 ± 289[4]	359 ± 216	<0.001
Folate (μg/d)	351 ± 133	306 ± 112	314 ± 131	275 ± 117	0.31
Vitamin B-12 (mg/d)	8.1 ± 4.2	7.8 ± 4.4	6.0 ± 3.4	5.7 ± 3.4	0.73
Riboflavin (mg/d)	2.07 ± 0.83[3,4]	1.73 ± 0.72[3]	1.70 ± 0.76[4]	1.54 ± 0.76	<0.001
Vitamin B-6 (mg/d)	1.78 ± 0.69	1.54 ± 0.65	1.49 ± 0.67	1.31 ± 0.69	0.10

[1] Sex × age group interaction (2-factor ANOVA if not otherwise noted).
[2] $\bar{x}$ ± SD (all such values).
[3] Significant difference between sexes within the age group, $P < 0.01$ (one-factor ANOVA with Bonferroni post hoc test).
[4] Significant difference between age groups within sex, $P < 0.01$ (one-factor ANOVA with Bonferroni post hoc test).
[5] Sex × age group interaction (logistic regression analysis).

adjusted for additional potential confounders, such as physical activity, plasma concentrations of folate and vitamin B-12, and serum concentration of creatinine.

For testing of the interaction between B vitamins and n−3 FA intake, we constructed a summary score for total B vitamin intake, which was calculated as the sum of quartile scores (quartiles 1–4) for intakes of folate and riboflavin and vitamins B-6 and B-12. Interaction between the quartiles of total B vitamin intake and the intake of these FAs was analyzed by multiple regression, which included a product term of the quartile of n−3 FA or VLC n−3 FA intake and the total B vitamin intake score. We also evaluated the intakes of n−3 and VLC n−3 FAs as tHcy predictors in strata of B vitamin intake by carrying out multiple regression analyses in the separate quartiles of B vitamin intake.

We initially conducted all analyses within the 4 age and sex groups. However, because we observed several significant interactions by age group, we combined men and women in the analyses, and we present the results both in all subjects and within the 2 separate age groups. Notable differences between sexes are mentioned in the text.

Gaussian generalized additive regression models (31), as implemented in S-PLUS for WINDOWS software (version 6.2; Insightful Corporation, Seattle, WA), were used to generate graphic representations of the dose-response relations between tHcy concentrations and the intake of different types of fat, after adjustment for age groups (47–49 or 71–74 y old), sex, energy intake, smoking, and coffee intake. On the y-axis, this nonparametric model generates a reference value of zero that approximately corresponds to the tHcy concentration associated with the mean intakes of different types of fat for all subjects. Multiple linear regression analyses were used to examine significant associations between the tHcy concentrations and the intake of different types of fat. For other analyses, we used SPSS for WINDOWS software (release 12.0.1; SPSS Inc, Chicago, IL). All P values are 2-sided, and values < 0.05 were considered significant.

RESULTS

Subject characteristics and dietary intake

Subject characteristics and dietary factors in the 4 age and sex groups are shown in **Table 1**. Mean plasma tHcy was higher in the older than in the younger age group within each sex group and higher in men than in women within each age group, but the differences were not significant. Older women had significantly higher BMIs than did younger women, and smoking was significantly more prevalent in the younger than in the older group ($P \leq 0.01$ for both; Table 1).

In general, food intake was significantly higher in men than in women and significantly higher in the younger than in the older group ($P \leq 0.01$ for both). The most important exception was that the older participants consumed more fish than did the younger (See Table S1 under "Supplemental data" in the current online issue at www.ajcn.org.). Intake of VLC n−3 FAs as a percentage of energy intake was higher in the older than in the younger subjects (data not shown). The use of fish-oil supplements was reported by ≈40% of the populations, whereas the use of multivitamins or other supplements that contained B vitamins was reported by ≈16% of the population. Approximately 9% of all subjects reported the use of both fish-oil and B vitamin supplements. Data on fish-oil use were based on use of ordinary cod liver oil (both as oil and as capsules) and the Triomar fish-oil capsule (Pronova Biocare, Bærum, Norway). None of the fish-oil supplements contained B vitamins.

Plasma total homocysteine and intake of fatty food items

The adjusted associations between tHcy and the fat-containing foods are presented in **Table 2**. There were inverse associations between intakes of fish, reduced-fat milk, skimmed milk, and vegetable oil and fish-oil supplement use and plasma concentrations of tHcy. Intakes of cream products, butter, and margarine were positively associated with plasma tHcy concentrations. The associations for lean fish and butter were significant only in the

TABLE 2
Mean (and 95% CI) plasma total homocysteine (tHcy) concentrations by quartile of selected food intakes in subjects who participated in the Hordaland Homocysteine Study

	All subjects (n = 5917)			Subjects 47–49 y old (n = 3032)			Subjects 71–74 y old (n = 2885)		
Dietary item intake categories[1]	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend
	μmol/L	%		μmol/L	%		μmol/L	%	
Fish, fatty									
1	10.9 (10.7, 11.0)			9.7 (9.5, 9.9)			12.2 (12.0, 12.5)		
2	11.0 (10.8, 11.1)			9.8 (9.6, 10.0)			12.3 (12.1, 12.6)		
3	10.7 (10.6, 10.9)			9.6 (9.4, 9.8)			12.0 (11.7, 12.3)		
4	10.7 (10.5, 10.8)	−1.8	0.02	9.6 (9.4, 9.8)	−1.0		11.9 (11.6, 12.2)	−2.5	
Adjusted[4]		−0.7	0.19		1.0			−1.7	
Fish, lean[5]									
1	11.0 (10.9, 11.2)			9.7 (9.5, 9.9)			12.6 (12.3, 12.9)		
2	10.8 (10.6, 11.0)			9.7 (9.5, 9.9)			12.1 (11.8, 12.4)		
3	10.7 (10.6, 10.9)			9.7 (9.5, 9.9)			12.0 (11.7, 12.3)		
4	10.5 (10.5, 10.8)	−3.9		9.6 (9.5, 9.8)	−1.0	0.59	11.8 (11.5, 12.0)	−6.3	<0.001
Adjusted[4]		−3.0			0	0.96		−5.6	<0.001
Milk, whole									
0.0 mL/d	10.8 (10.6, 10.9)			9.6 (9.4, 9.8)			12.1 (11.8, 12.3)		
0.1–1.0 mL/d	10.8 (10.6, 10.9)			9.7 (9.6, 9.9)			11.9 (11.7, 12.2)		
>1.0 mL/d	10.9 (10.7, 11.1)	1.1	0.26	9.6 (9.4, 9.8)	0		12.4 (12.1, 12.6)	2.5	
Adjusted[4]		1.5	0.14		0			2.5	
Milk, reduced-fat									
1	11.0 (10.9, 11.2)			9.9 (9.7, 10.1)			12.3 (12.0, 12.6)		
2	10.8 (10.7, 11.0)			9.8 (9.6, 10.0)			12.1 (11.8, 12.3)		
3	10.7 (10.6, 10.9)			9.6 (9.4, 9.8)			12.1 (11.8, 12.4)		
4	10.6 (10.5, 10.8)	−3.5	<0.001	9.5 (9.3, 9.6)	−4.0		12.0 (11.7, 12.3)	−2.4	
Adjusted[4]		−3.8	<0.001		−5.1			−3.3	
Milk, skimmed									
0.0 mL/d	10.9 (10.8, 11.0)			9.8 (9.6, 9.9)			12.2 (12.0, 12.4)		
10–150 mL/d	10.7 (10.5, 10.9)			9.5 (9.2, 9.8)			12.0 (11.7, 12.4)		
>150 mL/d	10.4 (10.2, 10.6)	−4.1	<0.001	9.3 (9.1, 9.6)	−5.1		11.6 (11.3, 12.0)	−4.9	
Adjusted[4]		−4.0	<0.001		−4.1			−4.9	
Cream, sour cream, ice cream									
1	10.7 (10.5, 10.9)			9.6 (9.4, 9.8)			12.0 (11.7, 12.3)		
2	10.6 (10.5, 10.8)			9.6 (9.4, 9.8)			11.8 (11.5, 12.1)		
3	10.8 (10.6, 11.0)			9.7 (9.5, 9.9)			12.1 (11.8, 12.3)		
4	11.1 (10.9, 11.3)	3.9	<0.001	9.9 (9.7, 10.1)	3.1		12.6 (12.3, 12.9)	5.0	
Adjusted[4]		4.0	<0.001		3.1			5.0	
Margarine									
1	10.6 (10.5, 10.8)			9.5 (9.3, 9.7)			11.9 (11.6, 12.2)		
2	10.8 (10.7, 11.0)			9.7 (9.5, 9.9)			12.1 (11.8, 12.4)		
3	10.8 (10.7, 11.0)			9.7 (9.5, 9.9)			12.2 (11.9, 12.5)		
4	11.0 (10.8, 11.1)	3.2	0.005	9.8 (9.6, 10.0)	3.2		12.3 (12.0, 12.6)	3.4	
Adjusted[4]		2.1[5]	0.08		3.2			2.5	
Butter, butter mixture[5]									
0.0 g/d	10.8 (10.7, 10.9)			9.7 (9.6, 9.8)			12.0 (11.8, 12.2)		
0.1–10 g/d	10.9 (10.7, 11.0)			9.6 (9.3, 9.8)			12.3 (12.0, 12.6)		
>10 g/d	10.8 (10.6, 11.0)	0.4		9.5 (9.2, 9.9)	−2.1	0.14	12.3 (12.0, 12.6)	2.5	0.05
Adjusted[4]		0.3			−2.1	0.19		1.7	0.06
Vegetable oils									
0.0 g/d	11.1 (10.9, 11.2)			9.9 (9.7, 10.2)			12.4 (12.1, 12.6)		
0.1–1.0 g/d	10.7 (10.6, 10.8)			9.6 (9.5–9.8)			11.9 (11.7, 21.2)		
>1.0 g/d	10.6 (10.4, 10.8)	−4.4	<0.001	9.6 (9.4, 9.8)	−3.0		11.7 (11.4, 12.0)	−5.6	
Adjusted[4]		−3.4	<0.001		−3.0			−4.9	
Fish-oil supplements									
Nonusers	10.9 (10.8, 11.0)			9.8 (9.6, 9.9)			12.3 (12.1, 12.5)		
Users	10.6 (10.5, 10.7)	−3.0	<0.001	9.6 (9.4, 9.7)	−2.0		11.8 (11.6, 12.1)	−4.1	
Adjusted[4]		−2.8	<0.001		−2.0			−4.1	

[1] The terms 1, 2, 3, and 4 for some categories represent quartiles, which were established by using 25th and 75th percentiles or the categories defined in Subjects and Methods.
[2] Multiple linear regression analysis adjusted for sex, age group (except within the age groups), energy intake, daily smoking (yes or no), and coffee intake.
[3] Difference in tHcy between the lowest and highest categories.
[4] Also adjusted for total intakes of vegetables, fruit, and berries.
[5] Significant age × fat type intake category interaction, $P = 0.05$.

older group. The magnitude of the differences in tHcy between the highest and lowest quartile of intake of these foods was 2.5–6.3%. The largest difference in tHcy concentrations was observed for lean fish intake in the older group.

The associations between tHcy and food items listed in Table 2 were also evaluated after additional adjustment for intakes of vegetables, fruit, and berries. Some associations between tHcy and selected foods became weaker after these adjustments, but in

The American Journal of Clinical Nutrition

TABLE 3
Least-squares mean (and 95% CI) plasma total homocysteine (tHcy) concentrations related to fat type intake in subjects who participated in the Hordaland Homocysteine Study

	All subjects (n = 5917)			Subjects 47–49 y old (n = 3032)			Subjects 71–74 y old (n = 2885)		
Dietary fat type intake categories[1]	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend	Adjusted geometric mean[2] (95% CI)	Differences across categories[3]	P for trend
	μmol/L	*%*		*μmol/L*	*%*		*μmol/L*	*%*	
Total fat									
1	10.4 (10.2, 10.6)			9.5 (9.3, 9.7)			11.5 (11.1, 11.8)		
2	10.7 (10.6, 10.9)			9.6 (9.4, 9.8)			11.9 (11.7, 12.2)		
3	10.8 (10.7, 11.0)			9.7 (9.5, 9.9)			12.2 (11.9, 12.5)		
4	11.3 (11.0, 11.5)	7.8	<0.001	10.0 (9.7, 10.3)	5.3		12.9 (12.5, 13.3)	12.2	
Adjusted[4]		4.5	0.02		2.1			8.6	
Adjusted[5]		5.1	0.008		2.1			8.6	
Saturated fat									
1	10.4 (10.2, 10.6)			9.4 (9.2, 9.7)			11.5 (11.2, 11.8)		
2	10.5 (10.5, 10.8)			9.6 (9.4, 9.8)			11.8 (11.5, 12.1)		
3	10.9 (10.7, 11.1)			9.7 (9.5, 9.9)			12.3 (12.0, 12.6)		
4	11.3 (11.1, 11.5)	8.8	<0.001	10.1 (9.8, 10.3)	7.4		12.9 (12.5, 13.3)	12.2	
Adjusted[4]		5.5	<0.001		4.2			8.6	
Adjusted[5]		6.1	<0.001		4.2			8.6	
Monounsaturated fat									
1	10.6 (10.4, 10.8)			9.7 (9.4, 9.9)			11.5 (11.2, 11.9)		
2	10.7 (10.6, 10.9)			9.6 (9.4, 9.8)			12.1 (11.8, 12.4)		
3	10.9 (10.7, 11.1)			9.8 (9.6, 9.9)			12.3 (12.0, 12.6)		
4	11.0 (10.8, 11.2)	4.3	0.005	9.8 (9.5, 10.0)	1.0		12.6 (12.2, 13.0)	9.6	
Adjusted[4]		1.8	0.20		1.0			6.0	
Adjusted[5]		2.9	0.05		0.0			6.9	
Polyunsaturated fat[6]									
1	10.7 (10.5, 10.9)			9.4 (9.2, 9.6)			12.1 (11.8, 12.4)		
2	10.8 (10.6, 10.9)			9.8 (9.6, 10.0)			11.9 (11.6, 12.1)		
3	10.8 (10.7–11.0)			9.6 (9.4–9.8)			12.3 (12.0–12.6)		
4	11.0 (10.8–11.2)	2.7		10.0 (9.7–10.2)	6.4	0.03	12.2 (11.9–12.5)	0.8	0.32
Adjusted[4]		1.8			5.3	0.08		0	0.54
Adjusted[5]		1.7			3.2	0.17		0	0.45
n–6 Polyunsaturated fatty acids[6]									
1	10.7 (10.5–10.8)			9.4 (9.2–9.6)			12.1 (11.8–12.4)		
2	10.7 (10.6–10.9)			9.7 (9.5–9.9)			11.9 (11.6–12.2)		
3	10.7 (10.6–10.9)			9.6 (9.4–9.8)			12.1 (11.9–12.4)		
4	11.1 (10.9–11.3)	4.2		10.1 (9.8–10.3)	7.4	0.002	12.4 (12.1–12.7)	2.5	0.13
Adjusted[4]		2.6			5.3	0.02		1.7	0.45
Adjusted[5]		2.1			4.2	0.10		0.8	0.53
n–3 Polyunsaturated fatty acids[6]									
1	10.9 (10.8–11.1)			9.8 (9.5–10.0)			12.3 (12.0–12.6)		
2	10.9 (10.8–11.1)			9.8 (9.6–10.0)			12.3 (12.0–12.5)		
3	10.8 (10.6–10.9)			9.6 (9.4–9.8)			12.2 (11.9–12.4)		
4	10.6 (10.4–10.8)	−2.8		9.6 (9.4–9.9)	−2.0	0.32	11.8 (11.5–12.1)	−4.1	0.03
Adjusted[4]		−1.3			0	0.98		−3.3	0.13
Adjusted[5]		−0.4			1.0	0.73		−1.7	0.38
Very-long-chain n–3 fatty acids[7]									
1	11.1 (10.9–11.2)			9.9 (9.7–10.1)			12.4 (12.2–12.7)		
2	10.8 (10.7–11.0)			9.7 (9.6–9.9)			12.1 (11.9–12.4)		
3	10.8 (10.6–10.9)			9.6 (9.4–9.8)			12.2 (11.9–12.4)		
4	10.5 (10.4–10.7)	−5.0	<0.001	9.5 (9.3–9.7)	−4.0		11.7(11.5–12.0)	−5.6	
Adjusted[4]		−3.8	<0.001		−3.1			−4.8	
Adjusted[5]		−2.2	0.06		−1.0			−4.8	

[1] The terms 1, 2, 3, and 4 for some categories represent quartiles, which were established by using 25th and 75th percentiles or the categories defined in Subjects and Methods.
[2] Multiple linear regression analysis adjusted for sex, age group (except within the age groups), energy intake, daily smoking (yes or no), and coffee intake.
[3] Difference in tHcy between the lowest and highest categories.
[4] Adjusted for sex, age group (except within the age groups), energy intake, daily smoking (yes or no), coffee intake, and folate intake.
[5] Adjusted for sex, age group (expect within the age groups), energy intake, daily smoking (yes or no), coffee intake, and intakes of folate, riboflavin, and vitamins B-6 and B-12.
[6] Significant age group × food intake category interaction, $P < 0.05$.
[7] Sum of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid.

general the associations remained significant (Table 2). These associations were not significantly changed when we adjusted for the total intake of B vitamins (folate, riboflavin, and vitamins B-6 and B-12) in addition to age group, sex, energy intake, smoking, and coffee intake (results not shown).

Plasma total homocysteine and types of fatty acids

The associations between adjusted tHcy and types of dietary fat are presented in **Table 3**. There were significant increasing trends in tHcy concentrations with increasing quartiles of total

The American Journal of Clinical Nutrition

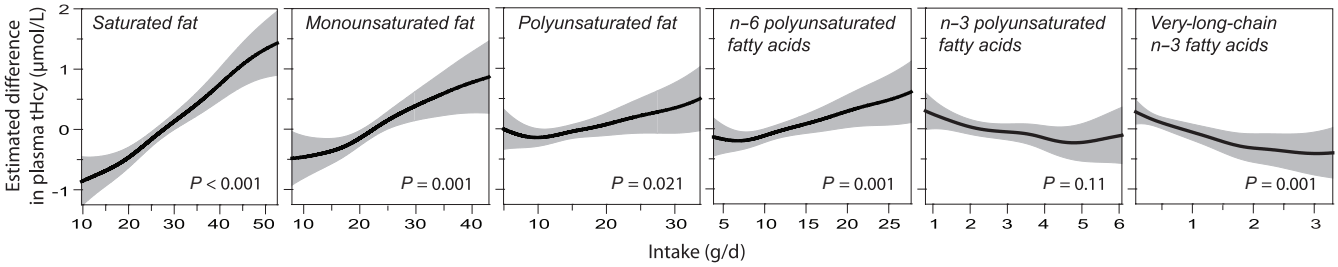

FIGURE 1. Estimated mean (and 95% CI) plasma total homocysteine (tHcy) concentrations according to the intake of types of fat after adjustment for sex, age group, energy intake, smoking, and coffee intake by additive Gaussian generalized regression model. $n = 5917$. Solid lines, the estimated dose-response curves; shaded areas, 95% CIs. *P* values are from corresponding multiple linear regression analyses. The lowest and highest 2.5 percentiles of fat intakes are not included.

fat, SFA, and MUFA intakes. Concentrations of tHcy were, on average, 4.3–8.8% higher in the highest intake quartile than in the lowest quartile. VLC n−3 FA intake was inversely associated with plasma tHcy concentration: it was 5% lower in the highest quartile than in the lowest quartile. In the younger subjects, we observed a significant increasing trend between tHcy and intake of both total and n−6 PUFAs. The intake of n−3 FAs was inversely associated with tHcy in the older group.

For comparison, tHcy differences across the extreme quartiles of folate intake were −13.6% and −15.9% in the younger and older groups, respectively. Geometric mean concentrations of plasma tHcy across increasing quartiles of folate intake were 10.3, 9.8, 9.6, and 8.9 μmol/L in the younger group and13.2, 12.3, 11.7, and 11.1 μmol/L in the older group (*P* for trend ≤ 0.001 and *P* for interaction between the age groups = 0.05; data not shown). Most of the associations between tHcy and types of fat were weakened by adjustment for the intake of folate or all 4 B vitamins. However, total fat and SFA intakes remained significant predictors of tHcy after adjustment for these variables. Moreover, the inverse association between plasma tHcy concentration and VLC n−3 FA intake remained significant after additional adjustment for folate intake (Table 3). These results for tHcy across quartiles of VLC n−3 FA intake were the same when we excluded users of fish-oil and B vitamin supplements from the analysis (data not shown).

Adjustment for plasma folate had effects similar to those of adjustment for folate intake. There were no marked changes when we adjusted for physical activity, plasma concentrations of vitamin B-12, or serum concentration of creatinine. The associations presented in Tables 2 and 3 varied slightly across the 4 age and sex groups and were strongest in younger men and older women. The association between plasma tHcy and SFA intake was particularly weak in younger women ($P = 0.11$; data not shown). There were no significant interactions with sex. The sex × age group × intake interactions were significant for intakes of whole and reduced-fat milk, cream products, butter and butter mixtures, and MUFA ($P = 0.04$, 0.03, 0.004, 0.001, and 0.03, respectively). These significant interactions were most likely due to the fact that the associations between plasma tHcy and these variables were weaker in younger women than in the other 3 age and sex groups.

The dose-response associations between tHcy and the types of fat in all subjects are shown in **Figure 1**. All of the associations presented were continuous, and there was no threshold effect.

We also tested for an interaction between the total intake of B vitamins (folate, riboflavin, and vitamins B-6 and B-12) and the total intake of n−3 fatty acids and VLC n−3 FAs for plasma tHcy concentrations. We found a significant ($P = 0.01$) interaction between VLC n−3 FAs and B vitamin intakes. When stratified by intake quartiles of B vitamin intake, the inverse trend between tHcy and VLC n−3 FA intake quartiles was significant only in the highest quartile of B vitamin intake. Difference in tHcy between the highest and lowest quartile of VLC n−3 FA intake within the highest quartile of B vitamin intake was −9.5% (*P* for trend = 0.001) (data not shown). When the users of fish-oil and B vitamin supplements were excluded, this significant inverse trend was maintained only in the highest quartile of B vitamin intake (difference between highest and lowest quartiles: −9.8%; *P* for trend = 0.005; data not shown).

DISCUSSION

In the present study, we found significant associations between fat intakes and plasma tHcy concentrations. There were some notable differences between the younger and the older subjects but few significant differences between the sexes. In particular, SFA intake showed a strong, positive association with plasma tHcy concentration. The difference in plasma tHcy across the extreme quartiles of SFA intake was 8.8%. As a comparison, the respective effect size of folate intake on plasma tHcy in this population was −13.6% in the younger and −15.9% in the older group. Intakes of total fat and MUFA also showed strong positive associations with plasma tHcy concentration in all groups, whereas intakes of PUFA and n−6 PUFA were positively associated in the younger age group, and those of marine n−3 FAs were inversely associated with plasma tHcy concentrations in all groups.

This positive association between dietary SFA and plasma tHcy has also been observed in a population-based study in Ireland (20). This association was strong and highly significant in the population of the present study. A possible biochemical explanation may be an increase in the formation of phosphatidylcholine via PEMT pathway as a response to dietary SFA (21, 22, 25). Phosphatidylcholine synthesis via the PEMT pathway has been shown to increase when rats were fed a coconut-oil diet, which is rich in SFA (25). It is possible that SFA intake in our study population has had an effect on phosphatidylcholine synthesis and also that it caused an increase in plasma tHcy concentrations (21, 22) similar to the increase caused by coconut oil in a rat study (25).

Our results suggest an inverse association between the intakes of VLC n−3 FA and tHcy. Dietary supplementation studies have reported conflicting results with respect to the effect of VLC n−3 FA on plasma tHcy. One study observed an increase in tHcy

The American Journal of Clinical Nutrition

concentrations after fish-oil supplementation in normolipidemic subjects (17). A 12-wk supplementation with fish powder (15) or fish oil (11) did not beneficially affect tHcy concentrations in hyperlipidemic subjects. However, 2 other studies found reductions in tHcy concentrations in hyperlipidemic (16) and cardiovascular disease (12) patients supplemented with fish oil. Finally, an inverse relation between plasma tHcy concentration and VLC n−3 FA concentration in serum phospholipids was observed in hyperlipidemic (10) and healthy (13) males. Our results are consistent with these latter studies.

The mechanism by which dietary VLC n−3 FA could affect tHcy has not been elucidated. However, it has been hypothesized that the effect could be due to modulation of gene expression in the enzyme or enzymes involved in the synthesis of homocysteine (13).

The intake of reduced-fat types of milk in all subjects and of lean fish in the older group in the present study were inversely related to plasma tHcy concentrations. Because these foods also are high in vitamin B, it is unclear whether the inverse tHcy association was due to vitamin B or dietary fats. We adjusted for B vitamin intake in one analysis and found evidence of an effect of these foods that was independent of vitamin B intake. A negative association between plasma tHcy and skimmed milk but not between plasma tHcy and fish intake in men was observed in oil platform workers by Oshaug et al (19). No adjustment was made for vitamin B intake in that study. Furthermore, the mean age of the platform workers was closer to that of the younger group in the present study, among whom no relation between fish intake and plasma tHcy concentrations was shown. In another study on the relation between dietary pattern and plasma tHcy, fish was a component of a tHcy-lowering diet in elderly subjects (32). Furthermore, in a controlled feeding study in adults, a diet with 0.7 servings of fish/d resulted in a significantly lower tHcy than was seen with a control diet with 0.3 servings of fish/d (33). However, the fish diet in that study included other components, such as fruit, vegetables, and dairy products, that differed from the control diet. Fish intake, in particular in the older group, was considerably higher in the population of the present study than in populations examined in other studies conducted in other countries (34–36). This difference in fish intake therefore may have given the present study a much higher power to find an effect than other studies had. The reason that we found an effect of lean but not of fatty fish may have been that lean fish was consumed to a much greater extent than fatty fish in the present study.

We observed that the inverse association between plasma tHcy and VLC n−3 FA intake was mainly weakened by adjustment for intakes of vitamins B-6 and B-12. Although this weakening could be due to confounding introduced by simultaneous consumption of other vitamin B-6– and B-12–rich foods, it also may be due to the fact that fish is a common main source of both VLC n−3 FAs and these B vitamins (37). Total fish intake contributed on the average to 56% of VLC n−3 FA intake, 45% of vitamin B-12 intake, and 16% of vitamin B-6 intake in this population. Fish-oil supplement use contributed on average to 35% of VLC n−3 FA intake. No fish-oil supplements with added B vitamins were available in Norway at the time of the data collection. Our results suggest that the inverse association between plasma tHcy and VLC n−3 FA intake is not dependent on the use of fish-oil or B vitamin supplements. Furthermore, our results may suggest that persons with high intakes of marine oils have other dietary and lifestyle factors related to lower plasma tHcy concentration.

De Bree et al suggested that dietary n−3 FAs reduce tHcy only when given in combination with B vitamins (18). Our finding that the inverse association between tHcy concentrations and VLC n−3 FA intake was observed only at the highest level of B vitamin intake, irrespective of fish-oil and B vitamin supplement use, was consistent with the hypothesis of de Bree et al.

The present study is the largest observational study to date on fat intake as a predictor of tHcy concentrations. It is also possible that the present study is the first with the power to detect a positive association with dietary saturated fat intake and an inverse association with VLC n−3 FA intake. Our results became weaker but were still observable after adjustments for other potential confounders, including B vitamins. Folate, fruit, and vegetable intakes estimated by FFQ in the present population have been found to be highly correlated with plasma folate concentrations (38). Moreover, previous validation studies of an FFQ similar to the one used here yielded high correlations between food records and the FFQ for vegetable and fruit intake (39). However, we still cannot completely exclude the possibility of residual confounding by these factors or the possibility that the observed associations were due to other confounders we have not adjusted for. Intervention studies are needed to confirm our findings.

We observed several associations that were weaker in younger women; in particular, the association between saturated fat and tHcy was not significant, whereas it was strong and highly significant in the other groups. We have no explanation for this weaker finding in the younger women. It has been suggested that female hormones may influence plasma tHcy concentrations (40). However, the extent to which this factor would have introduced random error in the tHcy measurements and obscured the associations in this group is not clear.

In conclusion, our results suggest an association between the dietary intake of saturated fat and plasma tHcy concentrations. High dietary intake of VLC n−3 FA was inversely associated with tHcy, but the relation was reduced when adjusted for B vitamin intake. Consumption of types of low-fat milk and of vegetable oils may be an important determinant of plasma tHcy concentrations. A diet low in saturated fat may reduce the tHcy concentration. The extent to which the effect is present in persons with a high B vitamin intake should be addressed in future studies.

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