

Evaluation of a Food Frequency Questionnaire with Weighed Records, Fatty Acids, and Alpha-Tocopherol in Adipose Tissue and Serum

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The authors examined the validity of a self-administered 180-item food frequency questionnaire in 125 Norwegian men aged 20–55 years who filled in the questionnaire and completed 14-day weighed records in fall 1995 to winter 1995/6. Spearman correlation coefficients between the two measurements ranged from 0.42 for percent of energy from fat to 0.66 for sugar intake (median $r = 0.51$). On average, 39% of the men were classified in the same quartile with the two methods, and 3% in the opposite quartile. Correlation coefficients between intake of fatty acids estimated from the questionnaire and the relative amounts of fatty acids in adipose tissue were: linoleic acid (18:2, n-6), $r = 0.38$; alpha-linolenic acid (18:3, n-3), $r = 0.42$; eicosapentaenoic acid (20:5, n-3), $r = 0.52$; and docosahexaenoic acid (22:6, n-3), $r = 0.49$. The correlations for these fatty acids between the total serum lipids and the diet were 0.16, 0.28, 0.51 and 0.52, respectively. The data suggest that very-long-chain n-3 fatty acids in adipose tissue and total serum lipids reflect the dietary intake of very-long-chain n-3 fatty acids to the same degree. No associations were observed between intake of alpha-tocopherol and concentration in adipose tissue and serum. *Am J Epidemiol* 1999;150:75–87.

adipose tissue; alpha-tocopherol; biomarkers; diet; fatty acids; questionnaires

There is a need for methods to measure long-term dietary intake with adequate accuracy. This is essential in national food consumption surveys, nutritional epidemiology, and as basis for nutritional policy. Food frequency questionnaires are commonly used in studies of habitual food intake among large populations (1–5).

Relative validation studies often have been used to evaluate the limitations of collected dietary data. With this approach, results from the test-method, for instance, a questionnaire, are evaluated against results from another dietary assessment method. Multiple weighed recording has been a widely adopted reference method (6). One of the limitations of this approach is the considerable individual day-to-day variation, which reduces the possibility to obtain a true measure of usual intake with few recording days (7). Moreover, weighed records have a major drawback related to underestimation (8).

An attractive alternative to relative validation is to compare results of a questionnaire with biochemical measurements (biomarkers). The primary advantage of this form of validation is that these measurements are objective and the sources of errors in the two methods will be independent. To be used in validation studies, biomarkers should be sensitive to dietary intake, and they should provide reproducible results.

Although suitable biomarkers are not available for many nutrients, studies have shown that the adipose tissue content of essential fatty acids as linoleic acid (18:2, n-6) and very-long-chain n-3 fatty acids may reflect long-term intake of these fatty acids (9–13). Furthermore, blood levels of alpha-tocopherol are reported to reflect intake of vitamin E in controlled studies (14, 15). Several authors (15–19) have already compared plasma concentration of alpha-tocopherol with vitamin E intake derived from dietary questionnaires. The validation studies with blood levels of alpha-tocopherol have primarily been performed in groups of people including supplement users, and little is known about the validity of the questionnaires in non-supplemented groups. Few studies have examined the relation between alpha-tocopherol in adipose tissue and diets with normal range of vitamin E intake (20, 21). Because it has been estimated that a large fraction of body tocopherol is contained in adipose tissue (22), and since there is a low turnover of tocopherol in adi-

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Abbreviations: BMR, basal metabolic rate; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EI/BMR, ratio between energy intake and BMR; EPA, eicosapentaenoic acid.

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pose tissue, this may be a better indicator of the long-term intake of alpha-tocopherol than blood concentrations.

In the present study, we compared nutrient intake estimated with our 180-item food frequency questionnaire against 14-day weighed records among a group of Norwegian men. Furthermore, we evaluated whether there was any relation between intake of fatty acids and alpha-tocopherol estimated from the questionnaire and the fatty acid and alpha-tocopherol values in serum and subcutaneous adipose tissue.

MATERIALS AND METHODS

Subjects and design

Healthy men who worked at Ørland flight-station, a military facility located on the west coast of Norway, were invited to participate in the study. The study was conducted during fall 1995 to winter 1995/6.

A total of 137 subjects received the quantitative food frequency questionnaire by mail at the beginning of the study. About one week later, they were instructed on how to keep weighed food records for 14 days (figure 1). Of the initial 137 participants, 125 completed the records. Adipose tissue and blood samples were taken, and weight and height were measured at the start of the first recording period.

The study protocol was approved by the regional ethical committee, and all participants gave written informed consent.

Food frequency questionnaire

The 11-page optical mark readable questionnaire was designed to capture the habitual food intake among adults, including questions about 180 food items grouped together according to the Norwegian meal pattern. The frequency alternatives vary from once a month to several times a day, and the portion size alternatives are ample (household units, e.g., slices, glasses, cups, pieces, spoons, deciliter). The

questionnaire in the present study was a revised version of the questionnaire previously described in detail (23). The main changes between the first version of the questionnaire and the one used in the present study are: a new layout, replacement of some food items (especially foods low in fat and sugar), and that the question about beverages, fruit, dessert, and cakes in the first version was separated into two questions. The questionnaire includes questions about frequency of use and portion size of seven types of dietary supplements (cod liver oil, cod liver oil and fish oil capsules, multi-vitamin/mineral mixtures, vitamin C, vitamin E, and iron). Questions about weight, height, physical activity, and smoking habits were also included. A more detailed description of these questions has been published elsewhere (4).

Weighed diet records

The 14-day weighed diet record was split into five shorter periods (3 + 3 + 3 + 3 + 2-day units) by 1-week intervals (figure 1). The men started to register their food intake the day after they were instructed how to keep weighed food records. The total 14-day period consisted of two Mondays, two Tuesdays, two Wednesdays, etc. (10 weekdays and 4 weekend days).

The men were provided with five blank diary forms (one for each registration period) and a digital scale with a precision of ± 1 g and a maximum capacity of 2,500 g. They were given both oral and written instructions on how to weigh and describe in detail the consumption of foods and beverages, and how to fill in the diary forms. Furthermore, the subjects were asked to monitor their "normal" food intake and to avoid any temptation to change the diet in order to loose weight or simplify the recording. The use of household measures was accepted when it was impossible to use the scale.

The diary form from the first 3-day period was checked for completeness immediately after finishing this first period, and each participant was contacted by telephone to clarify improper responses. The forms were coded by two nutritionists (L.F.A and K.S.).

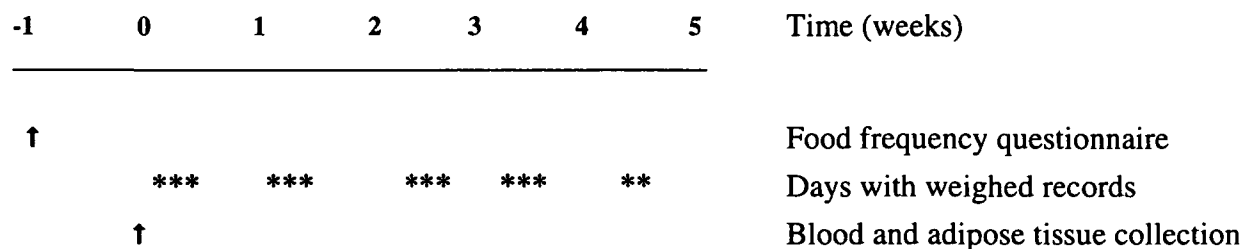


FIGURE 1. Design of the validation study to assess the validity of a self-administered 180-item food frequency questionnaire in a group of 125 Norwegian men aged 20–55 years who filled in the questionnaire and completed 14-day weighed records in fall 1995 to winter 1995/6.

Nutrient calculations

The daily intake of energy and nutrients was computed by using a food database and software system developed at the Institute for Nutrition Research, University of Oslo. The food database is mainly based on the official food composition table (24), and is continuously supplemented with data on new food items and nutrients. Cod liver oil and vitamin and mineral supplements were included in the nutrient calculations.

Body weight and height

Body weight and standing height were measured with the men wearing their indoor clothes (shirt and pants) and without shoes. Body mass index was calculated as body weight (kg)/height (m)².

Evaluation of energy intake using cut-off values

Estimates of basal metabolic rate (BMR) were calculated from standard formulas based on height, weight, age, and sex (25). The formulas for males aged 18–30 and 30–60 years were used. A comparison of energy intake with estimates of BMR can be used to calculate number of respondents in a dietary survey who may underreport their energy intake (26). Based on estimates of BMR with 95 percent confidence intervals and a diet recording period of 14 days, a ratio between measured energy intake and BMR (EI/BMR) below 1.12 for individual records may indicate underreporting. Furthermore, for individuals in a non-dieting population, it is suggested that a ratio between energy intake and estimated BMR of less than 1.35 most likely does not represent long-term habitual intake.

Samples

The adipose tissue biopsy specimen was obtained from the subcutaneous adipose tissue of the buttock by using a 14-gauge needle, a Venoject multi-sample luer adaptor (Terumo Corporation, Tokyo, Japan) and an evacuated blood tube (27). The samples were stored at –18°C for a maximum of 5 days and then transferred to –70°C in the original plastic adaptor.

An overnight fasting blood sample was drawn in the morning. The samples were centrifuged and stored at –18°C for a maximum of 5 days before transferring to –70°C.

Adipose tissue and fasting blood samples were obtained from 131 and 136 participants, respectively. Ten of the adipose tissue samples were too small for analysis of fatty acid pattern and alpha-tocopherol, and

one tissue sample had an unreasonably high percent of fat in the biopsy (weight of total fatty acids/total biopsy weight). Furthermore, one subject was excluded because he had used cholesterol-lowering drugs for a long period. In total, 119 adipose tissue samples and 135 blood samples were analyzed.

Analyses

Analyses of fatty acids in the adipose tissue and serum lipids were carried out at the National Public Health Institute, Helsinki, Finland, while analyses of alpha-tocopherol in adipose tissue and serum were done at the Institute for Nutrition Research, Oslo, Norway.

Fatty acids. Total serum lipids were extracted with dichloromethane/methanol (2/1; vol/vol) (28) and the adipose tissue biopsy in isopropanol was extracted with hexane/isopropanol (3/2; vol/vol) (29). The lipids from serum and adipose samples were transesterified to methyl esters with acidic methanol (30). Percent composition of the methylated fatty acids was determined using a HRGC 412 Micromat gas chromatograph (HNU-Nordion Instruments, Helsinki, Finland) with a 100 m long SP 2560 column (d 0.2 mm, phase layer 0.20 mm) (Supelco, Bellefonte, Pennsylvania) using a temperature program (168°C (50 minutes) – >210°C (45 minutes), 2°C/minute). We used split injection and helium as carrier gas. The fatty acids from 12:0 to 22:6 were determined with a Sunicom Workstation (Helsinki, Finland), and the area of the identified peaks was normalized to 100 percent.

Pooled serum ($n = 8$) or homogenous pig adipose tissue ($n = 7$) were used as control samples. The coefficient of variation for fatty acid peaks over 5 percent was 1.5–3 percent and for peaks under 5 percent it was 2–10 percent. For very small peaks (<0.2 percent) or partly overlapping peaks, the coefficient of variation was about 20 percent.

The serum and adipose tissue fatty acids are expressed in percent of total fatty acid methyl esters.

Alpha-tocopherol. Serum samples were extracted by diluting 200 μ liter serum with 900 μ liter cold isopropanol containing 10 mg/mL butylated hydroxytoluene (BHT) as antioxidant and the internal standard tocol. The adipose tissue samples were extracted by 1 ml cold isopropanol containing 10 mg/mL BHT and the internal standard tocol. The extracts were homogenized, vortexed, and centrifuged at 12,500 rpm for 10 minutes (10°C). 10 μ liter of the extract was injected into the HPLC system. The rest of the extract from the adipose tissue was sent to Finland on dry ice for analysis of fatty acids. We used a Hewlett-Packard 1100 HPLC system (Hewlett-Packard, Palo Alto, California) with a Shimadzu RF-530 fluorescence detector

(Shimadzu Corporation, Kyoto, Japan) set to 294 nm for excitation and 330 nm for emission. Elution of tocopherol was done with 100 percent methanol at a flow rate of 2 ml/min. The column was a 125 mm × 4 mm inner diameter purospher RP-18 e (Merck, Darmstadt, Germany). The mobile phase was continuously degassed with helium, and the temperature on the column was ambient.

The recovery was found to be almost 100 percent, and the detection limit was 2 pmol. The intra-assay reproducibility was 2 percent ($n = 6$) and the inter-assay reproducibility was 3.5 percent ($n = 10$).

Alpha-tocopherol in adipose tissue is expressed as µg/g of total fatty acid methyl esters. The concentration of fatty acid methyl esters in adipose samples was determined by adding a known amount of internal standard (19:0 methyl ester) to the extraction solution. The coefficient of variation of the internal standard method was 9 percent.

Cholesterol. Total cholesterol in serum was measured enzymatically at the Institute of Aviation Medicine, Oslo, by the Cholesterin CHOD-PAP-Method (Boehringer, Mannheim, Germany).

Statistical analyses

Statistical analyses were performed with StatView (Abacus Concepts, Inc., Berkeley, California) and Jump (SAS Institute, Inc., Cary, North Carolina). As the distributions of nutrient intake and food intake data were not normally distributed using the Shapiro-Wilks' W test, non-parametric statistical methods were chosen for most analyses. The sample medians and 25th and 75th percentiles were computed for nutrient intake, food intake, serum, and adipose tissue concentrations of fatty acids and alpha-tocopherol. The differences were tested with Wilcoxon's signed rank test (paired data), Mann-Whitney test (unpaired data), and Kruskal-Wallis test (table 6). The agreement between methods was analyzed by the method proposed by Bland and Altman (31), using a plot of the differences between two measurements against the average of the measurements. This type of plot shows the magnitude of disagreement, spot outliers, and any trends. Spearman rank correlation was used to compare two measurements instead of Pearson correlation because most of the nutrient and the fatty acid distributions were skewed. Ninety-five percent confidence intervals were calculated for the correlation coefficients (32). Within- and between-subject variances were determined from the 14-day records by analysis of variance (ANOVA). Agreement on category level between the questionnaire and the records was examined by classification of intakes divided into quartiles.

RESULTS

Food frequency questionnaire vs. 14-day weighed records

The characteristics of the 125 participants who completed the food frequency questionnaire and the 14-day weighed records are shown in table 1. Twenty-two men (17 percent) had a ratio between energy intake estimated from 14-day records and BMR at or below 1.12.

The intake of energy, protein, total fat, sugar, and polyunsaturated fatty acids measured by the questionnaire and by the diet records did not differ significantly (table 2). However, the percents of energy derived from macronutrients from the food questionnaire were significantly different from the weighed records values, except for the percent of energy derived from protein. Furthermore, the questionnaire gave significantly higher estimates for all micronutrients compared with the records, except for calcium. The number of persons with a difference in measured intake between the questionnaire and the diet records of less than 20 percent of the diet records ranged from 23 persons for vitamin D to 111 persons for percent of energy derived from protein. Most of the Bland and Altman plots showed a pattern similar to the plot of energy (figure 2). The plots did not show any systematic tendency for increase in difference between the two methods with increasing intake. Spearman correlation coefficient between pairwise measurements by diet records and by questionnaire ranged from 0.42 for percent of energy from fat to 0.66 for sugar intake (table 3), whereas the median coefficient was 0.51.

Table 4 shows how the questionnaire was able to classify individuals into the same quartile of intake estimated from the diet records and the questionnaire, and to misclassify into opposite quartiles. The proportion of subjects appearing in the same quartile varied

TABLE 1. Characteristics of Norwegian men who completed the food frequency questionnaire and 14-day weighed records, fall 1995 to winter 1995/6 ($n = 125$)

Characteristic	
Age (years), median (range)	38 (20–55)
Weight (kg), median (range)	81.5 (59–115)
Height (m), median (range)	1.80 (1.64–1.97)
BMI* (kg/m ²), median (range)	25.1 (20.5–34.2)
BMI (kg/m ²) >30 (%)	9
El _{WR} /BMR†, median (range)	1.38 (0.75–2.07)
El _{FFQ} /BMR‡, median (range)	1.29 (0.57–4.31)
Smokers (%)	20

* BMI, body mass index.

† The ratio of energy intake calculated from the 14-day weighed record (WR) and the estimated basal metabolic rate (BMR).

‡ The ratio of energy intake calculated from the food frequency questionnaire (FFQ) and the estimated BMR.

TABLE 2. Daily intake of energy and nutrients based on the food frequency questionnaire and weighed records among Norwegian men, fall 1995 to winter 1995/6 (*n* = 125)

Nutrient or energy intake	Median intake (25th percentile, 75th percentile)		<i>p</i> value†	No. of persons different <20%‡, <i>n</i> (%)
	FFQ*	WR*		
Energy§ (MJ)	10.2 (8.7, 12.3)	10.8 (9.1, 12.1)	NS*	71 (56)
Protein (g)	97 (79, 116)	95 (84, 111)	NS	75 (60)
Total fat (g)	89 (71, 119)	103 (86, 115)	NS	52 (42)
SFA* (g)	34.3 (26.7, 44.4)	40.5 (33.5, 45.9)	0.001	53 (42)
MUFA* (g)	32.1 (25.1, 41.3)	36.8 (31.0, 42.8)	0.025	55 (44)
PUFA* (g)	16.5 (11.7, 23.9)	17.5 (13.6, 21.0)	NS	45 (36)
Total carbohydrate (g)	299 (259, 361)	298 (240, 338)	0.006	73 (58)
Sugar (g)	51.1 (33.2, 80.2)	61.8 (36.6, 87.6)	NS	40 (32)
Alcohol (g)	6.2 (3.3, 8.9)	6.8 (2.4, 16.0)	0.001	57 (53)
Fiber (g)	23.0 (19.5, 27.8)	19.7 (16.9, 23.8)	<0.001	63 (50)
Vitamin A (µg)	1,721 (1,219, 2,373)	1,394 (1,041, 1,923)	<0.001	45 (36)
Vitamin D (µg)	9.1 (4.7, 15.8)	6.8 (4.5, 11.6)	0.001	23 (18)
Alpha-tocopherol (mg)	11.3 (7.9, 15.5)	9.4 (7.5, 12.0)	<0.001	38 (30)
Thiamin (mg)	1.6 (1.3, 2.1)	1.4 (1.2, 1.7)	<0.001	67 (54)
Riboflavin (mg)	2.2 (1.7, 2.8)	1.9 (1.5, 2.5)	0.021	58 (46)
Vitamin C (mg)	96 (74, 133)	78 (55, 123)	<0.001	44 (35)
Calcium (mg)	1,060 (787, 1,337)	997 (783, 1,225)	NS	57 (46)
Iron (mg)	13 (11, 16)	13 (11, 15)	0.045	67 (54)
Magnesium (mg)	384 (332, 448)	351 (306, 411)	<0.001	76 (61)
% energy from				
Protein	15.4 (14.4, 16.8)	15.6 (14.2, 17.3)	NS	111 (89)
Fat	32.4 (28.6, 36.5)	35.2 (32.7, 37.4)	<0.001	97 (78)
Carbohydrate	50.1 (46.2, 53.4)	46.8 (43.2, 49.4)	<0.001	102 (82)
Sugar	8.9 (6.1, 11.7)	9.8 (6.8, 12.5)	0.020	56 (45)
Alcohol	1.7 (0.9, 2.6)	2.0 (0.6, 4.5)	<0.001	60 (56)

* FFQ, food frequency questionnaire; WR, weighed records; NS, not significant; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

† The difference between WR and FFQ tested with Wilcoxon's signed rank test.

‡ Number of persons with a difference in measured intake between FFQ and WR <20% of WR.

§ To convert kJ to kcal use the factor 0.24 (1 MJ = 1,000 kJ).

from 33 percent for monounsaturated fatty acids to 52 percent for retinol equivalents, with a median of 39 percent. On average, 3 percent of the males were misclassified into extreme quartiles.

Fatty acids in diet and tissues

Median intakes of fatty acids expressed as g/day and as percent of total fat intake estimated with the questionnaire are shown in table 5 together with the distribution of fatty acids in adipose tissue. Table 5 also presents the relations between fatty acid intake and fatty acid pattern in adipose tissue. The highest correlations were observed for the very-long-chain n-3 fatty acids, eicosapentaenoic acid (EPA) (20:5, n-3) and docosahexaenoic acid (DHA) (22:6, n-3), when intake was expressed as percent of total fat intake ($r = 0.52$ and $r = 0.49$, respectively). Usually, the correlations between the fatty acids (expressed as percent of total fat intake) and the relative amount in adipose tissue were higher than correlations between the absolute amount of dietary fatty acids (g/day) and the relative amount in

adipose tissue. The correlations of saturated and monounsaturated fatty acid intake with the respective adipose tissue fatty acid proportion were weak, although sometimes statistically significant (table 5).

Linoleic acid (18:2, n-6), alpha-linolenic acid (18:3, n-3), and very-long-chain n-3 fatty acids in adipose tissue were significantly correlated with the respective dietary intakes (table 5). When the proportions of the corresponding fatty acids in adipose tissue were related to quartiles of the intake (fatty acids expressed as percent of total fat intake) (table 6), men who consumed fatty acids in the highest quartile had adipose tissue levels that were significantly higher than men who consumed fatty acids in the lowest or next lowest quartile.

Correlations between the fatty acids in total serum lipids and intake of fatty acids estimated from the food questionnaire are shown in table 7. The highest correlations were observed for the same fatty acids as in the adipose tissue, namely EPA (20:5, n-3) and DHA (22:6, n-3). The correlations between intake of fatty acids expressed as percent of total fat intake and the relative amount of the fatty acids in serum were usu-

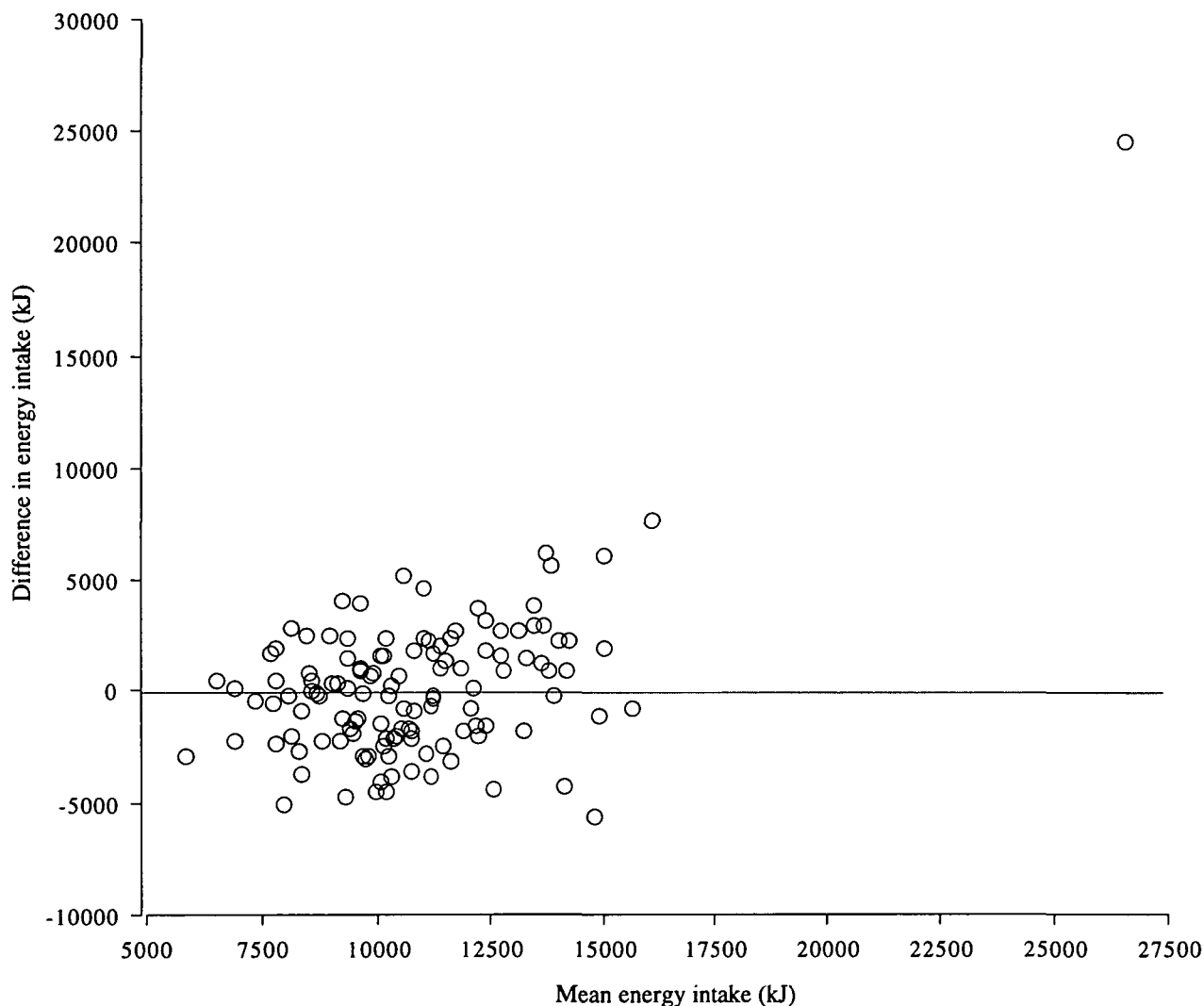


FIGURE 2. Difference in energy intake estimated with the food frequency questionnaire and the 14-day weighed records in 125 Norwegian men aged 20–55 years, fall 1995 to winter 1995/6, plotted against the mean of energy intake measured with the questionnaire and the weighed records.

ally higher than the correlations between the absolute amounts of dietary fatty acids and the relative amount of fatty acids in serum.

Alpha-tocopherol in diet and tissues

Spearman correlation coefficients between the intake of alpha-tocopherol and the concentration of alpha-tocopherol in adipose tissue and serum are displayed in the last line of tables 5 and 7, respectively. Dietary intake of alpha-tocopherol was neither correlated to the level of alpha-tocopherol in adipose tissue nor to lipid-standardized serum alpha-tocopherol. We analyzed the possibility that the questionnaire might classify individuals into the same quartile by the ques-

tionnaire and the concentration in adipose tissue or serum, and misclassify into opposite quartiles. Twelve percent of the men were misclassified into opposite quartiles, and 28 percent were classified into the same quartile by the questionnaire and the concentration in adipose tissue and serum (data not shown).

DISCUSSION

The present study was conducted to evaluate a self-administered food frequency questionnaire used in large studies in Norway (4, 33, 34). The questionnaire gave higher median values than the diet records for micronutrients. The same tendency for micronutrients was observed in the study of elderly Norwegian women using a similar questionnaire and having a sim-

TABLE 3. Spearman correlation coefficients (*r*) and 95% confidence intervals (CI) between intake of energy and nutrients based on the food frequency questionnaire and weighed records, and the ratio of within-subject and between-subject variances, among Norwegian men, fall 1995 to winter 1995/6 (*n* = 125)

Nutrient or energy intake	<i>r</i>	95% CI	Within-subject: between-subject
Energy* (MJ)	0.48	0.33–0.61	1.8
Protein (g)	0.44	0.29–0.57	1.0
Total fat (g)	0.46	0.31–0.59	1.3
SFA† (g)	0.44	0.29–0.57	1.1
MUFA† (g)	0.45	0.30–0.58	1.3
PUFA† (g)	0.52	0.38–0.64	1.2
Total carbohydrate (g)	0.57	0.44–0.68	1.6
Sugar (g)	0.66	0.55–0.75	1.3
Alcohol (g)	0.64	0.52–0.73	2.0
Excluding nondrinkers	0.53‡	0.38–0.66	
Fiber (g)	0.45	0.30–0.58	0.7
Vitamin A (μg)	0.62	0.50–0.72	0.7
Vitamin D (μg)	0.61	0.49–0.71	0.8
Alpha-tocopherol (mg)	0.46	0.31–0.59	0.8
Thiamin (mg)	0.54	0.40–0.65	0.8
Riboflavin (mg)	0.51	0.37–0.63	1.1
Vitamin C (mg)	0.50	0.36–0.62	0.8
Calcium (mg)	0.50	0.36–0.62	1.2
Iron (mg)	0.44	0.29–0.57	1.4
Magnesium (mg)	0.45	0.30–0.58	1.2
% energy from			
Protein	0.51	0.37–0.63	
Fat	0.42	0.26–0.56	
Carbohydrate	0.45	0.30–0.58	
Sugar	0.66	0.55–0.75	
Alcohol	0.62	0.50–0.72	

* To convert kJ to kcal, use the factor 0.24 (1 MJ = 1,000 kJ).

† SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

‡ Correlation excluding participants (*n* = 19) who reported no alcohol consumption.

TABLE 4. Classification of subjects by quartiles of calculated nutrient intake from the weighed records and the food frequency questionnaire among Norwegian men, fall 1995 to winter 1995/6 (*n* = 125)

Nutrient or energy intake	Correctly classified (%)	Grossly misclassified (%)
Energy (MJ)	35	4
Protein (g)	38	4
Total fat (g)	38	3
SFA* (g)	37	2
MUFA* (g)	33	2
PUFA* (g)	44	2
Carbohydrate (g)	39	2
Sugar (g)	50	2
Alcohol (g)	41	1
Fiber (g)	40	5
Vitamin A (μg)	51	2
Vitamin D (μg)	45	2
Alpha-tocopherol (mg)	34	3
Thiamin (mg)	46	5
Riboflavin (mg)	42	6
Vitamin C (mg)	50	5
Calcium (mg)	39	2
Iron (mg)	38	4
Magnesium (mg)	36	6
% energy from		
Protein	38	3
Fat	39	5
Carbohydrate	34	4
Sugar	52	2
Alcohol	41	2

* SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

higher median correlation coefficients were obtained. However, these studies are different in many aspects, and the correlation coefficients are not directly comparable.

The ability of the food frequency questionnaire to assign the subjects to the same quartile of the distribution by the diet records was on average 39 percent, comparable to previous findings (23). For the nutrients, we found a similar percent subject distribution to that reported by Bingham et al. (45) from their Oxford questionnaire.

In a validation study, the reference method used should be as accurate as possible. In the present study, 22 men had a ratio of energy intake to BMR from weighed records below 1.12, and it is most likely that these men underestimated their habitual intake. However, they were included in the validity analysis, and when calculating the association between nutrients estimated from the records and the questionnaire, the correlation coefficients were similar with and without the 22 men included (data not shown). Body mass index (kg/m²) was significantly higher in the

ilar design as the present study (23). Results from previous studies that have compared food frequency questionnaires (varying in number of food items and portion sizes alternatives) and food records are diverse. Some studies have reported measurements from questionnaires to be generally higher (35–39) and others have reported measurements from questionnaires not systematically different (2, 5, 40–42) from the corresponding diet records measurements.

The observed median crude correlation coefficient of 0.51 between the nutrient intake estimated from the questionnaire and the weighed records was lower than the median found in the study of elderly women (median *r* = 0.61) (23). The results from several US studies (2, 43) and a Danish study (41) were similar to ours, whereas for two Dutch studies (3, 44), a Finnish (38) and a Swedish study (39), somewhat

TABLE 5. Comparison of dietary fatty acids and alpha-tocopherol derived from the food frequency questionnaire (FFQ), and relative amount of fatty acids and alpha-tocopherol concentration in adipose tissue, among Norwegian men, fall 1995 to winter 1995/6 (*n* = 119)

Fatty acids or alpha-tocopherol intake	Median intake (25th percentile, 75th percentile)		Spearman correlation coefficient	95% CI†
	FFQ	Adipose tissue		
Saturated				
g/day	34.1 (26.7, 46.7)		0.21*	0.04 to 0.38
% of total fat	38.3 (36.2, 41.4)	29.9 (28.1, 31.1)‡	0.18	0.00 to 0.35
Monounsaturated				
g/day	32.2 (24.5, 41.3)		-0.21*	-0.38 to -0.03
% of total fat	35.5 (34.2, 37.1)	51.4 (49.5, 53.4)‡	0.11	-0.07 to 0.28
Polyunsaturated				
g/day	16.4 (12.3, 23.1)		0.26**	0.08 to 0.42
% of total fat	18.4 (16.1, 20.9)	15.4 (14.3, 16.6)‡	0.34***	0.17 to 0.49
Palmitic acid (16:0)				
g/day	16.8 (13.5, 22.0)		0.10	-0.08 to 0.28
% of total fat	18.5 (17.5, 20.3)	21.4 (20.3, 22.1)	0.12	-0.06 to 0.30
Oleic acid (18:1)				
g/day	26.4 (20.0, 34.8)		-0.15	-0.32 to 0.03
% of total fat	29.5 (28.3, 31.3)	41.5 (40.8, 43.0)	0.18	0.00 to 0.35
Linoleic acid (18:2, n-6)				
g/day	12.6 (9.2, 18.5)		0.31***	0.14 to 0.46
% of total fat	14.3 (11.7, 16.4)	13.4 (12.3, 14.5)	0.38***	0.22 to 0.52
Alpha-linolenic acid (18:3, n-3)				
g/day	1.56 (1.21, 2.42)		0.36***	0.19 to 0.51
% of total fat	1.82 (1.53, 2.22)	0.82 (0.70, 0.94)	0.42***	0.26 to 0.56
Eicosapentaenoic acid (20:5, n-3)				
g/day	0.28 (0.10, 0.51)		0.45***	0.29 to 0.58
% of total fat	0.26 (0.14, 0.54)	0.09 (0.02, 0.13)	0.52***	0.38 to 0.64
Docosapentaenoic acid (22:5, n-3)				
g/day	0.07 (0.04, 0.11)		0.26***	0.08 to 0.42
% of total fat	0.07 (0.05, 0.11)	0.24 (0.18, 0.29)	0.39***	0.23 to 0.53
Docosahexaenoic acid (22:6, n-3)				
g/day	0.46 (0.16, 0.74)		0.43***	0.27 to 0.57
% of total fat	0.42 (0.21, 0.76)	0.24 (0.19, 0.35)	0.49***	0.34 to 0.62
Alpha-tocopherol				
mg/day	11.4 (7.7, 15.5)		-0.05	-0.23 to 0.13
µg/g fat§		420.6 (216.7, 735.3)		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

† CI, confidence interval.

‡ Saturated fatty acids are the sum of 12:0, 14:0, 15:0, 17:0, 18:0, 20:0. Monounsaturated fatty acids are the sum of 14:1, 16:1tr, 16:1 n-9, 16:1 n-7, 17:1, 18:1tr, 18:1 n-9, 18:1 n-7, 20:1tr, 20:1, 22:1tr. Polyunsaturated fatty acids are the sum of 18:2 n-6, 18:3 n-3, 20:2 n-6, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3.

§ Total fatty acid methyl esters.

group of men with a ratio below 1.12 compared with the men who had a higher ratio. The median (25th percentile, 75th percentile) values were 26.5 (24.9, 28.8) and 24.8 (23.6, 26.8) ($p = 0.01$), respectively. It has been observed in a number of previous studies (23, 36, 44, 46, 47) that underreporting is more likely to occur among overweight than in normal-weight subjects. The energy intake estimated from the questionnaire showed that 55 percent of the participants had a ratio of energy intake to BMR of less than 1.35. This indicates that a large proportion probably underreported their usual food intake estimated from the questionnaire. We found a similar association between

underreporting and body weight as observed for the records.

The reference method in the present study should reflect the usual intake because it was used to evaluate the usual intake measured by the questionnaire. It has been shown that the number of days necessary to rank individuals with a given level of accuracy depends on the ratio of within- to between-subject variance in the specific group studied (7, 48). According to the ratio of within- to between-subject variances found in this study (table 3), 14 days of recording should be sufficient for satisfactory ranking of subjects in the present population.

TABLE 6. Relative amount of fatty acids in adipose tissue related to quartiles of fatty acid intake expressed as percent of total fat intake estimated with the food frequency questionnaire among Norwegian men, fall 1995 to winter 1995/6 ($n = 119$)

Fatty acids	Median intake (25th percentile, 75th percentile), by quartile			
	1	2	3	4
Linoleic acid (18:2, n-6)				
Intake (% of fat)	10.6 (9.6, 11.0)	13.1 (12.6, 13.5)	15.4 (15.0, 15.9)	18.1 (16.8, 19.2)
Adipose tissue (%)	12.4 (11.2, 13.4)*	13.4 (11.7, 14.5)	13.6 (12.8, 14.5)	14.1 (13.1, 15.3)†
Alpha-linolenic acid (18:3, n-3)				
Intake (% of fat)	1.35 (1.17, 1.40)	1.67 (1.59, 1.74)	2.03 (1.90, 2.11)	2.43 (2.29, 2.73)
Adipose tissue (%)	0.73 (0.65, 0.81)*	0.75 (0.64, 0.90)*	0.85 (0.76, 0.97)	0.90 (0.80, 0.99)†
Eicosapentaenoic acid (20:5, n-3)				
Intake (% of fat)	0.07 (0.05, 0.08)	0.21 (0.16, 0.24)	0.37 (0.32, 0.47)	0.88 (0.69, 1.11)
Adipose tissue (%)	0.03 (0.00, 0.08)*	0.07 (0.00, 0.12)*	0.11 (0.08, 0.12)	0.13 (0.10, 0.18)†
Docosapentaenoic acid (22:5, n-3)				
Intake (% of fat)	0.04 (0.03, 0.04)	0.06 (0.058, 0.068)	0.09 (0.08, 0.10)	0.15 (0.13, 0.18)
Adipose tissue (%)	0.20 (0.17, 0.25)*	0.22 (0.18, 0.26)	0.25 (0.20, 0.29)	0.29 (0.21, 0.34)†
Docosahexaenoic acid (22:6, n-3)				
Intake (% of fat)	0.14 (0.09, 0.17)	0.32 (0.28, 0.38)	0.55 (0.48, 0.62)	1.22 (0.93, 1.42)
Adipose tissue (%)	0.21 (0.15, 0.23)*	0.24 (0.18, 0.31)*	0.29 (0.22, 0.36)	0.38 (0.22, 0.45)†

* , † Values within a line with different superscript symbols are significantly different. Kruskal-Wallis test is used to compare the quartiles. If Kruskal-Wallis test is significant, the highest and the lowest quartile, and the highest and the next lowest quartile are tested with the Mann-Whitney test.

Fatty acids

Most authors (e.g., 49–53) have compared fatty acid intake with fatty acids in serum lipid fractions as cholesterol esters, phospholipids, and triglycerides. In the present study, we have used total serum lipids. Houwelingen et al. (54) found that the habitual intake of n-3 fatty acids, measured with a cross-check dietary history in 61 elderly Dutch men, was better reflected by the total serum lipids than by separate lipid fractions. This may be important, because the measurement of total serum fatty acids is far less time-consuming than analysis of lipid subfractions.

The intake of total fat, saturated, monounsaturated, and polyunsaturated fatty acids among the participants in the present study was similar to the intake found in a nationwide representative sample of Norwegian men (aged 20–55 years, $n = 1,061$) (4). The correlations we observed between the intake of EPA (20:5, n-3) and DHA (22:6, n-3) and the relative amounts of these fatty acids in total serum lipids are similar to the correlations found by van Houwelingen et al. (54) in serum lipids, and by Ma et al. (53) and Andersen et al. (55) in cholesterol esters and phospholipids. The last two studies used a modified Willett questionnaire and a questionnaire similar to the one in the present study, respectively, to estimate fatty acid intake.

The proportion of fatty acids in adipose tissue was similar to that found in two Danish studies (56, 57). Adipose tissue level was more closely correlated with the relative proportion of dietary fat intake (fatty acids expressed as percent of total fat intake) rather than the absolute intake. This is consistent with observations

from other groups (13, 58). The correlation coefficients for polyunsaturated fatty acids and the very-long-chain n-3 fatty acids in our study were similar to previous observations in studies using food frequency questionnaires to assess fatty acid intake (41, 58–61). The correlation for linoleic acid (18:2, n-6) in the present study was close to that observed by Tjønneland et al. (56), London et al. (58), and Garland et al. (61), but lower than observed by Hunter et al. (59). Few authors have looked at the correlation coefficient between dietary intake of alpha-linolenic acid (18:3, n-3) and the level of this fatty acid in adipose tissue. We found a significant correlation for alpha-linolenic acid (18:3, n-3) of similar magnitude to that observed in a small group of men (56).

Because it has been shown that weight changes may reduce the correlation between dietary intake and fat composition of adipose tissue (10, 12), we conducted some analyses excluding 37 men who reported change of weight of more than 3 kg during the last 2 years. Most of the correlations observed were similar to the correlations found in the overall data (not shown).

Although polyunsaturated fatty acids in tissues are derived from the diet, the association between proportion of polyunsaturated fatty acids in tissues and dietary intake is impaired by the complexity of metabolic processes—including desaturation, elongation, esterification, oxidation, and compartmentalization. For example, EPA (20:5, n-3) is a poorer substrate than all other examined fatty acids for esterification to cholesterol (62) and diacylglycerol (63). Moreover, some n-3 fatty acids are preferred substrates for certain enzymes (64), which leads to, for example, preferen-

TABLE 7. Comparison of dietary fatty acid and alpha-tocopherol derived from the food frequency questionnaire (FFQ) and serum level of fatty acids and alpha-tocopherol among Norwegian men, fall 1995 to winter 1995/6 (*n* = 135)

Fatty acids or alpha-tocopherol intake	Median intake (25th percentile, 75th percentile)		Spearman correlation coefficient	95% CI
	FFQ	Serum		
Saturated				
g/day	34.3 (27.5, 46.7)		-0.05	-0.22 to 0.12
% of total fat	38.3 (35.9, 41.4)	30.8 (29.9, 32.2)†	0.23**	0.06 to 0.38
Monounsaturated				
g/day	32.2 (25.5, 41.5)		-0.10	-0.26 to 0.07
% of total fat	35.5 (34.2, 37.1)	24.7 (22.6, 27.3)†	0.08	-0.09 to 0.25
Polyunsaturated				
g/day	16.4 (12.0, 23.4)		0.15	-0.02 to 0.31
% of total fat	18.4 (16.0, 20.8)	44.6 (41.4, 47.1)†	0.20*	0.03 to 0.36
Palmitic acid (16:0)				
g/day	16.8 (13.8, 22.2)		-0.08	-0.25 to 0.09
% of total fat	18.7 (17.5, 20.3)	21.6 (20.7, 22.7)	0.11	-0.06 to 0.27
Oleic acid (18:1)				
g/day	26.2 (20.6, 35.0)		-0.13	-0.29 to 0.04
% of total fat	29.5 (28.3, 31.3)	19.7 (17.8, 21.4)	0.09	-0.08 to 0.26
Linoleic acid (18:2, n-6)				
g/day	12.8 (8.8, 18.5)		0.18	0.01 to 0.34
% of total fat	14.2 (11.6, 16.4)	30.2 (27.9, 33.5)	0.16	-0.01 to 0.32
Alpha-linolenic acid (18:3, n-3)				
g/day	1.57 (1.18, 2.43)		0.31***	0.15 to 0.46
% of total fat	1.81 (1.53, 2.20)	0.62 (0.54, 0.75)	0.28***	0.12 to 0.43
Eicosapentaenoic acid (20:5, n-3)				
g/day	0.28 (0.09, 0.52)		0.50***	0.36 to 0.62
% of total fat	0.27 (0.12, 0.54)	1.17 (0.85, 1.90)	0.51***	0.37 to 0.63
Docosapentaenoic acid (22:5, n-3)				
g/day	0.07 (0.04, 0.11)		0.35***	0.19 to 0.49
% of total fat	0.07 (0.05, 0.11)	0.62 (0.55, 0.74)	0.38***	0.23 to 0.52
Docosahexaenoic acid (22:6, n-3)				
g/day	0.46 (0.16, 0.75)		0.49***	0.35 to 0.61
% of total fat	0.43 (0.21, 0.76)	2.91 (2.29, 3.84)	0.52***	0.39 to 0.63
Alpha-tocopherol				
mg/day	11.3 (7.9, 15.5)			
μmol/liter		24.5 (20.6, 30.6)	-0.10	-0.26 to 0.07
Alpha-tocopherol/cholesterol		4.45 (4.10, 4.91)	0.03	-0.14 to 0.20

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

† CI, confidence interval.

‡ Saturated fatty acids are the sum of 14:0, 15:0, 17:0, 18:0, 20:0, 22:0. Monounsaturated fatty acids are the sum of 16:1tr, 16:1 n-9, 16:1 n-7, 17:1, 18:1tr, 18:1 n-9, 18:1 n-7, 20:1. Polyunsaturated fatty acids are the sum of 18:2 n-6, 18:3 n-6, 18:3 n-3, 20:2 n-6, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3.

tial incorporation of n-3 fatty acids into some phospholipids. Furthermore, tissue fatty acids are usually expressed as proportions of total fatty acids; therefore, the denominator includes endogenously synthesized saturated and monounsaturated fatty acids. These proportions are inherently imperfect reflections of dietary fatty acid intakes. Another possible source of imperfect correlation between dietary fatty acids and tissue fatty acids is within-person biologic and dietary intake variability, and method variability (53).

For the very-long-chain n-3 fatty acids, we found that the correlation coefficients between dietary fatty acids intake (expressed as percent of total fat) and fatty

acid level in adipose tissue were similar to the coefficients between fatty acid intake and fatty acid level in serum. However, the correlations for dietary intake of polyunsaturated fatty acids and linoleic acid were highest with proportions in adipose tissue. The correlation between fatty acid levels in serum and in adipose tissue were highest for the n-3 fatty acids (alpha-linolenic acid (18:3, n-3), $r = 0.57$; EPA (20:5, n-3), $r = 0.54$; DPA (22:5, n-3) $r = 0.43$; DHA (22:6, n-3), $r = 0.69$). This indicates that the very-long-chain n-3 fatty acids in serum total lipids may be a similarly suitable marker for the intake of n-3 fatty acids as the content of these fatty acids in adipose tissue.

Alpha-tocopherol

No correlation was found between the serum concentration of alpha-tocopherol and alpha-tocopherol intake, similar to the observation in previous reports (15, 16, 18). Even when serum alpha-tocopherol was adjusted for serum concentration of cholesterol and triglyceride, we did not observe a significant correlation, which is in contrast to other reports (15, 17, 19, 65–67). However, in several of these studies, the intake of vitamin E included supplements and was much higher than in the present study. When the supplements were excluded from the analyses, the correlation coefficients were reduced (17, 19, 65, 66). In the Norwegian diet, cod liver oil is an important source of vitamin E intake. In the group of subjects who reported that they consumed vitamin E supplements or cod liver oil twice weekly or more ($n = 58$), the correlation between cholesterol-adjusted serum alpha-tocopherol and alpha-tocopherol intake was also low and non-significant ($r = 0.02$).

Information about the relation between dietary intake and adipose content of alpha-tocopherol is much more scarce than data on the relation between blood levels of alpha-tocopherol and intake (21, 68). We did not observe any correlation between the adipose level of alpha-tocopherol and dietary intake. Kardinaal et al. (21) found a nonsignificant correlation of 0.19 between adipose level of alpha-tocopherol and intake of vitamin E measured with a 95-item food questionnaire. When adjusting for sex and age, the coefficient increased to 0.24 ($p < 0.05$).

In the present study, 23 participants reported that they took vitamin E supplements 3–4 times per week. There was no difference in the concentration of alpha-tocopherol in adipose tissue between the group that consumed supplements and the group that did not consume vitamin E supplements. However, the correlation coefficient between dietary intake of alpha-tocopherol and the concentration in adipose tissue was higher in the group of men who took supplements ($r = 0.32$) than in the non-supplemented group ($r = -0.10$), but none of the correlations were significant. Thus, it seems as if the content of alpha-tocopherol in adipose tissue is a better marker for intake when the subjects have a high intake of vitamin E including supplements (69).

The present study did not show any significant relation between alpha-tocopherol concentration in adipose tissue and serum and the intake of alpha-tocopherol, which could be due to several factors. The relation will inevitably be confounded and attenuated by individual variations in absorption, availability, and metabolism (65). Furthermore, the use of vitamin E supplements may be overreported in the

questionnaire. A higher percent of the participants reported use of vitamin E supplement in the questionnaire than during the recording period. Moreover, the food composition data are of varying quality for alpha-tocopherol.

In summary, the 180-item food frequency questionnaire used in a population of healthy men aged 20–55 years may rank subjects adequately in regard to energy and most nutrients. However, the data also indicate that the median intake of micronutrients based on the questionnaire may be overestimated. Dietary intake of linoleic acid (18:2, n-6) and n-3 fatty acids estimated with the questionnaire was able to predict bioavailability of linoleic acid (18:2, n-6), alpha-linolenic acid (18:3, n-3), and especially EPA (20:5, n-3) and DHA (22:6, n-3). Moreover, our data indicate that the relative amounts of very-long-chain n-3 fatty acids in total serum lipids may be associated with the intake of these fatty acids to the same extent as the relative amounts of these fatty acids in adipose tissue.

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