Fatty acid composition of skeletal muscle reflects dietary fat composition in humans[1–3]

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ABSTRACT

Background: It is still unknown whether the fatty acid composition of human skeletal muscle lipids is directly influenced by the fat composition of the diet.

Objective: We investigated whether the fatty acid composition of the diet is reflected in the fatty acid profile of skeletal muscle phospholipids and triacylglycerols.

Design: Thirty-two healthy adults (25 men and 7 women) included in a larger controlled, multicenter dietary study were randomly assigned to diets containing a high proportion of either saturated fatty acids (SFAs) [total fat, 36% of energy; SFAs, 18% of energy; monounsaturated fatty acids (MUFAs), 10% of energy] or MUFAs (total fat, 35% of energy; SFAs, 9% of energy; MUFAs, 19% of energy) for 3 mo. Within each diet group, there was a second random assignment to supplementation with fish oil capsules [containing 3.6 g n−3 fatty acids/d; 2.4 g eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3)] or placebo. A muscle biopsy sample was taken from the vastus lateralis muscle after the diet period. Parallel analyses of diet and supplementation effects were performed.

Results: The proportions of myristic (14:0), pentadecanoic (15:0), heptadecanoic (17:0), and palmitoleic (16:1n−7) acids in the skeletal muscle phospholipids were higher and the proportion of oleic acid (18:1n−9) was lower in the SFA group than in the MUFA group. The proportion of total n−3 fatty acids in the muscle phospholipids was ≈2.5 times higher, with a 5 times higher proportion of eicosapentaenoic acid (20:5n−3), in subjects supplemented with n−3 fatty acids than in those given placebo. Similar differences were observed in the skeletal muscle triacylglycerols.

Conclusion: The fatty acid composition of skeletal muscle lipids reflects the fatty acid composition of the diet in healthy men and women. *Am J Clin Nutr* 2002;76:1222–9.

KEY WORDS n−3 Fatty acids, monounsaturated fatty acids, saturated fatty acids, skeletal muscle phospholipids, skeletal muscle triacylglycerols, diet, adults, fish oil

INTRODUCTION

It has been suggested that not only total fat intake but also dietary fatty acid composition plays an important role in the development of obesity, type 2 diabetes (1), and cardiovascular disease (2). The fatty acid composition of the diet is known to influence the fatty acid composition of stored and structural lipids in different body compartments, such as serum lipids (3–5), erythrocyte membranes (5), buccal epithelial cells (6), and adipose tissue (7, 8). Skeletal muscle is an essential tissue for whole-body energy metabolism, including insulin-stimulated glucose uptake (9) and fatty acid oxidation (10). The fatty acid composition of skeletal muscle phospholipids has in several human populations been related to peripheral insulin sensitivity (11–14) and obesity (13, 15). In general, an increased unsaturation of the muscle membrane fatty acids has been associated with improved insulin sensitivity; in particular, the proportion of n−3 fatty acids may have a beneficial role (16, 17). Increased intake of n−3 fatty acids has been suggested to reduce the incidence of coronary artery disease (18), and a high proportion of n−3 fatty acids in red blood cell membranes has been associated with a reduced risk of primary cardiac arrest (19).

Few studies have addressed whether the fatty acid composition of the diet influences the fatty acid profile of skeletal muscle phospholipids and triacylglycerols. The fatty acid composition of the cell membrane is a dynamic system, and the regulation mechanisms are not fully understood. Both genetic (20) and lifestyle-related factors, including diet (21, 22) and physical activity (23, 24), seem to play a part in determining the fatty acid composition of skeletal muscle phospholipids. Changes in the fatty acid profile of skeletal muscle phospholipids in response to changes in the fatty acid composition of the diet have been clearly shown in animals (17, 25–27). More recent data, comparing breast-fed and formula-fed infants, indicate that the fatty acid composition of the diet may modify the phospholipid fatty acid profile in human skeletal muscle as well (21, 22). The question of whether the fatty acid composition of the diet influences that of skeletal muscle triacylglycerols has so far been addressed only in animal studies (27, 28). The aim of the present study was to investigate whether a dietary intervention, with changes in the fatty acid composition of the diet, is reflected in the fatty acid composition of skeletal muscle phospholipids and triacylglycerols in middle-aged men and women.

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[2] Supported by grants from the Swedish Council for Forestry and Agricultural Research and the Swedish Nutrition Foundation. Food was supplied by Carlshamns mejeri AB, Van den Bergh Foods AB, and Svenska Nestlé AB.

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Received July 6, 2001.

Accepted for publication January 21, 2002.

TABLE 1
Fatty acid composition of the n−3 fatty acid capsules (fish oil) and the placebo capsules (olive oil)[1]

	n−3 Capsules	Placebo capsules
	% of total fatty acids	
16:0	1.7	9.2
16:1	2.0	0.7
16:4	0.9	—
18:0	0.9	4.0
18:1	9.5	79.6
18:2n−6	1.4	5.7
18:3n−3	1.2	0.6
18:4n−3	5.8	0.4
20:1n−9	—	—
20:4n−6	2.6	—
20:4n−3	1.7	—
20:5n−3	39.4	—
22:0	1.6	—
21:5n−3	1.5	—
22:5n−6	0.8	—
22:5n−3	3.7	—
22:6n−3	24.1	—

[1] Identified by gas chromatography.

SUBJECTS AND METHODS

Subjects

One hundred sixty-two healthy participants (86 men and 76 women) aged 30–65 y were included in a controlled, multicenter dietary study, the KANWU (Kuopio, Aarhus, Naples, Wollongong, Uppsala) study (29), with the main objective of examining whether changes in the fatty acid composition of the diet affect insulin sensitivity. Of the 34 subjects included in Uppsala, 32 subjects (25 men and 7 women) volunteered for a muscle biopsy at the end of the treatment period and were included in the present study. Subjects with impaired glucose tolerance were included in the KANWU study, whereas individuals with diabetes mellitus according to World Health Organization criteria (30) were not. Detailed information about the inclusion and exclusion criteria is published elsewhere (29). The participants were told to keep their degree of physical activity and alcohol consumption, if any, unchanged throughout the study. All the women were postmenopausal and were not receiving any hormone replacement therapy. All subjects gave their informed consent. The study was approved by the local ethics committee at the Medical Faculty of Uppsala University.

Study design

The subjects were randomly assigned to diets containing either a high proportion of saturated fatty acids (SFA diet; $n = 16$) or a high proportion of monounsaturated fatty acids (MUFA diet; $n = 16$) for 3 mo. Within the diet groups, there was a second random assignment in which one-half of the subjects in each diet group were supplemented with fish oil capsules (n−3 group, $n = 15$: SFA + n−3, $n = 7$, and MUFA + n−3, $n = 8$) and one-half were supplemented with placebo capsules (control group, $n = 17$: SFA + placebo, $n = 9$, and MUFA + placebo, $n = 8$). The n−3 capsules (Pikasol; Lube A/S, Hadsund, Denmark) contained 3.6 g n−3 fatty acids/d, of which 2.4 g was eicosapentaenoic (20:5n−3) and docosahexaenoic (22:6n−3) acids and 1.2 g was other n−3 fatty acids. The placebo capsules contained the same amount of olive oil. The complete fatty acid composition of the fish oil and placebo capsules is presented in **Table 1**.

The test period was preceded by a 2-wk stabilization period during which subjects consumed their habitual diets and were given placebo capsules. Routine clinical tests, according to the main protocol (29), including an oral-glucose-tolerance test, were carried out during this period. Blood samples were taken and anthropometric measurements were performed before and at the end of the dietary period. So that we could monitor dietary intake, the participants completed a 3-d weighed-food record (2 weekdays and 1 weekend day) once before and twice during the intervention period. In addition, dietary adherence was monitored through analysis of the fatty acid composition of serum cholesterol esters and phospholipids before and at the end of the intervention period. At the end of the diet period, a muscle biopsy sample was taken from the quadriceps (vastus lateralis) muscle for analysis of the fatty acid composition of phospholipids and triacylglycerols.

Diets and dietary analyses

Special fat products and food items in combination with dietary advice were given to the subjects for control of the fatty acid composition of the diet according to the KANWU study protocol (29). The SFA diet included butter and table margarine containing a relatively high proportion of SFAs. The MUFA diet included a spread and margarine containing high proportions of oleic acid (18:1n−9) derived from sunflower oil high in 18:1n−9 with negligible amounts of n−3 fatty acids. The subjects were not told which diet they were given. The participants were asked to reduce their intake of other food items with a high fat content and especially to avoid fatty fish and products including fish fatty acids. To ensure good adherence to the diet, the subjects met the same nutritionist for dietary advice at least twice each month during the study. All participants were instructed to eat a diet with the same proportions of macronutrients and with an energy level individually adjusted to maintain constant body weight. The target diets were calculated to contain 37% of energy as fat, with 17%, 14%, and 6% of energy as SFAs, MUFAs, and polyunsaturated fatty acids (PUFAs), respectively, in the SFA diet, and 8%, 23%, and 6% of energy as SFAs, MUFAs, and PUFAs in the MUFA diet. The achieved dietary intake was calculated from the weighed-food records by using the PC-KOST database (version 1-96; Swedish National Food Administration, Uppsala, Sweden) and a computerized calculation program (STOR MATS, version 4-0d; Rudans Lättdata; Västerås, Sweden). Data for margarine and other specially prepared foods were entered into this database for inclusion in the analysis.

Muscle sample

Muscle biopsy samples (15–30 mg) were obtained from the quadriceps (vastus lateralis) muscle under local anesthesia by using a Bergstrom needle and were immediately frozen in liquid nitrogen and stored at −70 °C until analyzed. The vastus lateralis is the most commonly investigated muscle with regard to fatty acid composition and the associations between insulin sensitivity and obesity (11–15).

Fatty acid composition

The fatty acid composition of serum phospholipids, cholesterol esters, skeletal muscle phospholipids, and triacylglycerols was determined by gas-liquid chromatography as previously described in detail (23). Briefly, the lipid esters were extracted in chloroform,

TABLE 2
Clinical characteristics of the participants in the 2 diet groups according to n−3 fatty acid supplementation[1]

	SFA group		MUFA group		*P* for main effect of	
	n−3 (*n* = 1 F, 6 M)	Control (*n* = 2 F, 7 M)	n−3 (*n* = 3 F, 5 M)	Control (*n* = 1 F, 7 M)	Diet[2]	Supplement[3]
Age (y)	53 ± 8	52 ± 5	51 ± 7	48 ± 11	NS	NS
Body weight (kg)	86.6 ± 15.8	81.7 ± 13.2	76.7 ± 10.9	82.5 ± 10.9	NS	NS
Height (cm)	180.3 ± 12.3	175.9 ± 9.2	176.0 ± 11.9	178.5 ± 7.6	NS	NS
BMI (kg/m^2)	26.4 ± 2.0	26.3 ± 2.8	24.7 ± 1.8	25.9 ± 3.1	NS	NS
Waist-to-hip ratio[4]	0.89 ± 0.06	0.92 ± 0.06	0.86 ± 0.06	0.92 ± 0.04	NS	0.02
Blood glucose, fasting (mmol/L)	5.36 ± 0.24	5.33 ± 0.48	5.63 ± 0.53	5.58 ± 0.43	NS	NS

[1] $\bar{x}$ ± SD. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.
[2] Differences between the SFA (*n* = 16) and MUFA (*n* = 16) diet groups.
[3] Differences between the n−3-supplemented (*n* = 15) and control (*n* = 17) groups.
[4] Significance calculated with a nonparametric test.

separated by thin-layer chromatography, transmethylated, and separated by gas-liquid chromatography on a capillary column. Butylated hydroxytoluene was added as an antioxidant during the fatty acid analyses. The fatty acids were identified by comparing each peak's retention time with those of methyl ester standards. The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. The activity of Δ^5-desaturase was estimated as the product-to-precursor ratio of the proportion of arachidonic acid (20:4n−6) to dihomo-γ-linolenic acid (20:3n−6) in skeletal muscle phospholipids. The sum of n−6 PUFAs [linoleic acid (18:2n−6), 20:3n−6, and 20:4n−6] and the sum of n−3 PUFAs [α-linolenic acid (18:3n−3), 20:5n−3, docosapentaenoic acid (22:5n−3), and 22:6n−3] in muscle phospholipids and triacylglycerols, respectively, were calculated. The proportions of 18:3n−3 in the muscle phospholipids and of 20:3n−6, 22:5n−3, and 22:6n−3 in the muscle triacylglycerols were too low to detect in several subjects. The individual proportions of these fatty acids are reported only for the subjects who had detectable values. In the calculated sum of n−3 and n−6 PUFAs, these values are included as zero.

Blood sampling and anthropometric measurements

Blood samples were drawn after the subjects had fasted for 12 h overnight. Body weight, height, and waist and hip circumferences were measured, and the waist-to-hip ratio and body mass index (in kg/m^2) were calculated as described in the main protocol of the KANWU study (29). Plasma glucose concentrations were analyzed by the glucose oxidase method (31).

Statistical methods

The data were analyzed statistically by using the SAS (version 8.0) and JMP (version 3.2) software programs (SAS Institute Inc, Cary, NC). The results are expressed as means ± SDs. For each outcome variable, the treatment differences were estimated from a statistical model (analysis of covariance) in which treatment categories (SFA or MUFA and the presence or absence of n−3 fatty acids) and their interactions were analyzed as explanatory factors, adjusted for the possible confounding effects of age, sex, and the baseline value of the outcome variable. For variables with skewed distributions (Shapiro Wilk's *W* test < 0.95), a logarithmic transformation was made to obtain normal distribution. When normality was not achieved by logarithmic transformation of the data, a nonparametric test was used (identified in the tables). For nonnormal variables, a test for interaction was performed by using the bootstrap percentile method with 20 000 replicated bootstrap samples (32). Because no interactions between diet and supplementation effects were observed (except for the proportion of 18:3n−3 in skeletal muscle phospholipids) and because one-half of the subjects in both diet groups were given n−3 supplements, the data were analyzed in 2 parallel ways. On the one hand, the total SFA group (*n* = 16) was compared with the total MUFA group (*n* = 16); on the other hand, the supplemented n−3 subjects (*n* = 15) were compared with the control subjects (*n* = 17). In this way, we get as large a sample size as possible for each treatment and increase the statistical power. For comparison of baseline data and for dietary intake before and during the study, the Student's unpaired test or Wilcoxon's rank-sum test was used. Relations between variables were analyzed by simple linear correlations: Pearson's correlation or the nonparametric Spearman's correlation. For all tests, two-tailed significance values were given with $P < 0.05$ regarded as statistically significant.

RESULTS

Clinical characteristics

There were no significant differences between the 2 diet groups (SFA and MUFA groups) in age, sex, body weight, height, body mass index, waist-to-hip ratio, or fasting plasma glucose concentrations at baseline (**Table 2**). For subjects in the n−3 and control groups, the only observed difference in clinical characteristics was in the waist-to-hip-ratio, which was higher in the control group. The clinical characteristics remained unchanged in each treatment group during the intervention period.

Dietary intake

The subjects' reported nutrient intakes before the study did not differ significantly between the 2 diet groups or between the n−3 and control groups (**Table 3**). During the intervention period, the reported fatty acid composition of the diet differed between the diet groups, with twice as much SFAs and one-half the amount of MUFAs in the SFA group than in the MUFA group. No significant differences were observed between the diet groups during the intervention period for intakes of total energy, macronutrients, or PUFAs. The reported mean intakes of fat and fatty acids during the study were close to the target values, indicating a valid design in monitoring the fatty acid composition of the diet. No significant differences in dietary fatty acid composition, as calculated from

TABLE 3
Calculated average dietary intake in the 2 diet groups, according to n−3 fatty acid supplementation, before and during the study period[1]

	SFA group				MUFA group				*P* for main effect of	
	n−3 (*n* = 7)		Control (*n* = 9)		n−3 (*n* = 8)		Control (*n* = 8)			
	Before	During	Before	During	Before	During	Before	During	Diet[2]	Supplement[3]
Energy (MJ)	10.5 ± 2.4	10.2 ± 2.2	9.8 ± 2.0	8.8 ± 1.1	9.3 ± 1.7	8.7 ± 1.9	10.7 ± 1.8	10.1 ± 2.0	NS	NS
Protein (% of energy)	14.7 ± 2.7	14.5 ± 1.6	14.8 ± 2.3	15.0 ± 1.5	15.9 ± 2.6	15.3 ± 1.5	14.6 ± 1.3	14.6 ± 1.3	NS	NS
Carbohydrate (% of energy)	47.9 ± 5.5	48.6 ± 2.0	51.4± 4.2	46.2 ± 3.8	49.3 ± 6.8	49.6 ± 4.0	47.5 ± 4.8	47.5 ± 1.4	NS	<0.05
Fat (% of energy)	36.3 ± 5.5	36.1 ± 1.6	29.1 ± 4.7	35.3 ± 5.02	31.6 ± 8.3	34.1 ± 3.4	35.2 ± 5.0	36.4 ± 2.4	NS	NS
SFAs (% of energy)[4]	16.1 ± 4.0	18.0 ± 1.0	12.2 ± 1.8	17.6 ± 2.4	13.3 ± 3.8	8.5 ± 0.6	14.8 ± 2.3	10.0 ± 1.4	<0.001	NS
MUFAs (% of energy)[4]	13.3 ± 1.5	10.1 ± 0.7	10.9 ± 2.2	10.4 ± 1.6	11.8 ± 3.4	19.3 ± 2.6	12.8 ± 2.3	19.4 ± 1.4	<0.001	NS
PUFAs (% of energy)	4.5 ± 0.4	4.6 ± 0.8	3.8 ± 0.9	4.2 ± 0.8	4.0 ± 1.0	3.9 ± 0.4	5.2 ± 1.0	4.3 ± 0.5	NS	NS
Alcohol (% of energy)[4]	1.1 ± 1.2	0.8 ± 0.9	4.8 ± 5.9	3.6 ± 3.4	3.2 ± 4.0	1.1 ± 1.7	2.6 ± 5.6	1.4 ± 1.6	NS	NS
Dietary fiber (g)	23 ± 6	23 ± 4	23 ± 6	18 ± 5	20 ± 6	20 ± 9	23 ± 6	23 ± 5	NS	NS
Cholesterol (mg)	360 ± 84	380 ± 117	282 ± 82	354 ± 72	396 ± 131	224 ± 89	402 ± 145	245 ± 54	<0.001	NS

[1] $\bar{x}$ ± SD. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. There were no significant differences between groups before the study period.
[2] Differences between the SFA (*n* = 16) and MUFA (*n* = 16) diet groups during the study.
[3] Differences between the n−3-supplemented (*n* = 15) and control (*n* = 17) groups during the study.
[4] Significance calculated with a nonparametric test.

the food records, were found between the n−3 and control groups (Table 3). However, a slightly higher proportion of carbohydrates (as a percentage of energy) was reported in the n−3 group.

Fatty acid composition of skeletal muscle phospholipids

In skeletal muscle phospholipids, the proportions of myristic (14:0), pentadecanoic (15:0), heptadecanoic (17:0), and palmitoleic (16:1n−7) acids were higher and the proportion of 18:1n−9 was lower in the SFA group than in the MUFA group (**Table 4**). The subjects in the n−3 group had higher proportions of total n−3 PUFAs and lower proportions of all the n−6 PUFAs than did those in the control group, followed by a lower ratio of n−6 to n−3 fatty acids. Compared with the control group, the n−3 group had a higher ratio of 20:4n−6 to 20:3n−6 (estimate of Δ^5-desaturase activity). No interactions were observed between the effect of diet and the effect of n−3 supplementation, except for the proportion of 18:3n−3 ($P < 0.05$). The results of the analyses of the fatty acid profile in skeletal muscle phospholipids are presented for 29 of the 32 total participants; muscle samples in 3 subjects were analyzed unsuccessfully.

Fatty acid composition of skeletal muscle triacylglycerols

In skeletal muscle triacylglycerols, the proportions of 14:0, palmitic acid (16:0), and 16:1n−7 were higher and the proportion

TABLE 4
Comparison of the fatty acid composition of skeletal muscle phospholipids between the 2 diet groups according to n−3 fatty acid supplementation[1]

	SFA group		MUFA group		*P* for main effect of	
	n−3 (*n* = 6)	Control (*n* = 9)	n−3 (*n* = 8)	Control (*n* = 6)	Diet[2]	Supplement[3]
	% of total fatty acids		*% of total fatty acids*		*P*	
14:0	0.97 ± 0.06	1.06 ± 0.13	0.80 ± 0.12	0.74 ± 0.15	<0.001	NS
15:0	0.31 ± 0.04	0.32 ± 0.10	0.22 ± 0.03	0.22 ± 0.03	<0.001	NS
16:0[4]	24.4 ± 1.8	23.9 ± 0.7	23.4 ± 1.9	23.4 ± 1.4	NS	NS
17:0[4]	0.43 ± 0.03	0.40 ± 0.04	0.36 ± 0.03	0.36 ± 0.02	<0.001	NS
18:0	14.9 ± 1.2	13.8 ± 0.7	14.1 ± 1.2	14.0 ± 0.8	NS	NS
16:1n−7	0.66 ± 0.19	0.92 ± 0.35	0.63 ± 0.19	0.56 ± 0.20	<0.01	NS
18:1n−9	10.3 ± 1.5	12.4 ± 1.6	13.7 ± 1.4	15.0 ± 1.4	<0.001	<0.001
18:2n−6	27.3± 1.6	30.2 ± 1.9	25.4 ± 2.1	29.8 ± 1.5	NS	<0.001
18:3n−3[4]	0.49 ± 0.29 [3]	0.37± 0.10 [5]	0.33 ± 0.08 [3]	0.30 ± 0.05 [4]	NS	NS
20:3n−6[4]	0.86 ± 0.11	1.28 ± 0.17	0.82 ± 0.08	1.23 ± 0.21	NS	<0.001
20:4n−6	8.39 ± 0.72	10.9 ± 1.0	8.94 ± 1.13	10.5 ± 1.2	NS	<0.001
20:5n−3[4]	5.14 ± 0.95	1.12 ± 0.20	5.28 ± 1.09	0.96 ± 0.06	NS	<0.001
22:5n−3[4]	2.29 ± 0.24	1.41 ± 0.10	2.03 ± 0.17	1.20 ± 0.12	NS	<0.001
22:6n−3[4]	4.22 ± 0.62	2.21 ± 0.36	4.22 ± 0.77	1.79 ± 0.20	NS	<0.001
Sum n−6	36.5 ± 1.9	42.4 ± 2.1	35.1 ± 2.7	41.6 ± 2.2	NS	<0.001
Sum n−3[4]	11.5 ±.1.8	4.79 ± 0.66	11.7 ± 1.8	4.14 ± 0.17	NS	<0.001
n−6:n−3[4]	3.3 ± 0.61	9.0 ± 1.2	3.1 ± 0.7	10.1 ± 0.9	NS	<0.001
20:4n−6:20:3n−6	9.9 ± 1.29	8.7 ± 1.4	11.1 ± 1.9	8.7 ± 1.5	NS	<0.01

[1] $\bar{x}$ ± SD; *n* in brackets. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid. *P* values are adjusted for age and sex. No interaction between diet and supplement effects was observed, except for the proportion of 18:3n−3, for which $P < 0.05$.
[2] Differences between the SFA (*n* = 15) and MUFA (*n* = 14) diet groups.
[3] Differences between the n−3-supplemented (*n* = 14) and control (*n* = 15) groups.
[4] Significance calculated with a nonparametric test.

TABLE 5
Comparison of the fatty acid composition of skeletal muscle triacylglycerols between the 2 diet groups according to n−3 fatty acid supplementation[1]

	SFA group		MUFA group		*P* for main effect of	
	n−3 (*n* = 7)	Control (*n* = 9)	n−3 (*n* = 8)	Control (*n* = 8)	Diet[2]	Supplement[3]
	% of total fatty acids		*% of total fatty acids*			
14:0	4.00 ± 0.40	3.50 ± 0.40	3.20 ± 0.54	3.18 ± 0.74	<0.01	NS
15:0	0.39 ± 0.07	0.34 ± 0.05	0.32 ± 0.07	0.31 ± 0.09	NS	NS
16:0	24.6 ± 1.3	24.0 ± 1.4	22.7 ± 2.2	23.0 ± 1.1	<0.01	NS
17:0	0.29 ± 0.08	0.26 ± 0.04	0.25 ± 0.04	0.25 ± 0.04	NS	NS
18:0	5.38 ± 1.93	4.48 ± 1.25	5.12 ± 1.28	5.08 ± 1.19	NS	NS
16:1n−7	6.80 ± 1.74	7.27 ± 1.50	5.77 ± 2.21	5.50 ± 1.60	<0.01	NS
18:1n−9	46.2 ± 2.0	48.7 ± 1.8	50.3 ± 1.7	51.4 ± 1.9	<0.001	<0.01
18:2n−6	9.97 ± 0.81	9.83 ± 0.95	10.2 ± 0.9	9.74 ± 1.17	NS	NS
18:3n−3[4]	0.82 ± 0.15	0.84 ± 0.12	0.82 ± 0.13	0.75 ± 0.27	NS	NS
20:3n−6[4]	0.15 ± 0.05 [6]	0.15 ± 0.03 [5]	0.16 ± 0.07 [5]	0.17 ± 0.59 [4]	NS	NS
20:4n−6[4]	0.35 ± 0.07	0.38 ± 0.07	0.35 ± 0.06	0.34 ± 0.09	NS	NS
20:5n−3	ND	ND	ND	ND		
22:5n−3[4]	0.57 ± 0.16 [6]	0.32 ± 0.06 [5]	0.59 ± 0.26 [6]	0.35 ± 0.02 [4]	NS	0.001
22:6n−3[4]	0.75 ± 0.17 [6]	0.34 ± 0.07 [5]	0.65 ± 0.25 [7]	0.40 ± 0.09 [4]	NS	0.001
Sum n−6[4]	10.4 ± 0.92	10.3 ± 0.93	10.6 ± 0.97	10.2 ± 1.16	NS	NS
Sum n−3	1.95 ± 0.71	1.21 ± 0.35	1.83 ± 0.67	1.12 ± 0.50	NS	<0.01
n−6:n−3	6.7 ± 4.9	9.3 ± 3.3	6.7 ± 3.3	10.6 ± 4.2	NS	<0.01

[1] $\bar{x}$ ± SD; *n* in brackets. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; ND, not detectable. *P* values are adjusted for age and sex. No interaction between diet and supplement effects was observed.
[2] Differences between the SFA (*n* = 16) and MUFA (*n* = 16) diet groups.
[3] Differences between the n−3-supplemented (*n* = 15) and control (*n* = 17) groups.
[4] Significance calculated with a nonparametric test.

of 18:1n−9 lower in the SFA group than in the MUFA group (**Table 5**). The subjects in the n−3 group had higher proportions of 22:5n−3 and 22:6n−3, giving them a higher proportion of total n−3 PUFAs, a lower ratio of n−6 to n−3 fatty acids, and a lower proportion of 18:1n−9 than in the control group. No interactions between the effect of diet and the effect of n−3 supplementation were found.

Adjustment for sex and age, as possible for the parametric variables only, did not change the observed differences in the fatty acid composition of the muscle phospholipids and triacylglycerols between the treatment groups. The only discrepancy was observed in the proportion of 18:1n−9 in the n−3 and control groups, which was significantly different between groups only after the adjustment. In addition, a significant difference in the proportion of 15:0 in muscle triacylglycerols between the SFA and MUFA groups was observed only without adjustment.

Fatty acid composition of serum lipid esters

Changes in the fatty acid composition of both serum phospholipids and cholesterol esters during the intervention reflected the dietary fatty acid profile in each treatment group. Effects of the diets and the n−3 supplementation on serum phospholipid fatty acid composition are shown in **Table 6**. Effects of the dietary treatment on the fatty acid composition of the serum phospholipids were similar to those presented in the main KANWU study (29). No significant difference was observed in the proportions of fatty acids in serum phospholipids at baseline either between the n−3 and control groups or between the 2 diet groups. The composition of fatty acids in serum phospholipids in the SFA and MUFA groups at baseline was as follows: 14:0, 0.45 ± 0.09% and 0.46 ± 0.09%; 15:0, 0.25 ± 0.04% and 0.23 ± 0.05%; 16:0, 30.8 ± 0.9% and 30.4 ± 1.1%; 17:0, 0.44 ± 0.04% and 0.43 ± 0.05%; stearic acid (18:0), 14.0 ± 0.8% and 14.1 ± 0.8%; 16:1n−7, 0.80 ± 0.18% and 0.79 ± 0.21%; 18:1n−9, 12.9 ± 0.70% and 12.7 ± 1.3%; 18:2n−6, 21.3 ± 2.8% and 21.8 ± 1.5%; 18:3n−3, 0.30 ± 0.06% and 0.34 ± 0.11%; 20:5n−3, 1.60 ± 0.84% and 1.60 ± 0.48%; and 22:6n−3, 4.66 ± 1.02% and 4.71 ± 1.02%.

Correlations of fatty acid patterns within subjects

Relations between the proportions of individual fatty acids in skeletal muscle phospholipids and those of the corresponding fatty acids in muscle triacylglycerols, serum phospholipids, and cholesterol esters, respectively, were calculated in the whole study group (n = 29). Positive correlations were observed mainly for the PUFAs (data not shown). The strongest correlations between the proportions of fatty acids in the muscle phospholipids and the corresponding fatty acid in muscle triacylglycerols, serum phospholipids, and cholesterol esters, respectively, were found for the proportion of 18:1n−9 ($r = 0.75$, $r = 0.75$, and $r = 0.75$, $P < 0.001$), 20:4n−6 ($r = 0.45$, $r = 0.80$, and $r = 0.60$, $P < 0.001$–0.05), and 22:6n−3 ($r = 0.68$, $r = 0.80$, and $r = 0.81$, $P < 0.001$).

DISCUSSION

The results of the present study show for the first time that the fatty acid profile of skeletal muscle lipids, both phospholipids and triacylglycerols, in healthy men and women differs in groups with different dietary fatty acid compositions. The n−3-supplemented subjects, who were given 2.4 g 20:5n−3 and 22:6n−3/d, equivalent to the consumption of ≈100–200 g fatty fish such as salmon, had proportions of long chain n−3 PUFAs in skeletal muscle phospholipids that were ≈2.5 times higher than those in control subjects. This difference may have been particularly obvious because the study diets were low in long-chain PUFAs (not including any fatty fish products). Whereas the supplemented group had a high proportion of n−3 fatty acids in the muscle lipids, the control subjects had a total of ≈4.5% n−3 fatty acids in the muscle

TABLE 6
Effects of 3 mo of a saturated fatty acid (SFA) or monounsaturated fatty acid (MUFA) diet and n−3 fatty acid supplementation on fatty acid composition in serum phospholipids[1]

	SFA group				MUFA group					
	n−3 (n = 7)		Control (n = 9)		n−3 (n = 8)		Control (n = 8)		P for main effect of	
	Baseline	Change[2]	Baseline	Change[2]	Baseline	Change[2]	Baseline	Change[2]	Diet[3]	Supplement[4]
	% of total fatty acids				*% of total fatty acids*					
14:0	0.44 ± 0.08	0.06 ± 0.08	0.46 ± 0.10	0.04 ± 0.11	0.47 ± 0.09	−0.11 ± 0.05	0.44 ± 0.10	−0.12 ± 0.06	<0.001	NS
15:0	0.25 ± 0.04	0.03 ± 0.03	0.24 ± 0.04	0.02 ± 0.04	0.24 ± 0.06	−0.02 ± 0.09	0.23 ± 0.05	−0.05 ± 0.04	<0.001	NS
16:0	30.5 ± 0.6	0.47 ± 0.63	31.0 ± 1.1	−0.05 ± 0.87	29.9 ± 1.2	−0.23 ± 1.31	30.9 ± 0.9	−1.56 ± 1.0	<0.01	NS
17:0	0.43 ± 0.04	0.06 ± 0.07	0.44 ± 0.04	0.0 ± 0.03	0.45 ± 0.03	−0.02 ± 0.04	0.40 ± 0.05	−0.02 ± 0.05	<0.01	<0.05
18:0	13.8 ± 0.6	0.65 ± 0.72	14.2 ± 0.9	−0.08 ± 0.57	14.1 ± 0.08	0.24 ± 0.75	14.1 ± 0.9	−0.12 ± 0.36	NS	<0.01
16:1n−7	0.79 ± 0.12	−0.15 ± 0.08	0.81 ± 0.22	0.02 ± 0.11	0.86 ± 0.14	−0.27 ± 0.08	0.72 ± 0.25	−0.11 ± 0.31	NS	<0.05
18:1n−9	13.0 ± 0.9	−2.75 ± 0.74	12.8 ± 0.5	−1.14 ± 0.62	13.2 ± 1.1	0.52 ± 1.16	12.3 ± 1.3	3.51 ± 0.85	<0.001	<0.001
18:2n−6	22.5 ± 3.2	−4.33 ± 3.47	20.4 ± 2.2	1.19 ± 1.37	21.6 ± 1.5	−5.31 ± 1.46	21.9 ± 1.6	−1.46 ± 1.13	<0.05	<0.001
20:3n−6	3.09 ± 0.51	−1.22 ± 0.59	3.31 ± 0.78	0.29 ± 0.35	3.18 ± 0.58	−1.21 ± 0.45	3.10 ± 0.54	0.91 ± 0.44	<0.05	<0.001
20:4n−6	7.81 ± 1.62	−0.71 ± 1.02	8.51 ± 1.22	0.72 ± 0.72	8.26 ± 1.09	−0.70 ± 0.71	8.17 ± 0.57	0.74 ± 0.94	NS	<0.001
20:5n−3	1.55 ± 0.95	4.78 ± 1.74	1.65 ± 0.80	−0.20 ± 0.48	1.50 ± 0.35	4.70 ± 1.80	1.70 ± 0.58	−0.72 ± 0.49	<0.05	<0.001
22:5n−3	1.11 ± 0.34	0.87 ± 0.50	1.16 ± 0.25	−0.08 ± 0.15	1.05 ± 0.12	0.50 ± 0.16	1.12 ± 0.10	−0.24 ± 0.18	<0.05	<0.001
22:6n−3	4.49 ± 1.29	2.51 ± 1.39	4.79 ± 0.81	−0.54 ± 0.65	4.75 ± 1.16	2.28 ± 0.68	4.67 ± 0.96	−0.58 ± 0.64	NS	<0.001

[1] $\bar{x}$ ± SD. The proportion of 18:3n−3 was not detectable in most of the subjects and is therefore not presented in the table. No significant difference was observed between the diet groups or between the n−3-supplemented and control groups at baseline. No interaction between diet and supplement effects was observed. P values are adjusted for age, sex, and baseline value.
[2] Absolute changes.
[3] Difference in changes (3 mo versus baseline) between the SFA (n = 16) and MUFA (n = 16) diet groups.
[4] Difference in changes (3 mo versus baseline) between the n−3-supplemented (n = 15) and control (n = 17) groups.

phospholipids, which is ≈2 percentage units lower than that observed in other Swedish populations (14, 23, 24). Similar results were reported in infants: breast-fed infants have higher proportions of n−3 PUFAs in their skeletal muscle phospholipids than do formula-fed infants, reflecting the lack of long-chain n−3 PUFAs in the infant formula used (21, 22).

We also found the fatty acid composition of skeletal muscle phospholipids to differ between the 2 diet groups, reflecting the dietary fatty acid profile. The SFA group had higher proportions of SFAs such as 14:0, 15:0, and 17:0 than did the MUFA group. This agrees with the results of earlier studies that observed correlations between milk fat consumption and the proportions of 15:0 and 17:0 in adipose tissue (33, 34) and between milk fat and butter intake and 15:0 acid in serum (34, 35). The proportion of 18:1n−9 in the skeletal muscle phospholipids was higher in the MUFA group than in the SFA group, apparently reflecting the high proportion of 18:1n−9 in the margarine used in the MUFA diet and the low proportion of SFAs in this diet.

The triacylglycerols had an ≈4 times higher proportion of 18:1n−9 and an ≈25% lower proportion of total n−6 PUFAs than did the phospholipids. The observed differences in the fatty acid profile of the skeletal muscle triacylglycerols between treatment groups, however, were similar to those for the phospholipids. When the n−3 and control groups were compared, a higher proportion of total n−3 PUFAs in the muscle triacylglycerols was observed in the supplemented subjects, in agreement with, but not so pronounced as, the observed differences in the muscle phospholipids. In contrast with the composition of the muscle phospholipids, there were no significant differences in the proportion of total n−6 PUFAs in the muscle triacylglycerols.

The effects of supplementation with n−3 fatty acids appeared to be different in different lipid structures, probably as a result of the different turnover time and a selective handling of different fatty acids. The influence of n−3 supplementation on muscle lipid fatty acid composition has not previously been studied in humans. In infants, the effects of feeding different fatty acids, including long-chain PUFAs, were more marked in the muscle than in the erythrocytes (22). The increased proportion of n−3 PUFAs in serum phospholipids in this study is similar to earlier observations (36). The difference between subjects who were or were not supplemented with n−3 fatty acids in the proportion of n−3 PUFAs in muscle phospholipids was approximately of the same magnitude as the difference seen in the serum phospholipids. The observed differences in n−3 PUFAs in muscle triacylglycerols are in line with those observed earlier in adipose tissue after supplementation with n−3 fatty acids (36).

The fatty acid profile of different body compartments reflects the preceding fatty acid composition of the diet at different points in time. The dietary fatty acid profile is reflected in serum triacylglycerols a few hours after a meal, whereas the fatty acid composition of serum phospholipids and cholesterol esters changes more slowly and reflects the fatty acid composition of the diet during the preceding weeks to months (3, 5). The kinetics of skeletal muscle phospholipid and triacylglycerol fatty acid composition in response to changes in dietary fatty acid composition is not known. The 3-mo intervention period used in the present study appears, however, to have been adequate for detecting diet-induced differences. It is possible that the differences would have been even more pronounced if the intervention had been continued longer.

Within the treatment groups, there were individual differences in the proportions of fatty acids in skeletal muscle, although no significant differences were seen in the fatty acid composition of the diet. For example, the proportions of 20:5n−3 and 22:6n−3 in skeletal muscle phospholipids varied widely in both the control (0.9–1.4% and 1.6–2.8%, respectively) and particularly in the n−3-supplemented (3.3–6.8% and 3.2–5.4%, respectively) subjects. These wide ranges were observed despite an identical daily

intake of 20:5n−3 and 22:6n−3 in the supplemented subjects and a negligible intake of fish fatty acids in the control subjects. Good compliance with the treatment diet and the n−3 supplementation was verified by the fatty acid pattern in the serum and by the weighed-food records. The observed individual variations are consistent with earlier observations in infants (21), suggesting both individual differences in activity of the fatty acid elongase and desaturase enzymes and a different ability to incorporate long-chain PUFAs into muscle membranes. The individual variations are further supported when looking at correlations between fatty acid compositions of different lipid esters within subjects.

Strong positive correlations were seen between the proportions of PUFAs in particular in skeletal muscle phospholipids and the corresponding fatty acid in muscle triacylglycerols, serum phospholipids, and cholesterol esters. The fact that the fatty acid composition of the diet influenced the fatty acid profile of skeletal muscle phospholipids may be important for several reasons. The phospholipid fatty acid composition of the cell membrane is known to influence membrane fluidity, membrane protein incorporation and enzyme activities, receptor functions (37), and skeletal muscle function (38). In human populations, the fatty acid composition of skeletal muscle phospholipids has been related to insulin resistance (11–14) and obesity (13, 15). Both insulin resistance and obesity have mainly been linked with a low proportion of long-chain PUFAs, a high proportion of 16:0, and a low ratio of 20:4 n−6 to 20:3 n−6. The KANWU study (29) is the first intervention study in healthy humans showing effects on insulin sensitivity of changes in dietary fatty acid composition. In that study, insulin sensitivity was impaired after the SFA diet compared with the MUFA diet. In the present study group, representing one-fifth of the total KANWU group, we did not observe any significant changes in insulin sensitivity between the 2 diet groups (data not shown), which is probably explained by the small sample size. No effect of n−3 supplementation on insulin sensitivity was observed in the KANWU study. Neither did we find any difference in insulin sensitivity between subjects with or without n−3 supplementation (data not shown).

To limit the inconvenience to the subjects, a muscle biopsy was not performed before the intervention. We have, however, no reason to believe that the randomly assigned groups differed in their fatty acid composition of the skeletal muscle at baseline. The clinical characteristics of the subjects (with the exception of a difference in waist-to-hip ratio between the n−3 and control groups), the fatty acid composition of the serum lipids, and dietary intake did not differ significantly between the groups at baseline. Thus, it is likely that the dietary treatment and the presence or absence of n−3 fatty acids explains the observed differences in fatty acid composition between the groups at the end of the study.

In conclusion, after a 3-mo dietary intervention study, the fatty acid composition of skeletal muscle phospholipids and triacylglycerols in healthy men and women clearly differed in groups with different dietary fatty acid compositions. The present results confirm earlier observations in animals and infants. Further studies are required to determine the time course of the dietary effects on the fatty acid profile of the skeletal muscle lipid structures and whether more extreme diets would give different results. Moreover, further investigations are needed to clarify the possible implications of these changes for the development and treatment of obesity and type 2 diabetes.

We thank our co-workers at the metabolic ward and the research laboratory for assistance during the study, Lars Berglund for support with the statistical analyses, and Marina Spoverud-Älvebratt for her valuable assistance in the metabolic ward kitchen. We are also very grateful to the subjects for their dedicated participation.

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