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Training affects muscle phospholipid fatty acid composition in humans

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Helge, Jørn W., Ben J. Wu, Mette Willer, Jens R. Daugaard, Leonard H. Storlien, and Bente Kiens. Training affects muscle phospholipid fatty acid composition in humans. *J Appl Physiol* 90: 670–677, 2001.—Training improves insulin sensitivity, which in turn may affect performance by modulation of fuel availability. Insulin action, in turn, has been linked to specific patterns of muscle structural lipids in skeletal muscle. This study investigated whether regular exercise training exerts an effect on the muscle membrane phospholipid fatty acid composition in humans. Seven male subjects performed endurance training of the knee extensors of one leg for 4 wk. The other leg served as a control. Before, after 4 days, and after 4 wk, muscle biopsies were obtained from the vastus lateralis. After 4 wk, the phospholipid fatty acid contents of oleic acid 18:1(n-9) and docosahexaenoic acid 22:6(n-3) were significantly higher in the trained (10.9 ± 0.5% and 3.2 ± 0.4% of total fatty acids, respectively) than the untrained leg (8.8 ± 0.5% and 2.6 ± 0.4%, $P < 0.05$). The ratio between n-6 and n-3 fatty acids was significantly lower in the trained (11.1 ± 0.9) than the untrained leg (13.1 ± 1.2, $P < 0.05$). In contrast, training did not affect muscle triacylglycerol fatty acid composition. Citrate synthase activity was increased by 17% in the trained compared with the untrained leg ($P < 0.05$). In this model, diet plays a minimal role, as the influence of dietary intake is similar on both legs. Regular exercise training per se influences the phospholipid fatty acid composition of muscle membranes but has no effect on the composition of fatty acids stored in triacylglycerols within the muscle.

exercise; one-leg model; enzyme activity; fiber types; lipids

PHOSPHOLIPIDS ARE THE MAJOR structural lipids of membranes. The physical structure of the phospholipids is strongly influenced by the subtypes of the two inherent fatty acids (41). Thus the properties of membrane function such as fluidity, permeability, and anchoring of membrane proteins depend considerably on membrane phospholipid fatty acid composition (21, 45).

There is evidence that the fatty acid composition of the muscle membrane is linked to the prevalence of two of the major lifestyle diseases, obesity and insulin resistance, with a higher proportion of more saturated fatty acids linked to adverse outcomes (44). It is therefore interesting to evaluate which factors influence muscle membrane composition and, in due course, whether these factors are involved and how they are involved in the progression toward these lifestyle diseases.

It is well known that dietary fatty acid profile plays an important role for the incorporation of fatty acids into the muscle membrane in humans (34, 44). The time course for these adaptations in humans is not clear; however, in rats, these changes in muscle membrane fatty acid composition occur within days and remodeling approaches a plateau within 3–4 wk (D. A. Pan and L. H. Storlien, unpublished observations). In addition to the effect of diet, there is some evidence that physical activity per se could also be a possible moderator of membrane phospholipid fatty acid composition. Thomas and colleagues (47) found a small but significantly lower content of palmitate (C16:0) and a trend toward a higher sum of C18–C20 fatty acids in vastus lateralis muscle when endurance-trained and sedentary male subjects were compared. However, diet was a completely uncontrolled variable. More recent work by Andersson and colleagues (3) demonstrated that 6 wk of low-intensity exercise training resulted in significant changes in muscle phospholipid fatty acid composition with a significant increase in oleic acid 18:1(n-9) and a decrease in arachidonic acid 20:4(n-6). Although great care was taken to attempt to control dietary fatty acid profile, the subjects were free-living. In addition, randomization of subjects unfortunately resulted in a sedentary group that was significantly older (44 ± 6 vs. 36 ± 8 yr) and, before initiation of training, somewhat less trained than the trained group (33.8 ± 5.6 sedentary vs. 39.5 ± 7.0 $ml \cdot min^{-1} \cdot kg^{-1}$ trained, $P = 0.069$, unpaired t-test).

The aim of this study was therefore to investigate the effects of regular exercise training on skeletal muscle phospholipid fatty acid composition in humans applying a one-leg training model in which the other leg serves as a reference. Thus dietary fatty acid composi-

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tion, initial level of training, and other relevant variables are perfectly controlled. Our working hypothesis was that regular training, primarily through its effect on substrate flux and substrate storage, induces an adaptive response in muscle membrane phospholipid fatty acid composition.

METHODS

Subjects. Seven healthy male subjects age 22 ± 1 years, height 178 ± 3 cm, and weight 74.3 ± 5.4 kg participated in the study. The subjects were fully informed of the nature, stresses, and possible risks associated with the study before they volunteered. The study was approved by the Copenhagen Ethics Committee.

Protocol. Before the experiment, subjects were accustomed to exercise in the one-leg knee extension ergometer (Fig. 1). Before the experiment, maximal work capacity (W_{max}) was determined for each leg separately as described by Andersen et al. (1). At the initiation of the training period, subjects arrived to the laboratory in an overnight-fasted state and had performed no rigorous physical activity over the last 48 h. After subjects rested for 20 min in a lying position, a needle biopsy from both legs was obtained from the vastus lateralis muscle under local anesthesia with lidocaine and with suction (5). Muscle biopsies were frozen within 5–10 s and stored at −80°C until further analysis. Over the ensuing 4 wk, the knee extensors of one leg were trained and the other leg served as a nontrained control. The subjects followed a supervised training program, which is described in detail below. Selection of the leg eligible for training was done by randomized stratification, such that the dominant and nondominant leg were similarly represented. The determination of leg dominance was done subjectively and subsequently confirmed by comparison of the one-leg W_{max} measured before the training period. After 3 (n = 1) or 4 (n = 6) days and after 4 wk of exercise training, another muscle biopsy was obtained from the vastus lateralis muscle of both legs. Maximal whole body oxygen uptake was determined on a Krogh bicycle ergometer using a standard progressive exercise test before and after the 4-wk intervention. Before and after the training period, thigh volume was determined from measurements of the length, three circumferences, and three skinfolds of the thigh; subsequently, this volume was used to calculate the leg quadriceps femoris muscle mass (27, 28).

Training. All subjects followed a supervised training program that was designed to enhance leg muscle endurance capacity. Training was performed in the one-leg knee extension ergometer four times a week during the first 2 wk and five times a week during the last 2 wk. Duration of the training was progressively increased by 10 min over the first seven training sessions, starting at 60 min on the first two sessions and reaching 120 min at the eighth training session. This duration was maintained for the remaining period of training. Each training session was initiated with a 10- or 15-min warm-up at 60% of W_{max} and ended with a 10-min active recovery period at 50% W_{max}. In the remaining time, subjects performed exercise that alternated between short and long intervals, exercising at 70–100% of W_{max} interspersed with short breaks, consisting of exercise at 50% W_{max}. After 14 days, W_{max} was measured for the trained leg so that training intensity could be adjusted if necessary. W_{max} was not measured in the control leg after 14 days to avoid any risk of a training effect. At every training session, heart rate, and thus training intensity, was monitored with a heart rate recorder (Polar Vantage NV, Polar Electro). During the training sessions, subjects had free access to water.

Dietary intake. Habitual daily energy intake and diet composition were determined in each subject using a 4-day dietary record (3 weekdays and 1 weekend day). All food and fluid intake were carefully weighed and registered to 1-g accuracy. During the latter 2 wk of the training period, subjects repeated the registration of daily energy and nutrient intake with a new 4-day dietary record. The energy intake and nutrient composition of the dietary intake were calculated by using a computer database (Dankost 2000, the Danish Catering Center, Copenhagen, Denmark).

Analyses. For the analysis of maximal oxygen uptake, expired air was sampled in Douglas bags and then the volume of air was measured in a Collins bell-spirometer (Collins W.E.). The fractions of oxygen and carbon dioxide were determined with paramagnetic (Servomex) and infrared (Beckmann LB-2) systems, respectively. Two gas samples with known compositions were used to calibrate both systems regularly. For the determination of whole body oxygen uptake, carbon dioxide excretion, and ventilation during the leg W_{max} test, an automated online system was applied (Medigraphics, CPX/D).

Biochemical analysis. For the determination of enzymatic activity, muscle biopsy samples were freeze-dried and all

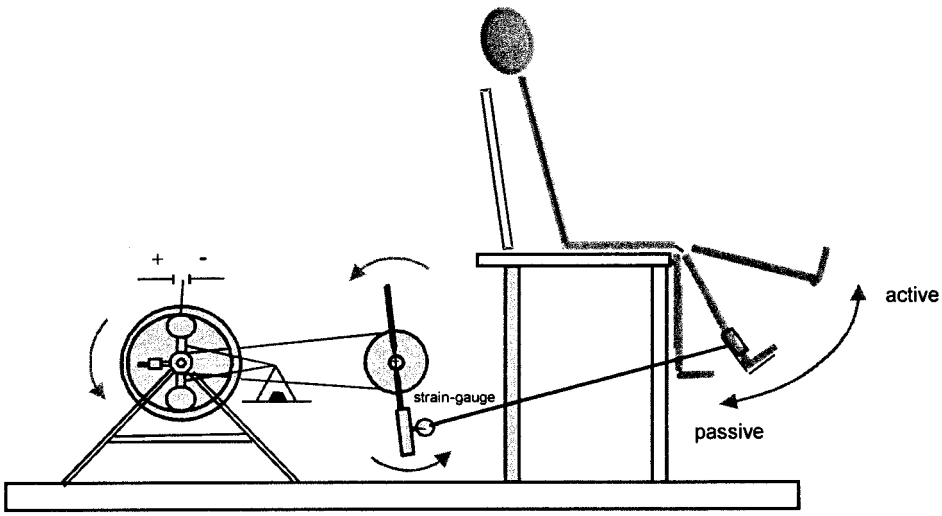

Fig. 1. Depiction of the one-leg extension model, as described by Andersen and Saltin (2).

connective tissue, visible fat, and blood were carefully dissected away under a stereomicroscope in a room with a temperature of 18°C and a relative humidity below 30%. The maximal activities of the enzymes β-hydroxy-acyl-CoA-dehydrogenase and citrate synthase were then determined fluorometrically according to Lowry and Passoneau (32). The extraction, derivatization, and quantification of the fatty acid components of muscle phospholipids and triacylglycerol have been described in full detail elsewhere (39). In brief, muscle tissue, ~50 mg wet weight, was homogenized in 2:1 (vol/vol) chloroform-methanol and total lipid extracts were prepared according to the method used by Folch et al. (12). Phospholipids were isolated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, MA). Fatty acids from the triaclyglycerol fraction or the phospholipid fraction were then transmethylated, and the methyl fatty acids were separated, identified, and quantified by gas chromatography. The content of individual fatty acids in the phospholipids and triacylglycerol extracted from the muscle was expressed as a percentage of the total fatty acids identified. Individual fatty acids that made up <1% of the total in all groups are not shown in Tables 3 and 4. Several indexes, the sum of saturated fatty acids, the sum of unsaturated fatty acids, the sum of monounsaturated fatty acids, the ratio between n-6 fatty acids and n-3 fatty acids (n-6/n-3), and the total percentage of long-chain polyunsaturated fatty acids (PUFA) with ≥20 carbon units (ΣC20–C22PUFA), were derived. In each subject, myosin heavy chain (MHC) composition and thus muscle fiber-type composition were analyzed before and after 4 wk only in the trained leg. MHC composition was analyzed as described in Ref. 6.

Statistics. Differences due to training and time were tested with a two-way variance analysis with repeated measurements. Wherever ANOVA revealed significant effects, a Student-Newman-Keuls test was used to discern differences between groups. Analyses were performed with SigmaStat statistical analysis system version 2.0 (Jandel), and statistical significance was set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Over the experimental period, body weight remained unchanged at 74.3 ± 5.4 kg. The habitual dietary energy intake was similar to the energy intake during the experimental period (Table 1). Although the 10% higher energy intake during the experimental period was not significantly different from the habitual intake, the extra 1.1 MJ/day consumed during the experiment actually does account for the calculated increased energy expended during the training of ~1.3 MJ/day. The nutrient composition was similar between the habitual diet and the diet consumed during the experiment (Table 1). Furthermore, there was no detectable change in the relative consumption of saturated, monounsaturated, and polyunsaturated fatty acids among subjects (Table 1).

Table 1. *Energy intake and dietary composition before and during the experiment*

	Before	During
Energy, MJ	11.1 ± 0.6	12.2 ± 0.9
Protein, E%	12 ± 1	12 ± 1
Carbohydrate		
E%	54 ± 2	56 ± 2
g	365 ± 23	374 ± 39
g/kg body wt	5.0 ± 0.1	4.9 ± 0.1
Fat, E%	32 ± 2	33 ± 2
%Saturated FA	44 ± 4	46 ± 5
%Monounsaturated FA	38 ± 3	37 ± 3
%Polyunsaturated FA	18 ± 1	17 ± 2

Values are means ± SE. FA, fatty acids; E%, percent of total energy content of diet.

Table 2. *Muscle mass and muscle enzymatic activity of vastus lateralis muscle before and after 4-wk adaptation to regular one-leg knee extension training*

	Before Adaptation		After 4-wk Adaptation	
	Untrained	Trained	Untrained	Trained
Quadriceps muscle mass, kg	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.2	3.1 ± 0.2*†
CS activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$	40 ± 2	40 ± 2	41 ± 2	48 ± 2*†
HAD activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$	26 ± 1	26 ± 2	25 ± 1	27 ± 1

Values are means ± SE. Muscle mass, citrate synthase (CS) activity, and β-hydroxy-acyl-CoA-dehydrogenase (HAD) activity in vastus lateralis muscle before and after 4 wk of training in the one-leg knee extension ergometer are provided. *$P < 0.05$, initial vs. after 4 wk; †$P < 0.05$, trained vs. untrained.

During the 4 wk, all subjects completed all 18 training sessions. After 4 wk, a significant time × training interaction was present for the estimated quadriceps femoris muscle mass, such that muscle volume in the trained leg was increased by 4% after training ($P < 0.05$, Table 2). Before the experiment, maximal one-leg W_{max} was 43 ± 4 and 44 ± 3 W in the untrained and the trained leg, respectively. After 2 wk of training, leg W_{max} was increased by 9% in the trained leg to 48 ± 4 ($P < 0.05$). Because W_{max} was not measured after 14 days in the untrained leg and after 28 days in either leg, a possible contralateral training effect on the untrained leg cannot be excluded. However, because enzyme activity and muscle mass remained unchanged after 4 wk in the untrained leg, there does not seem to be any effect of training on the contralateral leg. Before the training, neither citrate synthase activity nor β-hydroxy-acyl-CoA-dehydrogenase activity differed between legs. After 4 wk, citrate synthase activity was increased by 17% in the trained compared with the untrained leg ($P < 0.05$) and was significantly higher than before the training (Table 2). For the β-hydroxy-acyl-CoA-dehydrogenase activity, no significant effect of the training was observed after 4 wk (Table 2). Maximal whole body oxygen uptake remained unchanged at 3.5 ± 0.1 l/min, indicating that the one-leg training did not provide sufficient stimulus to induce a whole body cardiovascular adaptation. Muscle fiber composition of the vastus lateralis measured only in the thigh that was trained averaged 49 ± 4% MHC type I, 31 ± 5% MHC type IIA, and 20 ± 5% MHC type IIx (type IIB in old nomenclature). The presence of 20 ± 5% of MHC type IIx indicates that the subjects were initially rather untrained. After subjects completed the training, the fiber-type composition of the

vastus lateralis muscle was 50 ± 3% MHC type I, 36 ± 3% MHC type IIA, and 15 ± 4% MHC type IIx. Thus the fraction of type IIx fibers was significantly decreased with training. In a previous study, fiber type was unaltered in the untrained thigh after a period of one-leg training of the other leg (27, 28).

There was a significant effect of regular exercise training on the composition of the membrane phospholipid fatty acids. After 4 wk, the content of oleic acid 18:1(n-9), vaccenic acid 18:1(n-7), and docosahexanoeic acid 22:6(n-3) was increased in the trained compared with the untrained leg by 18, 14, and 14%, respectively ($P < 0.05$, Table 3). Of the composite measures of fatty acid composition, the sum of unsaturates (post hoc test reveals a difference only within the 28 days values, $P = 0.017$) was higher and the ratio (n-6/n-3) and the sum of saturates were significantly lower in the trained compared with the untrained leg after the training period (Table 3). The sum of monunsaturates and the ratio of C18:1(n-9) to C16:0 were significantly higher in the trained vs. the untrained leg throughout the experiment; however, the difference increased over time from 3% for both parameters initially to 13 and 38% after the 4-wk training, respectively. A graphic interpretation of the main relative differences in fatty acid composition induced by regular one-leg exercise training is shown in Fig. 2. There was no effect of time as a parameter on any of the individual fatty acids or the composite measures of muscle phospholipid fatty acid composition (Table 3). In contrast to the above, muscle triacylglycerol fatty acid composition was completely unaffected by regular exercise training (Table 4). However, a significant effect of time was noted for several of the triacylglycerol fatty acids. The content of stearic acid (18:0) was significantly decreased and that of oleic acid 18:1(n-9) significantly increased in both legs after 4 days, after which they remained unchanged (Table 4). Of the composite measures, the sum of unsaturates was increased after 4 days and no further changes were observed (Table 4). The ratio of C18:1(n-9) to C16:0 was not affected in the muscle triacylglycerol fatty acid pool (ratio not shown).

DISCUSSION

In the present study, the main finding was a significant training-induced increase in the relative content of oleic acid, vaccenic acid, docosahexanoeic acid, and the total sum of unsaturated fatty acids in the muscle membrane phospholipid fatty acids. In contrast, no effect of regular training was observed on fatty acid composition of triacylglycerols stored within the muscle. In this one-leg training model, dietary fatty acid intake does not confound the effects of exercise, providing evidence that exercise per se independently exerts an effect on muscle membrane phospholipid fatty acid composition.

In skeletal muscle, regular exercise induces a multitude of adaptations that enhance the oxidative capacity of the muscle cell and its ability to maintain homeostasis during sustained contractions (43). Maintenance of homeostasis is linked to the transport and control of ion and metabolite concentrations within the cell, both of which are influenced by membrane function, which, in turn, is modulated by membrane structural lipid composition. In this study, 4-wk regular exercise training induced changes in the muscle membrane phospholipid fatty acid profile; therefore, exercise training should be considered as a modulator of muscle phospholipid fatty acid composition. In humans, two studies have previously addressed this issue. Andersson and colleagues (3) studied the effect of 6-wk low-intensity exercise training and diet on muscle membrane lipid profile and compared those results with the results from a control group that consumed the same diet over the 6-wk period. Over the 6 wk of

Table 3. *Individual and composite measures of phospholipid fatty acid composition of quadriceps muscle in a trained and an untrained leg before and after 4-day and 4-wk adaptation to a one-leg training program*

Fatty Acid	Before		After 4 day		After 4 wk		Two-way ANOVA		
	Untrained	Trained	Untrained	Trained	Untrained	Trained	Train	Time	T×T
16:0	14.2 ± 0.9	14.8 ± 1.1	13.2 ± 0.5	13.6 ± 0.7	15.4 ± 1.5	11.7 ± 0.7	NS	NS	NS
18:0	12.3 ± 1.0	12.1 ± 0.8	10.6 ± 0.6	9.8 ± 0.3	10.6 ± 0.7	11.2 ± 0.8	NS	NS	NS
18:1n-9	8.6 ± 0.5	9.2 ± 0.6	9.1 ± 0.6	9.6 ± 0.7	8.8 ± 0.4	10.9 ± 0.5*†	<0.01	NS	<0.05
18:1n-7	2.2 ± 0.2	2.2 ± 0.2	2.4 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.5 ± 0.1*†	NS	NS	<0.02
18:2 n-6	32.7 ± 0.7	32.1 ± 0.8	32.2 ± 0.8	32.0 ± 0.6	32.3 ± 0.9	32.3 ± 0.5	NS	NS	NS
20:3 n-3	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	NS	NS	NS
20:4 n-6	17.9 ± 0.9	17.6 ± 1.1	19.9 ± 0.7	19.8 ± 0.4	18.7 ± 0.4	19.1 ± 1.0	NS	NS	NS
22:6 n-3	2.7 ± 0.4	2.8 ± 0.4	2.9 ± 0.4	3.0 ± 0.5	2.6 ± 0.4	3.2 ± 0.4*†	<0.04	NS	<0.04
ΣUnsaturated FA	73.1 ± 1.7	72.7 ± 1.2	75.5 ± 0.6	76.2 ± 0.5	73.6 ± 1.0	76.6 ± 1.1*	<0.02	NS	NS
ΣMonounsaturated FA	14.1 ± 0.5	14.5 ± 0.8‡	14.6 ± 0.6	15.7 ± 0.4‡	14.6 ± 0.3	16.5 ± 0.4‡	<0.01	NS	NS
C18:1n-9/C16:0	0.62 ± 0.05	0.64 ± 0.05	0.7 ± 0.1	0.72 ± 0.07	0.60 ± 0.05	0.96 ± 0.09*†	<0.01	NS	<0.04
(n-6)/(n-3)	12.2 ± 1.2	11.3 ± 1.4	11.9 ± 1.0	12.0 ± 1.1	13.1 ± 1.2	11.1 ± 0.9*	<0.02	NS	<0.03
ΣC20–22PUFA	25.6 ± 1.0	25.3 ± 1.0	27.6 ± 0.7	27.6 ± 0.7	25.9 ± 0.5	27.1 ± 1.2	NS	NS	NS

Values are means ± SE in %total fatty acids. Train, training effect; Time, time effect; T × T, training and time interaction; NS, not significant; Σunsaturated FA, sum of all unsaturated fatty acids; Σmonounsaturated FA, sum of all monounsaturated fatty acids; C18:1n-9/C16:0, ratio between oleic and palmitic acid; (n-6)/(n-3), ratio between all n-6 fatty acids and all n-3 fatty acids; ΣC20-22PUFA, sum of polyunsaturated long-chain fatty acids with 20–22 carbons. *$P < 0.05$, trained vs. untrained leg (within 4 wk); †$P < 0.05$, initial vs. 4 wk (within the trained leg); ‡$P < 0.05$, trained vs. untrained.

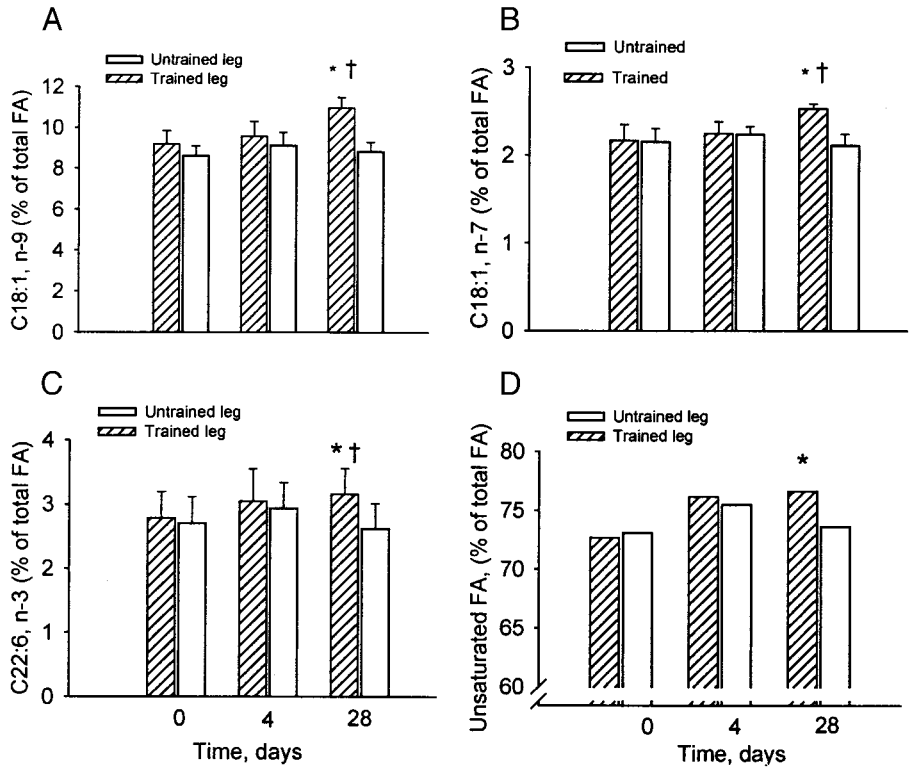

Fig. 2. Muscle membrane phospholipid fatty acid content of oleic acid C18:1(n-9) (*A*), vaccenic acid C18:1(n-7) (*B*), docosahexanoeic acid C22:6(n-3) (*C*), and the sum of unsaturated fatty acids (FA; %unsaturated fatty acids) (*D*) in vastus lateralis muscle in a trained and an untrained leg before and after 4 and 28 days of regular one-leg training. Values are means ± SE. *$P < 0.01$, trained vs. untrained. †$P < 0.01$, 4 wk vs. initial.

training, the muscle membrane content of arachidonic acid 20:4(n-6) decreased and that of oleic acid 18:1(n-9) increased significantly more in the trained than in the control group. This change matched the training-induced increase in the membrane content of oleic acid observed in the present study; however, in the present study, training did not affect arachidonic acid membrane content. Interestingly, the ratio of C18:1(n-9) to C16:0 was significantly higher after training in this study, which nicely compares with the trend toward an increase in the same ratio in the study by Andersson et al. (3). This increased ratio would suggest an increase in the Δ9-desaturase activities induced by training.

In a cross-sectional study of trained vs. untrained individuals, for which a strict dietary control was not performed, a small but significant decrease in the content of palmitate and a trend toward an increase in the sum of C18–C20 were apparent in trained compared with sedentary men (47). In contrast to the present study and the study by Andersson et al. (3), the study by Thomas and co-workers (47) reported no apparent increased membrane unsaturation in their trained group. Several explanations are available for this discrepancy. First, it is well known that dietary fatty acid composition is a very potent moderator of membrane fatty acid composition (39) and, because the trained group in the study by Thomas et al. (47) probably had a higher energy intake (8, 25), it is possible that the dietary fat intake before the muscle biopsy could have been quite different compared with that of the seden-

Table 4. *Individual and composite measures of triacylglycerol fatty acid composition of quadriceps muscle in a trained and an untrained leg before and after 4-day and 4-wk adaptation to a one-leg training program*

	Before		After 4 days		After 4 wk		Two-way ANOVA		
Fatty Acid	Untrained	Trained	Untrained	Trained	Untrained	Trained	Train	Time	T×T
16:0	19.1 ± 2.1	16.7 ± 1.7	19.3 ± 1.5	18.3 ± 1.6	17.7 ± 1.5	18.6 ± 1.3	NS	NS	NS
18:0	12.0 ± 3.3	16.6 ± 3.5	5.7 ± 0.7*	5.9 ± 1.0*	11.9 ± 3.1*	6.1 ± 1.0*	NS	<0.02	NS
16:1 n-7	5.5 ± 0.5	6.1 ± 0.8	5.9 ± 0.7	5.5 ± 0.5	5.1 ± 0.6	6.2 ± 0.7	NS	NS	NS
18:1 n-9	45.4 ± 1.7	42.1 ± 1.5	48.2 ± 1.3*	51.7 ± 1.8*	47.0 ± 2.5*	49.1 ± 1.4*	NS	<0.01	NS
18:1 n-7	3.4 ± 0.2	3.8 ± 0.2	3.9 ± 0.2	4.0 ± 0.3	3.6 ± 0.3	3.7 ± 0.2	NS	NS	NS
18:2 n-6	10.4 ± 0.4	10.0 ± 0.3	11.3 ± 0.4	10.4 ± 0.6	10.3 ± 0.6	10.9 ± 0.3	NS	NS	NS
20:4 n-6	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	NS	NS	NS
22:6 n-3	0.6 ± 0.1	0.8 ± 0.2	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	NS	<0.03	NS
ΣUnsaturated FA	68.3 ± 2.3	66.2 ± 2.0	74.3 ± 1.7*	75.4 ± 1.7*	69.8 ± 2.3*	74.5 ± 1.7*	NS	<0.01	NS
ΣMonounsaturated FA	54.9 ± 2.1	52.6 ± 1.7	59.0 ± 1.6	62.0 ± 1.6	56.4 ± 2.7	59.9 ± 1.6	NS	<0.01	NS
(n-6)/(n-3)	10.5 ± 1.6	6.9 ± 0.9	9.4 ± 0.6	10.9 ± 0.8	11.5 ± 2.1	7.7 ± 0.9	NS	NS	NS
ΣC20–22PUFA	2.3 ± 0.2	2.6 ± 0.4	3.0 ± 0.4	2.5 ± 0.3	2.2 ± 0.3	2.8 ± 0.2	NS	NS	NS

Values are means ± SE in %total fatty acids. *$P < 0.05$, values vs. initial values.

tary subjects; as such, the balance between exogenous and endogenous fatty acids might also have differed. Second, with the application of a cross-sectional design and thus selection of 10 experienced endurance runners, it cannot be excluded that these subjects differed from the control group in other aspects than their training habits and this may confound the interpretation of a training effect. However, another explanation for the small difference lies in the intriguing possibility that individuals who exercise regularly compensate for the preferential oxidation of unsaturated fatty acids (26, 31, 42) by modulating dietary fatty acid profile. They may do this merely as an associated lifestyle change, or it may reflect a homeostatic regulatory mechanism. In a recent study in rats, it was demonstrated that a heavy long-term exercise program, during which trained and sedentary rats consumed the same dietary fatty acids, resulted in more saturated muscle phospholipids, and this may confound the results of some studies (22). The explanation for the different effects of training between the present study, the study by Andersson et al. (3), and our previous study in rats (22) is not readily apparent; however, it is most likely due to both the species difference and the very arduous training program (60 min/day, 28 m/min, 10% incline, 6 days/wk) that the rats were subjected to in the former study.

In the present study, muscle triacylgycerol fatty acid composition was unaltered by regular exercise training, a finding confirmed in the study by Andersson et al. (3). This indicates that the effect of regular exercise training on muscle phospholipid fatty acid composition is not directly linked to changes in the muscle triacylglycerol fatty acid composition. However, in the present study, there were some effects of time on the muscle triacylglycerol fatty acid composition, and, interestingly, the changes were present already after 4 days of exercise training. A few studies have demonstrated that adipose tissue fractional recruitment of oleic acid is increased during both rest and exercise (24, 37, 49). Also, there is evidence (19), however not unequivocal (20), that the fractional uptake of oleic acid is increased during exercise of the human forearm muscle. In the present study, the fraction of oleic acid in muscle triacylglycerol was significantly increased by exercise training, indicating a preferential recruitment and uptake of oleic acid during and after exercise. However, the role of muscle triacylglycerol and possibly the role of the composition of fatty acids within the triacylglycerol pool in relation to training and exercise are not resolved. There is evidence that muscle triacylglycerol levels, determined in samples from vastus lateralis (38) or determined in soleus by nuclear magnetic resonance techniques (11, 30, 35), are inversely correlated to insulin sensitivity in humans. However, other studies that measured muscle triacylglycerol in vastus lateralis (29) or in tibialis anterior (40) failed to demonstrate the relation between muscle triacylglycerol and insulin sensitivity. There is good evidence that endurance training increases insulin sensitivity (11, 30, 35), and, in the context that long-term endurance training maintains (46) or even increases muscle triacylglycerol storage (23), there inevitably exists a conflict in relation to training and the underlying factors disposed for decreased insulin sensitivity. It has been speculated that endurance training alters intracellular location and distribution of muscle triacylglycerol toward a more uniform storage located around the mitochondria (50), and it is possible that this can explain the apparent discrepancy described above. However, further studies are needed to elucidate the complex interaction between training, insulin sensitivity, and muscle triacylglycerol storage and whether the composition of the fatty acids stored in the muscle triacylglycerol just reflects the dietary fat intake or indeed is of importance.

This study does not provide direct evidence to clarify the mechanism responsible for the effect of regular exercise training on the muscle phosholipid fatty acid profile. However, several possible explanations are presented. It is of course obvious to speculate that the change in muscle phospholipid fatty acid composition was due to the change in muscle fiber-type composition; however, there was absolutely no correlation between the change in fiber type and the changes in fatty acid composition; therefore, this does not seem to be the case. In addition to being a major component of the membrane structure, the phospholipid fatty acids constitute a major storage site for fatty acids (33). However, one study demonstrated that acute exercise will not lead to a decrease in membrane phospholipid content (13), suggesting that phospholipid fatty acids are not recruited for muscle metabolism and therefore do not contribute to the increased fat oxidation during exercise at the same absolute workload normally observed after endurance training. There is some evidence that prolonged adaptation to regular exercise training will lead to increased muscle membrane phospholipid content in humans (36) and rats (16). In humans, the 16% increase in phospholipid content was almost completely due to an increased phosphatidylcholine content, whereas, in rats, the training-induced increase was only present in red gastrocnemius and diaphragm and not due to increases in a single phospholipid class. In this study, we did not measure the total phospholipid content; therefore, it is not possible to exclude that a training-induced increase in phospholipids may have played a role in the observed change in muscle membrane phospholipid fatty acid composition.

It may be argued that some correlate of physical activity rather than the activity itself is responsible for the changes in phospholipid fatty acid composition. Gudbjarnason (18) showed that catecholamine stress, by repeated administration of epinephrine, can alter the fatty acid composition of cardiac phospholipids. In that study, catecholamine stress caused an increase in arachidonic acid 20:4(n-6) and docosahexaenoic acid 22:6(n-3) content and a decrease in linoleic acid 18:2(n-6), changes that were not detected in the present study. However, the increase in percentage of docosahexaenoic acid 22:6(n-3) was consistent between studies as was the decreased ratio of n-6 to n-3. Exercise per-

formed in the one-leg exercise model at submaximal levels only results in moderate increases in circulating catecholamine levels (28), which would be similar for both legs. However, an increased local concentration of norepinephrine from the greater nervous system activation of the exercising leg cannot be ruled out.

In the literature, there is evidence that insulin sensitivity is related to muscle phospholipid fatty acid composition (7, 44, 48). In one study, an inverse relationship between the muscle membrane content of palmitic acid (16:0) and insulin sensitivity was demonstrated (48). Also, there is evidence that an increased unsaturation and a decreased ratio of n-6 to n-3 fatty acids in the muscle membrane are compatible with an increased membrane fluidity, findings that have been linked to the presence of an increased number of insulin receptors and an increased insulin binding (14, 15, 17). Thus, in the present study, the increased ratio of oleic to palmitic acid [C18:1(n-9)/C16:0], the increased unsaturation, and the decreased n-6 to n-3 fatty acid ratio after exercise training indicate that regular exercise training may act to enhance insulin sensitivity through an effect on the membrane fatty acid composition. At first sight, this suggestion contradicts two studies performed in normal (10) and diabetic (4) human subjects in which insulin binding was not increased with either 10-wk one-leg training or 6-wk bicycle training. However, for every single measured point in both studies, the ratio for bound vs. free insulin was insignificantly higher in trained than in untrained muscle. Furthermore, although the 19% higher insulin receptor number after 6 wk of training of the insulin-dependent diabetes mellitus subjects was not significant, very recent data from a study in rats (9), in which insulin receptor function and number were increased after 1 and 5 days of exercise, suggest that insulin receptor number might increase with long-term training. Further studies must investigate the long-term role of exercise for membrane fatty acid composition and function and the mechanisms for this effect.

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