Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle

AGNETA ANDERSSON, ANDERS SJÖDIN, ROGER OLSSON, AND BENGT VESSBY
Departments of Geriatrics and Nutrition, Uppsala University, and PhysTest Scandinavia, S-751 25 Uppsala, Sweden

Andersson, Agneta, Anders Sjödin, Roger Olsson, and Bengt Vessby. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E432–E438, 1998.—The effects of low-intensity exercise on the fatty acid composition in skeletal muscle and in serum were studied in 19 sedentary, middle-aged Swedish men. During a 10-wk period, all subjects were given a standardized diet with an identical fat composition. After 4 wk on this diet, they were randomly allocated to a daily exercise program (55% peak oxygen uptake) or to continue to live a sedentary life for the remaining 6 wk. Aerobic capacity (submaximal bicycle test) and peripheral insulin sensitivity (hyperinsulinemic euglycemic clamp) improved with training, whereas the body weight as well as the body composition (underwater weighing and bioimpedance) were unchanged. The proportions of palmitic acid (16:0) and linoleic acid [18:2(n-6)] and the sum of n-6 fatty acids [18:2(n-6), 20:3(n-6), 20:4(n-6)] were decreased in skeletal muscle phospholipids, whereas the proportion of oleic acid [18:1(n-9)] was increased, by training. The fatty acid profile in skeletal muscle triglycerides remained unchanged. We conclude that regular low-intensity exercise influences the fatty acid composition of the phospholipids in skeletal muscle, which hypothetically may contribute to changes of the skeletal muscle membrane fluidity and influence the peripheral insulin sensitivity.

dietary fat quality; insulin sensitivity; physical activity; blood lipids

---

IMPAIRED INSULIN ACTION plays a central role in the pathogenesis and clinical course of several human diseases, including non-insulin-dependent diabetes mellitus, hypertension, and atherosclerotic cardiovascular disease (7). The insulin sensitivity is influenced by several genetic and environmental factors. Decreased insulin sensitivity is associated with conditions such as obesity (6), aging (5), and physical inactivity (20). Dietary factors, such as a high fat intake, may also play an important role in the development of insulin resistance, as recently reviewed by Storlien et al. (25). Habitual aerobic exercise will enhance the body's sensitivity to insulin (17, 20). The beneficial effects of such exercise on glucose metabolism may be explained by multiple factors, including an increase in muscle mass (29), the muscle blood flow and capillary density (19), and glycogen synthase activity (3), changes in number of insulin receptors (9), and activation of the glucose transport system (22). The exact mechanisms through which physical activity and diet interact to influence insulin action are not clearly understood.

Recent data have indicated that the fatty acid composition of skeletal muscle phospholipids may influence insulin sensitivity. This has been demonstrated in both rats (26) and humans (4, 21, 27). It has been documented in experimental animals (1) that the fatty acid composition in the skeletal muscle cell membrane can be influenced by dietary changes. The physical properties of the membrane are greatly influenced by the fatty acid composition of the phospholipids. In cultured cells an increase in the concentration of polyunsaturated fatty acids within cell membranes has been found to increase the membrane fluidity and the number of insulin receptors (11, 12). Converse effects have been observed when the concentration of saturated fatty acids in the membrane is increased (12, 13).

However, whether physical activity may influence the fatty acid composition in skeletal muscle membranes has not been examined in detail. The present study was undertaken to investigate the effect of low-intensity exercise on *1*) the fatty acid composition of the phospholipids and triglycerides in skeletal muscle, *2*) the fatty acid composition in the serum (phospholipids and cholesterol esters), and *3*) other metabolic parameters such as serum lipoproteins and the peripheral insulin sensitivity.

MATERIALS AND METHODS

Subjects. Healthy men of ages between 30 and 55 yr who had not engaged in any regular physical exercise training during the last year were invited to participate in this study. The subjects were recruited at the local employment exchange in Uppsala, Sweden. Most of them were unemployed construction workers. Twenty-two men agreed to enter the study. Of these, three did not complete the study period; one became ill, and two obtained employment that prevented them from continuing in the study. Ten of the remaining 19 subjects were tobacco users. They were asked not to alter their smoking or snuff habits during the study. Subjects with any kind of metabolic disease were excluded, as were persons with known excessive alcohol consumption or extreme dietary habits. Characteristics of the subjects are presented in Table 1.

Before entering the study, the subjects underwent an exercise electrocardiogram test to exclude coronary insufficiency of a degree that could be hazardous in an exercise program. None of the subjects used any medication during the study period, except one subject who was on citalopram (a serotonin-specific reuptake inhibitor) treatment during the last 6 wk of the study. The subjects were given free food but no monetary payment. The design of the study was approved by the local ethics committee, and all subjects gave their informed consent.

Experimental design. After 4 wk of a 10-wk standardized diet, the subjects were randomly assigned to an exercise group (EXE, $n = 10$) or to a sedentary group (SED, $n = 9$). The EXE group took part in a daily exercise program for 6 wk. The subjects in the SED group were asked not to make any changes in their physical activity during the 6-wk intervention period. Before entering the study, all participants recorded their food intake for 3 days: 2 weekdays and 1 day

0193-1849/98 $5.00 Copyright © 1998 the American Physiological Society

Downloaded from ajpendo.physiology.org on May 1, 2012

Table 1. *Characteristics of subjects at the beginning of the study (week 0)*

	EXE Group ($n = 10$)	SED Group ($n = 9$)
Age, yr	36 ± 8	44 ± 6*
Height, cm	183 ± 7	179 ± 4
Weight, kg	84.1 ± 15.0	83.0 ± 8.7
Body fat, %	23.9 ± 7.6	24.9 ± 8.2
BMI, kg/m^2	25.0 ± 3.7	26.0 ± 3.5
Waist-to-hip ratio	0.94 ± 0.05	0.95 ± 0.05
$\dot{V}O_{2peak}$, ml·min^{-1}·kg^{-1}	39.5 ± 7.0	33.8 ± 5.6
Tobacco users	4	6

Values are means ± SD for each group. BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen uptake. Body fat was measured by underwater weighing and bioimpedance. Significant differences between exercise (EXE) and sedentary (SED) groups: *$P = 0.016$.

during the weekend (weighed food record) to allow an estimation of their habitual dietary intake.

At the beginning (*week 0*), after 4 wk (*week 4*), and at the end of the study (*week 10*), the concentrations of serum lipoproteins were determined and the body composition was estimated. At the beginning and end of the intervention period (after *week 4* and *week 10*, respectively) the fatty acid composition of the serum phospholipids, cholesterol esters and the fatty acid composition of phospholipids and triglycerides in biopsy samples of skeletal muscle were determined. On these two occasions the peripheral insulin sensitivity was also evaluated and a submaximal bicycle test was performed.

Peak oxygen uptake ($\dot{V}O_{2peak}$) was determined at the beginning of the study during an incremental exercise test on an electronic bicycle ergometer (Monark 829E, Varberg, Sweden), with simultaneous measurements of respiratory gas exchanges (SensorMedics 2900Z, Anaheim, CA).

The submaximal bicycle test was performed on an electronic bicycle ergometer at a work load corresponding to 55% of the pretest $\dot{V}O_{2peak}$. The exercise duration was individually chosen in relation to the work load to correspond to ~2,900 kJ (EXE group: 115 ± 24 W and 81 ± 18 min; SED group: 98 ± 14 W and 91 ± 7 min). During the bicycle test, heart rate was monitored, using a light, portable microcomputer (Sport Tester PE 3000; Polar Electro, Kempele, Finland). The same equipment as that used in the $\dot{V}O_{2peak}$ test was used to measure oxygen uptake and carbon dioxide production. Whole blood lactate was measured at the beginning of the bicycle test, after 60 min, and at the end of the test by an enzymatic, photometric method in hemolyzed capillary blood (Dr. Lange apparatus, Boehringer Mannheim, Berlin, Germany).

Exercise program. The EXE group was instructed to work at an individually specific heart rate equivalent to that attained in the submaximal test (55% of pretest $\dot{V}O_{2peak}$). The exercise (bicycling and/or running/walking) corresponded to an extra energy turnover of ~1,900 kJ/day during the 1st wk and 2,900 kJ/day during the last 5 wk of the intervention period. The subjects were allowed to divide their exercise time into one or two workouts a day (no workout with a duration shorter than 30 min). During the workouts, the participants themselves monitored their heart rate with the same portable microcomputer as that used in the submaximal bicycle test. Midway through the intervention period, the EXE group performed the training at the metabolic ward on a bicycle at the same fixed work load as at the beginning of the training period. The heart rate was recorded, and the target heart rate used for setting the training intensity was adjusted according to this test. If the participants became ill or injured and were unable to exercise, this was recorded in a training diary.

Diet and nutrient calculations. The diet was composed to give all subjects the same qualitative nutritional intake, including an identical dietary fat quality. The energy level was individually adjusted to maintain a constant body weight throughout the study. The energy need was calculated for each subject, based on the basal metabolic rate according to World Health Organization/Food and Agriculture Organization/UNU 1985 and a PAL-factor (physical activity level for light daily activity) of 1.55 was used. During the intervention period, the energy intake in the EXE group was increased to meet the elevated energy need and maintain energy balance. The food provided during the study was based on a traditional Swedish diet. The menu was planned for 1 wk and repeated 10 times. It included breakfast, lunch, dinner, and snacks. All food was prepared in a metabolic ward kitchen. Twice a week the participants collected their food, which was prepared separately for each individual. They were asked to eat all food that was served. The standardized menu was prepared on four different energy levels (10.0, 11.7, 13.4, and 15.1 MJ). To adjust the energy intake more precisely, snacks (with a nutritional quality similar to that of the standardized menu) were added to the standardized menu. To make it more pleasant for the subjects, a list of food (low in fat) for free consumption up to 840 kJ/day was available to each participant. No more than ~15 g of alcohol per week was allowed during the study. The free consumption of alcohol and food had to be recorded by each subject.

The nutritional intake during the study period was calculated from the standardized menu with the addition of the free consumption, using the data base of the Swedish National Food Administration and a computerized calculation program (Dietist; Kost och Näringsdata, Stockholm, Sweden). In addition to the prescribed diet, the subjects were allowed to eat one "free dinner" every 2 wk. This consumption was not included in the nutrient calculations. Duplicate food portions corresponding to each day of the standardized menu were analyzed for their fat content (after homogenization, 10 g of the homogenate were extracted with 50 ml methanol, 100 ml chloroform, and 150 ml 0.2 mol sodium dihydrogen phosphate per liter; 10 ml of the chloroform phase were evaporated to dryness, and the total fat content was determined by weighing) and fatty acid composition (determined by gas-liquid chromatography, see below). This was repeated on two further occasions during the study period. The nutrient intake before entry into the study (*week −1*) was calculated on the basis of the 3-day weighed records, using the same data base and calculation program as described above.

Biochemical analyses. Venous blood samples were drawn in the morning after an overnight fast. No vigorous physical activity was allowed, and no smoking or snuff taking was permitted in the morning before the sampling. Very low density lipoproteins, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were isolated by a combination of preparative ultracentrifugation (14) and precipitation with a sodium phosphotungstate and magnesium chloride solution (23). Triglyceride and cholesterol concentrations were measured in serum and in the isolated lipoprotein fractions by enzymatic methods, using the IL Test Cholesterol Method 181618–10 and IL Test Triglyceride Enzymatic-colorimetric Method 181610–60 for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, MA).

The fatty acid compositions of serum phospholipids and cholesterol esters and of skeletal muscle phospholipids and triglycerides were determined by gas-liquid chromatography, as was the dietary fatty acid composition. For determination of the fatty acid composition in serum, 5 ml of methanol were added to 1 ml of serum. Chloroform (10 ml) containing 0.005%

butylated hydroxytoluene as an antioxidant was then added, followed by 15 ml of 0.2 mmol/l NaH_2PO_4 (2). After thorough mixing, the extract was left at +4°C for 1–4 days. The chloroform phase was evaporated to dryness under nitrogen, and the lipid residue was dissolved in chloroform. The lipid esters (triglycerides, cholesterol esters, and phospholipids) were separated by thin-layer chromatography and transmethylated at 60°C overnight after addition of 2 ml of 5% H_2SO_4 in methanol. The methyl esters were extracted into 3 ml of petroleum ether (boiling point 40–60°C) containing 0.005% butylated hydroxytolvene after addition of 1.5 ml of distilled water. The phases were separated after thorough mixing and centrifugation at 1,500 *g* for 10 min. The petroleum ether phase was pipetted off, and the solvent was evaporated under nitrogen. The methyl esters were then redissolved in 1 ml of Uvasol, grade hexane.

The fatty acid methyl esters were separated by gas-liquid chromatography on a 25-m wall-coated open tubular glass capillary column coated with SLP OV-351 (QUAD-REX), with helium as carrier gas. A Hewlett-Packard system consisting of GLC 5890, integrator 3396, and autosampler 7671 A was used. The fatty acids were identified by comparing the retention times with fatty acid methyl ester standard Nu Check Prep (Elysian, MN).

Muscle samples were obtained from the quadriceps femoris muscle (vastus lateralis) by a fine needle biopsy and immediately frozen in liquid nitrogen and stored at −70°C. Small pieces of skeletal muscle tissue (15–30 mg) were homogenized in 1 ml of physiological saline in a Kinematica Polytron PT 3000 homogenizer at 30,000 revolutions/min for 15 s on ice. The homogenized muscle tissue was extracted overnight by a solvent system containing 5 ml methanol, 10 ml chloroform containing 0.005% butylated hydroxytolvene, and 15 ml 0.2 mmol/l NaH_2PO_4. The chloroform phase was evaporated to dryness under nitrogen, after which the lipid esters were separated by thin-layer chromatography as described above, transmethylated, and analyzed by gas-liquid chromatography.

The coefficient of variation for determination of the proportions of fatty acids in skeletal muscle phospholipids was <10% for all the fatty acids, with the exception of palmitoleic acid [16:1(n-7)], heptadecanoic acid (17:0), and α-linolenic acid [18:3(n-3)], which were present in small amounts, with larger variations between the analyses (coefficient of variation 20, 28, and 44%, respectively). The coefficient of variation for the proportions of fatty acids in skeletal muscle triglycerides was 10% or less for all fatty acid with proportions >0.5%, with the exception of α-linolenic acid (13%).

Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique (8). The insulin (Actrapid R Human; Novo, Copenhagen, Denmark) infusion rate during the clamp study was 56 $mU \cdot m^{-2} \cdot min^{-1}$, resulting in a mean plasma insulin concentration of ~100 mU/l. Insulin sensitivity is expressed as the M value (M) and by the insulin sensitivity index (M/I). The M value represents the glucose uptake ($mg \cdot kg$ body $wt^{-1} \cdot min^{-1}$) during the last 60 min of the clamp test. The insulin sensitivity index (the amount of glucose metabolized per unit of plasma insulin, $mg \cdot kg$ body $wt^{-1} \cdot min^{-1}$ per mU/l multiplied by 100) is a measure of tissue sensitivity to insulin expressed per unit of insulin, obtained by dividing the mean glucose uptake by the mean insulin concentration during the last 60 min of the clamp test. The participants were asked to minimize their physical activity on the day before measurement of the insulin sensitivity, with the exception of the second occasion (*week 10*) in the EXE group, when the subjects were training as usual according to their training program on the day before the clamp test.

The serum insulin concentration was measured by an enzymatic immunological assay (Boehringer Mannheim) performed in an ES 300 automatic analyzer. Blood glucose concentrations were measured by a glucose oxidase assay.

Body composition. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m^2). The body composition (body fat content) was estimated using a three-compartment model (10) based on underwater weighing and bioelectrical impedance analysis (BIA). The measurements were performed in the morning after an overnight fast. Underwater weight was measured with the participant sitting totally submerged in a water tank. Lung volume was determined simultaneously by the helium dilution technique (Volograph 2000 Mijnhart Bunnik, the Netherlands). For BIA a multiple-frequency, bioresistance, body composition analyzer (XITRON 4000B; Xitron Tech, San Diego, CA) was used.

Statistical analyses. The data obtained in this investigation were analyzed statistically with use of the Statistical Analysis System. The results are expressed as the least square means ± SD. For continuous variables with normal distributions (in some cases after logarithmic transformation), an analysis of variance model with factors for intervention, subjects, and time was used. Comparisons were made of the analyses performed before and after the intervention period in each group and of the changes in the two groups during the intervention period. For variables that were measured only once during the study, e.g., the nutrient intake, an unpaired *t*-test was used for comparisons between the groups. The level of significance was set at $P < 0.05$.

RESULTS

Exercise. The subjects in the EXE group trained for 92 ± 9% of the planned workouts, according to training records and heart rate monitors. In this group there was a 10% decrease in mean heart rate (122 ± 8 vs. 135 ± 12 beats/min, $P = 0.0004$) at the submaximal bicycle test at *week 10* compared with *week 4*, leading to significantly different changes in between the two groups ($P = 0.012$). The increase in mean blood lactate concentration during the submaximal bicycle test was lower in the EXE group after the intervention period than before (0.1 ± 0.4 vs. 0.9 ± 1.4 mmol/l, $P = 0.031$). No changes were found in the SED group (0.3 ± 0.3 vs. 0.6 ± 0.4 mmol/l, $P = 0.30$).

Diet. Dietary intake before the subjects entered the study and dietary intake during the intervention period are presented in Table 2. The mean dietary intake before the study was identical in both the groups. The mean dietary intake during the intervention period shows no difference between the two groups regarding the proportions of macronutrients and the fatty acid composition of the diet. The energy intake was higher (13%, $P = 0.024$) in the EXE group than in the SED group during the intervention period. The increased intake of energy in the EXE group also meant a higher absolute amount of nutrients as dietary fiber and cholesterol. The relative fatty acid composition of the standardized diet, eaten during the intervention period, was also analyzed from duplicate portions. The proportions of saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) were 52.5, 34.6, and 13.1%, respectively. Ten and two-tenths percent of the PUFA were n-6 fatty acids, and 2.9% were n-3 fatty acids, including 2.1% α-linolenic acid.

Table 2. *Calculated average dietary intake before subjects entered the study (week −1) and during the intervention period (weeks 4–10)*

	Week −1		*Weeks 4–10*	
	EXE group	SED group	EXE group	SED group
Energy, MJ	9.6 ± 1.5	11.4 ± 3.2	15.2 ± 2.0	13.2 ± 1.3*
Protein, E%	15.7 ± 3.5	13.3 ± 2.0	12.9 ± 0.1	12.8 ± 0.2
Carbohydrate, E%	47.6 ± 6.4	46.4 ± 3.5	48.9 ± 0.3	49.2 ± 0.8
Fat, E%	34.8 ± 4.4	34.3 ± 7.0	37.5 ± 0.4	37.2 ± 0.8
Saturated fatty acids, E%	14.5 ± 3.5	13.9 ± 3.2	16.8 ± 0.2	16.7 ± 0.3
Monounsaturated fatty acids, E%	13.0 ± 1.8	13.4 ± 3.2	13.5 ± 0.2	13.4 ± 0.3
Polyunsaturated fatty acids, E%	5.2 ± 1.4	5.0 ± 1.3	4.9 ± 0.1	4.8 ± 0.1
Alcohol, E%	1.9 ± 1.6	6.1 ± 8.3	0.7 ± 0.3	0.7 ± 0.2
Dietary fiber, g	20 ± 7	22 ± 5	28 ± 3.0	25 ± 2.0*
Cholesterol, mg	340 ± 200	360 ± 140	501 ± 64	425 ± 43†

Values are means ± SD for each group. E%, percentage of total energy in the diet. Average dietary intake before the study was based on 3-day weighed food record and during the intervention period was based on the standarized menu and the free consumption. No significant differences were found between the two groups before they entered the study. Significant differences between EXE ($n = 10$) and SED ($n = 9$) during the intervention period: * $P = 0.02$, † $P = 0.008$.

Effects on body weight and body composition. The mean body weight, BMI, waist-to-hip ratio, and body fat in both groups remained unchanged during the study, indicating energy balance.

Effects of exercise on insulin sensitivity, fasting insulin, and fasting glucose. The insulin sensitivity, expressed as the M value, increased from 6.19 ± 1.64 to 7.60 ± 2.16 (+23%, $P = 0.001$) in the EXE group, whereas there were no changes in the SED group (5.94 ± 1.15 vs. 6.14 ± 1.42), leading to significantly different changes ($P = 0.034$) in between the two groups. The index of insulin sensitivity (M/I) increased by 29% ($P = 0.002$) in the EXE group. Fasting blood glucose and the serum insulin level did not change in either group.

Fatty acid composition in serum phospholipids and cholesterol esters. No changes were found in the fatty acid composition of the serum phospholipids during the intervention period. In the serum cholesterol esters there was a decrease (−11%, $P = 0.035$) in the proportion of di-homo-γ-linolenic acid [20:3(n-6)] in the EXE group (*weeks 4–10*), whereas the proportion of arachidonic acid [20:4(n-6)] decreased in the SED group (−10%, $P = 0.031$).

Skeletal muscle phospholipids. Two of the subjects in the EXE group and one in the SED group did not undergo both muscle biopsies. Thus the effects of the intervention on the fatty acid composition of the skeletal muscle phospholipids and triglycerides were calculated from eight subjects in each group. In the SED group no differences were detected in the fatty acid composition of the skeletal muscle phospholipids during the intervention period. In the EXE group, on the other hand, this composition showed a change from *wk 4* to *wk 10*, as presented in Table 3 and illustrated in Fig. 1. The proportions of palmitic acid (16:0) and linoleic acid [18:2(n-6)] decreased (−7%, $P = 0.012$, and −8%, $P = 0.016$, respectively), and the proportion of oleic acid [18:1(n-9)] was increased substantially (+15%, $P = 0.0001$) in this group, leading to a significant difference ($P = 0.007$) in changes between the two groups (*weeks 4–10*). The proportion of arachidonic acid [20:4(n-6)] tended to decrease ($P = 0.077$) in the EXE group, whereas in the SED group it exhibited an increasing trend, resulting in a significant difference in changes between the groups from *week 4* to *week 10* ($P = 0.048$).

There was a reduction by 7% ($P = 0.006$) in total n-6 fatty acids [18:2(n-6), 20:3(n-6), 20:4(n-6)] in the EXE group during the intervention period. Compared with changes in the SED group, this decrease was almost significant ($P = 0.066$). Adjustment for the small individual changes in body weight did not influence the above findings.

Skeletal muscle triglycerides. The fatty acid composition of the skeletal muscle triglycerides did not change

Table 3. *Effects of exercise on fatty acid composition (%) in skeletal muscle phospholipids*

	EXE Group			SED Group			Diff. in Changes from *Week 4* to *Week 10*
Fatty Acid	*Week 4*	*Week 10*	*P* value	*Week 4*	*Week 10*	*P* value	EXE-SED *P* Value
16:0	22.4 ± 2.6	20.9 ± 1.6	0.012*	22.1 ± 1.5	21.8 ± 1.0	0.57	0.13
17:0	0.58 ± 0.18	0.61 ± 0.25	0.80	0.58 ± 0.09	0.64 ± 0.23	0.61	0.84
18:0	14.0 ± 1.1	15.1 ± 2.1	0.19	14.0 ± 1.2	14.0 ± 1.4	0.95	0.32
16:1(n-7)	0.99 ± 0.29	1.04 ± 0.33	0.73	0.93 ± 0.35	1.06 ± 0.26	0.37	0.67
18:1(n-9)	11.4 ± 0.7	13.1 ± 0.9	0.0001*	12.7 ± 1.3	13.0 ± 1.4	0.35	0.007*
18:2(n-6)	31.4 ± 1.3	29.0 ± 2.2	0.016*	31.3 ± 1.8	30.3 ± 1.7	0.28	0.27
20:3(n-6)	1.45 ± 0.19	1.37 ± 0.18	0.25	1.11 ± 0.16	1.18 ± 0.18	0.32	0.14
20:4(n-6)	13.8 ± 0.9	12.8 ± 1.7	0.077	12.0 ± 1.2	12.5 ± 1.5	0.27	0.048*
20:5(n-3)	1.26 ± 0.24	1.57 ± 0.37	0.13	1.60 ± 0.37	1.64 ± 0.49	0.86	0.29
22:5(n-3)	1.77 ± 0.19	1.81 ± 0.29	0.81	1.92 ± 0.14	1.95 ± 0.26	0.90	0.96
22:6(n-3)	2.70 ± 0.41	3.20 ± 0.87	0.066	2.78 ± 0.76	3.08 ± 0.94	0.21	0.57
Σ n-6	46.6 ± 1.2	43.2 ± 3.5	0.006*	44.4 ± 1.8	43.9 ± 2.0	0.66	0.066
Σ n-3	5.23 ± 1.76	5.95 ± 1.68	0.22	6.12 ± 0.91	6.05 ± 1.28	0.91	0.34
n-6/n-3	11.1 ± 8.2	7.9 ± 2.7	0.13	7.3 ± 1.1	7.6 ± 1.9	0.90	0.24
Σ C20–22	19.9 ± 2.9	20.2 ± 2.6	0.82	18.3 ± 3.0	19.6 ± 1.7	0.25	0.51
20:5/20:4	0.09 ± 0.02	0.12 ± 0.03	0.16	0.13 ± 0.03	0.13 ± 0.05	0.96	0.27
20:4/20:3	9.6 ± 1.5	9.4 ± 1.1	0.59	10.9 ± 1.6	10.7 ± 1.1	0.63	0.98

Values are means ± SD for each group; $n = 8$ for both groups. * Significant difference (*weeks 4–10*).

Downloaded from journals.physiology.org/journal/ajpendo at (...) on May 17, 2024.

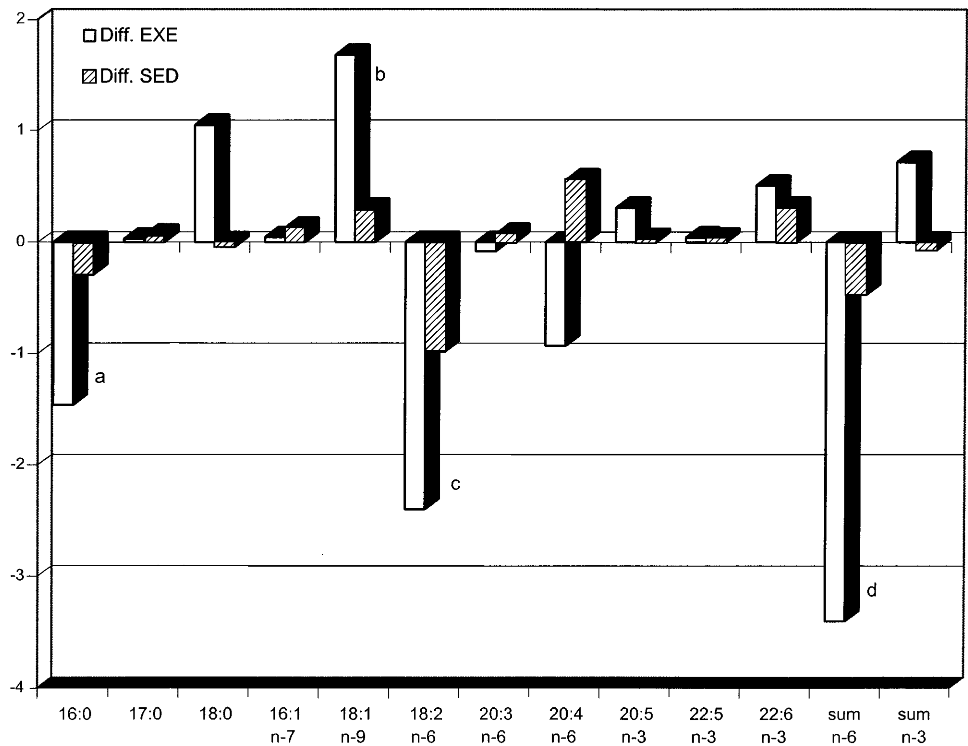

Fig. 1. Effects of exercise on fatty acid composition (%) in skeletal muscle phospholipids. Changes during intervention period (*weeks 4–10*) in each group presented as percentage units. EXE, exercise group; SED, sedentary group. Significant differences between *week 4* and *week 10*: [a]$P = 0.012$, [b]$P = 0.0001$, [c]$P = 0.016$, and [d]$P = 0.006$.

in either of the groups during the intervention period (Table 4).

Serum lipoprotein profile. The serum lipoprotein concentrations showed only minor changes, and there were no significant differences between the changes in the two groups. However, in both groups there was a slight reduction of the LDL-to-HDL ratio, due to a decrease in LDL cholesterol, which was significant in the SED group (−17%, $P = 0.0003$). There was a trend toward an increase in HDL cholesterol in the EXE group (+10%, $P = 0.064$), contributing to a decrease in the LDL-to-HDL ratio (−14%, $P = 0.031$).

DISCUSSION

Both diet and physical activity are known to influence the insulin sensitivity.

The fatty acid profile of the skeletal muscle phospholipids has earlier been shown to be related to variations in insulin sensitivity (4, 21, 26, 27) and is one factor that may contribute to both the diet- and exercise-induced changes in insulin sensitivity. In the present study the subjects in both groups were given a standardized diet with identical proportions of nutrients, including the proportion of dietary fatty acids. The energy intake was individually adjusted during the study, leading to a situation of energy balance, as indicated by an unchanged mean body weight and body composition in both groups. Six weeks of daily exercise at 55% of $\dot{V}O_{2peak}$ in the EXE group clearly increased the aerobic capacity. As expected, the physical activity also had a positive effect on the insulin action.

The major finding in the present study was the effect of regular low-intensity exercise on the fatty acid composition of the phospholipids in skeletal muscle. As far as we know this has never before been demonstrated in humans. In the EXE group we found that the proportion of palmitic acid (16:0) decreased and that of oleic acid [18:1(n-9)] increased, leading to a higher ratio of 18:1 to 16:0 (22%, $P = 0.0001$). Recently an inverse relationship between the proportion of palmitic acid (16:0) in skeletal muscle phospholipids and insulin sensitivity has been demonstrated (27). A higher proportion of 16:0 and a reduced level of PUFA are also found in the more insulin-resistant fast-twitch muscle fibers compared with the more insulin-sensitive slow-twitch muscle fibers in rats (18). In the present study the physical training also seemed to influence the contents of n-6 PUFA [18:2(n-6), 20:3(n-6), 20:4(n-6)] and n-3 PUFA [20:5(n-3), 22:5(n-3), 22:6(n-3)] in muscle phospholipids in different ways. In the EXE group there was a decrease in the proportion of n-6 PUFA (from 47 to 43%, $P = 0.006$). There was also a tendency toward a

Table 4. *Effects of exercise on fatty acid composition (%) in skeletal muscle triglycerides*

Fatty Acid	EXE Group		SED Group	
	Week 4	*Week 10*	*Week 4*	*Week 10*
15:0	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
16:0	25.6 ± 1.8	25.8 ± 1.5	26.0 ± 2.0	26.1 ± 2.4
18:0	6.6 ± 2.2	7.0 ± 1.9	7.7 ± 3.2	6.9 ± 2.7
16:1(n-7)	6.6 ± 1.3	6.1 ± 1.5	5.1 ± 1.9	5.7 ± 1.7
18:1(n-9)	48.8 ± 1.8	48.8 ± 1.7	48.5 ± 2.1	48.4 ± 2.6
18:2(n-6)	10.9 ± 1.2	10.9 ± 1.6	11.1 ± 0.9	11.3 ± 0.6
18:3(n-3)	1.0 ± 0.2	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.2
20:4(n-6)	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1

Values are means ± SD for each group; $n = 8$ for both groups. No significant changes were found within the groups (*weeks 4–10*) or when the changes were compared between the 2 groups (*weeks 4–10*).

reduction of the n-6-to-n-3 ratio due to the training (−29%, $P = 0.13$), although this decrease did not reach statistical significance.

The possibility to demonstrate significant changes of the minor fatty acid components, e.g., regarding the proportion of n-3 fatty acids in the skeletal muscle phospholipids, is restricted by the measurement error connected with the determination of some of the fatty acids present in low proportions.

The changes in the fatty acid profile of the skeletal muscle phospholipids due to exercise, with an increased ratio of 18:1(n-9) to 16:0, indicating an increased proportion of unsaturated fatty acids and an altered balance between n-6 and n-3 fatty acids, are compatible with an increased membrane fluidity. In cultured cells enriched with PUFA an increase in membrane fluidity associated with an increase in number of insulin receptors has been demonstrated (11). Decrease in insulin binding is observed in cultured cells exposed to saturated fatty acids (13). The changes in the fatty acid profile of the skeletal muscle phospholipids in the present study may theoretically contribute to an improvement in insulin sensitivity.

Whether the changes in fatty acid profile of the skeletal muscle phospholipids have contributed to the observed increase in insulin sensitivity cannot be determined from the results in the present study. The subjects of the EXE group were exercising according to the training program also the day before the last clamp test (*week 10*). The improvement in insulin action is therefore probably due to both the effects of training per se and the acute effect of the last bout of exercise. The fatty acid composition is modified over a longer time period and may mainly be connected with the exercise effect per se. To understand the relationships between the changes of the phospholipid fatty acid composition in skeletal muscle and the exercise-induced improvement of insulin sensitivity futher studies are needed. Several mechanisms, such as an increased muscle blood flow, capillary density, glycogen synthase activity, number of insulin receptors, and activation of glucose transport system, may also explain some of the exercise-induced improvement of insulin sensitivity seen in this study.

The mechanisms behind the changes of the fatty acid composition in skeletal muscle after physical activity, whether due to preferential oxidation of certain fatty acids, changes of transport rates or enzyme activities, or other causes cannot be determined from the results of the present study.

Earlier studies in rats have shown that long-term exercise can partially compensate for high-fat diet-induced insulin resistance (16). A recent study in rats (15) showed that training modified the membrane phospholipid fatty acid composition of muscle irrespective of diet intake. However, the changes in fatty acid profile observed in that study are not in line with our findings, which may be explained by differences between species or by methodological factors. For instance, the investigators showed a decrease in C20–22 PUFA, which rather suggests that the effect of training on the fatty acid composition of muscle phospholipids attenuates the exercise-induced improvement in insulin action. Other investigators (4, 21) have linked reductions in C20–22 PUFA in muscle phospholipids to impaired insulin action. We found no significant changes in C20–22 PUFA due to exercise in the present study.

In contrast to the present study and the work by Helge and colleagues (15), which both show a modification in the fatty acid profile of skeletal muscle phospholipids due to exercise, Kriketos and co-workers (18) observed little or no changes in the membrane lipid composition of exercising rats.

Another interesting finding in the present study is the divergent effects of exercise training on the fatty acid composition of skeletal muscle phospholipids and of skeletal muscle triglycerides. Although the training significantly influenced the fatty acid composition of skeletal muscle phospholipids, no such changes were seen in the skeletal muscle triglycerides. Apparently the body handles various pools of fatty acids in skeletal muscle in different ways in a situation of elevated energy turnover such as regular aerobic exercise.

The changes we found in the skeletal muscle phosholipids fatty acid composition due to training were not parallel with or subsequent to any changes in the fatty acid composition in the serum phospholipds or the serum cholesterol esters, neither during the first 4 wk (unpublished data) or during the exercise period. This indicates that the changes seen in the skeletal muscle phospholipid fatty acid profile were not a consequence of difference in the fatty acid composition between the habitual diet and the standardized study diet.

The serum lipoproteins were not significantly altered in the EXE group after the intervention period compared with the SED group. The constant body weight and body fat percentage may be one reason for not seeing any significant changes in HDL cholesterol (28). Another reason may be the limited training period or the low exercise intensity (24).

In conclusion, we found that regular, low-intensity physical exercise affected the relative fatty acid composition in skeletal muscle phospholipids in middle-aged, previously sedentary men but did not alter the fatty acid profile of the skeletal muscle triglycerides. In the muscle phospholipids we found a decrease in the percentage of palmitic acid (16:0) and an increase of oleic acid [18:1(n-9)]. We also found a decrease in n-6 PUFA after the training. The observed changes in fatty acid profile of the skeletal muscle phospholipds may have contributed to changes in the cell membrane fluidity. Futher investigations are required to clarify if these changes may be linked to the more efficient insulin-mediated glucose uptake by the muscles that is seen in endurance-trained subjects.

We express our gratitude to the subjects for devoted participation in the study. We are also grateful to Rawya Mohsen for performing the statistical analysis and to all co-workers at the metabolic ward and laboratories of the Departments of Geriatrics and Nutrition for their support.

The study was financially supported by the Swedish Dairy Council and the Swedish Medical Research Council.

Downloaded from ajpendo.physiology.org on May 17, 2011

Address for reprint requests: A. Andersson, Dept. of Geriatrics, Uppsala Univ., Box 609, S-751 25 Uppsala, Sweden.

Received 12 May 1997; accepted in final form 11 November 1997.

REFERENCES

1. **Ayre, K. J., and A. J. Hulbert.** Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. *J. Nutr.* 126: 653- 662, 1996.
2. **Boberg, M., L. B. Croon, I. B. Gustafsson, and B. Vessby.** Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. *Clin. Sci. (Colch.)* 68: 581–587, 1985.
3. **Bogardus, C., S. Lillioja, K. Stone, and D. Mott.** Correlation between muscle glycogen synthase activity and in vivo insulin action in man. *J. Clin. Invest.* 73: 1185–1190, 1984.
4. **Borkman, M., L. H. Storlien, D. A. Pan, A. B. Jenkins, D. J. Chisholm, and L. V. Campbell.** The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N. Engl. J. Med.* 328: 238–244, 1993.
5. **DeFronzo, R. A.** Glucose intolerance and aging: evidence for tissue insensitivity to insulin. *Diabetes* 28: 1095–1101, 1979.
6. **DeFronzo, R. A.** Insulin secretion, insulin resistance, and obesity. *Int. J. Obes.* 6, *Suppl.* 1: 73–82, 1982.
7. **DeFronzo, R. A., and E. Ferrannini.** Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and arterosclerotic cardiovascular disease. *Diabetes Care* 14: 173–194, 1991.
8. **DeFronzo, R. A., J. D. Tobin, and R. Andres.** Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* 237 (*Endocrinol. Metab. Gastrointest. Physiol.* 6): E214–E223, 1979.
9. **Dohm, G. L., M. K. Sinha, and J. F. Caro.** Insulin receptor binding and protein kinase activity in muscles of trained rats. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E170–E175, 1987.
10. **Forslund, A. H., A. G. Johansson, A. Sjödin, G. Bryding, S. Ljunghall, and L. Hambraeus.** Evaluation of modified multicompartment models to calculate body composition in healthy males. *Am. J. Clin. Nutr.* 63: 856–862, 1996.
11. **Ginsberg, B. H., T. J. Brown, I. Simon, and A. A. Spector.** Effect of the membrane lipid environment on the properties of insulin receptors. *Diabetes* 30: 773–780, 1981.
12. **Ginsberg, B. H., J. Jabour, and A. A. Spector.** Effects of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. *Biochim. Biophys. Acta* 690: 157–164, 1982.
13. **Grunfeld, C., K. L. Baird, and C. R. Kahn.** Maintenance of 3t3-L1 cells in culture media containing saturated fatty acids decreases insulin binding and insulin action. *Biochem. Biophys. Res. Commun.* 103: 219–226, 1981.
14. **Havel, R. J., H. A. Eder, and J. H. Bragdon.** The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34: 1345–1353, 1955.
15. **Helge, J. W., K. J. Ayre, A. Hulbert, B. Kiens, and L. H. Storlien.** Training modifies the phospholipid fatty acid membrane composition of rat muscle irrespective of dietary intake (Abstract). *Book Abstr. Congr. Frontiers Sport Sci., 1st Nice 1996,* p. 258–259.
16. **Kern, M., E. B. Tapscott, D. L. Downes, W. R. Frisell, and G. L. Dohm.** Insulin resistance induced by high-fat feeding is only partially reversed by exercise training. *Eur. J. Appl. Physiol.* 417: 79–83, 1990.
17. **Koivisto, V. A., H. Yki-Järvinen, and R. A. DeFronzo.** Physical training and insulin sensitivity. *Diabetes Metab. Rev.* 1: 445–481, 1986.
18. **Kriketos, A. D., D. A. Pan, J. R. Sutton, J. F. Y. Hoh, L. A. Baur, G. J. Cooney, A. B. Jenkins, and L. H. Storlien.** Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats. *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R1154–R1162, 1995.
19. **Lillioja, S., A. A. Young, C. L. Culter, J. L. Ivy, W. G. H. Abbott, J. K. Zawadzki, H. Yki-Järvinen, L. Christin, T. W. Secomb, and C. Bogardus.** Skeletal muscle capillary and fiber type are possible determinants of in vivo insulin resistance in man. *J. Clin. Invest.* 80: 415- 424, 1987.
20. **Mikines, K. J.** The influence of physical activity and inactivity on insulin action and secretion in man. *Acta Physiol. Scand.* 609, *Suppl.*: 1–43, 1992.
21. **Pan, D. A., S. Lillioja, M. R. Milner, A. D. Kriketos, L. A. Baur, C. Bogardus, and L. H. Storlien.** Skeletal muscle membrane lipid composition is related to adiposity and insulin action. *J. Clin. Invest.* 96: 2802–2808, 1995.
22. **Ploug, T., B. M. Stallknecht, O. Pedersen, B. B. Kahn, T. Ohkuwa, J. Vinten, and H. Galbo.** Effect of endurance training on glucose transport capacity and glucose transporter expression in rat skeletal muscle. *Am. J. Physiol.* 259 (*Endocrinol. Metab.* 22): E778–E786, 1990.
23. **Seigler, L., and W. T. Wu.** Separation of serum high-density lipoprotein for cholesterol determination: ultracentrifugation vs precipitation with sodium phosphotungstate and magnesium chloride. *Clin. Chem.* 27: 838- 841, 1981.
24. **Stein, R. A., D. W. Michielli, M. D. Glantz, H. Sardy, A. Cohen, N. Goldberg, and C. D. Brown.** Effects of different exercise training intensities on lipoprotein cholesterol fractions in healthy middle-aged men. *Am. Heart J.* 119: 277–283, 1990.
25. **Storlien, L. H., L. A. Baur, A. D. Kriketos, D. A. Pan, G. J. Cooney, A. B. Jenkins, G. D. Calvert, and L. V. Campbell.** Dietary fats and insulin action. *Diabetologia* 39: 621–631, 1996.
26. **Storlien, L. H., A. B. Jenkins, D. J. Chisholm, W. S. Pascoe, S. Khouri, and E. W. Kraegen.** Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and n-3 fatty acids in muscle phospholipid. *Diabetes* 40: 280–289, 1991.
27. **Vessby, B., S. Tengblad, and H. Lithell.** Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* 37: 1044–1050, 1994.
28. **Wood, P. D.** Physical activity, diet and health: independent and interactive effects. *Med. Sci. Sports Exerc.* 26: 838–843, 1994.
29. **Yki-Järvinen, H., and V. A. Koivisto.** Effects of body composition on insulin sensitivity. *Diabetes* 32: 965–969, 1983.