Acta Physiol Scand 2004, **181**, 199–208

Effect of chronic wheel running on the fatty acid composition of phospholipids and triacylglycerols in rat serum, skeletal muscle and heart

M. G. Nikolaidis,[1] A. Petridou,[1] A. Matsakas,[2] T. Schulz,[3] H. Michna[3] and V. Mougios[1]

1 *Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece*

2 *Institute of Morphology and Tumor Research, German Sport University Cologne, Cologne, Germany*

3 *Institute of Public Health Research, Technical University Munich, Munich, Germany*

Received 29 October 2003, accepted 10 February 2004.
Correspondence: V. Mougios, TEFAA, University of Thessaloniki, 541 24 Thessaloniki, Greece.

Abstract

Aim: The purpose of this study was to examine the effects of long-term wheel running on the fatty acid composition of phospholipids (PL) and triacylglycerols (TG) in rat serum, skeletal muscle (soleus and extensor digitorum longus) and heart.

Methods: To this end, the relevant tissues of 11 trained male Wistar rats were compared with those of 14 untrained ones.

Results: There were several significant differences between the two groups regarding the concentrations and percentages of individual fatty acids in serum PL and TG, with most differences appearing in the fatty acid distribution of PL. Monounsaturated fatty acids of muscle PL were significantly lower in the trained rats. Estimated elongase activity was significantly higher, whereas Δ^9-desaturase activity was significantly lower in the trained muscles. Monounsaturated fatty acids of PL were also significantly lower in the trained hearts. The fatty acid composition of PL in the skeletal muscles and the heart adapted to training in a comparable manner, whereas most of the changes in the fatty acid profile of TG were tissue-dependent. Judging from the magnitude of the effect sizes and the percentage differences between trained and untrained animals, there were many large effects of chronic exercise on the fatty acid composition of the tissues examined.

Conclusion: Long-term wheel running modified the fatty acid profile of PL and TG in rat serum, skeletal muscle and heart, and could thus be considered as a modulator of tissue fatty acid composition.

Keywords exercise, fatty acid profile, heart, lipid metabolism, serum, skeletal muscle, wheel running.

Studies addressing the effect of exercise, both acute and chronic, on the fatty acid composition of animal tissues have been appearing in the literature at a rising rate in recent years (for a review see Nikolaidis & Mougios 2004). The increased interest in this topic is probably due to the partial unraveling of the role of individual fatty acids in animal biochemistry and physiology. At the molecular level, individual fatty acids influence fundamental regulatory processes, such as ion homeostasis, gene expression, signal transduction and synthesis of lipid or lipid-derived messengers (Kogteva & Bezuglov 1998). These effects can then have significant impact on animal physiology. For example, there is now evidence linking insulin sensitivity to the fatty acid composition of skeletal muscle phospholipids (PL) (e.g. Borkman *et al.* 1993), while (in an exercise physiology context) increased dietary intake of ω3 fatty acids has been reported to decrease the endurance performance of rats (Ayre & Hulbert 1997) and salmons (McKenzie *et al.* 1998).

Skeletal muscle has been the most frequently studied tissue in terms of the effect of chronic exercise on its fatty acid composition (Thomas *et al.* 1977, Wirth *et al.* 1980, Kriketos *et al.* 1995, Andersson *et al.* 1998, 2000, Ayre *et al.* 1998, Mataix *et al.* 1998, Helge *et al.* 1999, 2001, Quiles *et al.* 1999, 2001, Szabó *et al.* 2002, Helge & Dela 2003). However, there is no consensus as to the effect of exercise, probably because of the near uniqueness of each of these studies in terms of type of exercise, species, subcellular fraction, lipid class and diet of the animals or humans examined. The same lack of consensus (with the same probable reasons) can be ascertained among the studies that have examined the effect of chronic exercise on the fatty acid composition of the heart (Wirth *et al.* 1980, Rocquelin *et al.* 1981, Tibbits *et al.* 1981, Ayre *et al.* 1998, Mataix *et al.* 1998, Demaison *et al.* 2000, Quiles *et al.* 2001) and plasma or serum (Hurter *et al.* 1972, Allard *et al.* 1973, Vihko *et al.* 1973, Wirth *et al.* 1979, 1980, Hambleton *et al.* 1980, Masumura *et al.* 1992, Andersson *et al.* 1998, 2000, Hashimoto *et al.* 1999, Quiles *et al.* 2003).

The aim of this study was to examine the effects of long-term wheel running (a stress-free exercise model) on the fatty acid composition of PL and triacylglycerols (TG) in rat serum, skeletal muscle and heart, in order to shed some new light on this controversial topic. Because there is evidence that the adaptive response of skeletal muscle to an exercise stimulus can be muscle-type specific (Holloszy & Coyle 1984), we included two skeletal muscles of different type in our investigation.

Methods

Animals

Thirty-five-male Wistar rats were purchased at the age of 7 weeks from Charles River Laboratories (Sulzfeld, Germany) and were housed under controlled environmental conditions (21 °C, 12 : 12-h light–dark cycle). The rats were allowed free access to water and standard rodent chow from Ssniff (Soest, Germany). The animals were maintained according to the European Union guidelines for the care and use of laboratory animals. The study design was approved by the Regional Administration of the city of Cologne (Bezirksregierung Köln).

Training

The animals were divided randomly into a trained ($n = 20$) and an untrained group ($n = 15$). The members of the trained group were housed individually in cages equipped with wheels where they exercised *ad libitum* for 8 weeks, while the members of the untrained group were housed individually in plain cages. The running activity of the trained group was recorded continuously through the DasyLab 5.0 data collection system from Datalog (Mönchengladbach, Germany).

Specimen collection

Upon completion of the training period, the 11 most active trained animals (having run, on average, over 2 km day^{-1}) and the 14 untrained animals (one died during the experimental period) were decapitated at approximately the same time of day (14:00–16:00 hours). Wheels and food had been removed from the cages 12 and 6 h earlier, respectively, to minimize the influence of the last exercise bout and the last feeding on the biochemical parameters of interest. Blood was collected promptly and left to clot at room temperature. The soleus and extensor digitorum longus (EDL) of the right hindlimb, as well as the heart (without the great vessels) were then removed as fast as possible. The tissues were ridden of visible fat, nerves and fasciae, and were immediately immersed in liquid nitrogen. Subsequently, they were stored at −80 °C. Upon clotting, the blood was centrifuged at 1500× ***g*** for 10 min. Serum was separated and stored at −80 °C as well.

Fatty acid analysis

On the day of analysis, the frozen tissues were pulverized with mortar and pestle in liquid nitrogen. The fatty acid composition of the specimens was determined by a combination of thin-layer chromatography (TLC) and gas chromatography (GC). One-half mL of serum or 30 mg of tissue powder were mixed with 2.5 mL of 2-propanol – heptane – 0.5 M H_2SO_4, 40 : 10 : 1 (v/v/v), after the addition of diheptadecanoyl phosphatidylcholine and triheptadecanoyl glycerol (both from Sigma, St Louis, MO, USA) as internal standards for the quantitation of PL and TG, respectively. After 10 min 1 mL of heptane and 1.5 mL of water were added, and the mixture was stirred vigorously in order to afford extraction of the lipids (Dole 1956).

The upper layer was then removed, condensed under a stream of nitrogen, and applied onto silica gel TLC plates (Sigma). The plates were developed with petroleum ether – diethyl ether – acetic acid, 130 : 20 : 1.5 (v/v/v), and lipid spots were located under ultraviolet light after spraying with a solution of dichlorofluorescein in ethanol. The spots corresponding to PL and TG were excised separately and incubated in 0.5 mL of methanolic sodium methoxide (Sigma) at 50 °C for 10 min. Then 0.5 mL of boron trifluoride (Fluka, Buchs, Switzerland) were added and incubation was repeated as before (Kramer *et al.* 1997). The fatty acid methyl esters thus produced were extracted with 1.5 mL of hexane and separated in a Hewlett Packard 5890 Series II gas chromatograph (Waldbronn,

Germany) equipped with a 30-m long AT-WAX capillary column from Alltech (Deerfield, IL, USA) and a flame ionization detector. The column temperature was programmed from 160 to 250 °C at 5 °C min^{-1}. The carrier gas was helium at a flow rate of 1 mL min^{-1} (at 160 °C). Methyl esters of individual fatty acids were identified in the chromatograms by comparing their retention times to those of pure methyl esters purchased from Sigma and were quantified by comparing the area under their peaks to that of methyl heptadecanoate (derived from the internal standards) with the aid of the HP 3365 ChemStation software from Hewlett Packard. Total PL and total TG concentrations were calculated as the sum of the corresponding acyl group concentrations divided by 2 and 3, respectively.

The fatty acid composition of rodent chow was determined as described for the tissue powders above, except that no internal standard was added and the extracted lipids were not separated by TLC.

Cholesterol determination

Serum total cholesterol (TC) was assayed by a photometric method using a kit from BEST (Athens, Greece). High-density-lipoprotein cholesterol (HDLC) was determined likewise after precipitation of the other lipoproteins with a dextran sulfate reagent from Konelab (Vantaa, Finland).

Citrate synthase assay

We assayed citrate synthase (CS) spectrophotometrically according to Srere (1969), as a marker of the oxidative capacity of skeletal muscle and the heart. A 10 mg of tissue powder were suspended in 250 μL (for skeletal muscle) or 1000 μL (for heart) of 175 mM KCl, 2 mM EDTA (pH 7.4), and centrifuged at 1500× ***g*** for 5 min. The assay solution consisted of 467 μL 100 mM Tris-HCl (pH 8.3), 67 μL 1 mM 5,5′-dithiobis-2-nitrobenzoate (DTNB), 33 μL 10 mM oxaloacetate, 100 μL 3 mM acetylcoenzyme A and 5 or 2.5 μL of skeletal muscle or heart supernatant, respectively. The rate of DTNB reduction by the coenzyme A formed was monitored at 412 nm, at 25 °C. CS activity is expressed in U g^{-1}, 1 U corresponding to 1 μmol of coenzyme A formed per min. All materials for the assay were purchased from Sigma.

Phosphofructokinase assay

We assayed phosphofructokinase (PFK) as a marker of the glycolytic capacity of skeletal muscle and the heart. The spectrophotometric assay was according to Ling *et al.* (1966), except that the homogenization buffer was after Baldwin *et al.* (1973). A 10 mg of tissue powder were suspended in 1000 μL of 100 mM potassium phosphate, 2 mM dithiothreitol, 0.5 mM ATP, 5 mM $MgCl_2$, 30 mM NaF (pH 8.0), and centrifuged at 1500× ***g*** for 5 min. The assay solution consisted of 125 μL 200 mM Tris-HCl (pH 8.0), 75 μL 20 mM ATP, 19 μL 200 mM $MgCl_2$, 75 μL 20 mM fructose 6-phosphate, 50 μL 2.4 mM NADH, 190 μL 200 mM KCl, 7.5 μL 100 mM dithiothreitol, 50 μL auxiliary enzyme solution (containing 8 U mL^{-1} of aldolase, 19 U mL^{-1} of triose phosphate isomerase, 2.4 U mL^{-1} of glycerol 3-phosphate dehydrogenase and 2 mg mL^{-1} of bovine serum albumin), 128 μL water, and 30 μL of soleus or heart supernatant. In the case of EDL, the volumes of water and supernatant were changed to 148 and 10 μL, respectively. The rate of NAD^+ formation was monitored at 340 nm, at 25 °C. PFK activity is expressed in U g^{-1}, 1 U corresponding to 1 μmol of fructose 6-phosphate converted per minute. All materials for the assay were purchased from Sigma.

Calculations and statistics

We have calculated the following indices of the fatty acid profile of PL and TG in each tissue: monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ω6 fatty acids, ω3 fatty acids, ω6/ω3, unsaturated to saturated ratio (U/S) and unsaturation index (UI; the average number of double bonds per fatty acid multiplied by 100). Additionally, we estimated fatty acid elongase and Δ^5-, Δ^6- as well as Δ^9-desaturase activities in the skeletal muscles and the heart through appropriate product-to-precursor ratios. These were 18 : 0/16 : 0 for elongase, 20 : 4ω6/20 : 3ω6 for Δ^5-desaturase, 18 : 3ω6/18 : 2ω6 for Δ^6-desaturase and 18 : 1ω9/18 : 0 for Δ^9-desaturase. The ratios were calculated from the sum of the concentrations of each fatty acid in PL and TG.

Values are expressed as mean values ± SD. The distribution of all dependent variables was examined by the Kolmogorov–Smirnov test and was found not to differ significantly from normal. Differences between untrained and trained animals were examined by unpaired Student's *t*-tests. To determine the meaningfulness of the effect of exercise on fatty acid composition, we calculated effect sizes as the difference between means divided by the SD of the untrained group. The level of statistical significance was set at $\alpha = 0.05$. The SPSS version 10.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

Diet

The fatty acid composition of the animals' diet is presented in Table 1. The major fatty acids were 16 : 0, 18 : 1ω9 and 18 : 2ω6, accounting for 89% of total.

Table 1 Percentage molar fatty acid composition of the rats' diet

Fatty acid	%
12 : 0	0.3
14 : 0	0.3
16 : 0	28.3
16 : 1ω7	0.2
18 : 0	3.9
18 : 1ω9	20.8
18 : 1ω7	1.8
18 : 2ω6	40.1
18 : 3ω3	2.9
20 : 1ω9	0.8
20 : 5ω3	0.6
Sum	100.0

Running activity

The Figure 1 illustrates the progression of the activity of the trained group during the experimental period. Wheel running activity increased rapidly up to the fourth week and fell slightly afterwards. Running activity was 5.2 ± 3.8 km day^{-1} during the entire training period.

Serum lipids

Fifteen fatty acids were detected in considerable amounts by GC, namely, myristate (14 : 0), palmitate (16 : 0), palmitoleate (16 : 1ω7), stearate (18 : 0), oleate (18 : 1ω9), cis-vaccenate (18 : 1ω7), linoleate (18 : 2ω6), γ-linolenate (18 : 3ω6), α-linolenate (18 : 3ω3), gondoate (20 : 1ω9), dihomo-γ-linolenate (20 : 3ω6), arachidonate (20 : 4ω6), eicosapentaenoate (20 : 5ω3), docosapentaenoate (22 : 5ω3) and docosahexaenoate (22 : 6ω3).

The total concentration of serum PL was significantly lower in the trained compared with the untrained rats by 10.6% (0.76 ± 0.11 vs. 0.85 ± 0.07 μmol mL^{-1}, $P = 0.018$). Total TG were borderline non-significantly lower in the trained animals by 24.5% (0.84 ± 0.39 vs. 1.11 ± 0.33 μmol mL^{-1}, $P = 0.072$). The effect of wheel running on the fatty acid composition of serum PL and TG is presented in Table 2. Concentrations of individual fatty acids are not presented (in any of the tissues examined), since most of the concentration changes were reflected in percentage changes. There were several significant differences between the two groups regarding the concentrations and percentages of individual fatty acids in serum PL and TG, with most differences appearing in the fatty acid distribution of PL, whereas there were no significant differences in any of the indices of the fatty acid profile calculated.

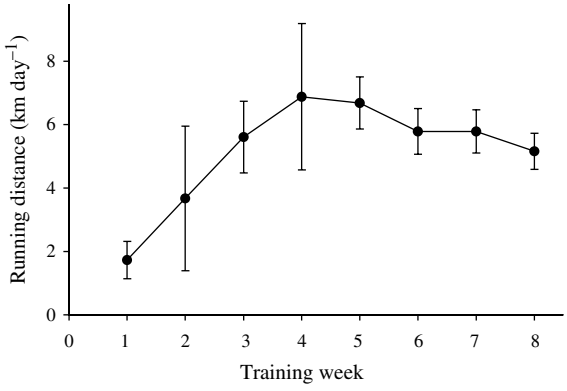

Figure 1 Weekly averages of the distance run daily by the training group (mean values ± SD).

Differences between the two groups with regard to serum TC, HDLC and their ratio (considered an atherogenic index) are presented in Table 3. TC and HDLC concentrations were not affected significantly, but their ratio decreased by 9.3% in the trained rats ($P = 0.002$).

Soleus lipids

The total concentration of soleus PL was similar in the trained and untrained rats (17.16 ± 2.17 vs. 18.37 ± 2.00 μmol g^{-1}). Again, total TG were borderline non-significantly lower in the trained rats (5.63 ± 3.36 vs. 8.26 ± 3.56 μmol g^{-1}, $P = 0.072$). The fatty acid profile of soleus PL and TG is presented in Table 4. There was a significant decrease in three of the four MUFA in PL, in terms of both concentration and percentage. As a result, the trained animals exhibited 10.9% lower MUFA ($P = 0.001$). The largest effect of exercise appeared on the percentage of 18 : 3ω6 in TG, which was higher in the trained rats by 54.9% ($P = 0.036$). Elongase activity was significantly higher, whereas Δ^9-desaturase activity was significantly lower in the trained animals.

EDL and heart lipids

The total concentration of EDL PL was similar in the trained and untrained rats (16.12 ± 2.36 vs. 14.31 ± 2.69 μmol g^{-1}). In EDL, total TG were non-significantly lower in the trained animals (0.64 ± 0.44 vs. 0.96 ± 0.62 μmol g^{-1}, $P = 0.143$). The fatty acid profile of PL and TG in EDL and heart, as well as the response of the fatty acid profile to training, was similar to that of soleus PL and TG (not shown). MUFA of PL in EDL were lower in the trained rats by 6.6% ($P = 0.023$), while elongase and Δ^9-desaturase activities differed significantly between the two groups in the same directions as in soleus.

Table 2 Molar percentage distribution of individual fatty acids and indices of the fatty acid profile in serum phospholipids and triacylglycerols of untrained and trained rats (mean values ± SD)

	Phospholipids			Triacylglycerols		
Fatty acid	Untrained	Trained	ES	Untrained	Trained	ES
14 : 0	0.18 ± 0.06	0.22 ± 0.09	0.60	0.89 ± 0.21	1.09 ± 0.35	0.97
16 : 0	19.04 ± 1.05	18.77 ± 1.36	−0.26	32.70 ± 1.67	31. 49 ± 1.82	−0.72
16 : 1ω7	0.54 ± 0.18	0.47 ± 0.13	−0.37	3.87 ± 1. 58	3.99 ± 1.83	0.07
18 : 0	23.32 ± 1.52	23.70 ± 1.91	0.25	2.34 ± 0.41	2.58 ± 0.36	0.59
18 : 1ω9	4.37 ± 0.57	4.75 ± 0.79	0.65	19.36 ± 2.84	18.65 ± 3.14	−0.25
18 : 1ω7	4.19 ± 0.70	3.37 ± 0.41*	−1.17	3.95 ± 0.64	3.76 ± 0.85	−0.29
18 : 2ω6	19.68 ± 2.10	22.15 ± 3.28*	1.17	30.57 ± 3.94	31.38 ± 4.21	0.20
18 : 3ω6	0.60 ± 0.06	0.69 ± 0.05*	1.62	0.47 ± 0.15	0.47 ± 0.15	0.04
18 : 3ω3	0.11 ± 0.04	0.16 ± 0.05*	1.35	1.20 ± 0.43	1.43 ± 0.29	0.55
20 : 1ω9	0.43 ± 0.11	0.40 ± 0.09	−0.26	0.67 ± 0.32	0.42 ± 0.16*	−0.76
20 : 3ω6	0.96 ± 0.26	1.06 ± 0.25	0.38	0.28 ± 0.07	0.29 ± 0.11	0.12
20 : 4ω6	22.27 ± 2.30	19.85 ± 3.48*	−1.05	2.07 ± 0.63	2.51 ± 1.33	0.70
20 : 5ω3	0.21 ± 0.07	0.25 ± 0.09	0.66	0.52 ± 0.17	0.61 ± 0.28	0.54
22 : 5ω3	1.05 ± 0.22	1.11 ± 0.21	0.27	0.43 ± 0.17	0.53 ± 0.18	0.55
22 : 6ω3	3.04 ± 0.42	3.04 ± 0.50	0.01	0.68 ± 0.30	0.80 ± 0.25	0.42
Sum	100.00	100.00		100.00	100.00	
Indices						
MUFA (%)	9.5 ± 1.2	9.0 ± 1.1	−0.43	27.8 ± 4.8	26.8 ± 5.3	−0.22
PUFA (%)	47.9 ± 1.3	48.3 ± 1.8	0.30	36.2 ± 4.7	38.0 ± 6.1	0.38
ω6 (%)	43.5 ± 1.7	43.8 ± 1.6	0.14	33.4 ± 4.4	34.6 ± 5.4	0.29
ω3 (%)	4.4 ± 0.6	4.6 ± 0.7	0.27	2.8 ± 0.9	3.4 ± 0.9	0.58
ω6/ω3	10.0 ± 1.6	9.7 ± 1.3	−0.20	13.2 ± 4.8	10.7 ± 2.0	−0.52
U/S	1.35 ± 0.07	1.35 ± 0.11	−0.08	1.79 ± 0.14	1.85 ± 0.15	0.46
UI	168 ± 5	164 ± 10	−0.82	112 ± 8	117 ± 12	0.61

ES, effect size; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, unsaturated/saturated; UI, unsaturation index.
*Significantly different from untrained ($P < 0.05$).

In heart, total PL concentration was lower in the trained rats by 8.0% (45.99 ± 4.71 vs. 50.00 ± 4.73 μmol g^{-1}, $P = 0.046$). The total concentration of heart TG was similar in the trained and untrained animals (0.61 ± 0.59 vs. 0.62 ± 0.28 μmol g^{-1}). MUFA of PL in heart were lower in the trained rats by 13.7% ($P = 0.002$). There were several significant differences in heart regarding individual fatty acids between the two groups, most of them appearing in PL.

Table 3 Serum cholesterol concentrations and atherogenic index in untrained and trained rats (mean values ± SD)

	Untrained	Trained
TC (mmol L^{-1})	1.54 ± 0.18	1.47 ± 0.25
HDLC (mmol L^{-1})	1.10 ± 0.13	1.15 ± 0.17
TC/HDLC	1.40 ± 0.11	1.27 ± 0.11*

TC, total cholesterol; HDLC, high-density-lipoprotein cholesterol.
*Significantly different from untrained ($P < 0.05$).

Enzymes

Wheel running did not affect the activities of CS and PFK in any tissue (Table 5). It is noticeable, however, that, compared with their untrained counterparts, the trained animals had higher activities in the skeletal muscles (on average, by 8.2%) and lower activities in the heart (on average, by 5.8%).

Discussion

We found that wheel running did not change significantly the enzyme activities that we chose as markers of the oxidative and glycolytic capacity in any of the tissues studied. Studies that employed the same mode of exercise have reported increased CS activity in trained skeletal muscles (Henriksen & Halseth 1995, Kriketos *et al.* 1995, Sexton 1995), no difference from untrained muscles (Cheng *et al.* 1997, Noble *et al.* 1999, Podolin *et al.* 1999) or both results depending on the muscle analysed (Rodnick *et al.* 1992). PFK has been studied less in relation to training. Although we did not find any

Table 4 Molar percentage distribution of individual fatty acids and indices of the fatty acid profile in soleus phospholipids and triacylglycerols of untrained and trained rats (mean values ± SD)

	Phospholipids			Triacylglycerols		
Fatty acid	Untrained	Trained	ES	Untrained	Trained	ES
14 : 0	0.28 ± 0.10	0.28 ± 0.08	0.03	2.43 ± 0.29	2.12 ± 0.30*	−1.08
16 : 0	10.08 ± 1.05	9.69 ± 0.96	−0.37	28.81 ± 1.28	28. 09 ± 1.64	−0.56
16 : 1ω7	0.86 ± 0.24	0.63 ± 0.09*	−0.97	8.42 ± 2.87	5.79 ± 1.54*	−0.92
18 : 0	21.62 ± 1.89	21.95 ± 1.83	0.18	3.22 ± 0.44	3.83 ± 0.37*	1.38
18 : 1ω9	3.78 ± 0.47	3.31 ± 0.14*	−0.99	20.84 ± 1.37	20.27 ± 0.85	−0.42
18 : 1ω7	3.44 ± 0.25	3.21 ± 0.18*	−0.87	3.58 ± 1.01	3.66 ± 0.32	0.08
18 : 2ω6	34.41 ± 2.06	35.10 ± 2.62	0.34	28.57 ± 3.89	31.84 ± 3.30*	0.84
18 : 3ω6	0.35 ± 0.04	0.36 ± 0.05	0.39	0.23 ± 0.08	0.36 ± 0.13*	1.52
18 : 3ω3	0.26 ± 0.04	0.31 ± 0.07	1.13	1.76 ± 0.22	1.73 ± 0.23	−0.17
20 : 1ω9	0.17 ± 0.04	0.19 ± 0.03	0.35	0.28 ± 0.06	0.33 ± 0.05*	0.89
20 : 3ω6	0.58 ± 0.10	0.61 ± 0.07	0.34	0.14 ± 0.07	0.16 ± 0.07	0.34
20 : 4ω6	11.04 ± 1.25	11.13 ± 1.08	0.07	1.22 ± 0.28	1.28 ± 0.56	0.22
20 : 5ω3	0.20 ± 0.10	0.21 ± 0.16	0.07	0.09 ± 0.05	0.10 ± 0.05	0.16
22 : 5ω3	3.07 ± 0.25	3.06 ± 0.28	−0.06	0.19 ± 0. 07	0.21 ± 0.10	0.32
22 : 6ω3	9.88 ± 1.18	9.96 ± 1.22	0.07	0.21 ± 0.07	0.24 ± 0.11	0.49
Sum	100.00	100.00		100.00	100.00	
Indices						
MUFA (%)	8.2 ± 0.8	7.3 ± 0.3*	−1.17	33.1 ± 3.7	30.0 ± 2.2*	−0.83
PUFA (%)	59.8 ± 2.7	60.7 ± 2.8	0.35	32.4 ± 4.2	35.9 ± 3.2*	0.84
ω6 (%)	46.4 ± 2.0	47.2 ± 2.9	0.42	30.2 ± 4.0	33.6 ± 3.1*	0.88
ω3 (%)	13.4 ± 1.3	13.5 ± 1.3	0.10	2.3 ± 0.3	2.3 ± 0.3	0.08
ω6/ω3	3.5 ± 0.3	3.5 ± 0.5	0.12	13.5 ± 1.9	14.9 ± 1.5	0.72
U/S	2.15 ± 0.29	2.15 ± 0.26	0.00	1.91 ± 0.13	1.95 ± 0.17	0.30
UI	200 ± 11	202 ± 9	0.14	104 ± 6	109 ± 5	0.79
Elongase†	0.86 ± 0.25	1.10 ± 0.30*	0.97			
Δ^5-desaturase†	18.0 ± 2.5	17.2 ± 1.4	−0.34			
Δ^6-desaturase†	0.009 ± 0.001	0.010 ± 0.002	0.85			
Δ^9-desaturase†	0.76 ± 0.27	0.55 ± 0.21*	−0.77			

ES, effect size; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, unsaturated/saturated; UI, unsaturation index.

*Significantly different from untrained ($P < 0.05$).

†Calculated as product-to-precursor ratios (described in the text) from the sum of the concentrations of the appropriate fatty acids in phospholipids and triacylglycerols.

relevant study with wheel running as the exercise stimulus, two studies with treadmill running reported increased PFK activity in trained rat skeletal muscle (Gillespie *et al.* 1982, Troup *et al.* 1986), whereas four other studies with treadmill running (Baldwin *et al.* 1977, Noble & Ianuzzo 1985, Hilty *et al.* 1989, Tikkanen *et al.* 1995) and two with swimming (Harri & Valtola 1975, St-Pierre *et al.* 1988) reported no changes in PFK activity.

The effect of wheel running on CS activity in the heart has not been studied extensively. The relevant studies have reported either increased activity in trained rats (Henriksen & Halseth 1995) or no difference from untrained ones (Noble *et al.* 1999, Duncan *et al.* 2000). We found no study investigating the effect of chronic exercise on PFK activity in the heart.

Although the lack of significant changes in CS and PFK activities suggests that the oxidative and glycolytic capacity of the two skeletal muscles and the heart were not affected by wheel running, the training stimulus was sufficient to induce several other adaptive events. This is shown by the many changes found in the concentrations and percentages of individual fatty acids, as well as by the more favourable lipidemic profile of the trained rats. A favourable lipidemic profile has been generally reported in the literature for wheel-trained rats (Lopez *et al.* 1975, Starzec *et al.* 1983, Fukuda *et al.* 1991, Suzuki & Machida 1995, Sakamoto *et al.* 1998). It is also worth mentioning that male rats covering the same distance in wheels for the same period as in the present study increased time to exhaustion by 52% and maximal oxygen uptake by 12% (Lambert & Noakes 1990).

Table 5 Enzyme activities (U g^{-1} wet tissue) in skeletal muscles and heart of untrained and trained rats (mean values ± SD)

	Soleus		EDL		Heart	
	Untrained	Trained	Untrained	Trained	Untrained	Trained
CS	32.7 ± 5.5	33.1 ± 4.4	20.1 ± 4.6	22.0 ± 4.9	84.5 ± 16.2	80.2 ± 19.0
PFK	10.4 ± 3.3	11.6 ± 2.1	47.3 ± 10.8	52.4 ± 6.8	13.8 ± 2.2	12.9 ± 2.0

EDL, extensor digitorum longus; CS, citrate synthase; PFK, phosphofructokinase.

The vast majority (63 of 65) of the effect sizes that accompanied significant differences in fatty acid composition and its indices are considered to be large (i.e. being 0.8 or higher) according to the classification of Cohen (1988). To further explore the meaningfulness of these differences, we calculated them as percentages relative to the respective values of the untrained group. These percentages were, on average, 24.4, 22.2, 24.7 and 26.0 in serum, soleus, EDL and heart, respectively. Therefore, either in terms of SD units (i.e. effect size) or in terms of percentage change, the effects of chronic exercise on fatty acid composition could be considered large in this study.

To our knowledge, there are three studies that investigated the effect of exercise on the fatty acid distribution of serum PL, all in humans (Allard *et al.* 1973, Andersson *et al.* 1998, 2000). Only two of them reported a limited number of disparate training effects (Allard *et al.* 1973, Andersson *et al.* 2000), which, in addition, are different from the ones found in the present study. Differences in species and exercise mode are probable reasons for these discrepancies. In terms of exercise mode, the human studies employed moderate-intensity continuous exercise, whereas wheel running is considered high-intensity intermittent exercise (Yancey & Overton 1993).

As for the fatty acid composition of serum TG, the sole difference found in this study (20 : 1ω9 being lower in the trained rats) cannot be compared with the findings of the only other relevant study (Allard *et al.* 1973), since it does not present data on this fatty acid. Unfortunately, most of the studies addressing the effect of chronic exercise on the fatty acid composition of plasma or serum lipids have either not separated lipid classes at all (Wirth *et al.* 1979, 1980, Hambleton *et al.* 1980, Masumura *et al.* 1992, Hashimoto *et al.* 1999, Quiles *et al.* 2003) or not separated PL and TG (Hurter *et al.* 1972, Vihko *et al.* 1973), thus preventing comparison with the present study.

We found comparable training-induced changes in the percentage fatty acid composition of PL in the type I (soleus) and type II (EDL) muscles that we examined. Comparison with the relevant literature (Thomas *et al.* 1977, Kriketos *et al.* 1995, Andersson *et al.* 1998, 2000, Ayre *et al.* 1998, Helge *et al.* 1999, 2001, Helge & Dela 2003) is very difficult, since there is no consensus among the studies, irrespective of the species examined.

Of the aforementioned studies, the one by Kriketos *et al.* (1995) is similar to ours in design. They trained rats in wheels for 45 days and determined the fatty acid profile of PL in soleus and EDL. The only differences found between trained and untrained animals were the lower percentages of 22 : 6ω3 and (sum of) ω3 fatty acids only in soleus of the trained animals (Kriketos *et al.* 1995). Whether this disagreement is due to the fact that Kriketos *et al.* (1995) used female rats or to baseline differences in the fatty acid profile (e.g. soleus PL of the untrained animals contained 16% 18 : 2ω6 and 23% 20 : 4ω6, as opposed to 34 and 11%, respectively, in our study) cannot be asserted with certainty.

We found markedly lower (although not statistically justified) total TG concentrations in soleus (by 31.9%) and EDL (by 33.7%) of the trained animals. Most of the relevant studies have found lower TG concentrations in skeletal muscle of trained rats (Froberg 1969, Kaciuba-Uscilko *et al.* 1981, Oscai *et al.* 1982), although no differences between trained and untrained rats have also been reported (Lee *et al.* 2002).

Wheel running had a remarkable effect on the fatty acid composition of skeletal muscle TG. No studies addressing the effect of chronic exercise on the fatty acid profile of skeletal muscle TG in any species other than humans have been published. Studies on humans have reported limited and contradictory effects of exercise (Andersson *et al.* 1998, 2000, Helge *et al.* 2001, Helge & Dela 2003).

We found the PL content of the heart to be lower in the trained animals, in contrast with Rocquelin *et al.* (1981), who found higher PL in trained rats. However, our data are in agreement with Grollman & Costello (1972), who reported lower total lipids in the heart of trained rats (considering that PL are by far the major lipid class in the heart, it is rather improbable that a decrease in total lipids will not be due to a decrease in PL).

Most of the changes in the fatty acid composition of heart PL with training (i.e. the decrease in the percentages of all MUFA) were similar to those found in the skeletal muscles. The relevant studies (Ayre *et al.* 1998, Demaison *et al.* 2000) reported no changes

except for a decrease of 20 : 3ω6 in trained rats (Ayre *et al.* 1998).

With the exception of two extreme and reverse changes in the percentages of two minor fatty acids (16 : 1ω7 being lower and 22 : 5ω3 being higher in trained animals), the fatty acid profile of heart TG was resistant to training. We are not aware of any relevant study.

What are the mechanisms behind the reported changes in fatty acid composition? We believe it is very difficult to answer this question at this stage. Potential control points of the fatty acid composition of tissues include lipid biosynthesis, degradation, transport and permeation through cellular membranes. We know very little about whether and how exercise affects these processes.

Conclusions

Long-term wheel running modified the fatty acid profile of PL and TG in rat serum, skeletal muscle and heart, and could thus be considered as a modulator of tissue fatty acid composition. The fatty acid composition of soleus, EDL and heart PL adapted in a comparable manner (lower MUFA), whereas most of the changes in the fatty acid profile of TG were tissue-dependent. Changes in the indices of activity of two enzymes involved in fatty acid biosynthesis (elongase and Δ^9-desaturase) were in the same direction in the three tissues (upward and downward, respectively). In addition, the magnitude of the adaptive capacity of the fatty acid profile of skeletal muscle TG to wheel running was comparable with that of PL. Judging from the magnitude of the effect sizes and the percentage differences between trained and untrained animals, there were many large effects of chronic exercise on the fatty acid composition of the tissues examined. The biological significance of these effects is unknown. Further studies are warranted to confirm the findings of the present study and delineate their physiological implications.

References

Allard, C., Alteresco, M., Ferguson, R.J., Chaniotis, L., Choquette, G. & Skinner, J. 1973. Changes in adipose tissue and increased serum cholesterol of coronary patients following training. *Can Med Assoc J* **109**, 194–197.

Andersson, A., Sjödin, A., Olsson, R. & Vessby, B. 1998. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. *Am J Physiol* **274**, E432–E438.

Andersson, A, Sjödin, A., Hedman, A., Olsson, R. & Vessby, B. 2000. Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men. *Am J Physiol* **279**, E744–E751.

Ayre, K.J. & Hulbert, A.J. 1997. Dietary fatty acid profile affects endurance in rats. *Lipids* **32**, 1265–1270.

Ayre, K.J., Phinney, S.D., Tang, A.B. & Stern, J.S. 1998. Exercise training reduces skeletal muscle membrane arachidonate in the obese (fa/fa) Zucker rat. *J Appl Physiol* **85**, 1898–1902.

Baldwin, K.M., Winder, W.W., Terjung, R.L. & Holloszy, J.O. 1973. Glycolytic enzymes in different types of skeletal muscle: Adaptation to exercise. *Am J Physiol* **225**, 962–966.

Baldwin, K.M., Cheadle, W.G., Martinez, O.M. & Cooke, D.A. 1977. Effect of functional overload on enzyme levels in different types of skeletal muscle. *J Appl Physiol* **42**, 312–317.

Borkman, M., Storlien, L.H., Pan, D.A., Jenkins, A.B., Chisholm, D.J. & Campbell, L.V. 1993. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med* **28**, 238–244.

Cheng, B., Karamizrak, O., Noakes, T.D., Dennis, S.C. & Lambert, E.V. 1997. Time course of the effects of a high-fat diet and voluntary exercise on muscle enzyme activity in Long-Evans rats. *Physiol Behav* **61**, 701–705.

Cohen, J. 1988. *Statistical Power Analysis for the Bevavioural Sciences*. Lawrence Erlbaum, Hillsdale, NJ.

Demaison, L., Blet, J., Sergiel, J.P., Gregoire, S. & Argaud, D. 2000. Effect of dietary polyunsaturated fatty acids on contractile function of hearts isolated from sedentary and trained rats. *Reprod Nutr Dev* **40**, 113–125.

Dole, V.P. 1956. A relation between non-esterified fatty acids in plasma and the metabolsim of glucose. *J Clin Invest* **35**, 150–154.

Duncan, N.D., Williams, D.A. & Lynch, G.S. 2000. Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. *Clin Sci* **98**, 339–347.

Froberg, S.O. 1969. Metabolism of lipids in blood and tissues during exercise. In: J.R. Poortmans (ed.) *Biochemistry of Exercise*, pp. 100–113. Karger, Basel.

Fukuda, N., Tojho, M., Hidaka, T., Sho, H. & Sugano, M. 1991. Reciprocal responses to exercise in hepatic ketogenesis and lipid secretion in the rat. *Ann Nutr Metab* **35**, 233–241.

Gillespie, A.C., Fox, E.L. & Merola, A.J. 1982. Enzyme adaptations in rat skeletal muscle after two intensities of treadmill training. *Med Sci Sports Exerc* **14**, 461–466.

Grollman, S. & Costello, L. 1972. Effect of age and exercise on lipid content of various tissues of the male albino rat. *J Appl Physiol* **32**, 761–765.

Hambleton, P.L., Slade, L.M., Hamar, D.W., Kienholz, E.W. & Lewis, L.D. 1980. Dietary fat and exercise conditioning effect on metabolic parameters in the horse. *J Anim Sci* **51**, 1330–1339.

Harri, M.N. & Valtola, J. 1975. Comparison of the effects of physical exercise, cold acclimation and repeated injections of isoprenaline on rat muscle enzymes. *Acta Physiol Scand* **95**, 391–399.

Hashimoto, M., Shinozuka, K., Tanabe, Y. *et al.* 1999. Hypotension induced by exercise is associated with enhanced release of adenyl purines from aged rat artery. *Am J Physiol* **276**, H970–H975.

Helge, J.W. & Dela, F. 2003. Effect of training on muscle triacylglycerol and structural lipids: a relation to insulin sensitivity? *Diabetes* **52**, 1881–1887.

Helge, J.W., Ayre, K.J., Hulbert, A.J., Kiens, B. & Storlien, L.H. 1999. Regular exercise modulates muscle membrane phospholipid profile in rats. *J Nutr* **129**, 1636–1642.

Helge, J.W., Wu, B.J., Willer, M., Daugaard, J.R., Storlien, L.H. & Kiens, B. 2001. Training affects muscle phospholipid fatty acid composition in humans. *J Appl Physiol* **90**, 670–677.

Henriksen, E.J. & Halseth, A.E. 1995. Adaptive responses of GLUT-4 and citrate synthase in fast-twitch muscle of voluntary running rats. *Am J Physiol* **268**, R130–R134.

Hilty, M.R., Groth, H., Moore, R.L. & Musch, T.I. 1989. Determinants of VO_2max in rats after high-intensity sprint training. *J Appl Physiol* **66**, 195–201.

Holloszy, J.O. & Coyle, E.F. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* **56**, 831–838.

Hurter, R., Peyman, M.A., Swale, J. & Barnett, C.W. 1972. Some immediate and long-term effects of exercise on the plasma-lipids. *Lancet* **30**, 671–674.

Kaciuba-Uscilko, H., Dudley, G.A. & Terjung, R.L. 1981. Muscle LPL activity, plasma and muscle triglycerides in trained thyroidectomized rats. *Horm Metab Res* **13**, 688–690.

Kogteva, G.S. & Bezuglov, V.V. 1998. Unsaturated fatty acids as endogenous bioregulators. *Biochemistry (Mosc)* **63**, 4–12.

Kramer, J.K.G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.D. & Yurawecz, M.P. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* **32**, 1219–1228.

Kriketos, A.D., Pan, D.A., Sutton, J.R. *et al.* 1995. Relationships between muscle membrane lipids, fiber type and enzyme activities in sedentary and exercised rats. *Am J Physiol* **269**, R1154–R1162.

Lambert, M.I. & Noakes, T.D. 1990. Spontaneous running increases VO_2max and running performance in rats. *J Appl Physiol* **68**, 400–403.

Lee, J.S., Bruce, C.R., Tunstall, R.J., Cameron-Smith, D., Hugel, H. & Hawley, J.A. 2002. Interaction of exercise and diet on GLUT-4 protein and gene expression in type I and type II rat skeletal muscle. *Acta Physiol Scand* **175**, 37–44.

Ling, K.H., Paetkau, V., Marcus, F. & Lardy, H.A. 1966. Phosphofructokinase. *Methods Enzymol* **9**, 425–429.

Lopez, A., Rene, A., Bell, L. & Hebert, J.A. 1975. Metabolic effects of exercise. I. Effect of exercise on serum lipids and lipogenesis in rats. *Proc Soc Exp Biol Med* **148**, 640–645.

McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G. & Randall, D.J. 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. *Fish Physiol Biochem* **19**, 111–122.

Masumura, S., Furui, H., Hashimoto, M. & Watanabe, Y. 1992. The effects of season and exercise on the levels of plasma polyunsaturated fatty acids and lipoprotein cholesterol in young rats. *Biochim Biophys Acta* **8**, 292–296.

Mataix, J., Quiles, J.L., Huertas, J.R., Battino, M. & Mañas, M. 1998. Tissue specific interactions of exercise, dietary fatty acids and vitamin E in lipid peroxidation. *Free Rad Biol Med* **24**, 511–521.

Nikolaidis, M.G. & Mougios, V. 2004. Effects of exercise on the fatty acid composition of blood and tissue lipids. *Sports Med* (in press).

Noble, E.G. & Ianuzzo, C.D. 1985. Influence of training on skeletal muscle enzymatic adaptations in normal and diabetic rats. *Am J Physiol* **249**, E360–E365.

Noble, E.G., Moraska, A., Mazzeo, R.S. *et al.* 1999. Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training. *J Appl Physiol* **86**, 1696–1701.

Oscai, L.B., Caruso, R.A. & Wergeles, A.C. 1982. Lipoprotein lipase hydrolyzes endogenous triacylglycerols in muscle of exercised rats. *J Appl Physiol* **52**, 1059–1063.

Podolin, D.A., Wei, Y. & Pagliassotti, M.J. 1999. Effects of a high-fat diet and voluntary wheel running on gluconeogenesis and lipolysis in rats. *J Appl Physiol* **86**, 1374–1380.

Quiles, J.L., Huertas, J.R., Mañas, M., Battino, M. & Mataix, J. 1999. Physical exercise affects the lipid profile of mitochondrial membranes in rats fed with virgin olive oil or sunflower oil. *Br J Nutr* **81**, 21–24.

Quiles, J.L., Huertas, J.R., Mañas, M., Ochoa, J.J., Battino, M. & Mataix, J. 2001. Dietary fat type and regular exercise affect mitochondrial composition and function depending on specific tissue in the rat. *J Bioenerg Biomembr* **33**, 127–134.

Quiles, J.L., Huertas, J.R., Ochoa, J.J., Battino, M., Mataix, J. & Mañas, M. 2003. Dietary fat (virgin olive oil or sunflower oil) and physical training interactions on blood lipids in the rat. *Nutrition* **19**, 363–368.

Rocquelin, G., Juaneda, P. & Cluzan, R. 1981. Influence of physical training on the effects of dietary oils on cardiac morphology and phospholipids in rats. *Ann Nutr Metab* **25**, 350–361.

Rodnick, K.J., Henriksen, E.J., James, D.E. & Holloszy, J.O. 1992. Exercise training, glucose transporters, and glucose transport in rat skeletal muscle. *Am J Physiol* **262**, C9–C14.

Sakamoto, S., Minami, K., Niwa, Y. *et al.* 1998. Effect of exercise training and food restriction on endothelium-dependent relaxation in the Otsuka Long-Evans Tokushima fatty rat, a model of spontaneous NIDDM. *Diabetes* **47**, 82–86.

Sexton, W.L. 1995. Vascular adaptations in rat hindlimb skeletal muscle after voluntary running-wheel exercise. *J Appl Physiol* **79**, 287–296.

Srere, P.A. 1969. Citrate synthase. *Methods Enzymol* **13**, 3–5.

St-Pierre, D.M., Leonard, D., Houle, R. & Gardiner, P.F. 1988. Recovery of muscle from tetrodotoxin-induced disuse and the influence of daily exercise. 2. Muscle enzymes and fatigue characteristics. *Exp Neurol* **101**, 327–346.

Starzec, J.J., Berger, D.F. & Hesse, R. 1983. Effects of stress and exercise on plasma corticosterone, plasma cholesterol, and aortic cholesterol levels in rats. *Psychosom Med* **45**, 219–226.

Suzuki, K. & Machida, K. 1995. Effectiveness of lower-level voluntary exercise in disease prevention of mature rats. I. Cardiovascular risk factor modification. *Eur J Appl Physiol Occup Physiol* **71**, 240–244.

Szabó, A., Romvári, R., Fébel, H., Bogner, P. & Szendrõ, Z. 2002. Training-induced alterations of the fatty acid profile of rabbit muscles. *Acta Vet Hung* **50**, 357–364.

Thomas, T.R., Londeree, B.R., Gerhardt, K.O. & Gehrke, C.W. 1977. Fatty acid profile and cholesterol in skeletal muscle of trained and untrained men. *J Appl Physiol* **43**, 709–713.

Tibbits, G.F., Nagatomo, T., Sasaki, M. & Barnard, R.J. 1981. Cardiac sarcolemma: compositional adaptation to exercise. *Science* **11**, 1271–1273.

Tikkanen, H.O., Naveri, H.K. & Harkonen, M.H. 1995. Alteration of regulatory enzyme activities in fast-twitch and slow-twitch muscle-fibers in low-intensity endurance-trained rats. *Eur J Appl PhysiolOccup Physiol* **70**, 281–287.

Troup, J.P., Metzger, J.M. & Fitts, R.H. 1986. Effect of high-intensity exercise training on functional capacity of limb skeletal muscle. *J Appl Physiol* **60**, 1743–1751.

Vihko, V., Sarviharju, P.J. & Suominen, H. 1973. Effect of endurance training on concentrations of individual plasma free fatty acids in young men at rest and after moderate bicycle ergometer exercise. *Ann Med Exp Biol Fenn* **51**, 112–117.

Wirth, A., Neermann, G., Eckert, W., Heuck, C.C. & Weicker, H. 1979. Metabolic response to heavy physical exercise before and after a 3-month training period. *Eur J Appl Physiol* **12**, 51–59.

Wirth, A., Heuck, C.C., Holm, G. & Björntorp, P. 1980. Changes in the composition of fatty acids of total lipids in various tissues and serum due to physical training and food restriction in the rat. *Scand J Clin Lab Invest* **40**, 55–62.

Yancey, S.L. & Overton, J.M. 1993. Cardiovascular responses to voluntary and treadmill exercise in rats. *J Appl Physiol* **75**, 1334–1340.

