Exercise alters the profile of phospholipid molecular species in rat skeletal muscle

Todd W. Mitchell,1,3 Nigel Turner,1,2 A. J. Hulbert,1,4 Paul L. Else,1,2 John A. Hawley,5 Jong Sam Lee,5 Clinton R. Bruce,5 and Stephen J. Blanksby3
1Metabolic Research Centre and Departments of Biomedical Science, 2Chemistry, and 4Biological Sciences, University of Wollongong, Wollongong, New South Wales 2522; and 5Exercise Metabolism Group, School of Medical Sciences, RMIT University, Bundoora, Victoria 3083, Australia

Submitted 31 March 2004; accepted in final form 11 June 2004

Mitchell, Todd W., Nigel Turner, A. J. Hulbert, Paul L. Else, John A. Hawley, Jong Sam Lee, Clinton R. Bruce, and Stephen J. Blanksby. Exercise alters the profile of phospholipid molecular species in rat skeletal muscle. J Appl Physiol 97: 1823–1829, 2004. First published June 18, 2004; doi:10.1152/japplphysiol.00344.2004.—We have determined the effect of two exercise-training intensities on the phospholipid profile of both glycolytic and oxidative muscle fibers of female Sprague-Dawley rats using electrospray-ionization mass spectrometry. Animals were randomly divided into three training groups: control, which performed no exercise training; low-intensity (8 m/min) treadmill running; or high-intensity (28 m/min) treadmill running. All exercise-trained rats ran 1,000 m/session for 4 days/wk for 4 wk and were killed 48 h after the last training bout. Exercise training was found to produce no novel phospholipid species but was associated with significant alterations in the relative abundance of a number of phospholipid molecular species. These changes were more prominent in glycolytic (white vastus lateralis) than in oxidative (red vastus lateralis) muscle fibers. The largest observed change was a decrease of ~20% in the abundance of 1-stearoyl-2-docosahexaenoyl-phosphatidylethanolamine [PE(18:0/22:6); P < 0.001] ions in both the low- and high-intensity training regimes in glycolytic fibers. Increases in the abundance of 1-oleoyl-2-linoleoyl phosphatidic acid [PA(18:1/18:2); P < 0.001] and 1-alkenylpalmitoyl-2-linoleoyl phosphatidylyethanolamine [plasmalogen PE (16:0/18:2); P < 0.005] ions were also observed for both training regimes in glycolytic fibers. We conclude that exercise training results in a remodeling of phospholipids in rat skeletal muscle. Even though little is known about the physiological or pathophysiological role of specific phospholipid molecular species in skeletal muscle, it is likely that this remodeling will have an impact on a range of cellular functions.

exercise training; electrospray-ionization mass spectrometry; fatty acids; insulin sensitivity

PHOSPHOLIPIDS ARE THE MAJOR structural component of biological membranes and, as such, have significant influence on their physical properties. Alterations in membrane phospholipid composition are known to influence a diverse range of cellular functions, from the properties of membrane-bound enzymes to cell growth (28, 41). Furthermore, it has been established that the fatty acid (FA) composition of skeletal muscle phospholipids is linked to metabolic disorders, such as obesity (35, 42) and insulin resistance (8, 26). In general, saturated FAs are associated with insulin resistance, whereas polyunsaturated FAs (PUFAs), particularly n-3 PUFAs, improve insulin-stimulated glucose uptake in skeletal muscle (8, 10, 40, 43).

The FA composition of skeletal muscle phospholipids is altered by diet (1, 5, 36) and, to a lesser extent, by exercise training (2, 3, 24, 27). It has also been established that alterations in the concentration of phospholipid head group classes occur in response to endurance training (18). A recent study in healthy humans by Helge and Dela (25) found that exercise training was associated with minor increases in phospholipid long-chain PUFAs in skeletal muscle that were correlated with leg glucose uptake during a hyperinsulinemic clamp (r = 0.57, P < 0.04, n = 8). Clore et al. (11) have also reported correlations between the PUFAs content of phosphatidylcholine (PC) but not phosphatidylethanolamine (PE) and glucose disposal during a hyperinsulinemic-euglycemic clamp in vastus lateralis of healthy humans (r = 0.58, P < 0.01, n = 27). Yet another recent report has established that a large number of insulin-sensitive changes in phospholipid molecular species occur in diabetic rat myocardium (21). Collectively, the results of these studies suggest that changes in muscle membrane lipid composition, particularly phospholipid molecular species, may be an important determinant of insulin sensitivity.

We have recently reported that no significant exercise-induced changes are observed in the skeletal muscle FA profile of chow-fed rats (44). Despite the constancy of the FA profile, extensive membrane remodeling (i.e., changes in phospholipid speciation) cannot be ruled out. Nevertheless, we are unaware of any data describing changes in whole phospholipid molecular species in response to exercise training in skeletal muscle.

Recent advances in the analysis of molecular species derived from biological sources through electrospray-ionization mass spectrometry (ESI-MS) (23, 37) provide us with the ability to rapidly screen for relative changes in phospholipid populations brought about by environmental factors such as diet or exercise (37). For rapid analysis of phospholipid mixtures, positive ion ESI-MS may be used to study neutral phospholipids, i.e., PC and negative ion analysis for acidic phospholipids, i.e., phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), and PE. ESI-MS has a number of advantages over traditional methods of membrane lipid analysis such as 1) high sensitivity, 2) very little sample handling, and 3) the ability to structurally characterize whole phospholipid molecular species. In the present investigation, we have exploited this technique to provide the first detailed analysis of changes in phospholipid molecular species associated with exercise training in rodent skeletal muscle.

PHOSPHOLIPIDS ARE THE MAJOR structural component of biological membranes and, as such, have significant influence on their physical properties. Alterations in membrane phospholipid composition are known to influence a diverse range of cellular functions, from the properties of membrane-bound enzymes to cell growth (28, 41). Furthermore, it has been established that the fatty acid (FA) composition of skeletal muscle phospholipids is linked to metabolic disorders, such as obesity (35, 42) and insulin resistance (8, 26). In general, saturated FAs are associated with insulin resistance, whereas polyunsaturated FAs (PUFAs), particularly n-3 PUFAs, improve insulin-stimulated glucose uptake in skeletal muscle (8, 10, 40, 43).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We have extended the scope of this study by analyzing both glycolytic [white vastus lateralis (WVL)] and oxidative [red vastus lateralis (RVL)] muscle fibers. Previous reports have demonstrated differences in the FA profile of these muscle fiber types, with the insulin-sensitive type I and type IIa fibers containing a greater proportion of long-chain PUFAs (32) and a higher ratio of stearate (18:0) to palmitate (16:0) (17) than type IIb fibers. There is also evidence that exercise-induced changes in phospholipid head group classes differ in oxidative and glycolytic fibers, with increases in total PE content in the oxidative and total PI content in the glycolytic fibers (18). By adding these parameters to our study, we are able to provide the first set of data describing the relative differences in the profile of phospholipid molecular species between muscle fiber types.

It is reasonable to expect a differing response to exercise intensity in the two muscle types, as glycolytic fibers are primarily activated during more intense exercise, whereas oxidative fibers are activated at lower intensity levels (4, 16, 39). Accordingly, we have also examined two exercise intensities (high vs. low) to investigate whether different levels of muscle activation result in differential alterations in the profile of phospholipid molecular species in the two fiber types.

METHODS

Animal care, dietary treatments, and exercise-training program.

Eighteen female Sprague-Dawley rats with an initial body mass of 90–100 g were obtained from the Animal Resource Centre, Monash University, Melbourne, Victoria, Australia, and housed two per cage. Rats were kept at a constant 22°C and 50% 2±% relative humidity, with a 12:12-h light-dark cycle (light 0700–1900). The rats were fed a standard laboratory chow diet (64% energy of energy (E%) carbohydrate, 20 E% protein, and 16 E% fat) (Ridley Agriproducts, Victoria, Australia). The FA composition of the diet was determined by using gas chromatography (GC), as previously described (36), and is presented in Table 1. All experimental procedures were approved by the Animal Experimentation Ethics Committee of RMIT University.

In the first week of the training protocol, all animals were familiarized with exercise by running on a motorized treadmill at 16 m/min for 10 min/day on a custom-built, eight-lane motorized treadmill in the hours before dark. The treadmill was not equipped with any form of electric shock device. After 1 wk, the rats were randomly divided into three groups: a control sedentary group (Sed, n = 6) that performed no specific training, a low-intensity group (Low, n = 6), and a high-intensity group (Vmax, n = 6). The Sed group performed 10 min of treadmill running at a speed of 16 m/min for 2 days/wk for 8 wk for familiarization purposes. The Low and Vmax groups both had exercise intensity gradually increased over 4 wk until they could complete 1,000 m of treadmill running 4 days/wk. The Low group then performed 125 min of running at 8 m/min for 4 days/wk, and the Vmax group 36 min at 28 m/min for 4 days/wk for the next 4 wk. Vmax group 36 min at 28 m/min for 4 days/wk for the next 4 wk. The Low program was chosen because previous investigations have shown this speed to elicit ~45% of maximal O2 uptake in rats, an intensity at which lipid oxidation predominates over carbohydrate oxidation (13). This was an important factor in our laboratory’s original study examining the effect of high-fat and high-carbohydrate diets on endurance and substrate utilization (33). We chose to examine the metabolic adaptations induced by a running program that all animals could complete without the use of external motivation (e.g., electric shock). Furthermore, we sought to maximize this stimulus so that the effects of the two distinct training programs could be examined. In determining the appropriate speed for the Vmax group, pilot testing revealed that all rats could run at a speed of 28 m/min (a velocity 3.5 times faster than the speed of running in Low), which corresponds to ~75% of maximal O2 uptake (13).

Animal death and tissue preparation. After the 8-wk training program, animals were euthanized by heart removal under anesthesia (intraperitoneal injection of pentobarbital sodium at 60 mg/kg body wt) 48 h after their last training bout. The muscles from the right hindlimb were exposed, and the RVL (16% type I, 33% type IIa, 50% type IIb fibers) and WVL (100% type IIb fibers) (12) were dissected out, frozen in liquid nitrogen, and then stored at −80°C until analysis.

Phospholipid extraction. All solvents used in the lipid analysis were of ultra-pure grade, purchased from Merck (Kilsyth, Victoria, Australia) and Crown Scientific (Moorebank, NSW, Australia). Analytical grade butylated hydroxytolene was purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Skeletal muscle lipids were extracted by standard methods (15) using ultra-pure grade chloroform-methanol (2:1 vol/vol) containing 0.01% butylated hydroxytolene as an antioxidant. Phospholipids were separated by solid-phase extraction on Strata SI-2 silica cartridges (Phenomenex, Pennant Hills, NSW, Australia). A small amount (50 μl) of the phospholipid extract was used for mass spectrometry analysis, as outlined in this study.

Mass spectrometry. ESI-MS analyses were performed on a Micromass Q-ToF2 (Micromass, Manchester, UK), equipped with an electrospray ion source and controlled by Micromass Masslynx version 3.5 software operated on a Compaq professional workstation AP200. Cone voltage was set to 70 V for negative and 30 V for positive ions. The capillary charge was set to 2,800 V and multichannel plate detector at 2,300 V. The source was heated to 80°C, and desolvation temperature was set to 120°C. For negative ion analysis, the pH of the sample was increased to 10 by the addition of ammonia. To facilitate the formation of lithium adducts for positive ion characterization, lithium iodide was added (~20 nmol/μl). Positive ion analysis was used to monitor neutral phospholipids (PC), whereas negative ion analysis was used for acidic phospholipids (PA, PS, PG, PI, and PE). Samples were infused (20 μl/min) by using a Harvard syringe pump, and phospholipids were detected in the mass-to-charge ratio (m/z) of 650 to 920. Typically, 100–120 spectra were averaged for each phospholipid extract. Tandem mass spectra (ESI-MS/MS) were obtained by using argon as the collision gas at energies ranging from 32 to 45 eV. The phospholipid molecular species are presented as the two-letter acronym of the head group followed by the two FA chains (number of carbons and number of double bonds) in parentheses.

Each mass spectrum was normalized as a percentage of the total phospholipid ions observed within the mass range (m/z 650~920) after correction for isotope contributions. Although absolute phospholipid concentrations cannot be determined by this method, owing to differences in the ionization efficiency of the different head groups, relative changes in individual phospholipid molecular species between exercise groups and fiber types were assessed. For a more detailed explanation of the determination of species abundance, see Refs. 22 and 37.

Table 1. Fatty acid composition of the diet

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>%Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>12.7</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>2.4</td>
</tr>
<tr>
<td>18:0</td>
<td>3.4</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>19.8</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>39.6</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>5.8</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.9</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>2.5</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.7</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>11.3</td>
</tr>
</tbody>
</table>
PHOSPHOLIPID SPECIATION AND EXERCISE IN RATS

Adaptations.

Table 2. Neutral phospholipid-molecular species

<table>
<thead>
<tr>
<th>Molecular Species</th>
<th>m/z (M + Li)</th>
<th>WVL</th>
<th>RVL</th>
<th>WVL</th>
<th>RVL</th>
<th>WVL</th>
<th>RVL</th>
<th>T</th>
<th>F</th>
<th>T×F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC(16:0/18:1)</td>
<td>766</td>
<td>10.7±0.3</td>
<td>10.3±0.3</td>
<td>10.9±0.2</td>
<td>11.4±0.4</td>
<td>10.2±0.3</td>
<td>11.8±0.2†</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>PC(16:0/18:2)</td>
<td>764</td>
<td>19.5±0.9</td>
<td>18.3±1.2</td>
<td>22.9±0.5</td>
<td>20.4±1.0</td>
<td>21.8±0.9</td>
<td>20.9±0.6</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PC(16:0/20:4)</td>
<td>788</td>
<td>29.7±1.1</td>
<td>28.2±1.3</td>
<td>27.1±0.4</td>
<td>25.3±0.4</td>
<td>26.9±0.6</td>
<td>25.7±0.3</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PC(16:0/22:6)</td>
<td>812</td>
<td>18.3±1.2</td>
<td>20.0±0.9</td>
<td>16.3±0.4</td>
<td>18.1±0.3</td>
<td>16.7±0.5</td>
<td>18.6±0.5</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PC(18:0/18:2)</td>
<td>792</td>
<td>6.9±0.4</td>
<td>9.7±0.9</td>
<td>7.8±0.3</td>
<td>10.1±1.4</td>
<td>8.7±0.7</td>
<td>8.6±0.7</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PC(18:0/20:4)</td>
<td>816</td>
<td>6.9±0.5</td>
<td>5.7±0.5</td>
<td>7.4±0.7</td>
<td>6.8±0.4</td>
<td>8.1±0.9</td>
<td>6.6±0.3</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>PC(18:1/20:4)</td>
<td>814</td>
<td>8.0±0.3</td>
<td>7.8±0.3</td>
<td>7.7±0.2</td>
<td>7.8±0.4</td>
<td>7.7±0.2</td>
<td>7.9±0.3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean normalized ion intensities ± SE after correction for isotope effects (n = 6 per group). m/z, Mass-to-charge ratio; M, molecular mass; Li, lithium; Sed, sedentary; Low, low-intensity training (8 m/min); Vmax, maximal voluntary running intensity (28 m/min); WVL, white vastus lateralis; RVL, red vastus lateralis; T, training; F, fiber; PC, phosphatidylcholine; NS, not significant. *Significantly different from Sed, P < 0.05. †Significantly different from WVL, P < 0.05.

Fig. 1. Change in mean relative abundance of phospholipid molecular species after exercise in white (A) and red vastus lateralis (B). Only species with a significant effect of exercise training are displayed (9 in a total of 31 major species observed). Low, low-intensity training (8 m/min); Vmax, maximal voluntary running intensity (28 m/min); PA, phosphatidic acid; PE, phosphatidylethanolamine; PI, phosphatidylinositol; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid; 22:5, docosapentaenoic acid; 22:6, docosahexaenoic acid.

Statistical analysis. Data analysis was performed by using a two-way ANOVA, with training and fiber type as fixed factors. Where ANOVA revealed a significant effect, Tukey’s post hoc test was administered to identify differences between training groups. Significance was accepted at the level of P < 0.05, and results are presented as means ± SE. All statistical analyses were performed by using JMP version 4.0 statistical software (SAS Institute, Cary, NC). Figure 1 was produced by using Microsoft Excel 2000.

RESULTS

Compliance with training programs and anthropometric adaptations. All animals in the exercise-training groups completed each of the prescribed training sessions. There were no significant differences in the body mass of animals among the three groups at the end of the 8-wk experimental period (range 248–272 g). The effect of exercise training intensity on endurance running capacity and enzyme activity for these rats has been reported previously (33). Briefly, endurance running capacity was increased following training at both exercise intensities. Changes in the activities of the enzymes citrate synthase, β-hydroxy-acetyl-CoA dehydrogenase, and carnitine palmitoyl transferase, however, were minimal (33).

Phospholipid composition. A comparison between exercise training and the observed phospholipid profile is presented in Tables 2 and 3 (neutral and acidic phospholipids, respectively). A comparison of significant training-induced changes in phospholipid profile between the glycolytic and oxidative fibers is displayed in Fig. 1.

Training effects. No novel molecular species were observed in glycolytic or oxidative muscles after exercise training. Nevertheless, a number of significant changes in the profile of both neutral (Table 2) and acidic (Table 3) phospholipids were observed following exercise training. Increases in the abundance of PA[16:0/linoleic acid (18:2)] (P < 0.05), PA[18:1/18:2] (P < 0.001), plasmenyl PE[16:0/18:2] (P < 0.005), PI[18:022:5] (P < 0.01), and PC[16:0/18:2] (P < 0.01) ions were all observed with training. Exercise training was also associated with decreases in the abundance of PE[18:0/22:6] (P < 0.001), PC[16:0/20:4] (P < 0.005), and PC[16:0/22:6] (P < 0.05) ions.

Many of the main training effects were observed to occur in the WVL (Fig. 1). The largest effect of exercise was found to be significant decreases in PE[18:0/22:6] ions (P < 0.05). This decrease was seen in both the Low and Vmax training groups compared with the Sed animals (Table 3). Conversely, increases were observed in PA[16:0/18:2] (P < 0.05), PA[18:1/18:2] (P < 0.05), and plasmenyl PE[16:0/18:2] (P < 0.05) in both the Low and Vmax groups.

Similar trends to those found in white vastus were also observed in red vastus; however, only one significant training-induced alteration in the phospholipid profile was observed (Fig. 1). This was an increase in the abundance of PC[16:0/18:1] (P < 0.05) ions in the Vmax but not the Low training regime compared with the Sed animals. Even though a number of differences were observed between the Sed group and the two training groups, no statistically significant difference was observed between the two training intensities.
**DISCUSSION**

The FA composition of skeletal muscle phospholipids is altered in both humans (2, 3, 27) and rats (24, 32, 44) after chronic (≥4 wk) exercise training. Here we provide novel data describing the effect of exercise training on skeletal muscle phospholipid molecular species profile using ESI-MS. This investigation characterized relative changes in the phospholipid profile of both oxidative (RVL) and glycolytic (WVL) muscle fibers with low and high-intensity exercise training. We have also characterized, for the first time, differences in the profile of phospholipid molecular species between these two muscle fiber types.

Previous studies have reported that long-chain n-3 PUFAs are higher in the insulin-sensitive type I and type IIa fibers (32) and that type I fibers have a preference for stearic acid (18:0) over palmitic acid (16:0) (7, 17). The results of the present study reveal that these differences are also manifest in whole phospholipid PA and PE species (Table 3). Even though the phospholipid profile was found to be similar in both the oxidative and glycolytic muscles, some differences in the abundance of particular molecular species were observed. The largest differences between the two muscles are a higher level of PE(18:0/22:6) and a lower level of plasmenyl PE(18:0/20:4) in oxidative fibers. There is also some level of similarity between the phospholipid profile of the skeletal muscles reported here and that of rodent cardiac muscle reported previously (21). That is, the most abundant phospholipid molecular species are similar in each muscle, with the exception of cardiolipin and PC(18:0/20:4), which were found in higher abundance in cardiac muscle than in the skeletal muscles examined here.

An important finding is that exercise training is associated with alterations in the relative abundance of a number of phospholipid molecular species in rat skeletal muscle. The results also demonstrate that exercise-induced changes in skeletal muscle membranes are not facilitated through the creation of novel molecular species but rather through the regulation of molecular species already present in the membrane. These data suggest that this regulation is occurring predominantly in PC, PE, and PA species. The greatest effect of exercise training on an individual phospholipid molecular species was a reduction
in PE(18:0/22:6) in glycolytic fibers. Interestingly, analogous reductions in this same species have been observed with aging (6) and thermal adaptation (9, 30) in the brain, indicating that this molecule is regularly targeted during physiological adaptation to a variety of stressors.

It is possible that some of the observed changes may be partially explained by exercise-induced changes in muscle morphology, such as mitochondrial biogenesis, and changes in fiber type and hypertrophy. It is unknown whether mitochondrial biogenesis was a major contributor, although increases in mitochondrial content would result in an increase in the relative abundance of cardiolipin, which is found almost exclusively in the mitochondria of eukaryotes (19), and this was not observed. Indeed, our laboratory previously reported only small increases in the activities of several mitochondrial enzymes (citrate synthase, β-hydroxy-acyl-CoA dehydrogenase, and carnitine palmitoyl transferase), with little or no additive effect of the more intense training protocol (33). Furthermore, it would be expected that the phospholipid composition of the two muscles would become more alike if WVL were affected by a shift from type IIB/x fibers to type IIA fibers, when, in fact, the opposite occurred. This suggests that fiber-type changes may also not have been a major contributor to the observed differences. Although there was no significant difference in body mass between the exercised and Sed rats, the lean mass was not measured, and, therefore, the synthesis of phospholipids to accommodate increases in muscle mass cannot be ruled out as a significant contributor to the observed changes in phospholipid profile.

Glycolytic and oxidative muscles are known to be differentially activated by the level of exercise intensity (4, 16, 39). Phospholipid profile, however, did not appear to reflect this differential activation in either fiber type, despite the fact that exercise training itself had a large effect on skeletal muscle phospholipid composition (Table 1). Nevertheless, this lack of difference between exercise intensities was similar to what we observed for a number of other muscle parameters (e.g., enzyme activity) in these same animals (see Ref. 33). Accordingly, we acknowledge that the stimulus exerted by the higher intensity training protocol may have been insufficient to exert additional effects above those achieved through the Low regimen.

The large number of exercised-induced changes in phospholipid molecular species observed in the present study are in contrast to previous reports of only minor alterations in FA profile with exercise training (24, 32, 44). In a previous report on the GC analysis of these same phospholipid extracts (44), we reported that exercise training resulted in a small (nonsignificant) increase in linoleic acid [18:2(n-6)] content and slight reductions in the content of both docosahexaenoic acid [22:6(n-3)] and arachidonic acid [20:4(n-6)]. The finding that, in the present study, we can detect changes in whole phospholipids but not in FAs at first appears contradictory but may be rationalized by the serious dilution effect of grouping all FAs into species, the two most abundant phospholipids found in rat skeletal muscle membranes (7). These phospholipids are distributed asymmetrically within the plasma membrane, with PC being the most abundant species in the outer leaflet and PE the inner leaflet (38). Thus the observed changes in the concentrations of these two species may be the consequence of a specific response by each membrane layer to exercise training.

The observed reductions in phospholipids containing long-chain PUFAs is potentially the result of lipid peroxidation, as previous data have shown an increase in free radical and hydroperoxide production in rodent skeletal muscle mitochondria during exercise (34). This rationale is also chemically sensible as long-chain PUFAs have a greater propensity to undergo oxidation due to the greater number of activated bisallylic carbon centers on the acyl chain (20). This may result in the removal of these peroxidized FAs by phospholipase A2, as it has a greater affinity for peroxidized than normal phospholipids (14). Whereas it is possible that this alone may explain the reduction in the relative abundance of long-chain PUFAs and increase in less unsaturated FAs, the fact that the increase in the latter is occurring predominantly in PA species suggests that there may be a specific replacement with shorter chain, less unsaturated PUFAs as a protective action against further free radical attack.
Reports that long-chain PUFAs are reduced in rodent skeletal muscle with exercise (24, 32) tend to contradict the increases observed in human muscle (3, 27) and would suggest a deleterious effect on insulin sensitivity (8, 25, 26). Whereas reductions in phospholipids containing long-chain PUFAs were also observed in the present study, another interesting finding is the different effect of exercise training on the phospholipid profile of glycolytic and oxidative muscle fibers. Whereas only a small difference in speciation between the WVL and RVL was observed in sedentary animals, it was far greater following 8 wk of exercise training, predominantly due to the change in glycolytic muscle fibers. These fibers have been shown to have very little insulin-stimulated glucose uptake (29), and, as a result, the observed reductions in long-chain PUFAs would not likely have a major impact on insulin sensitivity. Of note was that the abundance of PE(18:0/22:6) was significantly higher in the insulin-sensitive oxidative muscle than in the glycolytic muscle. Furthermore, this level did not decrease after exercise training in the oxidative as it did in the glycolytic muscle. As high levels of membrane 22:6(n-3) content have been linked to increases in insulin sensitivity (10, 40), it poses the question as to whether this molecule is selectively spared in the insulin-sensitive tissue.

In conclusion, this study has demonstrated that rat skeletal muscle phospholipids undergo a significant rearrangement under the influence of exercise training, which is in contrast to the minor changes in FA profile previously observed in these rodents (44). We have also demonstrated that this rearrangement is more prominent in glycolytic than oxidative fibers. Despite this, information regarding the role of particular phospholipid molecular species in physiological regulation is limited, and, therefore, additional work is required to determine the functional significance of the observed changes. Further research in this area will prove to be highly informative as to the role of biological membranes in metabolism and their adaptation to various factors, e.g., exercise and diet, and their impact on disease states, such as insulin resistance and the metabolic syndrome.

GRANTS

This study was funded by an RMIT Faculty Research Grant (to J. A. Hawley), an Australian Research Council grant (to A. J. Hulbert and P. L. Else), and a University of Wollongong University Research Council Grant (to S. J. Blanksby).

REFERENCES


