Exercise training reduces skeletal muscle membrane arachidonate in the obese (fa/ fa) Zucker rat

KERRY J. AYRE,1 STEPHEN D. PHINNEY,2 ANNA B. TANG,2 AND JUDITH S. STERN2,3
1Department of Biomedical Science, University of Wollongong, New South Wales 2522, Australia;
2Department of Internal Medicine, University of California, Sacramento 95817; and
3Department of Nutrition, University of California, Davis, California 95616

Ayre, Kerry J., Stephen D. Phinney, Anna B. Tang, and Judith S. Stern. Exercise training reduces skeletal muscle membrane arachidonate in the obese (fa/ fa) Zucker rat. J. Appl. Physiol. 85(5): 1898–1902, 1998.—Compared with the lean (Fa/−) genotype, obese (fa/ fa) Zucker rats have a relative deficiency of muscle phospholipid arachidonate, and skeletal muscle arachidonate in humans is positively correlated with insulin sensitivity. To assess the hypothesis that the positive effects of exercise training on insulin sensitivity are mediated by increased muscle arachidonate, we randomized 20 lean and 20 obese weanling male Zucker rats to sedentary or treadmill exercise groups. After 9 wk, fasting serum, three skeletal muscles (white gastrocnemius, soleus, and extensor digitorum longus), and heart were obtained. Fasting insulin was halved by exercise training in the obese rat. In white gastrocnemius and extensor digitorum longus (fast-twitch muscles), but not in soleus (a slow-twitch muscle) or heart, phospholipid arachidonate was lower in obese than in lean rats (P < 0.001). In all muscles, exercise in the obese rats reduced arachidonate (P < 0.03, by ANOVA contrast). We conclude that improved insulin sensitivity with exercise in the obese genotype is not mediated by increased muscle arachidonate and that reduced muscle arachidonate in obese Zucker rats is unique to fast-twitch muscles.

THE OBESE (fa/ fa) Zucker rat exhibits a range of metabolic aberrations, including hyperlipidemia, hyperglycemia, adipocyte hypertrophy, and hyperinsulinemia (4). These characteristics are similar to those found in type II (non-insulin-dependent) diabetes mellitus, making the obese Zucker rat a useful animal model for the study of insulin resistance and diabetes.

Skeletal muscle is the principal site of insulin-mediated glucose uptake, and chronic aerobic exercise training has been shown to improve the severe insulin resistance found in obese Zucker rats (5, 9, 20) and to reduce plasma insulin (22). In obese humans, physical training decreased basal and glucose-induced plasma insulin levels and improved sensitivity to insulin (6). These improvements in muscle response to insulin with exercise training are specific for exercise intensity and fiber type (affecting mostly fast-twitch fibers) and appear to occur only in muscles that are substantially recruited during exercise (5). The mechanism through which exercise training improves the skeletal muscle insulin resistance of the obese Zucker rat has yet to be fully elucidated (10).

Compared with lean Zucker rats, obese rats have been reported to have low levels of arachidonate [20:4(n–6)] in skeletal muscle, liver, and heart phospholipid (PL) (8, 16, 21). A decreased proportion of specific polyunsaturated fatty acids (including arachidonate) in skeletal muscle PL is associated with decreased insulin sensitivity in obese Zucker rats (13) and in humans (3). These findings suggest that the specific fatty acid composition of skeletal muscle PL may influence the action of insulin and thus contribute to the variations in insulin sensitivity in animals and humans.

The aim of this study was to determine whether the beneficial effect of exercise on hyperinsulinemia in the obese Zucker rat is due to an increase in arachidonate levels in their muscle PL. We examined the tissue-specific effect of chronic exercise on muscle PL arachidonate content (as well as other constituent fatty acids) in fast- and slow-twitch skeletal muscles and also in heart from lean and obese Zucker rats.

MATERIALS AND METHODS

A total of 40 weanling male rats [20 heterozygous lean (Fa/−) and 20 obese (fa/fa)] were randomly assigned to sedentary or exercise groups for 9 wk. The rats were maintained on a 12:12-h light-dark cycle with lights off at midnight, and they were fed ad libitum a synthetic diet containing soy oil (11% of energy) as the only fat source (19). Food intake and body mass were recorded throughout the study. Rats were exercised toward the end of the dark cycle, commencing at 9 AM, on a motor-driven treadmill (Stanhope Scientific, Davis, CA). Final speed was 20 m/min for 1 h/day, 6 days/wk, following the protocol of Applegate and Stern (1). Although this target was readily achieved with the lean animals, the obese animals took more time to reach this goal and required more tending during the training sessions to maintain the pace.

After a 14-h fast and 26 h after the last exercise training period, rats were killed by decapitation. Blood was collected, and three hindlimb muscles [soleus, extensor digitorum longus (EDL), and white gastrocnemius] and the heart were rapidly removed and frozen at −80°C. Plasma glucose and insulin levels were determined using the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH) and an RIA (23), respectively.

Muscle PL fatty acids were analyzed from 4 of the 9–10 animals in each group completing the protocol. Lipids were extracted from minced muscle in a glass tissue grinder according to Folch et al. (7). The PL fraction was separated from the other lipids by TLC on a prepacked silica gel H plate. The fatty acids were cleaved and methylated using 5% (wt/vol) acetyl chloride in methanol. The fatty acid methyl esters were separated by high-resolution capillary gas chroma-
tochromatography (model 5890, Hewlett-Packard, Sunnyvale, CA) with a 50 m × 0.25 mm fused silica-bonded column (model 007 FFAP, Quadrex, New Haven, CT). Peaks were identified by comparison with authentic standards (Nu Chek Prep, Elysian, MN, and Supelco, Bellefonte, PA).

Statistical analysis. To test for heterogeneity in muscle PL fatty acid composition between the obese and lean rats, we performed two-factor ANOVAs without replication for each fatty acid (PC, SAS, Cary, NC), with contrasts to assess genotype, exercise, and genotype × exercise interactions. For muscle arachidonate, the primary focus of this project, P < 0.05 was considered statistically significant. Because data are reported for 12 fatty acids per muscle, however, P < 0.004 would be more appropriate for significance for fatty acids other than arachidonate. Similar ANOVAs were done to assess differences in food intake, weight, and plasma glucose and insulin; again, P < 0.05 was the threshold for significance. To test for correlations between arachidonate and insulin levels and food intake, simple correlation coefficients were calculated.

RESULTS

Final body weight, food intake, and plasma glucose and insulin levels were significantly higher in the obese than in the lean rats (P < 0.0001 in each case; Table 1). When the effect of exercise across both groups was assessed by ANOVA, the sedentary rats were significantly heavier than the exercised rats (P < 0.03). Exercise also caused a dramatic decrease (46%, P < 0.001) in plasma insulin levels in the obese rats (which, as expected, were 10 times higher than in the lean rats). There was not a consistent effect of exercise on plasma glucose levels, inasmuch as there was no significant difference between the obese exercise and sedentary groups but a significantly higher level in the lean exercise group than in the lean sedentary controls.

In the white gastrocnemius and EDL (fast-twitch muscles), PL 20:4(n–6) was lower in the obese than in the lean genotype (P ≤ 0.0001 and 0.002, respectively), but this was not seen in the soleus (slow-twitch) or heart (Table 2). Because of the divergent effect of exercise across genotypes, exercise training, assessed as a separate variable, significantly changed the PL arachidonate level only in the soleus (P = 0.04). However, in all four muscles (Table 2), exercise in the obese animals reduced the proportion of PL arachidonate, whereas exercise in the lean rats caused it to increase (P ≤ 0.03 for all 4 muscles by genotype × exercise contrast; Fig. 1). Interestingly, in the lean rats the effects of exercise on soleus and heart PL arachidonate were less marked than the effects on the EDL and white gastrocnemius.

In addition to the effects of genotype and exercise on muscle PL arachidonate, numerous other fatty acids were affected as well. Palmitoleate [16:1, reported as the sum of two isomers 16:1(n–9) and 16:1(n–7)] was consistently elevated in the obese compared with the lean animals, but its response to exercise was not consistent within genotypes. Linoleate [18:2(n–6)] was consistently higher in skeletal muscle PL of obese than of lean rats and rose further with exercise in the obese rats but had the opposite response in the white gastrocnemius and EDL of the lean rats. Linoleate in the heart PL was paradoxically lower in the obese than in the lean rats and rose only in the obese rats with exercise.

One of the most consistent fatty acid responses to exercise in all muscles studied was seen for dihomo-γ-linolenate [20:3(n–6)], which fell significantly in all muscles (except lean EDL) in both genotypes with exercise. Because it was consistently and dramatically higher in the muscle PL of the obese than of the lean genotype, however, the exercise did not result in a decline of the obese values to the level seen in the sedentary lean animals, and thus this abnormality of the obese genotype was not fully corrected by the exercise.

Other consistent observations were the higher proportions of Mead acid [20:3(n–9)] and eicosapentaenoate [20:5(n–3)] in the muscle PL of the obese genotype (significant for 3 of 4 muscles for each), although these two anabolic products of the desaturase pathway showed little if any change in response to exercise. Docosahexaenoate [22:6(n–3)], the end product of n–3 fatty acid metabolism, did not differ between genotypes in white gastrocnemius PL, was lower in obese than in lean in EDL and soleus, but was higher in obese than in lean heart. Exercise had no significant effect on 22:6(n–3) in any muscle tested.

To assess the previously reported relationship between muscle arachidonate and fasting insulin (3), correlations were done by regression for plasma insulin and PL arachidonate for each of the four muscles. Only the white gastrocnemius appeared to show a negative correlation (r = −0.52, P < 0.01), but the validity of this simple regression is suspect because the insulin values are not normally distributed across the two genotypes. Interestingly, however, there were negative correlations between muscle PL 20:4(n–6) and food intake, but

Table 1. Body weights, food intake, and fasting plasma insulin

<table>
<thead>
<tr>
<th></th>
<th>Lean Sed</th>
<th>Lean Ex</th>
<th>Obese Sed</th>
<th>Obese Ex</th>
<th>P (by ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>111 ± 3</td>
<td>107 ± 3</td>
<td>146 ± 6</td>
<td>142 ± 6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.8 ± 0.2</td>
<td>5.7 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>6.2 ± 0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>80 ± 14</td>
<td>72 ± 17</td>
<td>1,361 ± 192</td>
<td>776 ± 114</td>
<td>0.0001</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>878 ± 17</td>
<td>872 ± 26</td>
<td>1,115 ± 20</td>
<td>1,150 ± 18</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE of 9–10 rats/group. Sed, sedentary animals; Ex, exercise animals; Geno, genotype effect; Ex, exercise effect.
only in the fast-twitch muscles, EDL ($r = -0.71, P < 0.01$) and white gastrocnemius ($r = -0.85, P < 0.001$).

**DISCUSSION**

Exercise is accepted as a positive factor in overall health, and it is viewed as particularly useful in the management of diabetes and obesity. Chronic exercise training has been shown to reduce insulin resistance, as was the case in this study, reducing the fasting plasma insulin values for the obese Zucker rats. In addition, insulin sensitivity has been reported to be positively correlated with serum (11) and muscle PL arachidonate (3), and thus in this study we anticipated that exercise training might increase muscle PL arachidonate. Although there was a consistent trend toward increased muscle 20:4(n-6) in the lean exercised rats,
we saw the opposite effect in the obese exercised rats, leading to a significant genotype × training ANOVA contrast in all four muscles studied. These data indicate a dichotomy in the metabolic response of muscle to training at the level of PL fatty acid composition between lean and obese genotypes, despite marked reductions in fasting insulin levels of the obese genotype in response to training. Thus the reduction in fasting insulin that we observed cannot be attributed to increased muscle PL arachidonate content. Furthermore, to the extent that normal levels of muscle PL arachidonate are physiologically important, exercise does not benefit this variable in the obese Zucker rat.

Another important observation in the study is the lack of a genotype difference in slow-twitch skeletal muscle and heart muscle PL arachidonate in the sedentary animals. We and others have reported reduced proportions of arachidonate in liver and skeletal muscle PL from obese Zucker rats compared with lean rats (2, 8, 13, 16, 19). In the case of prior reports of muscle PL, however, the muscles analyzed were fast-twitch or mixed-fiber type. When examined separately in this study, the relative arachidonate deficit in PL was seen exclusively in the fast-twitch muscles, whereas the slow-twitch muscle PL from the two genotypes contained similar proportions of this fatty acid. Indeed, in soleus and heart PL, arachidonate was proportionately greater in the obese sedentary than in the lean sedentary animals, although these differences were not significantly different by ANOVA (perhaps because of our small sample size). This indicates that the maldistribution of arachidonate in the obese genotype, consistently seen in the liver and less profoundly in the plasma (16), is highly selective for individual organs or cell types rather than a uniform observation in all tissue PL fractions. Wahle et al. (21) reported reduced arachidonate in the heart PL of obese Zucker rats compared with lean animals, but we did not confirm this observation in the present study.

One concern with the technique used in this study and in prior reports is the assumption that PL extracted from the whole muscle cell accurately reflects plasma membrane composition (which is the presumed site of action of insulin). In reality, myocytes contain a variety of other membranes, including endoplasmic reticulum, mitochondria, and nuclear envelope. With endurance training, muscle mitochondria increase in number (17), and thus their proportionate contribution to total cellular PL would also increase. Inasmuch as mitochondrial membranes differ from other cellular membranes in fatty acid composition (18), training could alter the fatty acid composition of total muscle PL without necessarily being any change in plasma membrane composition. If the training regimen did alter the cellular ratio of mitochondrial to plasma membranes, this response would be expected to be more pronounced in predominantly slow-twitch muscle rich in mitochondria, such as soleus, and less pronounced in fast-twitch muscles, such as EDL and white gastrocnemius. Nonetheless, we saw similar lowering of arachidonate in both types of muscle with exercise in the obese animals, making this explanation for our results unlikely.

An additional point of interest that is yet to be explained is why the leptin-receptor defect that causes obesity in the Zucker fa/ fa rat leads to such a marked and complex alteration in fatty acid metabolism and distribution. Others have hypothesized reduced activity of Δ6- and Δ5-desaturase enzymes in the Zucker obese rat (2, 8), and we did note consistently greater proportions of 20:3(n–6) in the PL from all four muscles of obese animals than from lean animals. Although this might imply a relative holdup of fatty acid anabolism at Δ5-desaturase, it cannot explain the marked proportional increases in 20:3(n–9) and 20:5(n–3) observed in the PL for three of four muscles from the obese groups. These two fatty acids are also products of Δ5-desaturase, and their consistent elevations in liver and serum lipid fractions as well have led us to the alternative hypothesis of a marked increase in flux of all fatty acid families (n–6, n–3, and n–9) through Δ5- and Δ5-desaturase in the obese Zucker rat (16) and in the BSB mouse as well (15). In this hypothetical model, arachidonate is lost from the PL at an accelerated rate, accumulating in serum and liver cholesteryl esters, whereas the n–3 and n–9 products remain in the PL pool (12). This evidence for an elevated flux of both families of essential fatty acids (plus the nonessential n–9 family) through this anabolic pathway implies a dynamic stress on arachidonate metabolism associated with a variety of genes involved in the pathogenesis of obesity. At the level of muscle membranes, this stress appears to be exacerbated, rather than alleviated, by exercise in the obese Zucker genotype.

Also of possible interest in this study are the negative correlations between food intake and fast-twitch muscle...
PL arachidonate. This implies that a systemic effect of this selective distribution of arachidonate impacts on regulation of appetite or that variations in food intake due to some other cause can effect a remarkably fine-tuned secondary response in muscle membrane arachidonate. Although we have reported sharp increases in serum PL arachidonate with energy restriction (14), which implies that serum PL arachidonate is responsive to energy balance, we have also demonstrated that enhancing arachidonate production by feeding 18:3(n–6) reduces food intake in the obese Zucker rat (16, 19). Thus it is not a foregone conclusion that food intake is the independent variable in this equation.

In conclusion, the maldistribution of PL arachidonate in the obese Zucker rat was not uniform across all muscle types, being evident only in fast-twitch muscle, but not in slow-twitch skeletal muscle and heart. Chronic exercise induced changes in muscle PL arachidonate that were consistent across all four muscles studied, but the response differed by genotype. Whereas exercise induced an increase in arachidonate in muscle PL of lean animals, it was significantly decreased with exercise induced an increase in arachidonate in muscle PL arachidonate. This implies that a systemic effect of this selective distribution of arachidonate impacts on fine-tuned secondary response in muscle membrane arachidonate due to some other cause can effect a remarkably remarkable effect.

References


