Effect of long-term exercise on skeletal muscle lipid composition

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The ability of trained muscle to sustain activity longer than untrained muscle is unquestioned. The physically trained person is able to perform a fixed work load with less oxygen debt and lower blood lactate levels than the untrained (6, 7). These observations suggest that physical training induces adaptive changes in muscle which alter its energy substrate during exercise (4). Animal studies on the effect of training and exercise have yielded variable results which may have depended on diet, size of work load, duration of training, duration of exercise, or species of animal trained. To limit these problems we have studied human volunteers who trained the quadriceps muscles of one leg by daily isotonic exercise, the opposite leg serving as an untrained control. At the end of the training period the vastus lateralis of both control and trained legs was biopsied under local anesthesia and metabolic alterations induced by physical conditioning were measured.

Methods

Ten male prison volunteers ranging in age from 22 to 44 years were subjects for this study. They were in good health and ate a diet ad libitum which supplied approximately 44% of calories as carbohydrate, 42% as fat, and 14% as protein. The quadriceps muscle group of one leg selected at random was exercised daily by lifting a weighted quadriceps boot from 90° to the horizontal every 3 sec to exhaustion for a period of 39 days (range 31-58 days). Boot weight was progressively increased from 20 to 35 lb. as endurance increased. At the end of the training period bilateral vastus lateralis muscle biopsies were performed aseptically under local anesthesia (1% lidocaine without epinephrine). Care was taken to avoid muscle infiltration at the biopsy site. Muscle samples were placed in cold 0.15 M sodium chloride, and fascia, blood, and excess adipose tissue were carefully removed. The muscle samples (weighing approximately 0.5 g) were quickly removed, blotted, and weighed. Duplicate aliquots of about 50 mg were placed in tared vials and dried in vacua at 60-80 °C until constant weights were obtained.

The remaining muscle was divided with scissors and homogenized in 20 ml (1 volume) cold 0.15 M NaCl using a glass grinder. Two milliliters of the homogenate were removed for determination of protein as previously described (17). The remaining homogenate was stirred vigorously with 5 volumes of redistilled methanol. Fifteen minutes later 5 volumes of chloroform were added with shaking. After 30 min an additional 5 volumes of chloroform were added and sufficient 0.1 M KCl to achieve the proportions of 8:4:3 chloroform-methanol-KCl. After mixing, standing, and chilling, the clear lower layer was removed and the upper layer was reextracted twice more with chloroform. The lower layers were pooled, reduced to dryness at 30-35 °C in vacuo and dissolved in 10 ml CHCl₃-MEOH 2:1. Lipids were stored at -10 °C until assayed. Assays were carried out within 72 hr in all cases. Total lipid phosphorus, phospholipid distribution, and cholesterol were determined in duplicate as reported previously (17). Triglyceride was isolated by thin-layer chromatography on silica gel-G (identified by comparison with authentic tripalmitin, Applied Science Laboratories, State College, Pa.), eluted three times with 10 ml chloroform, and estimated by determination of the glycerol liberated by periodate oxidation (9). Authentic
tripalmitin assayed by this method gave recoveries of 97 ± 4%. Comparable values for triglyceride were also obtained by the method reported by Masoro and coworkers (16). Free fatty acid was determined on a phosphorus-free extract according to the Trout, Estes, and Friedberg modification of the method of Dole (21).

Cell fractions were prepared from 5.0-g muscle biopsy samples removed from three adult males undergoing hip surgery under ether anesthesia. These specimens were homogenized and prepared by differential centrifugation following the scheme of Marinetti et al. (14). An RC-2 refrigerated centrifuge with SS-34 rotor (Beckman Instruments, Spinco Division, Norwalk, Conn.) and a Beckman model L ultracentrifuge with SS-34 rotor (31) were used. Fractions were defined operationally as follows: 1) cell homogenate; 2) 600 X g; 20 min—cell fragments, myofibrils, and nuclei; 3) 26,000 X g; 1 min—mitochondria; 4) 60,000 X g; 60 min—microsomes; and 5) 60,000 X g—supernatant. The composition of the fractions was confirmed by light and electron microscopy. All fractions were extracted three times with chloroform-methanol by the method described above so that comparisons could be made with whole muscle homogenate values in our study. The extraction method of Marinetti et al. (14) was not followed.

RESULTS

Considerable variation between subjects was noted in almost all determinations. Particularly striking were the variations between individuals in muscle water (Table 1), total phospholipid, and triglyceride content (Table 2). Since we have used paired samples from the same individual in this study, the normal variation in composition is partially compensated. The wide variation of results, however, makes clear the dangers of comparison between individuals within this study group and especially between our results and those of other studies. Muscle water content showed surprisingly random variation averaging 80.0% of wet weight with a range of 74.4-86.6%. Because of this wet weight variability all lipid values are expressed on a dry weight basis, the values of protein and wet and dry weights being given for comparison in Table 1. Although muscle hypertrophy and increased protein concentration might have been expected, the changes in protein concentration (Table 1) were not consistent and there was considerable variability. Contractile proteins were not estimated but may have accounted for this variance. Muscle glycogen, however, was increased in the trained muscle as compared to controls in a parallel study (5).

Results of lipid analyses are summarized in Fig. 1. This figure indicates that there is a considerable range of normal variation in individual human muscle phospholipid, phosphatidyl choline (lecithin), and cholesterol content. Triglyceride and total lipid values, although not shown in Fig. 1, were even more variable. Figure 1 does not indicate paired values obtained from control and trained legs in the same individual; in Table 2, however, paired comparisons are made of phospholipid, phosphatidyl choline, cholesterol, triglyceride, and free fatty acid values obtained from control and trained muscle in the same subject. Compared in this way training produced highly significant increases in total phospholipid and phosphatidyl choline and decreases in total muscle cholesterol (values reported include cholesterol plus cholesterol ester). Alterations in the principal lipid membrane components (cholesterol and phosphatidyl choline) are emphasized in Fig. 2 which indicates that the phosphatidyl choline-to-cholesterol ratio increases as a result of physical conditioning. Table 2 also indicates that this ratio is consistently increased in the trained muscle of each subject. Triglyceride also increased in the trained muscle but the variation in triglyceride content suggests that variable amounts of adipose tissue may have been present in the muscle biopsy specimen.

The observed alterations in lipid composition suggested that an increase in membranous cell organelles had occurred, therefore, cell fractions were prepared from larger muscle samples from three untrained subjects. The ratio, micromoles phosphatidyl choline to micromoles cholesterol, was then calculated for homogenate (2.30) and fractions which were predominantly nuclei and myofibrils (1.57), mitochondria (2.55), microsomes (2.25), and the nonparticulate supernatant (0.88). Cholesterol levels were lowest in the mitochondrial fraction (1.2 μmoles/g dry weight muscle). The data of Marinetti et al. (14) and Stoneburg (20) were recalculated to yield phosphatidyl choline-to-cholesterol ratios which were highest (6.60) for the mitochondrial fraction and lower (0.66, 1.60, and 0.79, respectively) for nuclear-myofibrillar, microsomal, and supernatant fractions.

### Table 1. Change in muscle dry weight-to-wet weight ratios and protein content after prolonged exercise training

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control Mean ± SEM</th>
<th>Trained Mean ± SEM</th>
<th>Change* t test†</th>
<th>Protein, mg/g dry wt Control</th>
<th>Trained</th>
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<td>2.4</td>
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* Change = trained−control. † The Student t test of paired observations and probability of a larger value, sign disregarded; NS = probability > 0.05.
### TABLE 2. Changes in lipid composition of vastus lateralis after long-term exercise training

<table>
<thead>
<tr>
<th>Subject</th>
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<th>Trained</th>
<th>Change</th>
<th>Control</th>
<th>Trained</th>
<th>Change</th>
<th>Control</th>
<th>Trained</th>
<th>Change</th>
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<td>-1.85</td>
<td>1.76</td>
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<td>28.4</td>
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<td>24.1</td>
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<td>+9.9</td>
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<td>9.07</td>
<td>-3.90</td>
<td>1.90</td>
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<td>60.3</td>
<td>+2.9</td>
<td>24.6</td>
<td>27.7</td>
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<td>10.03</td>
<td>8.92</td>
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<td>10.2</td>
<td>45.3</td>
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Mean ± SEM: +7.34 ±2.10, +5.05 ±1.33, +2.54 ±0.48, +1.43 ±0.15, +2.29 ±0.53, -1.68 ±1.03.

* The Student t test of paired observations and probability of a larger value, sign disregarded; NS = probability > 0.05. § Dash (−) indicates missing value.

**DISCUSSION**

The data presented indicate that an increase of phospholipid (principally phosphatidyl choline) and a concomitant decrease in cholesterol occurs in muscle in response to physical training. These findings suggest that an increase in phospholipid might have occurred in muscle in response to physical training. Marinetti et al. (14) showed that the phosphatidyl choline/cholesterol ratio of pig heart mitochondria is higher than that of other cell fractions. We have, therefore, interpreted the changes in phosphatidyl choline and cholesterol as consistent with the hypothesis that an increase in number or size of mitochondria occurred in response to physical training. A reduction in cholesterol would not be expected on the basis of mitochondrial proliferation alone, but might be associated with changes in energy metabolism.

The mean value ± SEM for phosphatidyl choline/cholesterol was 1.68 ±0.10 in the control group and 1.03 ±0.01 in the trained group. The phosphatidyl choline/cholesterol ratio was significantly higher in the trained group (p < 0.001).

**Table 2: Variability in lipid content observed in control and trained vastus lateralis.**

**Figure 1: Variability in lipid content observed in control and trained vastus lateralis.**
EXERCISE EFFECT ON SKELETAL MUSCLE LIPID

seen if muscle cholesterol were directly lowered or its concentration relatively decreased by proliferation of nonlipid containing elements. Cholesterol may have been altered in response to uncontrolled serum cholesterol changes although such a systemic change would be expected to affect the untrained muscle also. Palladin (19) reports increased “free cholesterol” as a result of physical conditioning, but we have not been able to compare our results with his because we lack details of his experimental methods and results. Microscopic studies are now in progress to determine the number and size distribution of mitochondria and myofibrillar elements in trained muscle.

There is other recent evidence that muscle mitochondria are increased in response to physical training: 1) Astrand (1) cites recent studies which indicate that trained muscle metabolizes fat more rapidly than untrained, thus sparing glycogen during exercise; 2) oxidation of long chain fatty acids by muscle occurs primarily in mitochondrial enzyme systems (13); 3) Holloszy (10) has shown that physical training in rats increases the respiratory enzyme activity and protein content of skeletal muscle mitochondria, increases mitochondrial electron transport capacity, and increases the ability of muscle to oxidize pyruvate. Thus, on inferential grounds, lipid changes consistent with increased mitochondrial number or size might be expected as a result of physical conditioning of somatic muscle.

The importance of intracellular lipid as a source of energy for muscle contraction remains unclear. In a recent review Carlson (3) presents data which indicate that acute exercise reduces the muscle content of intracellular esterified fatty acid, mainly triglycerides. The work of Issekutz et al. (12) and Neptune et al. (18) support Carlson’s findings. However, these lower post-exercise triglyceride values in rats contradict those reported by Hultman (11) and Masoro (16) in primates. Hultman observed no change in needle-biopsy muscle triglyceride after exercise but emphasized that large variations were found. Masoro found that 5 hr of muscular stimulation did not decrease cellular triglyceride levels in monkeys; however, it is possible that the failure of triglycerides to fall with acute exercise may have been due to relatively low levels of triglycerides in the untrained monkeys (3.2 ± 0.61 μmoles/g wet weight). We also found low levels of triglycerides (2.5-4.2 μmoles/g wet weight) in 5 of 10 control (untrained) muscle biopsies. Calculation of the energy available from “endogenous” lipid stores indicates that the trained muscle acquired an average 3.6 cal/100 g wet weight addition to stores which ranged from 0.2 to 27/cal per 100 g wet weight in the control, untrained muscle. The data in the present study show a wide range of values for “intracellular” triglycerides in both the control and trained muscles. So great is this variation that interpretation of the results is hazardous; however, all subjects studied showed a net increase in triglyceride in the trained muscle as compared to the control. We emphasize that apparent alterations in intracellular triglyceride stores may be due to contamination by adipose tissue incompletely removed in spite of our best efforts.

The concentrations of human muscle free fatty acids reported here are significantly greater than those found in rats by other investigators. In rat muscle we also have found lower levels of free fatty acids, comparable to those reported by Masoro (15) and by Garland and Randle (8). It appears then that this difference in fatty acid levels may be due to the difference in species.

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