Effects of Training and Detraining on the Distribution of Cholesterol, Triglyceride, and Nitrogen in Tissues of Albino Rats

By Edward W. Watt, Merle L. Foss, and Walter D. Block

ABSTRACT
The effects of standardized exercise training and detraining on the content of cholesterol, triglyceride, and nitrogen in blood, myocardium, skeletal muscle, and epididymal fat of rats were studied. Forty-eight 90-day-old male rats were randomly assigned to four groups (two control groups, one trained group, and one trained and detrained group). Trained rats were subjected to 8 weeks of moderate running in motor-driven wheels; detraining was effected by discontinuing the running program for 8 weeks. Rats were provided a standard diet and water ad libitum. Trained rats displayed lower body weights and resting bradycardia. Training lowered (P<0.01) serum cholesterol, serum triglyceride, and adipose triglyceride levels but had no effect on these lipids in heart or skeletal muscle. These lowered lipid levels persisted after 8 weeks of detraining even though body weight was regained rapidly. Total nitrogen content was higher (P<0.01) in myocardial and triceps muscles of both trained and trained-detrained rats. Heart cholesterol and triglyceride levels were unchanged by detraining, but skeletal muscle cholesterol content was lowered. The results indicate that the changes in lipid content associated with training and detraining are tissue specific: reduction occurred in some tissues (serum and adipose tissue), although the lipid content of others (skeletal and heart muscle) was independent of training status, body weight, or circulating lipid levels.

KEY WORDS
skeletal muscle  exercise  adipose tissue  lipids  heart

Numerous studies indicate that physical activity lowers serum lipid concentrations in man (1-3) and experimental animals (4-13). In the limited number of studies relating changes in tissue lipid content with physical activity, variable results have been reported for liver and skeletal muscle. The effects of detraining on tissue lipid levels have been reported by only one investigator (2).

This experiment was undertaken to determine the effects of a standardized exercise training program followed by a period of detraining on the content of lipid fractions in the blood and various other tissues of albino rats. Total nitrogen was also determined for myocardial and skeletal muscle in an attempt to associate lipid changes with protein changes. All analytical data for tissues except blood were expressed on a dry-weight basis.

Methods
Sixty-two 90-day-old male rats of the Sprague-Dawley strain were preliminarily tested in motor-driven exercise wheels (modification of Waham LC-34 activity cage) to determine their willingness and ability to run. These tests consisted of four exercise sessions, each lasting 5 minutes, at 0.53 mph. Forty-eight rats were randomly
selected to establish four groups (N = 12 per group). Group 1 rats were nontrained 8-week controls, and group 2 rats were trained for 8 weeks. Group 3 rats were nontrained 16-week controls, and group 4 rats were trained for 8 weeks and detrained for 8 weeks. The subject pool for groups 2 and 4 consisted of all "runners" (N = 21) and the "nonrunners" (N = 17). Rats from the same group were caged (8 \times 11 \times 8 inches) in pairs in light-regulated (12 hours of light, 12 hours of dark) and temperature-regulated (23 \pm 2°C) quarters. Cage position was randomized. All rats were fed a standard diet (Rockland mouse/rat diet containing 56.2% carbohydrate, 24.3% protein, and 4.2% fat) and water ad libitum throughout the study.

Rats of groups 2 and 4 were run each day for 8 weeks, and those of groups 1 and 3 were retained in their home cages. Running wheel speed was 0.58 mph. The duration of exercise was progressively increased from 20 to 90 minutes by the following approximate schedule: a 10-minute increase after each of the first 4 weeks with a 30-minute increase after the fifth week so that duration was 90 minutes during the final 3 weeks. The total distance run by each rat in trained groups was approximately 33 miles. The rats in group 4 were then detrained by discontinuing their running sessions and returning them to a life of cage inactivity for 8 weeks. Training effectiveness was verified through measurements of resting heart rates by a procedure similar to that used by Tipton (14).

All rats of each group (N = 12) were fasted for 12 hours and then killed by guillotine. Blood samples were drained into 17 \times 100-mm Falcon tubes, and the serum was separated and stored at 4°C. The heart, right triceps, and a sample from the epididymal fat pad were quickly removed, freed of extraneous adipose and connective tissue, and the serum was separated and stored at -20°C. Serum and tissue samples were analyzed for cholesterol by the method of Abell et al. (15). Tissue extracts were prepared as follows. A sample of tissue (0.05-0.2 g) was placed in a 50-ml centrifuge tube and extracted with petroleum ether thoroughly mixed. Then 10 ml of petroleum ether was added, thoroughly mixed, and allowed to stand for 5 minutes until two distinct liquid layers separated. The supernatant petroleum ether layer was then transferred to a 50-ml Erlenmeyer flask. Two additional extractions with petroleum ether were made and added to the original extract, and the total extract was evaporated to dryness under reduced pressure in a 60°C water bath. To dissolve the residue, 10 ml of an acetone-alcohol mixture (1:1) was added. Two separate 4-ml samples of the extract were pipetted into separate 25-ml volumetric flasks and evaporated to dryness under reduced pressure in a 60°C water bath. The flasks were stoppered and stored at room temperature for later analyses.

Triglyceride determinations for serum and tissue samples were done by the method of Block and Jarrett (16). Tissue extracts were prepared as follows. A sample of tissue (0.05-0.20 g) was placed in a 50-ml centrifuge tube and extracted for total lipid by the procedure of Folch et al. (17). Extraction of the tissue was repeated with 10 ml and then with 5 ml of extractant to ensure complete removal of all lipid. The extract was pooled and evaporated to dryness under reduced pressure in an atmosphere of nitrogen. The residue was dissolved in 10 ml of isopropanol and analyzed for triglycerides. Total nitrogen was determined by the standard micro-Kjeldahl procedure (18).

Means and standard errors were calculated for the data on all variables measured, and comparisons between groups were made using two-way analysis of variance as described by Snedecor (19). Comparisons within groups for body weights and resting heart rates across time were made using a paired t-test according to Dixon and Massey (20). Significant differences were determined for the 0.05 and 0.01 levels.

**Results**

**Body Weights.—**Following 8 weeks of training, the mean body weight of group 2 rats was 91.3 g less (P < 0.01) than that of the nontrained 8-week control rats (group 1). At the same 8-week period in the experiment, the mean body weight of the group 4 rats was 36.4 g lower (P < 0.01) than that of the nontrained 16-week control rats (group 3). The rats in group 4 gained weight rapidly during the 8-week period of detraining, and their final weight at death equaled that of the nontrained 16-week control rats (group 3) (Table 1).

**Resting Heart Rates.—**After 8 weeks of exercise training, the mean resting heart rates of rats in groups 2 and 4 were 44 and 33 beats/min slower (P < 0.01), respectively, than the corresponding mean rates of nontrained control rats in groups 1 and 3. After 8
weeks of detraining, the mean resting heart rate of group 4 rats was elevated (P < 0.01) so that there was no significant difference between the mean rates of the nontrained control rats in group 3 and the trained-detrained rats in group 4 at 16 weeks (Table 2).

Serum Cholesterol and Triglyceride.—Serum cholesterol and triglyceride levels were significantly lower (P < 0.01) in group 2 rats after 8 weeks of training than they were in the nontrained control rats of group 1. Following 8 weeks of detraining, the serum cholesterol and triglyceride levels of group 4 rats remained significantly lower (P < 0.05) than those in the comparable nontrained control rats of group 3 (Table 3).

Consideration of changes in mean levels of both serum lipids and body weight (Tables 1 and 3) indicated that higher lipid levels were associated with heavier rats in groups 1, 2, and 3. A departure from this trend was observed in the detrained rats (group 4), since mean serum cholesterol and triglyceride levels remained lowered even though mean body weight increased greatly.

Tissue Cholesterol.—There was a decrease (P < 0.05) in the cholesterol content of the triceps of the trained-detrained group 4 rats compared with that of the control group 3 rats. No significant differences were found for any of the other tissues regardless of experimental group (Table 3).

Tissue Triglyceride.—The levels of triglyceride in epididymal fat of the trained groups (2 and 4) were lower (P < 0.05) than the levels in the corresponding nontrained control groups (1 and 3). There were no significant differences in heart and triceps triglyceride levels in any of the other experimental or control groups (Table 3).

Heart and Triceps Nitrogen.—After training, the total nitrogen levels in heart and triceps muscles were greater (P < 0.01) in group 2 rats than they were in the sedentary control rats of group 1. Following 8 weeks of detraining, the total nitrogen levels of these muscles were also greater (P < 0.01) in group 4 rats than they were in the sedentary control rats of group 3 (Table 3).

Ratios of Cholesterol to Nitrogen and of Triglyceride to Nitrogen.—The triceps muscles of the detrained group 4 rats displayed a significant decrease (P < 0.05) compared with

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**TABLE 1**

<table>
<thead>
<tr>
<th>Time (wk)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>455.8 ± 4.2</td>
<td>445.1 ± 5.6</td>
<td>450.5 ± 11.3</td>
<td>447.0 ± 9.8</td>
</tr>
<tr>
<td>8</td>
<td>549.7 ± 10.5*</td>
<td>458.4 ± 10.9†</td>
<td>523.8 ± 20.9*</td>
<td>487.4 ± 6.4†</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>567.2 ± 20.5</td>
<td></td>
<td>586.2 ± 11.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. N = 12 for all groups.
*Significant difference (P < 0.01) within groups.
†Significant difference (P < 0.01) between groups.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Time (wk)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>325.0 ± 5.9</td>
<td>333.0 ± 3.6</td>
<td>340.0 ± 3.9</td>
<td>335.0 ± 3.7</td>
</tr>
<tr>
<td>8</td>
<td>340.0 ± 6.7</td>
<td>296.0 ± 4.5†</td>
<td>339.0 ± 7.4</td>
<td>306.0 ± 4.2†</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>335.0 ± 6.1</td>
<td></td>
<td>331.0 ± 5.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. N = 12 for all groups.
*Significant difference (P < 0.01) between groups.
†Significant difference (P < 0.01) within groups.
TABLE 3

Cholesterol, Triglyceride, and Nitrogen in Serum and Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (mg/100 ml)</td>
<td>121.7 ± 4.6</td>
<td>104.9 ± 3.1</td>
<td>125.4 ± 9.3</td>
<td>97.2 ± 4.6†</td>
</tr>
<tr>
<td>Heart (mg/g)</td>
<td>5.6 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>5.8 ± 0.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Triceps (mg/g)</td>
<td>2.5 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.2 ± 0.04</td>
<td>2.1 ± 0.1†</td>
</tr>
<tr>
<td>Epididymal fat (mg/g)</td>
<td>0.7 ± 0.03</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.03</td>
</tr>
</tbody>
</table>

Triglyceride

| Serum (mg/100 ml)       | 105.6 ± 13.3    | 75.2 ± 3.9*     | 117.3 ± 13.9    | 83.8 ± 8.3†     |
| Heart (mg/g)            | 10.1 ± 1.5      | 9.3 ± 1.0       | 9.0 ± 2.6       | 5.7 ± 0.9       |
| Triceps (mg/g)          | 7.8 ± 0.9       | 6.5 ± 0.8       | 12.6 ± 1.7      | 9.9 ± 1.8       |
| Epididymal fat (mg/g)   | 979.4 ± 3.0     | 968.1 ± 3.9†    | 976.5 ± 3.6     | 965.9 ± 3.5      |

Total Nitrogen

| Heart (mg/g)            | 131.8 ± 0.8     | 136.2 ± 0.5*    | 128.1 ± 0.5     | 130.8 ± 0.5*    |
| Triceps (mg/g)          | 132.7 ± 0.6     | 139.7 ± 0.6*    | 129.1 ± 0.2     | 132.7 ± 0.7*    |

Values are means ± se. N = 12 for all groups, and mg/g is expressed as dry weight.
*Significant difference (P < 0.01) between groups.
†Significant difference (P < 0.05) between groups.

Discussion

The lower body weights observed for trained rats occurred with ad libitum feeding, suggesting that energy expenditure equaled or exceeded energy intake. These results support the findings of other rat exercise experiments employing ad libitum feeding (4, 21) as well as those using equalized food consumption (22, 23). Hanson et al. (21) suggested that such lower body weights reflect a decreased food intake due to appetite depression as a result of exercise. In support, Simko et al. (24) showed depressed food intake and feces excretion in rats following 86 days of swim training.

The observation that rats in groups 2 and 4, after 8 weeks of forced running, displayed a significant resting bradycardia agrees with reports (14, 23) on rats similarly trained in running wheels. The bradycardia evident in group 4 was not present after 8 additional weeks of detraining. These data indicate that the training program, though of moderate

TABLE 4

Ratios of Lipids to Nitrogen in Tissues of Experimental Rats

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol to total N,</td>
<td>0.0425 ± 0.0005</td>
<td>0.0421 ± 0.0006</td>
<td>0.0461 ± 0.0007</td>
<td>0.0445 ± 0.0007</td>
</tr>
<tr>
<td>Heart</td>
<td>0.0191 ± 0.0006</td>
<td>0.0186 ± 0.0004</td>
<td>0.0174 ± 0.0003</td>
<td>0.0159 ± 0.0004*</td>
</tr>
<tr>
<td>Triglyceride to total N,</td>
<td>0.0770 ± 0.0112</td>
<td>0.0680 ± 0.0071</td>
<td>0.0710 ± 0.0195</td>
<td>0.0440 ± 0.0065</td>
</tr>
<tr>
<td>Heart</td>
<td>0.0680 ± 0.0071</td>
<td>0.06470 ± 0.0038</td>
<td>0.0680 ± 0.0126</td>
<td>0.0759 ± 0.0126</td>
</tr>
</tbody>
</table>

Values are means ± se. N = 12 for all groups.
*Significant difference (P < 0.05) between groups.

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intensity, effectively produced a training adaptation that was subsequently lost during detraining.

Various studies indicate that plasma cholesterol levels, measured at rest in nontrained rats, increase with age. Approximate values of 25 mg/100 ml (4), 62 and 84 mg/100 ml (11, 24), and 249 mg/100 ml (7) have been published for young, middle-aged, and old rats, respectively.

Our initial values of 121 mg/100 ml are for 170-day-old rats. The lowered serum cholesterol levels measured after training support some (5-7, 25), but not all (24, 26), previous reports. A new finding in this study indicates that training may provide some protection against age-related serum cholesterol elevation. Although this protection may not be permanent, serum cholesterol levels of trained rats did remain lowered for as long as 8 weeks. Since the trained rats gained weight rapidly during this 8-week detraining period, these findings conflict with the viewpoint that increased serum cholesterol levels are related to body weight and larger fat stores. In fact, the retention of lowered blood cholesterol levels by the detrained rats may relate to their having less body fat after training, resulting in a diversion of acetyl coenzyme A from cholesterol to fat synthesis during the detraining period. Other investigators have suggested that an increased rate of cholesterol oxidation by muscle cells during exercise (27) or a greater excretion of fecal sterols (28) may explain the lower blood cholesterol levels of trained animals killed at rest.

Training did not alter the cholesterol concentration (mg/g dry tissue) in skeletal muscle, myocardium, or adipose tissue in this study. Detraining also had little effect, since only the cholesterol content of the triceps was lower. Comparison of our tissue cholesterol values with those of other workers is difficult because of the variety of units used to express both dry- and wet-weight concentrations. Allowing some error in conversion, our triceps muscle cholesterol level of 2.5 mg/g is nearly twice that reported by Fröberg (11) for white (1.1 mg/g) and red (1.4 mg/g) samples of rat gastrocnemius muscles excised at rest. Acute exercise (1-3 hours of running) did not alter these latter cholesterol values. Morgan et al. (13), using human muscle biopsy techniques, reported that training lowers the cholesterol content of vastus lateralis muscle. Their values ranged from 2.8 to 5.3 mg/g in untrained subjects and from 2.4 to 3.5 mg/g in trained subjects. Data on changes in the concentration of cholesterol in adipose and heart tissue with training are limited. Our data indicate that epididymal fat contains about 0.7 mg cholesterol/g tissue and that heart contains 5.6 mg/g. Our values for heart are twice those reported for younger rats by Fröberg (11), who also reported that acute exercise does not alter heart cholesterol levels. Conversion of other data (7, 11, 29) indicates that liver cholesterol levels are 3.5-5.5 times greater than our levels for skeletal muscle. The degree to which the above differences are related to differences in species, techniques, subject age, or muscle fiber composition remains to be shown.

The effect of training and detraining on serum triglyceride levels was essentially identical to that seen for cholesterol, i.e., serum triglyceride was significantly reduced with training and remained low with detraining. Reported plasma triglyceride values range from 5-7 mg/100 ml for middle-aged rats (11) to 22-43 mg/100 ml for young rats (4) and 38 mg/100 ml for old rats (7) at rest, with values ranging from 2 to 26 mg/100 ml after acute exercise (4) or training (7). Our plasma triglyceride values of 106 and 57 mg/100 ml for control and trained rats, respectively, are considerably higher than the previously reported values, probably because of variations in techniques and conditions before death. Carlson and Fröberg (7) reported lower triglyceride levels in heart and red skeletal muscle samples from rats after acute exercise. The data of Morgan et al. (13) indicate a lowering of muscle triglyceride levels with training, but their data are questionable due to their large variability. Our data indicate that on a mg/g dry weight basis, the triglyceride concentrations in cardiac and
skeletal muscles are unchanged with training. This finding is apparent even when concentrations are compared on a mg/mg basis through the calculation of ratios of lipid to total nitrogen (Table 4). Descriptive comparisons of group mean ratios indicate a pattern of consistently smaller ratios for trained and detrained groups, which undoubtedly reflect essentially unchanged lipid levels accompanied by large increases in nitrogen. The significant increases in total nitrogen are most likely due to an increased number of myofibrillar or sarcoplasmic proteins, as shown by others (30, 31) for trained rats.

In contrast to muscle tissue, the epididymal fat from both trained and detrained rats displayed significantly lower triglyceride levels. These lower triglyceride values and those measured for serum of trained rats are probably due to an increased mobilization and utilization of fatty acids to meet increased energy expenditures during training. Exercise has been shown to increase lipolysis, resulting in a greater release of free fatty acids from adipose stores (32). Simko and Chorvathova (33) have reported that trained rats may have a lower level of free fatty acids in their plasma at rest and a lower, less prolonged elevation during subsequent exercise. Increases in fatty acid efflux from plasma during exercise can be related to an increased uptake and utilization by working muscles (34, 35). The work of Carlson (32) suggests that an increased perfusion of muscle tissue during exercise may be the immediate cause of the increased efflux of free fatty acids from plasma. Increases in the amount or the activity of lipoprotein lipase may also account for a portion of the increased efflux of plasma fatty acids to peripheral tissue. This enzyme is thought to regulate the peripheral uptake of fatty acids (36) and has been reported to increase with exercise in the myocardial tissues of rats (37).

This study indicates that alterations in tissue triglyceride and cholesterol levels with training and detraining are tissue and lipid specific. Although reductions occurred in some tissues (serum and adipose tissue), the levels in others (skeletal and heart muscle) remained unchanged with training. It appears, therefore, that muscle lipid levels may be independent of training status, body weight, or circulating lipid levels.

References


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