

# Metabolic Adaptation With Physical Training: <sup>14</sup>C-Acetate Incorporation Into Tissue Lipids

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Forty-eight rats were fed ad libitum, fasted 24 hr, rested 48 hr, and injected i.p. with 40  $\mu$ Ci of <sup>14</sup>C-acetate/100 g body weight. Twenty-four rats had followed a progressive physical training program for 12 wk and 24 rats acted as their controls. Following this injection, the rats were sequentially sacrificed at 5-, 10-, 15-, and 20-min intervals and total cholesterol (TC), free cholesterol (FC), and triglyceride (TG) specific activity and concentrations were measured from serum, liver, triceps, and heart tissue. Curves relating specific activity to the time point data were fitted by the method of least squares. Comparison of these curves revealed that serum, liver, and triceps TC and FC specific activity

were significantly higher in the trained rats. In contrast, corresponding TC and FC concentrations for these three tissues varied. Liver TC level was significantly less for the trained group, probably due to a reduction in the esterified moiety, since liver FC measures were unchanged. Training resulted in significantly lower TC concentrations in the selected tissues studied even though specific activity curves appeared similar for both groups. Our conclusions are that lipid metabolic adaptation; studied in vivo, occurs in tissues with training, but that these adaptations are not uniform across tissues, lipid moieties, or measurement parameters.

**P**REVIOUS STUDIES on the effectiveness of physical training in altering lipid metabolism in experimental animals have primarily been concerned with changes in serum concentration or, to a lesser extent, with alterations in tissue lipid levels. Regardless of the tissue studied equivocal results have been obtained. For example, there have been reports of decreases in serum lipid concentrations<sup>1-3</sup> as well as no differences<sup>4-6</sup> following training. In a limited number of training studies measuring tissue lipid concentration changes conflicting results have also been reported for the liver,<sup>6,7</sup> for skeletal muscle,<sup>3,4,8</sup> and heart muscle.<sup>3,9</sup> These discrepancies in lipid concentrations make it difficult to discern whether true adaptations in lipid metabolism occur with training. Furthermore, if adaptations in lipid metabolism do occur with training, determinations of changes in lipid concentrations alone do not give an indication of the underlying mechanisms.

Since lipid concentrations may reflect the combined effects of both biosynthesis and degradation it seems reasonable that assessment of at least two of these three important parameters must be made if tissue adaptations to training are to be identified and better understood. In this study the hypothesis that physical training causes no adaptations in functionally different tissues as

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reflected by alterations in measurements of lipid metabolism was tested. This was accomplished by comparing  $^{14}\text{C}$ -acetate incorporation into total cholesterol (TC), free cholesterol (FC), and triglycerides (TG) within selected tissues from trained and control animals, and by subsequently relating these estimates to measures of lipid concentrations in these tissues.

### MATERIALS AND METHODS

Forty-eight 60-day-old Sprague-Dawley rats, selected for their willingness to run, were randomly assigned to two groups designated as physically trained or controls. Throughout the 12-wk experimental period the animals were housed in an environmentally controlled room (22°C, 12 hr light, 65% relative humidity) and were provided water and commercial chow ad libitum. Animals were weighed weekly to determine body weight changes.

Animals in the trained group were subjected to physical training that consisted of running on motor-driven wheels for durations progressively increased from 20 to 90 min/session, at a speed of 0.93 kph (0.58 mph), 6 days/wk for a period of 12 wk. Control animals were handled in the same manner except that they were not run. Prior to training and 24 hr following the last regular training session, resting heart rates were determined according to the method of Tipton<sup>10</sup> to verify training effectiveness. Animals were then subjected to maintenance training before being sacrificed over a 3-day period.

Forty-eight hours after the last maintenance training session, animals were fasted 24 hr and administered intraperitoneally 40  $\mu\text{Ci}$  of  $^{14}\text{C}$ -acetate/100 g body weight.<sup>7,11</sup> Animals were randomly assigned to four subgroups to allow for standardized radioisotope injection and sacrifice procedures. Following the injections, animals in each subgroup were decapitated at 5-, 10-, 15-, or 20-min intervals. Blood was drained into small polypropylene tubes, allowed to clot, and centrifuged, and the separated serum was stored at  $-4^\circ\text{C}$ . The heart, liver, and tripeps were quickly removed, freed of blood clots and fascia, rinsed with distilled water, blotted, frozen, dried by lyophilization, and ground to a fine mixture.

TC, FC, and TG concentrations were determined for serum and the dried tissues.<sup>12,13</sup>  $^{14}\text{C}$ -acetate incorporation into TC and FC was determined on the isolated digitonide precipitate. TG were separated from other lipid fractions by thin-layer chromatography (TLC) on Polygram Silica Gel G sheets (Brinkman Instruments, Westbury, N.Y.) utilizing two modified solvent systems.<sup>14,15</sup> The first solvent mixture contained petroleum ether, 2-butanone, 2-pentanone, and glacial acetic acid (170:15:15:3). The second mixture contained petroleum ether, ether, and glacial acetic acid (90:10:1). The TLC plates were dried and sprayed with a solution of Bromphenol blue (40 mg Bromphenol blue/100 ml in 0.01 *N* NaOH) to determine the location of the TG. This area was scraped from the TLC plate and radioactivity was determined by the method of Shaw and Harlan.<sup>16</sup> All radioactivity determinations were corrected for quenching with appropriate internal standards.

Significant differences in lipid concentrations for the various tissues studied were determined using a one-way analysis of variance. Differences in incorporation of  $^{14}\text{C}$ -acetate into lipids of serum and tissues were determined through a one-way analysis of covariance to adjust for the concomitant variate, time. Prior to this analysis specific activity values were transformed to square-root units. Since radioactivity counts tend to follow a Poisson distribution, these transformations tend to make the variances more independent of the mean and more homogenous. Specific activity values were therefore defined as the square root of disintegrations/min/ $\mu\text{Ci}$  injected/100 ml serum or gram of dry tissue analyzed ( $\sqrt{\text{dpm}/\mu\text{Ci}/100 \text{ ml}}$  or  $\sqrt{\text{dpm}/\mu\text{Ci}/\text{g}}$ ). In investigating differences in specific activity for the four time periods, polynomial curves were fitted to each set of data separately by least squares. The curves for control and trained groups were tested for differences at the 5% level (using a multivariate test on all the parameters). If the curves were not significantly different, the data from both groups were pooled and a combined curve fitted and reported. If the curves were significantly different, the separately best fitting curves were reported.

A pair *t* test was used to determine significant differences in resting heart rates and body weights. A level of  $\alpha = 0.05$  was used to indicate statistical significance throughout the study.

## RESULTS

Weekly mean body weights for control and trained animals indicated that weight gain was suppressed in the trained group throughout the study. This suppression was apparent after 2 wk of physical training with greatest retardation occurring during periods of adjustment to increases in work duration. The rate of weight gain for the control group was linear throughout the study with body weights finally plateauing 3 wk prior to sacrifice. After 12 wk of training, group mean body weights were significantly different at 547 and 435 g for control and trained groups, respectively.

Prior to training there was no significant difference between group mean resting heart rates. After training, a significantly lower mean resting heart rate was found for the trained group, with this value also being significantly lower than their initial mean rate. These results indicated that the progressive training program was effective in producing resting bradycardia in the trained group (mean reduction of 23 beats/min), an adaptation commonly associated with an improved level of cardiovascular function.

Table 1 contains data describing the effects of physical training on the concentrations of TC, FC, esterified cholesterol (EC), and TG in the tissues studied. EC was calculated as the difference between the analytically determined TC and FC values. Physical training resulted in significantly lower TG concentrations in all the tissues studied but had variable effects on cholesterol concentrations in these tissues. For example serum cholesterol levels were not significantly

**Table 1. Total, Free, and Esterified Cholesterol and Triglyceride Concentrations (Mean  $\pm$  SEM) in Serum, Liver, Triceps, and Heart Tissues of Control and Trained Rats**

	Control Group	Trained Group
Serum*		
Total cholesterol	67.3 $\pm$ 1.6	70.9 $\pm$ 2.4
Free cholesterol	10.7 $\pm$ 1.8	12.8 $\pm$ 1.3
Esterified cholesterol	57.1 $\pm$ 2.0	58.1 $\pm$ 1.9
Triglycerides	42.8 $\pm$ 3.4	33.3 $\pm$ 1.9†
Liver‡		
Total cholesterol	10.3 $\pm$ 0.3	9.2 $\pm$ 0.3†
Free cholesterol	7.0 $\pm$ 0.2	7.1 $\pm$ 0.2
Esterified cholesterol	3.4 $\pm$ 0.2	2.1 $\pm$ 0.1†
Triglycerides	15.0 $\pm$ 1.4	11.0 $\pm$ 0.8†
Triceps‡		
Total cholesterol	3.0 $\pm$ 0.1	3.1 $\pm$ 0.1
Free cholesterol	2.5 $\pm$ 0.1	2.8 $\pm$ 0.1†
Esterified cholesterol	0.5 $\pm$ 0.03	0.5 $\pm$ 0.04
Triglycerides	31.9 $\pm$ 2.3	23.5 $\pm$ 2.6†
Heart‡		
Total cholesterol	6.1 $\pm$ 0.1	6.5 $\pm$ 0.1 †
Free cholesterol	4.6 $\pm$ 0.1	4.8 $\pm$ 0.2
Esterified cholesterol	1.5 $\pm$ 0.1	1.7 $\pm$ 0.2
Triglycerides	6.6 $\pm$ 0.7	4.5 $\pm$ 0.5†

\*Concentrations in serum are expressed as mg/100 ml.

†Significance difference ( $p < 0.05$ ) between groups.

‡Concentrations in these tissues are expressed as mg/g dry tissue weight.

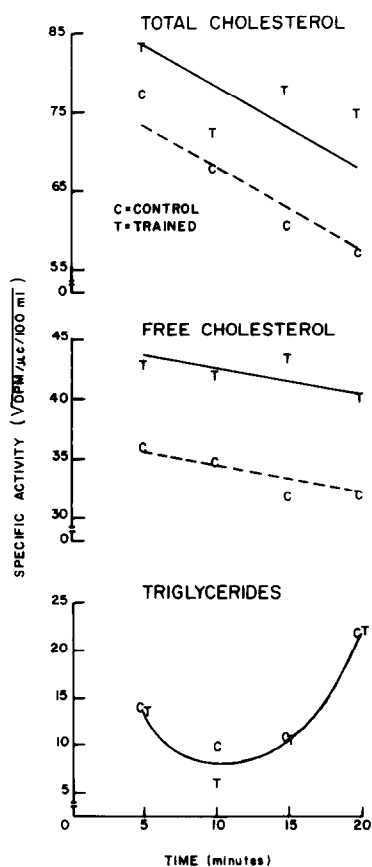


Fig. 1. Mean specific activity of serum lipids of control (C,  $N = 6$ ) and trained (T,  $N = 6$ ) rats in specified times after  $^{14}\text{C}$ -acetate injection.

different in the two groups, while significantly lower values for liver TC and EC were found in trained animals. In contrast to these lowered concentrations for liver, the triceps and heart muscle showed significantly higher values for FC and TC, respectively.

Figs. 1-4 present curves of specific activity of  $^{14}\text{C}$ -acetate incorporation into TC, FC, and TG of serum, liver, triceps, and heart tissues. It should be noted that the curves were fitted to all the data, not merely to the means. Thus, for example, even though the means for the trained group for liver TC appeared visually to suggest a quadratic curve, the quadratic term was not significantly different from zero ( $p > 0.05$ ) when the curve was fitted. In addition to the estimated curves, the subgroup means ( $N = 6$ ) were plotted at each time point, using designations of C for control and T for trained animals.

These  $^{14}\text{C}$ -acetate incorporation results indicate that training caused alterations which were specific both to lipids and to tissues. For example, a consistent pattern of greater incorporation into TC and FC was found for serum, liver, and triceps tissues from trained animals, but not for hearts from these same animals. In contrast, similar TG incorporation curves were found for all tissues except heart where separate curves were plotted, mainly because of the exceedingly high specific activity level at the 5-min point.

Except for liver FC, peak incorporation of acetate into various cholesterol

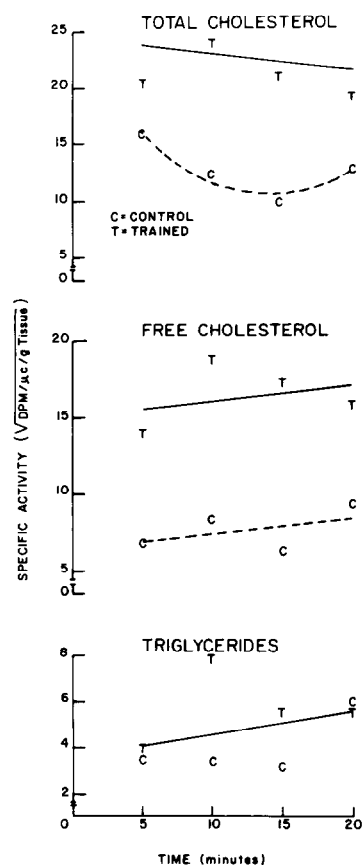


Fig. 2. Mean specific activity of liver lipids of control (C,  $N = 6$ ) and trained (T,  $N = 6$ ) rats at specified times after  $^{14}\text{C}$ -acetate injection.

forms in all tissues studied occurred at 5 min and then displayed a significant decline ( $p < 0.05$ ) with time for both groups. The TG incorporation curves were different from the cholesterol plots, since peak values occurred either at the 20-min time point or remained fairly constant with time. The only exception was that of heart TG specific activity for trained animals as mentioned above.

#### DISCUSSION

The results of this experiment demonstrate that moderate-intensity physical training causes lipid metabolic adaptations in selected tissues, apparent only through a combined quantitation of both concentrations and incorporations for the same lipid moieties. Furthermore, these adaptations reflected the influence of training, rather than the effects of acute exercise or dietary modification, since injection of  $^{14}\text{C}$ -acetate was made into rested and fasted animals. In addition, the observation that acetate is more readily available as a precursor to cholesterol than to TG verified the finding of Dupont.<sup>17</sup> Other important indicators of lipid metabolic changes found to accompany training were the consistently higher level of specific activity for TC and FC which persisted over the time intervals studied for serum, liver, and skeletal muscle tissues. Another demonstration of training effects was the substantial decrease in TG concentra-

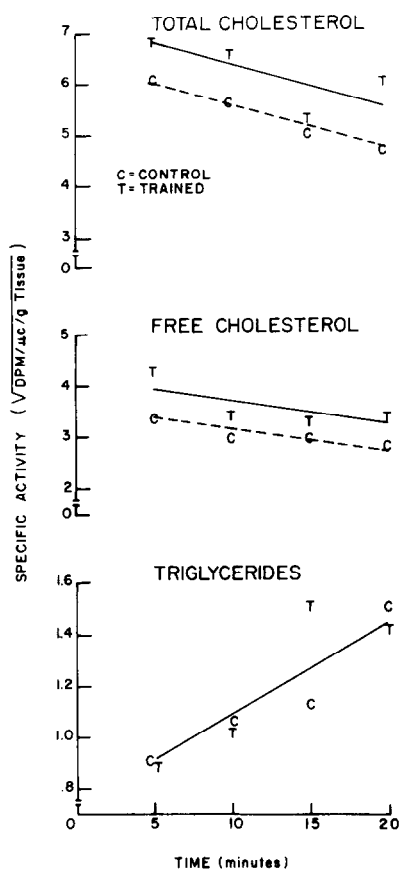


Fig. 3. Mean specific activity of triceps lipids of control (C, N = 6) and trained (T, N = 6) rats at specified times after  $^{14}\text{C}$ -acetate injection.

tions in all tissues studied. In addition to lipid changes, other established adaptations to physical training were apparent, such as resting bradycardia<sup>3,10,18</sup> and lowered body weights while on an ad libitum diet.<sup>2,3,19</sup>

Although tissue lipid changes were frequently observed, all tissues did not display similar adaptations to training. Evidence for this nonuniformity in adaptation to training was apparent when contrasts of liver and extrahepatic cholesterol concentrations were made. In this study, the level of liver TC was significantly reduced by training, a finding documented by others,<sup>5,20,21</sup> and this reduction in liver TC appeared to be due to reduction in the esterified moiety since liver FC measures were unchanged. Such reductions were not found for extrahepatic tissues. The serum results were consistent with earlier studies,<sup>2,5,6,22</sup> showing that training does not significantly reduce serum TC levels in the rat, while the greater concentration of FC found in skeletal muscles of trained animals confirms the report of Palladin.<sup>23</sup> The results however, that heart tissue from trained animals contained elevated TC levels is inconsistent with the findings of Watt et al.<sup>3</sup> and Froberg,<sup>4,24</sup> who reported unaltered myocardial cholesterol concentrations following either physical training or acute exercise. The explanation for these different findings is unknown but may relate to differences in training intensity or resting status prior to sacrifice.

A dissimilar response of hepatic tissue was also apparent when measures of

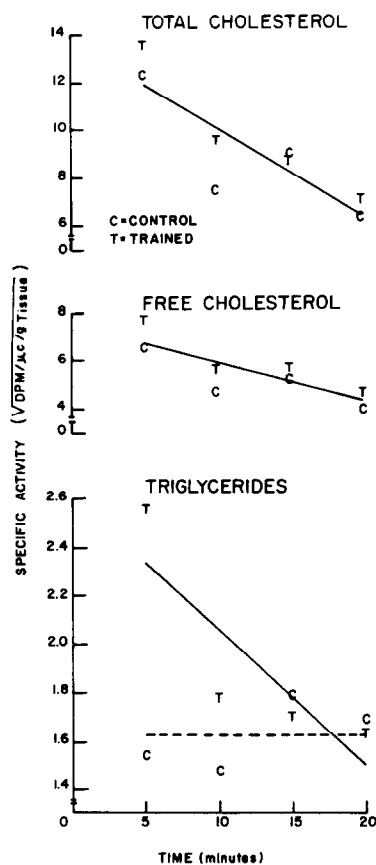


Fig. 4. Mean specific activity of heart lipids of control (C, N = 6) and trained (T, N = 6) rats at specified times after  $^{14}\text{C}$ -acetate injection.

concentration and specific activity were compared. For example, liver tissue from trained animals was found to have lower TC levels in the presence of a greater incorporation of  $^{14}\text{C}$ -acetate into TC. This suggests that the lower hepatic TC concentration found with training is not due to suppressed hepatic cholesterogenesis, but involves some other cholesterol-lowering mechanism. These findings confirm reports of other investigators<sup>7,11</sup> and are compatible with evidence that training increases conversion of cholesterol to bile acids<sup>21,25</sup> and increases fecal steroid excretion.<sup>5,21,26</sup> In contrast, concentrations of liver FC were the same for both groups, even though measures of incorporation were greater for trained animals. This finding suggests that a more rapid liver degradation or removal of FC also occurs with training. Taken together, these results would indicate a more rapid turnover of all liver cholesterol moieties in trained animals, but with predominant turnover in the CE fraction.

While the evidence for training adaptations is apparent for liver cholesterol metabolism, lipid metabolism of serum and skeletal and heart muscle tissues merit special comment. It is impossible to postulate from our data to what extent biosynthesis took place in muscle tissue or to what extent specific activity was due to free exchange with surrounding serum which had equilibrated with synthesized cholesterol from the liver. However, our results showing that with training there are increased quantities of heart TC with no increased specific

activity suggests that training alters cholesterol efflux but has little effect on influx. In comparison, the elevated levels of skeletal muscle FC in the presence of higher specific activity suggests that skeletal muscle adapts differently to training than does heart muscle. Furthermore, the finding of greater triceps TC specific activity in the presence of no changes in triceps TC levels for trained animals suggests enhanced influx and efflux for skeletal muscle in trained as compared to control animals. This finding is of special interest since it suggests that training promotes cholesterol metabolism in skeletal muscle.

The responses of selected tissues to training are especially apparent in the TG results. If only concentration data are considered, the interpretation might be that metabolic adaptations to training are rather general, since all tissues displayed similar reductions. Conversely, if only TG specific activity measures are considered, all of the tissues studied might be judged resistant to adaptive changes. The major findings that lower TG levels existed in various tissues of trained animals at rest without direct evidence of differences in precursor incorporation suggests enhanced metabolic utilization. In contrast to our findings of unchanged  $^{14}\text{C}$ -acetate incorporation into TG of rat skeletal muscles, *in vitro* results indicate that human muscle has an increased ability to incorporate palmitate into intracellular TG<sup>27</sup> and that homogenates of rat muscle have a greater capacity to esterify glycerol-3-phosphate<sup>28</sup> following training. These discrepancies are difficult to explain, but might be due to differences in methods, i.e., *in vivo* versus *in vitro*. Our results suggest that the endogenous hypotriglyceridemia seen with training cannot be attributed to a reduction in hepatic TG production, a finding consistent with the report of Askew et al.<sup>28</sup>

In conclusion, these data emphasize the need for specifically relating training-induced lipid metabolic adaptations to individual tissues rather than generalizing isolated findings across a variety of tissues, especially if the tissues have known different functional responsibilities. Furthermore, physical training resulted in significant reductions in TG levels of all tissue studied, but had variable influence on cholesterol concentrations.

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