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Effects of Exercise Detraining on Lipid Storage in Rats

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ABSTRACT

This study was done to determine if regular exercise followed by detraining would result in an elevation of lipids in serum and body tissue. The regular exercised (RE) rat group trained by swimming for 70 minutes four times per week for the entire 21 weeks of the exercise protocol. The detrained (DE) group trained with equal duration of exercise for 16 weeks, then did not swim for the remaining five weeks to detrain and the control (CO) group did not exercise. At the end of the study, no significant differences were found among the three groups for serum TC, HDL-C, and TG. However, significant differences were observed in body weight gained and in skin lipid. The trained group had the least amount of stored fat in the form of triglyceride (4.9%) and the control group had the most (10.2%). These findings suggest that cessation of training (detraining) reverses the effects of exercise on lipids stored in tissues.

Key words: exercise, detraining, lipids, rats

INTRODUCTION

Coronary heart disease (CHD) is the chief cause of death in the United States killing more Americans than all forms of cancer combined (1,2). The cause of CHD has been strongly correlated to an atherogenic life-style which can be characterized by a diet excessive in saturated fat, calories and salt, unrestrained weight gain, and a sedentary existence. The risk of developing CHD has been shown to be approximately twice as likely in sedentary people as those who were vigorously active (3). Unrestrained weight gain tends to exacerbate all these atherogenic factors predisposing one to blood vessel blockage (4). In the Framingham heart study, the rates of CHD after 14 years of follow-up were inversely related to physical activity in men but not women. This relation was independent of age, blood pressure, smoking, and cholesterol level (3). Results of numerous clinical investigations show that management of blood lipids and lipoproteins through regular, aerobic exercise significantly benefits cardiovascular health. For exam-

ple, Superko (5) showed that regular exercise decreases total cholesterol (TC) and triglyceride (TG) levels while increasing levels of high-density lipoprotein cholesterol (HDL-C) in humans. Additional potential benefits from physical activity involve helping to maintain body weight, acting as a substitute for smoking, improving glucose-insulin dynamics, and lessening depression, anxiety, and stress (6). Direct correlations with regular exercise and a lower risk of CHD have encouraged many Americans to begin an exercise regimen (7,8,9). However, after a period of time, many people quit exercising for a variety of reasons. This reduction in exercise following a period of regular exercise is known as detraining. Little is known about the effects of detraining on serum lipid levels and lipid storage. Therefore, the objective of this study was to evaluate the effects of exercise detraining in rats by measuring the following factors: body weights, serum TC, HDL-C, TG levels, and storage of lipids.

METHODS

Subjects

Male Fischer 344 rats, 5-6 week-old (86-115 gram), were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and all procedures were approved by the institutional animal care review board. Diagnostic kits were obtained from Sigma Chemical Company (St, Louis, MO) for the spectrophotometric determination of serum TC (#352), HDL-C (#352-3), and TG (#336). The TG procedure was also used for the determination of triglyceride extracted from skin flaps. All water used for spectrophotometric assays was de-ionized and then filtered through a Millipore water system.

All animals were housed, in pairs, at the animal care facility located in the Department of Biological Sciences, Illinois State University, Normal, IL. The room temperature was kept at a constant 23°C with a 12 hour light/dark cycle beginning at 700h. All the animals were fed a standard rat chow diet obtained from Harlan Teklad (Madison, WI) containing 4% fat, and water ad libitum.

Exercise Protocol

Rats were randomly assigned to one of three groups (eight rats/group of exercised rats and seven rats in control group). Swimming was the method of physical training utilized in this study. Rats swam as a group in 30-35 gallon waste cans filled 3/4 full with 35°C tap water. Water was drained after each swim and the buckets rinsed thoroughly. Animals were exercised beginning between 1030h and 1200h.

The regular exercise (RE) group trained by swimming for 70 minutes four times per week for the entire 21 weeks of the exercise protocol. The detrained group (DE) trained with equal duration of exercise as the regularly exercised group for the first 16 weeks. This group of rats then discontinued swimming (detrained) for the remaining five weeks of the study. The control group (CO) of rats did not swim during the experiment and were not exercised in any manner. The group was cage confined but handled four days per week in order to standardize any handling effect.

All exercising rats were conditioned to the water for four days before training began by increasing the time swimming in the water from 15, 30, 45, to 60 minutes per session. The rats were then swum 70 minutes per day on Mondays, Tuesdays, Thursdays, and

Fridays. At the completion of each swim, rats were towel dried and returned to their cages. Body weights were recorded for all rats at the beginning of each week before swimming. Animals were fasted overnight and sacrificed 24 hours after the final exercise session. Rats were anesthetized by diethyl ether inhalation. Rats were decapitated and a heart puncture was performed using a 5cc syringe and a 18 gauge needle into the beating heart to remove approximately 2 mL blood from each rat. Blood samples were dispersed into two 1.5 mL polypropylene tubes and allowed to clot for 30 minutes at ambient temperature. The clotted blood was then centrifuged, using a Beckman microcentrifuge, at 12,000 xg for five minutes in a 4°C cold room . The serum was removed and stored at -20°C. Serum samples were analyzed for TC, HDL-C, and TG using the Sigma diagnostic kits. Stomachs were removed, opened, washed with saline and visually inspected. No ulcerations were observed in any of the rats. Other organs (liver, kidneys, adrenal glands) were also removed, weighed and inspected. A skin flap (8-10 cm^2) (which contained fur, skin, muscle, and adipose) was taken from the abdominal area of each rat. The hair was removed by shaving with an electric razor and the flap stored at -20°C.

Lipid was extracted from these samples to evaluate the percent lipid. Prior to extraction, the tissue samples were lyophilized for 24 hours to remove water. Preliminary work indicated that removal of water increased the yield of lipid extracted by our procedure. The lipid extractions were performed by soxhlet extraction using a Kimax soxhlet 34/45 with matching condenser, round bottom flask, and petroleum ether (125mL, 60-80^o range) as solvent. The lyophilized tissue, cut into small pieces (approximately 0.5 cm^2) was placed into a Whatman cellulose extraction thimble (30mm x 80mm). The tissue was extracted for 24 hours with a recycle time of 15 minutes. The petroleum ether was removed by rotoevaporation, then by nitrogen gas until a constant weight of the lipid extract was obtained. In preliminary work, a second 24 hour soxhlet extraction of the tissue under the same conditions yielded less than 0.5 % additional lipid. Consequently, a single extraction of each tissue was deemed sufficient. Data for the extracted lipid are reported as gram lipid per 100 g dry tissue weight x 100 and is, therefore, referred to as percent.

The lipid extract was evaluated by thin layer chromatography (TLC) which indicated a major portion of the lipid extracted was in the form of triglycerides. Cholesterol and sterol ester were also identified (relative to authentic standards). The TLC plates were Redi-Plate™ Silica-Gel G TLC plates (20 cm^2) (Fisher Scientific Company, Pittsburgh, PA). The solvent system used for TLC was petroleum ether (60-80^o):diethyl ether: glacial acetic acid (80:20:1) (*v/v/v)*. Lipids were visualized as black spots following use of a 5% sulfuric acid in methanol spray and gentle heating of the TLC plate.

Statistical Analysis

Variance of weekly body weights, final body weights, and percent lipid extracted from lyophilized skin flaps were compared across groups using analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) post test. Differences were considered significant at $p < 0.05$. Means, standard deviations (SD) or standard errors of the mean (SEM) are reported.

RESULTS

Body weights for each group at week 1, 16, and 21 of the experiment were determined. Statistically all three groups of rats had the same body weight at the beginning of the experiment with the RE, DE, and CO groups averaging weights of 152 g ± 11, 146 g ± 9, and 154 g ± 12, respectively. After 16 weeks of training, before the detraining had begun, the mean body weight gain per group was 174 ± 13, 174 ± 18, and 204 ± 17 grams for the RE, DE, and CO respectively. The control group was statistically heavier than the RE and DE groups (Table 1). The percent weight gain per week was about 15 percent higher for the CO group relative to the other rats. Following a five week period of detraining the body weight gains for the groups (relative to week one) were determined to be 197 ± 13, 210 ± 22, and 236 ± 17 grams for the RE, DE, and CO groups, respectively. The CO group was significantly heavier (by about 17%) than the other two groups which did not differ significantly. The final body weights for each group were 349 ± 2, 356 ± 3 and 390 ± 3 g for the RE, DE, and CO groups, respectively. There were no significant weight differences between groups in any of the organ weights (data not shown).

Analysis of total serum lipid concentrations showed no significant cholesterol differences between groups with a mean ± S.D. for the RE, DE, and CO of 41 ± 1.3, 41 ± 3.5 and 41 ± 4.0 mg/dL, respectively. In addition, no significant differences were observed in serum levels of high density lipoprotein-cholesterol concentrations with mean ± S.D. for the RE, DE, and CO which were 28 ± 1.9, 26 ± 3.1, and 28 ± 2.7 mg/dL, respectively. Similarly, there were no differences in serum triglyceride concentrations between the three groups with the RE = 49 ± 11, the DE = 46 ± 10, and the CO = 45 ± 7.4 mg/dL (Table 1).

Table 1. Comparisons of Body Weights, Serum Lipids, and Skin Lipids After 21 Weeks

Parameter	Regular Exercise	Detrained	Control
Body Weight (g)	349 ± 2[a]	356 ± 3[a]	390 ± 3[b]
Serum Triglyceride (mg/dL)	49 ± 11	46 ± 10	45 ± 7.4
Serum Cholesterol (mg/dL)	41 ± 1.3	41 ± 1.3	41 ± 1.3
Percent Skin Triglyceride	4.9 ± 1.6[c]	6.4 ± 2.4[c]	10.2 ± 2.8[d]
Percent extracted Skin Lipid	18.1 ± 0.6[e]	23.9 ± 1.1[e]	29.2 ± 1.0[f]

Values with the same letters are not significantly different at $p < 0.5$

Levels of extracted lipid per gram of dry skin tissue (mean ± S.E.M.) showed the RE group had 18.1 ± 0.6, the DE group 23.9 ± 1.1 and the CO group 29.2 ± 1.0 percent. The CO group is statistically higher (about 30%) than both the RE and DE group which were not statistically different. Examination of the extracted lipid by TLC identified the major component as triglyceride (data not shown). The percent triglyceride extracted per dry skin flap for the RE was 4.9 ± 1.6, the DE was 6.4 ± 2.4, and the CO was 10.2 ± 2.8 which is almost doubled the values of the other two groups. Analysis yielded significant differences between the CO group and the DE or RE groups with no significant difference observed between the RE and DE groups. The percentage of water per skin weight was the same for all three groups (data not shown).

DISCUSSION

A significant training effect was observed on body weight as predicted and our results are similar to those of other studies (1,12). The exercised rats gained 15 % less weight than their sedentary controls on an ad libitum diet of water and standard rat chow. The only difference between the two groups of rats was in levels of physical activity. This significant effect indicates that the training regimen is sufficiently intense to lead to physiological effects although it is much less intense that other reports in the literature (15). The weight gain of 390 grams by the control group of rats at 196 days is equivalent to that shown by Yu, et al., (11) for rats under conditions of no physical exercise.

A detraining effect was observed on body weight. Before the detraining began, the exercised and detrained rat groups had gained weight equally on a week by week basis. The detrained rats gained, on average, 50 % more weight per week than the exercised rats during the first three weeks of detraining. The detrained rats gained weight at a rate which was approximately 20 % faster than the control rats during the first three weeks of detraining. This was unexpected. A possible explanation for this is that the detrained rats were consuming a high number of calories per day while exercising. When these rats were not forced to swim, they continued to consume a large caloric intake, but did not utilize the large number of calories. Consequently, the rats stored the excess calories as tissue fat (triglycerides and cholesterol esters). This suggests that quitting exercise leads to loss of the positive effects on weight control of exercise. This also suggests that the physiological mechanisms controlling weight, hunger and energy metabolism are processes that are slow to adjust.

These results are in agreement with those published by Arnold and Richard (12) which showed approximately a 50% increase in weight in detrained rats as compared to exercised rats after 27 days of inactivity. We suggest that the majority of increased weight gain in the detrained group is attributable to an increase of stored triglycerides in the adipose tissue. As expected, soxhlet extraction of the skin flaps showed that the control rats were storing more lipid (both triglyceride as well as total lipid) per gram of dry tissue weight than the detrained rats and exercised rats. We interpret these data as an indication of the difference in relative percent body fat between the three groups of rats. While not significantly different the RE group had less TG than the DE group demonstrating a trend to greater storage upon detraining.

The RE rats did not accumulate much stored fat in the form of triglyceride in the adipose tissue because of the rigorous training protocol. These data strongly suggest that with regular exercise there is less body weight gain as lipid as compared to the controls. Further, upon cessation of exercise an increase of stored body fat is observed. These findings suggest that detraining increases body weight in the form of stored tissue lipids. In the exercised group of rats, a considerable portion of the lipid extract appeared to be in the form of sterol ester (as estimated by the relative spot sizes of various lipids seen by thin layer chromatography, data not shown). A likely explanation for the observed differences in the type of stored fat is that the exercising rats are esterifying the cholesterol to a greater extent and thereby storing it in adipose and perhaps other tissues such as the

liver. Clearly more work on effects of exercise on the LCAT system as well as various esterases would be of interest.

Serum levels of triglyceride or cholesterol were not significantly different between groups. This is in contrast to the work by others. Simonelli and Eaton (16) reported a reduction in triglyceride secretion as a consequence of chronic exercise. Durstine et al. (17) measured serum lipids on the Zucker rats in response to an endurance running program. They reported a significant reduction (in obese but not lean rats) in serum triglycerides after an 18 week exercise protocol. However, they reported that serum cholesterol levels increased significantly ($p < 0.01$) in the trained obese relative to the sedentary obese rats. For their lean rats, the cholesterol levels did not change in the sedentary relative to the trained rats after 18 weeks.

Since body weight clearly increased in the control group relative to the RE group in our study, there appears to be a net flux of lipid from dietary calories to storage depots such as skin adipose. Upon cessation of the training, the body weight increases (DE group) seen are related to increased storage of lipids. As yet, the regulatory mechanisms which leads to this net storage are not well understood but may be related to changes in hormone sensitive lipase activity. Hormone sensitive lipase is important in the mobilization of triglycerides from adipose (13). Exercise may stimulate this activity while sedentary life style may inhibit this activity. In addition, our data demonstrate that the training effects are lost upon detraining and at about the same rate as they were gained during training. We suggest that a longer detraining time would result in more significant differences between the RE and DE groups and little differences between the CO and the DE groups. Others such as Ghaemmaghami et al. (15) reported reduction in LDL cholesterol levels in rats that swam for 2-6 hours per day, 5 days per week, for 5 weeks. It is possible that a more intensive training regimen might have effectively lowered the serum lipid levels. Our data also suggest that the rat is an inappropriate model to study some risk factors for coronary heart disease such as serum triglycerides and cholesterol levels. This is likely related to the observations that rats have higher HDL-cholesterol levels than humans (14).

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