PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 162–166 (1981)

Changes in Plasma Lipids and Lipolytic Activity during Recovery from Exercise of Untrained Rats (41040)

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Abstract. The activities of lipoprotein lipase in adipose tissue and heart homogenates, and epinephrine-stimulated lipase of adipose tissue were determined at the end of an exercise bout and during recovery from exercise of untrained rats. Concurrently, triglyceride and free fatty acid concentrations of plasma were measured. Lipoprotein lipase activity in adipose tissue homogenates was depressed at the end of the exercise bout and remained depressed 24 hr later. Heart lipoprotein lipase activity was significantly elevated 24 hr after the exercise bout. Basal activity of epinephrine-stimulated lipase (without epinephrine) was significantly elevated at the end of the exercise bout, remained elevated for 6 hr, but returned to control levels 12 hr later. In the presence of epinephrine, however, the activity of the enzyme was elevated at the end of the bout and throughout the 18-hr period of recovery after the exercise bout. Plasma triglyceride concentrations tended to decrease with exercise and the subsequent periods of rest with significant differences observed for the 12- and 18-hr-rested groups. Free fatty acid levels were significantly elevated immediately after exercise but returned to control levels 6 hr after the exercise bout. These findings show that certain changes in lipid metabolism resulting from a single bout of exercise, persist for some time after the termination of the exercise bout.

Changes in plasma triglyceride levels resulting from exercise have been reported to occur in both humans and experimental animals (1). Holloszy *et al.* (2) reported that a single or repeated bouts of exercise resulted in lowered plasma triglyceride levels in normal, middle-aged men. Naryan and Calhoun (3) reported that chronic exercise of rats decreased serum triglycerides and very-low-density lipoproteins. Gyntelberg *et al.* (4) reported that exercise caused a reduction in the triglyceride content of very-low-density lipoproteins of patients suffering from type IV hyperlipoproteinemia, regardless of whether or not the increase in caloric expenditure resulting from exercise was compensated for by an increase in food intake.

There is evidence to suggest that the decrease in plasma triglyceride levels resulting from exercise may be related to an increased rate of delipidation of the triglyceride-rich particles during the exercise bout. Jones and Havel (5) demonstrated that triglycerides ([^{14}C]palmitate labeled) of chylomicrons were "cleared" from plasma more rapidly in exercising rats than in rested controls. Furthermore, Askew *et al.* (6) found that the activity of lipoprotein lipase of heart and muscle was elevated following exhaustion of untrained rats. Similarly, lipoprotein lipase activity was reported to be elevated by endurance training in rats (7) and humans (8).

To date, little information is available to document the changes in plasma lipid levels or tissue lipolytic activity after recovery from a single bout of exercise. In this paper, we report the changes in plasma levels of triglyceride and free fatty acids as well as the changes in adipose tissue hormone-sensitive lipase, and heart and adipose tissue lipoprotein lipase at exhaustion and during recovery from exhaustion.

Methods. The results in this manuscript are from two similar experiments, designated I and II. In both experiments, male Holtzman rats weighing 250–300 g (average age 62 days at the time of sacrifice) were housed in individual cages. In experiment I, rats were divided into the following groups: (i) exercised; (ii) exercised 6 hr rested; (iii) exercised 12 hr rested; (iv) exercised 18 hr rested. Two groups of animals were used in experiment II: (i) exercised; and (ii) exercised 24 hr rested. Each group of exercised

0037-9727/81/020162-05$01.00/0

animals (not previously trained) had a corresponding control group of comparable weight that remained sedentary in the cages. The exercised animals were run on a motor-driven treadmill (0% grade) at a speed of 28 m/min. The animals were run for 2 hr or until exhausted, whichever came first. Animals that got exhausted in less than 1 hr were not used. Approximately 90% of the animals ran longer than 1 hr, and the average running time was 115 ± 10 min ($\bar{x}$ ± SD). During the exercise period, food was withheld from the control groups. When the exercised animals were resting, both the exercised and control animals were allowed free access to food (Wayne Lab Blox, Allied Mills Inc., Chicago, Ill.) and water. All animals were kept on a 12-hr dark–light cycle, with the lights turned off at 6 PM. Animals were exercised at the same time of day (between 7 and 10 AM) in a room maintained at a temperature of 68°F. At the end of the desired regimen, the experimental animals and their corresponding controls were sacrificed at the same time by decapitation.

The following were performed in experiment I: (a) assay of epinephrine-stimulated lipase in adipose tissue and (b) determination of plasma triglyceride and free fatty acid levels. In experiment II, the activity of lipoprotein lipase was measured in heart and adipose tissue homogenates of control, exercised, and exercised 24-hr-rested animals. Following the desired regimen, both experimental and control animals were sacrificed by decapitation and blood was collected in heparinized test tubes. Epidiymal fat pads and heart were excised and kept in 0.15 *M* KCl over ice and used shortly afterwards.

Lipids were extracted from plasma by the method of Folch *et al.* (9). Triglycerides and fatty acids were separated by thin-layer chromatography and quantitated as previously described (10).

Epinephrine-stimulated lipase (ESL) activity was assayed by a modification of the method of Ho *et al.* (11). Epididymal fat pads were incubated in Krebs–Henseleit (12) buffer, pH 7.3, containing 5% fatty acid free bovine serum albumin, for 1 hr at 37°. Tissue to buffer concentration was 1:5 (w/v). Epinephrine was present at 20 μg/ml buffer for one pad while the contralateral pad served as a nonephinephrine control (basal activity). Following incubation, an aliquot of the buffer surrounding the pad was removed and the released free fatty acids were extracted and titrated by the method of Dole and Meinertz (13). Enzyme activity is expressed as μeq FFA released/hr/g tissue.

For the assay of lipoprotein lipase, tissues were homogenized in Chapell–Perry (14) buffer, pH 7.4.[1] Tissue to buffer ratio was 1:10 (w/v) for heart and 1:5 (w/v) for adipose tissue. Both tissues were homogenized in a hand-held glass–glass homogenizer, the homogenates centrifuged at 600*g* for 10 min, and the supernatant fraction was retained. Lipoprotein lipase was assayed in duplicate as described earlier (15). Reactions were linear with respect to time of incubation and enzyme concentration. Following a 60-min incubation period, the reaction was terminated and the liberated fatty acids were extracted and quantitated as described above (13). Lipoprotein lipase activity is expressed as μeq FFA released/hr/mg protein or g tissue. Protein concentration was determined by the biuret method (16) for heart and by the Lowry method (17) for adipose tissue.

The results of these experiments were analyzed by a one-way analysis of variance (18). Comparisons of means were tested for significant differences by a Newman–Keuls procedure (18). The level of statistical significance chosen for these experiments was $P < 0.05$.

Results. The results of the analyses of plasma triglycerides and free fatty acids are shown in Table I. Triglyceride concentrations tended to decrease immediately after the exercise bout and throughout the subsequent rest periods of the exercised animals with significant differences observed in the 12- and 18-hr rested groups. The variations within the control values may be due

[1] A pH of 7.4 was used in this study although recently several investigators have used a pH of 8.2 for maximum LPL activity. While we do not have comparative data at these two pH, Fielding (19) has shown that the maximal activity is over a broad range of pH (from 7.4 to 8.5).

TABLE I. PLASMA TRIGLYCERIDE AND FREE FATTY ACID CONCENTRATIONS OF CONTROLS, EXHAUSTED, 6-hr-RESTED, 12-hr-RESTED, AND 18-hr-RESTED RATS

Animals	Triglycerides	Free fatty acids
	μg/ml plasma	
Control	671 ± 59[a]	177 ± 7.0
Exhausted	544 ± 55	264 ± 16*
Control	505 ± 80	183 ± 9.0
6 hr rested	414 ± 45	194 ± 4.0
Control	702 ± 97	200 ± 13
12 hr rested	413 ± 66*	191 ± 9.0
Control	1200 ± 128	202 ± 11
18 hr rested	700 ± 75*	175 ± 7.0

[a] Values are $\bar{x}$ ± SEM of at least eight samples.
* Denotes statistically significant differences ($P < 0.05$) between the experimental group and its corresponding control group.

to diurnal fluctuations. Free fatty acid levels were significantly elevated immediately after the exercise bout, but returned to control levels after 6 hr of rest following the exercise bout.

The changes in the basal (nonepinephrine) and epinephrine-stimulated lipolytic activity of adipose tissue are shown in Fig. 1. Basal lipolytic activity was significantly elevated immediately after exercise and remained elevated 6 hr later. Following 12 hr of rest, basal lipolytic activity returned to control levels. Epinephrine-stimulated lipolysis in the experimental groups tended to be higher than that of the controls with statistically significant differences observed immediately after the exercise bout and in the group that was rested 18 hr.

Changes in lipoprotein lipase (LPL) activity of adipose tissue and heart homogenates are shown in Table II. Adipose tissue LPL activity was significantly depressed immediately after the exercise bout and remained depressed 24-hr later. In heart homogenates, no significant changes between the exercised group and the controls were observed. However, LPL activity was significantly higher in the exercised 24-hr-rested group than the control group.

Discussion. Lipids play a prominant role as energy sources during exercise. The fatty acids that are provided to the tissues are derived from several sources: circulating triglycerides as constituents of chylomicrons and very-low-density lipoproteins, fat depot stores in adipose tissue, and the lipids stored in the tissues themselves. In this study, we aimed at examining the changes in LPL-mediated uptake of fatty acids from circulating triglycerides and mobilization of fatty acids from adipose tissue immediately after an exercise bout and during recovery from that bout. The question we wanted to answer was this: how long after a single bout of exercise does it take an animal to return to the original state?

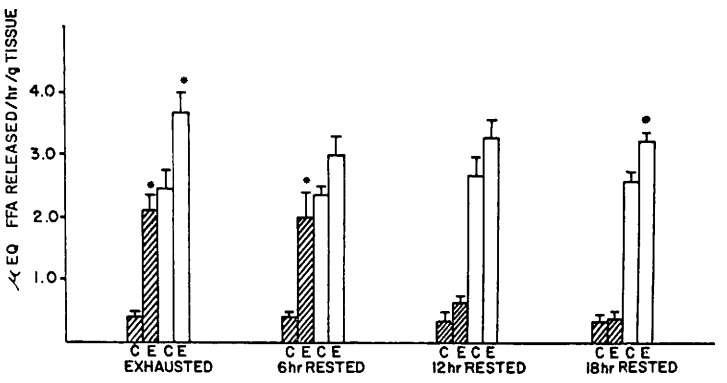

FIG. 1. Epinephrine-stimulated lipase activity in control (C) and experimental (E) groups. The hatched bars represent basal enzyme activity (without epinephrine) while open bars represent epinephrine-stimulated activity (with epinephrine). Asterisk denotes significant differences between the experimental group and its corresponding control. Values are mean ± SEM of at least eight samples.

TABLE II. LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE TISSUE AND HEART HOMOGENATES OF CONTROL, EXHAUSTED, AND 24-hr-RESTED RATS

	μEq FFA released/hr/ mg protein	μEq FFA released/h/ g tissue
Adipose tissue		
Control	1.86 ± 0.17[a]	20.0 ± 2.0
Exhausted	1.32 ± 0.15*	13.0 ± 1.2*
24 hr rested	1.00 ± 0.10*	10.6 ± 1.1*
Heart		
Control	0.28 ± 0.02	26.7 ± 2.0
Exhausted	0.32 ± 0.02	31.3 ± 2.3
24 hr rested	0.41 ± 0.02*	37.4 ± 1.9*

[a] Values are $\bar{x}$ ± SEM of at least eight observations.
* Denotes statistically significant differences ($P < 0.05$).

The changes we observed in plasma free fatty acid concentrations (Table I) are in agreement with those of Hagenfeldt and Wharen (20) in man, but are at variance with those of Oscai (21), who reported that serum free fatty acids of rats were elevated for as long as 96 hr after a 2-hr swim. Our results show that plasma-free fatty acids were elevated immediately after the exercise bout but returned to control levels 6 hr later. This occurred despite an increase in basal ESL activity (Fig. 1) which lasted for 6 hr after the exercise bout. The return of free fatty acid concentration to control levels after 6 hr despite the evidence of enhanced mobilization may be an indication of increased uptake of free fatty acids by tissue in our animals which were subjected to a rigorous exercise regimen.

The changes in ESL activity (both basal and stimulated) are noteworthy. The elevated levels in basal enzyme activity at exhaustion could conceivably be due to the elevation in catecholamine levels during the exercise bout. However, it is well documented that catecholamine levels which are elevated during the exercise bout return to resting levels 30–60 min after work (22–24). Thus the sustained increase in ESL activity for at least 6 hr after termination of the exercise bout might be due to stimulation of the enzyme through another mechanism not involving catecholamines. This is further supported by our findings of the changes in the activity of ESL upon stimulation with epinephrine *in vitro*. Epinephrine-stimulated lipolysis was invariably higher in the experimental groups than the control groups when this activity was measured in the presence of excess epinephrine. The added increment in lipolytic activity in the exercised groups of rats could be due to increased adipose tissue blood flow due to a release of a sympathetic vasoconstrictor (20), or to the elaboration of a factor(s), other than epinephrine, that causes the added increment in the activity of the enzyme when animals are exercised.

Lipoprotein lipase (LPL) hydrolyzes the triglycerides of chylomicrons and VLDL on the lumenal surface of the endothelial cells of the capillaries. Under conditions of increased energy needs, such as starvation, the activity of LPL is increased in muscle but is depressed in adipose tissue (25). There is evidence to suggest that the changes in LPL activity may be related to changes in insulin or glucagon levels. Pykalisto *et al.* (26) reported an increase in LPL activity of adipose tissue of humans in the fed state, which was dependent on the increment of serum insulin above basal levels. Borensztajn *et al.* (27) showed that when starved rats were fed glucose, heart LPL activity decreased while that of adipose tissue increased. Glucagon administration to these animals at the time of feeding prevented the decline in heart LPL activity, but had no effect on the adipose tissue enzyme.

The changes in LPL activity in response to exercise that we observed (Table II) and that were reported by others resemble the

changes seen in starvation. In this study we found that adipose tissue LPL activity was significantly depressed by exhaustion and remained so 24 hr later. Although no significant changes in LPL activity were observed in heart homogenates of the exhausted animals, LPL activity was significantly elevated after 24 hr of recovery. Similarly, Borensztajn *et al.* (7) reported that LPL activity in muscle homogenates of exercise-trained rats was elevated 24 hr after the last exercise bout. Askew *et al.* (6) reported that LPL activity in heart and muscle was elevated following exhaustion of untrained rats. Similar results were reported by Nikkila *et al.* (28, 29). In contrast, Lithell *et al.* (30) showed that in nonfasting human subjects performing heavy physical exercise for 1 hr LPL activity of adipose tissue increased, but that of muscle was unchanged, despite the fact that insulin concentration dropped dramatically during the exercise bout.

Taken all together, the results of the present study indicate that exercise increases lipolysis in adipose tissue and LPL-mediated uptake of fatty acids from circulating lipids by heart. At the same time, uptake of fatty acids by adipose tissue was decreased as evidenced by the decrease in LPL activity of that tissue. Our results further show that the acute effects of exercise persist for at least 24 hr after the exercise bout, possibly in response to hormonal changes that may be brought about by the exercise regimen.

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Received April 17, 1980. P.S.E.B.M. 1980, Vol. 166.