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Effect of Exercise on Liver Protein Loss and Lysosomal Enzyme Levels in Fed and Fasted Rats (40890)

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Abstract. Exhaustive treadmill running caused a significant decrease in hepatic protein levels. The rate of protein loss was 0.23 g/hr. This protein loss was accompanied by an increase in the activities of free (nonsedimentable) cathepsin D and arylsulfatase, suggesting the involvement of lysosomes. A 24-hr fast lowered the liver protein levels by about 20%. Plots of liver protein levels versus the time it took to reach exhaustion showed that the absolute rate of protein loss was the same for both the fed and the fasted rats. Since the initial protein levels were lower in the fasted rats, their relative rate of protein loss was greater. The level of activity of free (nonsedimentable) cathepsin D and arylsulfatase increased as the time to exhaustion increased in both the fed and fasted animals. The increase was severalfold greater in the fasted animals suggesting that fasting alters the structure of lysosomes in a way that enhances the increase in lysosomal fragility caused by exercise.

Although it is generally accepted that carbohydrates and fats are the primary sources of energy during exercise (1, 2), there is mounting evidence that proteins may also serve in that capacity. It was reported that both nitrogen excretion (3–6) and plasma urea (7) were increased by exercise. Reports of an increased capacity to oxidize leucine in trained rats (8) and increased $^{14}CO_2$ production from [^{14}C]leucine in exercising rats (9, 10) suggest that the rate of amino acid oxidation is increased during exercise. Amino acids may also serve as glucose precursors since gluconeogenesis is increased during exercise (11, 12).

The amino acids metabolized during exercise could be supplied by depletion of the plasma and tissue free amino acid pools, utilization of tissue proteins, or both. Preliminary evidence of utilization of tissue proteins was obtained from our studies which showed that exercise decreases the protein level of liver (13).

Like exercise, a 24-hr fast also lowers the level of liver proteins in the rat by 13 to 25% (14, 15) and in general puts the animal in a catabolic state, i.e., depleted glycogen stores, increased mobilization of fats, decreased insulin, and increased glucagon levels. Interestingly a second day of fasting resulted in only a small further loss of liver protein (14, 15). The increased proteolysis during fasting is accompanied by an increase in the levels of free (nonsedimentable) lysosomal enzymes (16).

Since either exercise or fasting leads to loss of liver protein, we have investigated the combined effects of exercise and fasting on protein levels and lysosomal enzyme activities to determine whether the protein loss is additive in the two catabolic states and also to establish whether fasting alters the effect of exercise on protein metabolism in the liver.

Materials and methods. Animals. In the first experiment (Table I), male Holtzman rats weighing approximately 300 g were divided into two groups: (1) a rested control group and (2) a group that was exhaustively exercised and sacrificed immediately. Exhaustively exercised animals were run on the treadmill at 30 m/min until they could no longer continue to run and could not right themselves when placed on their backs.

In the second experiment (Figs. 1–3) male Holtzman rats weighing approximately 200 g were preconditioned to run on a motor driven treadmill (10) by running twice a week for 3 weeks at 25 m/min for 1 hr. The animals were then randomly di-

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TABLE I. LYSOSOMAL ENZYME LEVELS IN RAT LIVER AFTER EXERCISE[a]

	Control	Exercised
Cathepsin D		
(μg hemoglobin degraded/min/g liver)		
Total	172 ± 4(10)	187 ± 7(10)
Free	41.0 ± 2.6(9)	56.5 ± 6.0(9)[b]
Arylsulfatase		
(μmole nitrocatechol released/min/g liver)		
Total	1.70 ± 0.03(5)	2.03 ± 0.18(6)[b]
Free	0.46 ± 0.07(5)	1.09 ± 0.13(6)[c]

[a] Values are means ± SEM with the number of observations in parentheses. Run time was 111 ± 14 min.
[b] Indicates statistical significance at $P < 0.05$ when compared to the control group.
[c] Indicates statistical significance at $P < 0.01$ when compared to the control group.

vided into four groups; (1) fed–rested, (2) fed–exercised, (3) fasted–rested, and (4) fasted–exercised. Food was withheld from the two fasted groups 24 hr prior to sacrifice. Half of the fed and half of the fasted rats were exhausted as described above. The appropriate fed or fasted control was always sacrificed at the same time as an exercised rat.

Materials. Nitrocatechol, potassium nitrocatechol sulfate, benzoyl-DL-arginine-2-naphthylamide hydrochloride, and methemoglobin were obtained from Sigma Chemical Company. [*Methyl*-^{14}C]methylated methemoglobin was obtained from New England Nuclear Corporation. All other chemicals were reagent grade (Fisher) and used without further purification.

Assay procedures. The livers were removed at the time of sacrifice, rinsed with isotonic KCl, and stored on ice for a maximum of 3 hr. A portion of each liver was homogenized (1:10 w/v) with a motor-driven glass–Teflon homogenizer (five passes) in a 1.0 m*M* Tris (pH 7.4) buffer that contained 0.25 *M* sucrose. The use of a Dounce homogenizer would have reduced the levels of free lysosomal enzymes, however, in this experiment we were interested in the fragility of the lysosomes, and the more rigorous homogenization by the motor driven homogenizer provided a measure of the mechanical fragility of the lysosomes. The protein concentration of this

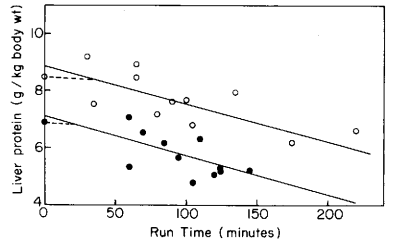

FIG. 1. Effect of exercise time on liver protein levels in fed (open circles) and fasted (filled circles) rats. The run time is the time to exhaustion. The zero time points represent averages of control (sedentary) animals whose values are 8.43 ± 0.18 and 6.83 ± 0.20 g liver protein/kg initial body wt for fed and fasted rats, respectively. The solid lines were calculated by the method of least squares using only animals that ran for 1 hr or more. The dashed lines through the zero time point was added to help visualize a possible induction period during the early stages of exercise.

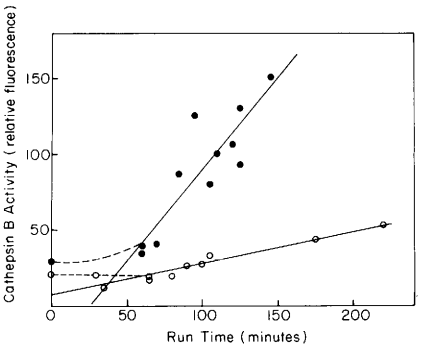

FIG. 2. Effect of exercise time on free cathepsin B levels in the livers of fed (open circles) and fasted (filled circles) animals. The zero time values are averages of sedentary animals whose values are 22 ± 3 and 29 ± 1 fluorescence units/assay for fed and fasted animals, respectively. The comments on the characters and lines are the same as in Fig. 1.

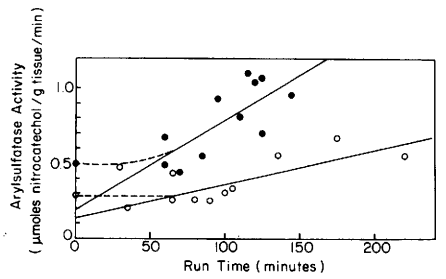

FIG. 3. Effect of exercise time on free arylsulfatase activity in fed (open circles) and fasted (filled circles) rats. The zero time points are averages of sedentary controls whose values are 0.28 ± 0.01 and 0.50 ± 0.04 μ moles/g tissue/min for fed and fasted animals, respectively. The comments on the characters and lines are the same as in Fig. 1.

homogenate was determined by the biuret method (17) using bovine serum albumin (Sigma Fraction V) as a standard. The remainder of the homogenate was centrifuged at 20,000*g* for 10 min and the supernatant was assayed for free (nonsedimentable) lysosomal enzyme activity. Total lysosomal enzyme activity was measured as described above except that the buffer also contained 0.2% Triton X-100.

Cathepsin D activity was assayed by a modification of the procedure of Anson (18, 19). Liver supernatant (0.05 ml) was mixed with 0.05 ml of 0.15 *M* citrate buffer (pH 3) containing 2 mg/ml methemoglobin and 0.2 μCi/ml [*methyl*-^{14}C]methylated methemoglobin. After incubating at 37°C for 30 min, the reaction was stopped by adding 0.075 ml of 13% TCA. The precipitate was removed by centrifugation at 80,000*g* for 30 sec in a Beckman airfuge, and 0.1 ml of the supernatant was counted in a liquid scintillation counter (Beckman, LS 233). Arylsulfatase activity was assayed as described by Barrett and Heath (19). Cathepsin B activity was measured by using a modification of the fluorometric method of Maskrey *et al.* (20). Since the 2-naphthylamine needed to construct the standard curve was unavailable (due to its carcinogenicity), the activity of cathepsin B is expressed in relative fluorescence units/assay. The fluorescence spectrophometer (Perkin–Elmer 512) was set to 100 fluorescence units with a fresh solution of 1.0 m*M* *p*-phenylenediamine in ethanol. The assay procedure was as follows: 0.5 ml of diluted liver supernatant (diluted 1:10 v/v with homogenization medium) was incubated at 37°C with 1.5 ml of 0.1 *M* phosphate buffer (pH 6.0) containing 1.33 m*M* dithiothreitol. The reaction was initiated by adding 0.04 ml of 100 m*M* Bz-DL-Arg–L-naphthylamide in dimethylsulfoxide, and terminated after 20 min by adding 4 ml of ethanol. After centrifugation at 800*g* for 10 min the fluorescence of the supernatant was determined (excitation 345 nm, emission 403 nm). The fluorescence increased linearly for at least 30 min. All enzyme assays were carried out within 5 hr of the time the rat was sacrificed.

Calculations. For statistical analysis of the data in Table I, a one-way analysis of variance was performed. The lines in Figs. 1–3 were subjected to linear regression analysis and the significance of the correlation coefficients was determined as directed by Edwards (21).

Results. Since lysosomes have been implicated in protein degradation in the liver (22, 23), we measured the effect of exercise on both the liver protein levels and the activities of lysosomal marker enzymes in our first experiment. The decrease in liver protein from 8.22 ± 0.16 g protein/liver/kg initial body wt in the rested animals to 7.32 ± 0.15 g protein/liver/kg initial body wt in the exhausted rats agrees with our previous report (13). The effect of exercise on the activity of both free and total cathepsin D and arylsulfatase is shown in Table I. The consistant observation for both enzymes was that exercise caused an increase in the activities of free (nonsedimentable) cathepsin D and arylsulfatase.

In our first experiment, we observed that the animals that ran longer before becoming exhausted tended to have higher levels of free lysosomal enzyme activity; this had the effect of increasing the variance within the exercised group (the SEM of the values for the exercised group are all greater than those of the control group in Table I). The length of time it takes an animal to reach exhaustion can be eliminated as a variable in the determination of protein and lysosomal enzyme levels by plotting the mea-

sured quantity vs run time to exhaustion. Thus the effect of exercise on hepatic protein loss and lysosomal enzyme activity is determined from the slope of the line of such plots.

In the second experiment the effect of a previous fast on hepatic protein loss and "free" lysosomal enzyme activity during exhaustive exercise was determined and the data are presented as plots of the measured quantity vs time to exhaustion. A plot of liver protein levels vs the length of time it took each animal to reach exhaustion (Fig. 1) produces a line of slope 0.013 g protein/liver/kg/min (r 0.77, $P < 0.005$) for fed rats and 0.014 g protein/liver/kg/min (r 0.58, $P < 0.025$) for fasted rats. Thus the amount of protein lost from both fed and fasted rats is the same during a bout of exercise.

In order to ascertain if the activities of "free" lysosomal enzymes were different in fed and fasted rats, the activities of the lysosomal marker enzymes cathepsin B and arylsulfatase were measured and the results are shown in Figs. 2 and 3. The activity of cathepsin B increased as the time to exhaustion increased in the fed rats (r 0.98, $P < 0.005$), but increased at a faster rate in the fasted rats (r 0.88, $P < 0.005$). The least squares line for the fasted rats in Fig. 2 is 5.3 times steeper than for the fed rats. In a similar manner, Fig. 3 reveals that the level of arylsulfatase increased 2.6 times faster with time to exhaustion in the fasted (r 0.73, $P < 0.005$) than in the fed (r 0.77, $P < 0.005$) rats. Thus the increase in the activities of both lysosomal enzymes during exercise is severalfold greater in the fasted–exercised rats than in the fed–exercised rats.

Discussion. The results of the present study confirm our observation that liver protein levels are lower immediately after a bout of exercise (13). Whether the decrease in the level of liver protein after exhaustive exercise is a result of an increase in the rate of liver protein degradation or a decrease in the rate of synthesis cannot be directly ascertained from these experiments. However, since lysosomes are involved in liver protein degradation (22, 23), and are known to become more fragile during increased proteolysis (24), our findings of increased activities of free lysosomal enzymes during exercise (Table I) suggest that the rate of protein degradation may be increased.

The previous findings that exercise caused an increase in plasma urea (7), increased urea excretion (4, 5), and increased production of $^{14}CO_2$ from [^{14}C]leucine (9, 10) would suggest that the amino acids produced by protein degradation are utilized during exercise. This protein loss is quite sizable amounting to about 0.23 g/hr for a 300-g rat. The mechanism of the loss of this liver protein is unknown, however, an upper limit on the energy available for exercise can be calculated by assuming that all the lost protein is utilized for energy production. The lost protein could produce 0.94 kcal/hr (4 kcal/g protein). Since an exercising rat expends approximately 5 kcal/hr (net caloric expenditure was calculated for a 300-g rat running at 28.5 m/min) (25), the caloric value of the liver protein lost could amount to as much as 19% of the energy utilized during exercise.

A 24-hr fast decreased the level of liver protein by 19% which is in agreement with the results of others (14, 15). It is well established that a second 24 hr of fasting results in a much slower rate of liver protein loss (14, 15). Thus it was surprising to find that the absolute rate of loss of liver protein during exercise was the same in both fed and fasted rats (Fig. 1). In fact, the percentage of liver protein lost as a function of time to exhaustion is increased in the fasted rats since the initial liver protein levels are lower. Thus it seems that exercise causes the liver to lose protein at a constant rate which is independent of the prior catabolic state of the animal, and it maintains this rate by increasing the relative rate of protein loss.

Since increased proteolysis is associated with lysosomes that become larger (26) and more fragile (24), resulting in the detection of higher levels of free lysosomal enzymes after homogenization, the levels of free cathepsin B (Fig. 2) and arylsulfatase (Fig. 3) were determined in the livers of fed and fasted rats. Although only slightly higher initially, the levels of the free lysosomal enzymes as a function of time to exhaustion increased several times faster in the fasted

than in the fed rats. This may be due to the greater relative rate of protein loss from the livers of the fasted rats or to changes in the lysosome caused by fasting.

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