The role of lysosomes in exercise-induced hepatic protein loss

George J. KASPEREK, G. Lynis DOHM, Hisham A. BARAKAT, Paul H. STRAUSBAUCH,
Donald W. BARNES and Rebecca D. SNIDER

School of Medicine, East Carolina University, Greenville, NC 27834, U.S.A.

(Received 18 May 1981/Accepted 9 October 1981)

Previous reports have shown that exercise causes a loss of liver protein. The purpose of the present study was to elucidate the mechanism of this exercise-induced protein loss. Exercise caused: (1) an increase in mechanical and osmotic lysosomal fragility; (2) a significant loss of hepatic water, glycogen, protein, phospholipid and RNA; (3) loss of protein from the soluble, mitochondrial and microsomal fractions; (4) loss of mitochondrial, microsomal and cytosolic, but not lysosomal, enzyme activity; (5) an increase in the number of autophagic vacuoles; (6) an increase in the lysosomal size. Taken together, these results suggest that the autophagolysosomal system is responsible for the exercise-induced hepatic protein loss.

Although the effect of exercise on skeletal-muscle biochemistry has been extensively studied (for review see Holloszy & Booth, 1976), there is a paucity of information on the effect of exhaustive exercise on the liver. Dohm *et al.* (1978) reported that exercise caused a 12% decrease in rat liver weight, which was partially accounted for by the loss of glycogen (Fell *et al.*, 1980) and protein (Dohm *et al.*, 1978; Kaspersek *et al.*, 1980). The purpose of the present study was to examine the mechanism underlying the exercise-induced protein loss.

Although protein degradation has been well studied (for review see Goldberg & St. John, 1976), the precise mechanism remains uncertain. Ballard (1977) proposed that protein degradation can occur via one of two pathways, one which is system-limited and the other is substrate-limited. The substrate-limited degradation system, whose location in the cell is unknown, degrades different proteins at different rates, and is believed to be responsible for the degradation of proteins with short turnover times. The system-limited degradation system, also called the autophagolysosomal system, involves the engulfment of cell constituents by membranes to form autophagic vacuoles. The autophagic vacuoles fuse with lysosomes to form autophagolysosomes, in which the engulfed contents, including proteins, are degraded (Dean & Barrett, 1976). In this system, the rates of breakdown of all proteins are expected to be similar and regulated mainly by the number of autophagic vacuoles. In contrast, Dean (1980) suggested that lysosomes are involved in most degradation processes and that protein degradation is regulated at several discrete steps in one pathway.

Cellular autophagy, which is a component in both Ballard's (1977) and Dean's (1980) models, occurs in normal as well as protein-catabolic states (Ericksen, 1969). In protein-catabolic states, increased autophagy is often accompanied by an increase in lysosomal fragility (Neely *et al.*, 1974).

Increased hepatic lysosomal fragility was associated with both starvation (Desai, 1969) and glucagon-induced autophagy (Deter & de Duve, 1967). Since exhaustive exercise caused increased mechanical fragility of the lysosomes of rat liver (Kaspersek *et al.*, 1980; Loegering *et al.*, 1975), it seemed possible that the autophagolysosomal system was involved in liver protein loss. In the present study, the specificity of the exercise-induced protein loss was determined by comparing the amount of protein and enzyme activities in several subcellular fractions from exercised animals with the same parameters in control animals. The effect of exercise on the fragility and ultrastructure of lysosomes was also measured. Taken together, our results lead us to conclude that exercise increases the rate of protein degradation via the autophagolysosomal system.

Experimental

Animals

Male Holtzman rats weighing approx. 250 g were placed into either a rested control group or an exercised group that was run on a treadmill at 28 m/min (0% grade) for a specified length of time or until exhausted. All exercised animals, except those used in the lysosomal-fragility and ultrastructural studies, were allowed free access to food and water. Exhaustion was defined as the point at which the

animals could no longer continue to run, and most animals ran for 4–5 h. In the lysosomal-fragility experiments, food was removed from all the animals near the end of the light-cycle, which was approx. 15 h before the start of the experiment. The animals were exercised for 30, 60, 120 or 180 min. Ultrastructural studies were performed on the livers of animals exercised for 180 min. In all experiments, control rats were killed at the same time as the exhausted rats.

Assay procedures

In the subcellular-fractionation studies the livers were quickly removed, rinsed in ice-cold 0.154 M-KCl, and homogenized (1:3, w/v) in a glass/Teflon Potter–Elvehjem homogenizer in unbuffered 0.25 M-sucrose. Mitochondria were separated by the method of Hoskins & Mackenzie (1961), and a sample was removed and disrupted by sonication. Microsomal fraction was prepared from the 10000g supernatant, and the contents of cytochrome *b*₅ and cytochrome *P*-450 (Omura & Sato, 1964) as well as the activities of aminopyrine *N*-demethylase (Nash, 1953) and aniline hydroxylase (Imai *et al.*, 1966) were determined as described by Barnes *et al.* (1979).

For the assay of lipogenic enzymes, a portion of the liver was homogenized (1:5, w/v) in a glass/glass Dounce homogenizer in 0.05 M-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA and 1 mM-dithiothreitol. The homogenate was centrifuged at 105000g for 60 min and then filtered through four layers of cheesecloth. This supernatant was assayed for fatty acid synthetase (Plate *et al.*, 1968), ATP citrate lyase (Takeda *et al.*, 1969), 'malic' enzyme (Hsu & Lardy, 1969) and glucose 6-phosphate dehydrogenase (Langdon, 1966) activities. The activity of phosphoenolpyruvate carboxykinase was determined in whole homogenates by the method of Opie & Newsholme (1967), and that of succinate dehydrogenase by a modification of the method of King (1967).

The fragility of the liver lysosomes was determined by a modification of the method of Deter & de Duve (1967). One portion of the liver was gently homogenized (five strokes) in 1.0 mM-Tris/HCl buffer, pH 7.4, containing 0.25 M-sucrose with a glass/glass Dounce homogenizer. This homogenate was divided into three portions: (1) one portion (total non-sedimentable) was made 0.3% (v/v) with respect to Triton X-100; (2) the second portion (free non-sedimentable) was diluted with an equal volume of 0.225 M-sucrose; (3) the third portion (osmotically stressed) was diluted with an equal volume of water, then incubated at 0°C for 30 min, after which the sucrose molarity was returned to 0.225 M by the addition of a concentrated sucrose solution. All three portions were then centrifuged at 20000g for

10 min, and the supernatants were assayed for cathepsin B activity by the method of Maskrey *et al.* (1977) as modified by Kasperek *et al.* (1980), and for β -*N*-acetylglucosaminidase activity as described by Barrett & Heath (1977), with benzyloxycarbonyl-DL-arginine 2-naphthylamide and 4-methylumbelliferyl *N*-acetyl- β -D-glucosaminide respectively as substrates. All assays were performed under conditions of substrate saturation and were linear with time.

The protein concentrations of the microsomal, mitochondrial and soluble mitochondrial fractions were determined by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V; Sigma Chemical Co.) as standard, and the biuret method as described by Gornall *et al.* (1949) was used on all other fractions. The DNA content was determined by the method of Labarca & Paigen (1980), and the RNA content by the method of Wannemacher *et al.* (1965). The water content was determined gravimetrically. Lipids were extracted with chloroform/methanol (2:1, v/v) and determined gravimetrically. Phospholipid content was determined by the method of Bartlett (1959). The content of neutral lipids was calculated by subtracting the phospholipid content from the total lipid content.

After anaesthesia with sodium pentobarbital (5.0 mg/100 g body wt.), a small portion of the liver was quickly frozen between tongs cooled with solid CO₂/acetone and pulverized in a percussion mortar. The glycogen content was analysed by the method of Lo *et al.* (1970).

Comparisons between exercised and rested groups were made by using an analysis of variance. The significance level in these comparisons was set at $P < 0.05$.

Tissue preparation for ultrastructural studies

Liver tissue for electron microscopy was fixed by perfusion *in situ* with 2.0% (v/v) glutaraldehyde fixative buffered with 0.1 M-sodium cacodylate buffer, pH 7.4, as follows. The animal was killed by cervical dislocation within 1 min of completing the exercise protocol. The abdomen was quickly opened and a perfusion cannula inserted into the inferior vena cava. Perfusion fluid was run at a rate of 2 ml/min after rupture of the portal vein. The liver lobes began to blanch after 2 min of perfusion and were firm to palpation within 4 min. Perfusion was continued for a total of 15 min. Sections of liver were then taken from the median lobe, cut into 1 mm blocks and immersed in 2% glutaraldehyde/0.1 M-cacodylate buffer, pH 7.4, for 24 h. Tissue pieces were washed in 0.1 M-cacodylate buffer, pH 7.4, and post-fixed in 1% osmium tetroxide/1.5% potassium ferricyanide for 1 h at 4°C. Tissue was subsequently dehydrated in graded ethanol and embedded in Epon resin as described by Luft (1961). Sections were cut

on a Reichert OmU-3 ultramicrotome. Areas of the sections showing mid-zone hepatocytes (areas five cells away from portal and central areas) were selected for further study (Loud, 1968). Thin sections were stained with uranyl acetate and lead hydroxide by standard techniques and examined in a Philips 201 electron microscope. Random survey photographs at low magnification ($\times 4500$) were taken. These areas were then photographed at higher magnification ($\times 10000$) to evaluate alterations in morphology induced by exercise, to observe autophagocytic vesicles, and to enumerate the number and size of lysosomal profiles. The size distribution of lysosomal profiles, which includes dense bodies, residual bodies and autophagosomes, was determined by measuring the average linear dimension of each profile. For circular profiles this was the diameter of the profile, and for oval or irregular profiles it was the average of the largest linear dimension and the width. Data were analysed by counting actual numbers of profiles found on random photographs of exercised and control animals and then plotting lysosomal profile size as a function of frequency. Light-microscopy was performed on Haematoxylin/Eosin-stained sections from paraffin-embedded glutaraldehyde-perfused tissues.

Results

Lysosomal-fragility studies

The activities of the lysosomal enzymes cathepsin B and β -N-acetylglucosaminidase were measured in the supernatants of liver homogenates that had been treated with detergent (total non-sedimentable), not treated (free non-sedimentable) or osmotically stressed (hypo-osmotic non-sedimentable) before centrifugation. Table 1 shows that the total lysosomal activity is not altered during the exercise bout. The free non-sedimentable lysosomal enzyme ac-

tivity is a measure of the extralysosomal enzyme activity and could result from lysosomal enzymes that originated outside of the lysosome and/or those released from the lysosome by homogenization. We previously reported (Kasperek *et al.*, 1980) that the free lysosomal activity was increased at exhaustion. The percentage non-sedimentable activity is calculated by dividing the free non-sedimentable activity by the total non-sedimentable activity and multiplying by 100. Table 1 shows that the percentage non-sedimentable lysosomal activity is significantly elevated at 2 h and remains elevated for the duration of the exercise bout. To determine if the liver lysosomes of the exercised animals became more osmotically fragile during exercise, the liver homogenates were diluted to hypo-osmotic conditions and incubated for 30 min before restoration to iso-osmotic conditions. The results (hypo-osmotic non-sedimentable activity) are best represented by calculating the osmofragility, which is the change in the percentage non-sedimentable activity [(% hypo-osmotic non-sedimentable activity) - (% free non-sedimentable activity)]. Fig. 1 shows that as little as 30 min of exercise caused the liver lysosomes to become more osmotically fragile, and this exercise-induced osmofragility was maintained during the remainder of the exercise bout.

If liver or other cells were damaged during exercise and the lysosomes were leaking enzymes, these enzymes should appear in the plasma. Table 1 shows that there was no elevation in the amount of β -N-acetylglucosaminidase in the plasma, and in other experiments (G. J. Kasperek, unpublished work) no increase in plasma β -N-acetylglucosaminidase activity 24 h after exercise was observed.

Changes in liver constituents as a result of exercise

In order to assess the effect of exercise on the gross composition of liver, its major constituents were measured; the results are presented in Table 2.

Table 1. Effect of the length of exercise time on lysosomal enzymes activities

For experimental details see the text. Values are means $\pm$ S.E.M. for at least 11 animals per group. A statistically significant difference ($P < 0.05$) between rested and exercised animals is shown by an asterisk (*).

Exercise time (min)	...	0	30	60	120	180
Cathepsin B						
Total non-sedimentable (fluorescence units/min per g of liver)		3220 $\pm$ 90	3260 $\pm$ 180	3060 $\pm$ 100	3360 $\pm$ 180	3050 $\pm$ 80
Percentage non-sedimentable		5.43 $\pm$ 0.37	5.78 $\pm$ 0.38	7.70 $\pm$ 0.90	12.1 $\pm$ 1.5*	12.3 $\pm$ 0.9*
β-N-Acetylglucosaminidase						
Total non-sedimentable (μ mol/min per g of liver)		2360 $\pm$ 210	2580 $\pm$ 170	2500 $\pm$ 90	2560 $\pm$ 160	2440 $\pm$ 160
Percentage non-sedimentable		13.3 $\pm$ 1.4	14.4 $\pm$ 1.1	14.4 $\pm$ 1.1	19.9 $\pm$ 1.4*	21.7 $\pm$ 2.0*
Plasma (μ mol/ml of plasma)		249 $\pm$ 10	261 $\pm$ 7	269 $\pm$ 10	244 $\pm$ 12	234 $\pm$ 7

Fig. 1. Effect of the length of exercise time on lysosomal osmofragility

The increased lysosomal osmofragility was determined by measuring the increase in the percentage of non-sedimentable cathepsin B (○) and β -N-acetylglucosaminidase (□) after hypo-osmotic stress. For full details see the Experimental section. Each point represents the mean for at least 11 animals, and a statistically significant difference ($P < 0.05$) between rested and exercised groups is shown by an asterisk (*).

A single bout of exercise caused a 24% loss of liver weight. This loss was due to significant decreases in water (21%), glycogen (96%), phospholipid (11%), RNA (15%) and protein (11%) content.

To determine if there was selective loss of liver proteins, the protein content of various subcellular fractions was measured. Table 2 shows that exercise caused an average decrease in the liver protein of 11% and that there was a statistically significant loss from all subcellular fractions measured. The activity of several enzymes was measured in order to determine if individual proteins within a particular subcellular fraction were selectively lost. The data in Table 3 reveal that the activities of three of the four microsomal enzymes, the mitochondrial enzyme and four of the five cytosolic enzymes measured were lower in the livers of the exercised animals. The activities of two lysosomal enzymes were not significantly changed by an exercise bout. One must exercise caution when interpreting data of this kind, since changes in activity may represent an activation or deactivation of an enzyme and thus not be representative of the protein amounts. However, taken together, the data in Table 3 indicate that enzymes are lost from all of the subcellular fractions measured, with the exception of the lysosomal fraction.

The loss of liver protein as a function of running time is shown in Fig. 2. We had previously reported that liver protein loss correlated with the time it took an animal to reach exhaustion (Kasperek *et al.*, 1980). Examination of Fig. 2 reveals that exercise-induced protein loss is not a function of exhaustion, but starts early in the exercise bout and continues as the bout proceeds, becoming statistically significant at

Table 2. Liver constituents of rested and exercised rats

For experimental details see the text. Values are means $\pm$ s.e.m. for at least nine animals per group. The units are g/liver per kg body wt. A statistically significant difference ($P < 0.05$) between rested and exercised groups is shown by an asterisk (*).

	Rested	Exercised
Liver wt.	46.5 $\pm$ 0.8	35.5 $\pm$ 0.6*
Water	32.5 $\pm$ 0.8	25.6 $\pm$ 0.5*
Glycogen	3.32 $\pm$ 0.10	0.14 $\pm$ 0.10*
Neutral lipid	1.03 $\pm$ 0.04	1.07 $\pm$ 0.03
Phospholipid	0.80 $\pm$ 0.03	0.71 $\pm$ 0.03*
DNA	0.169 $\pm$ 0.008	0.178 $\pm$ 0.007
RNA	0.40 $\pm$ 0.10	0.34 $\pm$ 0.01*
Total protein	8.22 $\pm$ 0.16	7.32 $\pm$ 0.15*
Soluble protein	4.42 $\pm$ 0.07	4.12 $\pm$ 0.08*
Total mitochondrial protein	1.54 $\pm$ 0.005	1.31 $\pm$ 0.04*
Soluble mitochondrial protein	0.834 $\pm$ 0.03	0.739 $\pm$ 0.03*
Microsomal protein	1.14 $\pm$ 0.02	0.89 $\pm$ 0.03*

EXPLANATION OF PLATE 1

Electron micrograph of starved control rat liver showing portion of hepatic parenchymal cell
Characteristic dense bodies (db) are evident most prominently in the pericanalicular region. Magnification $\times 7500$.

EXPLANATION OF PLATE 2

Electron micrograph of liver from starved exercised rat liver showing portion of hepatic parenchymal cell
Autophagocytic vacuoles (av) are evident along with pleomorphic dense bodies (db). Note the general increase in lysosomal size, their variability in shape and the heterogeneity of content. Magnification $\times 7500$.

Table 3. *Effect of exercise on the activities of several enzymes in liver*

For experimental details see the text. Values are means $\pm$ S.E.M. for at least ten animals per group. The units are $\mu\text{mol}/\text{min}$ per liver per kg body wt. for all the enzymes, except cathepsin B, where they are fluorescence units/min per liver per kg body wt. The values for cathepsin B and β -N-acetylglucosaminidase were determined from animals that had exercised for 3 h. All the other values are from animals exercised to exhaustion. A statistically significant difference ($P < 0.05$) between rested and exercised animals is shown by an asterisk (*).

	Rested	Exercised
Microsomal enzymes		
Aminopyrine <i>N</i> -demethylase	11.0 ± 0.3	$7.8 \pm 0.4^*$
Aniline hydroxylase	0.80 ± 0.05	0.77 ± 0.03
Cytochrome <i>b</i> ₅	0.48 ± 0.02	$0.41 \pm 0.01^*$
Cytochrome <i>P</i> -450	0.99 ± 0.04	$0.75 \pm 0.04^*$
Mitochondrial enzyme		
Succinate dehydrogenase	690 ± 40	$510 \pm 30^*$
Cytoplasmic enzymes		
Fatty acid synthetase	32 ± 2	$24 \pm 2^*$
Glucose 6-phosphate dehydrogenase	100 ± 6	$78 \pm 5^*$
ATP citrate lyase	110 ± 6	$71 \pm 5^*$
'Malic' enzyme	146 ± 9	$43 \pm 3^*$
Phosphoenolpyruvate carboxykinase	43 ± 4	41 ± 3
Lysosomal enzymes		
Cathepsin B	28900 ± 700	25600 ± 1200
β -N-Acetylglucosaminidase	19.7 ± 1.6	20.3 ± 0.8

Fig. 2. *Effect of the length of exercise time on total rat liver protein*

For full details see the Experimental section. Each point represents the mean for at least 11 animals, and a statistically significant difference ($P < 0.05$) between rested and exercised groups is shown by an asterisk (*).

2 h. In this experiment, a 3 h exercise bout caused a 12% loss of liver protein.

Effect of exercise on ultrastructural appearance and distribution of lysosomal elements

Light-microscopy of sections revealed no significant differences in liver morphology between starved control animals and starved exercised animals. In both rested and exercised rats the parenchyma was intact, with the sinusoids appearing slightly distended. Most of the erythrocytes had been removed by perfusion; however, there were small focal areas where perfusion was apparently inadequate, and clumps of erythrocytes could be seen within the sinusoids. Glycogen was absent from both control and exercised animals as determined by light-microscopy. Small lipid-containing vacuoles could be seen in both control and exercised animals, with no significant differences in vacuole size or distribution. Electron micrographs of liver from control and from exercised animals revealed no significant change in numbers of total lysosomes. However, there was a marked difference in the number of autophagocytic vacuoles found in exercised when compared with control animals. Random micrographs from control animals showed the absence of autophagocytic vacuoles (Plate 1). In contrast, random photographs of exercised animals invariably showed one or more autophagocytic vacuoles per photograph (Plate 2). A quantitative

Fig. 3. Size-frequency distribution of lysosomal profiles from random electron micrographs of mid-zonal parenchymal cells from liver of starved control and starved exercised (3 h) rats

For full details see the Experimental section. Percentage frequency is plotted versus average diameter of lysosomal profiles in arbitrary units. Each unit = 77 nm. The plot for control animals (—) represents average diameters of 160 profiles measured in random photographs; the plot for exercised animals (----) represents average diameters of 250 lysosomal profiles.

determination showed that the exercised animals exhibited 8.0 autophagic vacuoles per $1000\mu\text{m}^2$ of mid-zone hepatocyte area, in contrast with less than 0.5 autophagic vacuole per $1000\mu\text{m}^2$ in the controls. These vacuoles contain a variety of cytoplasmic components, the most conspicuous of which are enclosed mitochondria, but which also include microbodies, rough endoplasmic reticulum, membranous material and an occasional intact lysosome. However, most of the autophagocytic vacuoles showed contents at an increased stage of degradation, in which identity of the enclosed organelles could not be positively identified. Electron micrographs from exercised animals also showed increased numbers of enlarged residual-type bodies representing the end stage of lysosomal digestion. Other comparative ultrastructural differ-

ences between exercised and control animals included increased morphological variability of mitochondria, close association of mitochondria with adjacent rough endoplasmic reticulum and a tendency for the Golgi apparatus to assume a more concave shape. Control (starved) animals showed occasional glycogen particles, but none were seen in exercised animals. No apparent differences in peroxisomes, lipid droplets or other cellular components were noted.

When size distribution of lysosomal profiles was compared between the two groups of animals, a marked increase in lysosomal variability and size was noted in exercised compared with control animals (Fig. 3). Control animals show a small and relatively homogeneous population of lysosomes. In the exercised animals there is a general increase in the size of relatively regular-appearing dense bodies, along with increased numbers of enlarged heterogeneous pleomorphic secondary lysosomes, dense residual bodies, autophagosomes and autophagolysosomes.

Discussion

The increase in the percentage non-sedimentable lysosomal activity found in exhausted rats (Kasperek *et al.*, 1980) could result from (a) an increase in the amount of newly synthesized lysosomal enzymes not yet sequestered into the lysosome (Decker *et al.*, 1980), (b) the release of lysosomal enzymes *in vivo* from damaged lysosomes or autophagolysosomes (Decker *et al.*, 1980), or (c) the release of lysosomal enzymes from autophagolysosomes ruptured by homogenization *in vitro* (Deter & de Duve, 1967). Since the total lysosomal enzyme activity was not increased, the first possibility was ruled out. It was more difficult to choose between the last two possibilities, since they differ only in the timing of the release of lysosomal enzymes. Exercise causes the liver cells to become more permeable, releasing cytoplasmic enzymes into the plasma (Loefering *et al.*, 1975). If the lysosomal enzymes are released from the lysosome into the cytosol, we might expect to find them in the plasma, since Welman *et al.* (1978) found increased plasma activities of lysosomal enzymes after myocardial infarction, a condition known to release lysosomal enzymes *in vivo*. Since no increase in the activity of β -N-acetylglucosaminidase was detected immediately after exercise (Table 1) or 24 h later (G. J. Kasperek, unpublished work), no irreversible cell damage was suspected. The increase in lysosomal osmofragility with exercise also suggested that exercise caused the lysosomes to become more fragile but not to rupture until they were stressed. Since the increase in lysosomal fragility occurred

gradually during the exercise bout, it was not the result of an abrupt event that occurs at exhaustion. Thus our data support the hypothesis that exercise causes an increase in the number of autophagic vacuoles, the fusion of which with primary lysosomes results in a redistribution of the lysosomal enzymes into the more fragile autophagolysosomes. This process of autophagy increases as the duration of the exercise increases in a manner that parallels the loss of liver protein.

This autophagolysosomal hypothesis was tested biochemically and ultrastructurally. At the biochemical level, we reasoned that the autophagic process should not be selective and should thus lead to the decrease in other liver components in addition to protein and glycogen. Although 99% of this weight loss was accounted for by the loss of water, glycogen and protein, there was also a significant loss of RNA and phospholipid. The constancy of the DNA content suggested that the liver weight loss was due to a decrease in the weight of each liver cell rather than the loss of liver cells.

If the autophagolysosomal system is responsible for the increased loss of protein during exercise, the loss should be from all protein fractions, since this pathway is characterized as being non-specific. Exercise caused the loss of cytoplasmic protein, total mitochondrial protein, soluble mitochondrial protein and microsomal protein from rat liver. Furthermore, exercise resulted in the lowering of the activity of enzymes in the cytoplasm, mitochondria and endoplasmic reticulum, suggesting that exercise non-specifically removed protein from these fractions. The activities of the two lysosomal enzymes cathepsin B and β -N-acetylglucosaminidase were not significantly lowered by exercise, which is also consistent with the involvement of the autophagolysosomal system in exercise-induced protein degradation. The constancy of the activity of phosphoenolpyruvate carboxykinase could be explained by an exercise-induced activation of a smaller amount of enzyme (Huston *et al.*, 1975).

Although a precise morphometric ultrastructural study was not performed, examination of the micrographs revealed the presence of more autophagic vacuoles in the parenchymal cells of the exercised rats than in the controls. In addition, exercise caused an increase in lysosomal size. This is due largely to increased numbers of secondary lysosomes containing non-identifiable dense material, which we suspect are the remnants of cellular constituents degraded by the autophagolysosomal system. Thus the exercise-induced redistribution of lysosomal enzyme activity into the non-sedimentable fraction is analogous to the redistribution caused by glucagon (Deter & de Duve, 1967) and ischaemia (Cole *et al.*, 1971), and is unlike the redistribution caused by starvation, where no

increase in the number of autophagic vacuoles was observed (Pfeifer, 1973).

The signal that causes autophagy is not known with certainty. The autophagy in rat liver perfused with unsupplemented media can be decreased by the addition of amino acids and insulin (Neely *et al.*, 1974). Glucagon-induced autophagy can be suppressed by certain glucogenic amino acids, with glutamine being the most effective (Schworer & Mortimore, 1979). During exercise the plasma insulin concentration is low and that of glucagon high (Fell *et al.*, 1980), and this hormonal status could induce autophagy. In addition, plasma and liver concentrations of glutamine are lowered by exercise (Dohm *et al.*, 1981), and this could also induce autophagy. Thus the results of the present study are consistent with our hypothesis that the protein loss, as well as the loss of other liver constituents, is due to autophagy increased by lower than normal concentrations of insulin and glutamine, and by a higher than normal concentration of glucagon.

This work was supported in part by a grant from North Carolina United Way. We acknowledge the technical assistance of Mr. E. B. Tapscott, Ms. N. Sehgal and Mr. D. S. Kerr.

References

- Ballard, F. J. (1977) *Essays Biochem.* **13**, 1–37
- Barnes, D. W., Morahan, P. S., Loveless, S. & Munson, A. E. (1979) *J. Pharmacol. Exp. Ther.* **298**, 392–398
- Barrett, A. J., Heath, M. F. (1977) in *Lysosomes: A Laboratory Handbook*, 2nd edn. (Dingle, J. T., ed.), pp. 19–145, Elsevier/North-Holland Biomedical Press, Amsterdam and New York
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Cole, S., Matter, A. & Karnovsky, M. J. (1971) *Exp. Mol. Pathol.* **14**, 158–175
- Dean, R. T. (1980) in *Degradative Processes in Heart and Skeletal Muscle* (Wildenthal, K., ed.), pp. 3–30, Elsevier/North-Holland Biomedical Press, New York
- Dean, R. T. & Barrett, A. J. (1976) *Essays Biochem.* **12**, 1–40
- Decker, R. S., Poole, A. R. & Wildenthal, K. (1980) *Circ. Res.* **46**, 485–494
- Desai, I. D. (1969) *Can. J. Biochem.* **47**, 758–790
- Deter, R. L. & de Duve, C. (1967) *J. Cell Biol.* **33**, 437–449
- Dohm, G. L., Puennte, F. R., Smith, C. P. & Edge, A. (1978) *Life Sci.* **23**, 845–850
- Dohm, G. L., Beecher, G. R., Warren, R. Q. & Williams, R. T. (1981) *J. Appl. Physiol.* **50**, 41–44
- Erickson, J. L. E. (1969) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Fell, H. B., eds.), pp. 345–394, American Elsevier Publishing Co., New York
- Fell, R. D., McLane, J. A., Winder, W. W. & Holloszy, J. O. (1980) *Am. J. Physiol.* **238**, R328–R332

- Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–776
- Holloszy, J. O. & Booth, F. W. (1976) *Annu. Rev. Physiol.* **38**, 273–291
- Hoskins, D. D. & Mackenzie, C. G. (1961) *J. Biol. Chem.* **236**, 177–183
- Hsu, R. Y. & Lardy, H. A. (1969) *Methods Enzymol.* **13**, 230–235
- Huston, R. L., Weiser, P. C., Dohm, G. L., Askew, E. W. & Boyd, J. B. (1975) *Life Sci.* **17**, 369–376
- Imai, Y., Ito, A. & Sato, R. (1966) *J. Biochem. (Tokyo)* **60**, 417–428
- Kasperek, G. J., Dohm, G. L., Tapscott, E. B. & Powell, T. (1980) *Proc. Soc. Exp. Biol. Med.* **164**, 430–434
- King, T. E. (1967) *Methods Enzymol.* **10**, 322–332
- Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352
- Langdon, R. G. (1966) *Methods Enzymol.* **9**, 126–131
- Lo, S., Taylor, J. C. & Taylor, A. W. (1970) *J. Appl. Physiol.* **28**, 234–236
- Loefering, D. J., Bonin, M. J. & Smith, J. J. (1975) *Exp. Mol. Pathol.* **22**, 242–251
- Loud, A. V. (1968) *J. Cell Biol.* **37**, 27–46
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Luft, J. H. (1961) *J. Biophys. Biochem. Cytol.* **9**, 409–414
- Maskrey, P., Pluskal, M. G., Harris, J. B. & Pennington, R. J. T. (1977) *J. Neurochem.* **28**, 403–409
- Nash, T. (1953) *Biochem. J.* **55**, 416–421
- Neely, A. N., Nelson, P. B. & Mortimore, G. E. (1974) *Biochim. Biophys. Acta* **338**, 458–472
- Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239**, 2370–2378
- Opie, L. H. & Newsholme, E. A. (1967) *Biochem. J.* **103**, 391–399
- Pfeifer, U. (1973) *Virchows Arch. B* **12**, 195–211
- Plate, C. A., Joshi, V. C., Sedgewick, B. & Wakil, S. J. (1968) *J. Biol. Chem.* **243**, 5439–5445
- Schworer, C. M. & Mortimore, G. E. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3169–3173
- Takeda, Y., Suzuki, F. & Inoue, H. (1969) *Methods Enzymol.* **13**, 153–160
- Wannemacher, R. W., Banks, W. L. & Wunner, W. H. (1965) *Anal. Biochem.* **11**, 320–326
- Welman, E., Selwyn, A. P., Peters, T. J., Colbeck, J. F. & Fox, K. M. (1978) *Cardiovasc. Res.* **12**, 99–105