Biochem. J. (1980) **188**, 255–262
Printed in Great Britain

Effect of Exercise on Synthesis and Degradation of Muscle Protein

G. Lynis DOHM,* George J. KASPEREK,* Edward B. TAPSCOTT* and Gary R. BEECHER†
**Department of Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27834, U.S.A., and †Protein Nutrition Laboratory, Nutrition Institute, Science and Education Administration, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.*

(*Received 13 August 1979*)

Several reports have shown that amino acid utilization via oxidation and gluconeogenesis is increased during exercise. The purpose of this study was to investigate whether these changes are accompanied by alterations in protein synthesis and degradation in the muscle of exercising rats. One group of rats was made to swim for 1 h and then protein synthesis and protein degradation were measured in a perfused hemicorpus preparation. Protein synthesis was decreased and protein degradation was increased in exercised rats compared with sedentary control rats. Exercise also decreased amino acid incorporation by isolated polyribosomes from muscle. Measurement of several muscle proteinase activities demonstrated that exercise had no effect on alkaline proteinase or Ca^{2+}-activated proteinase. However, the free (unbound) cathepsin D activity was elevated in muscle of exercised rats, whereas the total activity of cathepsin D was unchanged. This increase in the proportion of free cathepsin D activity suggests that lysosomal enzymes may be involved in the increased protein degradation that was observed.

Several reports have been published which demonstrate that protein metabolism is altered by exercise. Gontzea *et al.* (1974, 1975) showed that exercise decreased nitrogen balance (increased nitrogen excretion) of men on a diet providing adequate energy supply. Haralambie & Berg (1976) found that plasma urea is increased by exercise, and Décombaz *et al.* (1979) showed that urea production was increased by 44% after a 100km run. Dohm *et al.* (1977*b*) demonstrated that trained rats have a greater capacity to oxidize leucine than their untrained counterparts. In preliminary communications White & Brooks (1977) and Askew *et al.* (1979) reported that $^{14}CO_2$ production from [^{14}C]leucine was greater in exercising than in sedentary rats. All of these reports seem to suggest increased amino acid oxidation during exercise, despite the opinion of many investigators that catabolism of protein makes no contribution to energy expenditure (Åstrand & Rodahl, 1970).

Another role that amino acids may play during exercise is as gluconeogenic precursors. Results of Huston *et al.* (1975) and Munoz-Clares *et al.* (1979) suggest that gluconeogenesis is increased during exercise. Felig & Wahren (1971) observed that alanine is released by exercising muscle and is then taken up by liver, to be converted into glucose. Chang & Goldberg (1978) showed that the carbon atoms of alanine come primarily from pyruvate, whereas the amino group is supplied by transamination with branched-chain amino acids. The alanine (and possibly other glucogenic amino acids) released during exercise may be taken up by the liver and kidney for gluconeogenesis.

Since amino acid utilization is increased during exercise, it is important to determine the source of the amino acids that are being used. Two possible ways to supply amino acids for utilization during exercise would be depletion of plasma and tissue free amino acid pools and depletion of tissue proteins. Haralambie & Berg (1976) found that plasma tyrosine increased during exercise, and they suggested that protein breakdown was increased during exercise. Décombaz *et al.* (1979) found a decrease in most free amino acids in plasma after a 100km race and conclude that amino acid utilization occurs at the expense of the amino acid pool. We have reported that muscle and liver protein contents were lowered by exercise in rats (Dohm *et al.*, 1978). However, since measuring protein depletion as a difference in protein contents of two groups (rested and exercised) involves determination of a small difference between two large quantities (with large variability), we have investigated the effect of an acute exercise bout on protein synthesis and protein degradation in a perfused muscle preparation. The results show that

0306-3283/80/040255-08$01.50/1

exercise causes a decrease in muscle protein synthesis and an increase in muscle protein degradation.

Experimental

Animals

Two different types of exercise were used in the various experiments; swimming and treadmill running. For the experiments involving swimming, male Holtzman rats weighing 300–325 g were divided into a rested group and an exercised group that was made to swim for 1 h immediately before the experiment. Four rats were put into a plastic tank (40 cm diameter and 40 cm deep) filled with water at 32°C. Putting several rats in the tank at one time causes them to swim continuously rather than float.

When treadmill running was selected as a form of exercise, male Holtzman rats weighing 200–225 g were made to run on a motor-driven treadmill (Dohm *et al.*, 1977*a*) at 30 m/min until they were exhausted. We have defined exhaustion as the point at which the rats cannot right themselves when placed on their backs. On our treadmill, untrained rats can be made to run to exhaustion and the running time varies between 1 and 3 h. An appropriate control (rested) group was always killed at the same time as the exercised rats.

Assay of protein synthesis and degradation

The muscle perfusion system that was used was modelled after those described by Ruderman *et al.* (1971) and Jefferson (1975). After anaesthesia with sodium pentobarbital (5.0 mg/100 g body wt.), a midline abdominal incision was made and the skin reflected. The intestines were carefully reflected to permit access to the great vessels, two ligatures were placed about the lower colon (about 3 cm apart) and the segment of colon between them was excised. The aorta and vena cava were carefully separated just below the insertion of the iliolumbar vessels and ligatures were placed loosely around each vessel. A ligature was placed about the internal spermatic vessel and the testes were excised. The iliolumbar vessels and the left renal vessels were also ligated securely. Loose ligatures were placed about the aorta just anterior to the left renal vein and about the vena cava just posterior to the junction with the right renal vein. Another ligature was placed around both great vessels just anterior to the junction with the iliolumbar vessels. Ligatures about the aorta and vena cava were quickly tightened and the animal was transected just anterior to the last secured ligature. The hemicorpus was transferred to the perfusion chamber (maintained at 35°C) and cannulae were quickly inserted into the aorta and vena cava. Flow of the perfusion media was started as soon as possible and the interruption of oxygenation of the hemicorpus was less than 2 min from the ligation of the aorta until flow was re-established.

The hemicorpus was perfused with Krebs/Henseleit solution (Krebs & Henseleit, 1932) containing 5.5 mM-glucose, 0.15 mM-pyruvate, 4% (w/v) bovine serum albumin, washed human erythrocytes at a haematocrit of approx. 25, and the 20 common amino acids at 5 times their plasma concentration (Beecher *et al.*, 1979), except tyrosine, which was 15 times normal plasma concentration. The erythrocytes were obtained from blood-bank time-expired (22-day-old) blood which had been rejuvenated by the procedure of Valeri (1974) as recommended by Rennie & Holloszy (1977). The flow rate was maintained at 10–12 ml/min. The perfusate from the first 5 min of the perfusion was collected and retained. The remainder of the perfusate was recirculated for the duration of the perfusion. The perfusion medium was oxygenated in a Silastic tube oxygenator, which consisted of 12 Silastic tubes in parallel (35 cm long, 1 mm in diameter and 0.2 mm wall thickness) which were suspended in another tube flushed with oxygen.

To determine the rate of protein synthesis in perfused muscle, [^{3}H]tyrosine ([3,5-*ring*-^{3}H]tyrosine; New England Nuclear, Boston, MA, U.S.A.) was added to the perfusion medium (0.25 μCi/ml of perfusate) and incorporation of radioactivity into muscle protein was determined. Muscle was quickly removed, rinsed in ice-cold 0.15 M-KCl, pressed through a tissue press (Harvard Apparatus, Mills, MA, U.S.A.), and homogenized in 0.03 M-potassium phosphate, pH 7.4 (0.1 g of muscle/ml of buffer) in a glass/Teflon homogenizer. A portion of the homogenate was kept to determine total muscle protein and the remainder was fractionated into soluble, myofibrillar and insoluble (stromal) fractions by a modification of the method of Helander (1957). The fractionation procedure was carried out at 0–4°C to prevent proteolysis. The homogenate was centrifuged at 15000 *g* for 10 min and the precipitate washed with an equal volume of 0.03 M-potassium phosphate (pH 7.4). The two supernatants (S1 and S2) contained the soluble protein fraction. To the precipitate was added 1.1 M-KI containing 0.3 M-potassium phosphate (pH 7.4) in a volume equal to the volume of homogenate being fractionated. The precipitate was resuspended and the mixture was incubated overnight in a shaking bath at 4°C and then centrifuged at 15000 *g* for 10 min. The supernatant (M1) was decanted and the precipitate was resuspended in an equal volume of 1.1 M-KI containing 0.3 M-potassium phosphate (pH 7.4). After incubation at 4°C for 1 h the mixture was centrifuged and the supernatant (M2) decanted. The precipitate was resuspended in 0.3 M-KOH and incubated at 55°C for approx. 3 h, and this

treatment resulted in a homogeneous solution, which is referred to as the insoluble (stromal) fraction. This insoluble fraction probably does not contain appreciable amounts of collagen, as suggested by Helander (1957), because most of the connective tissue is trapped on the screen when the muscles are pressed, and in addition very little precipitate remains after incubation in 0.3 M-KOH, as would be expected for collagen.

Incorporation of [^{3}H]tyrosine into muscle protein was determined for the total homogenate, fraction S1 (sarcoplasmic), fraction M1 (myofibrillar) and the insoluble fraction. A portion of each sample was taken for protein analysis by the biuret method (Gornall *et al.*, 1949) and the protein of a second 2.0ml sample was precipitated with 8.0ml of 10% (w/v) trichloroacetic acid. The precipitate was filtered in a Hoefer filter apparatus (Hoefer Apparatus Co., San Francisco, CA, U.S.A.) by using Whatman 42 ashless filter paper of 25mm diameter. The precipitate was washed with 3 x 2.0ml of 10% trichloroacetic acid, and then the filter paper and precipitate were dried and burned in a Packard model 306 sample oxidizer (Packard Instrument Co., Downers Grove, IL, U.S.A.). The ^3H_2O was collected in the scintillation cocktail prepared by Packard for the 306 oxidizer and the radioactivity was counted in a Beckman LS 233 liquid-scintillation counter. Counting efficiency was determined by the external-standardization method. The protein-synthesis rate was calculated by dividing the rate of incorporation of [^{3}H]tyrosine (d.p.m./h per mg of protein) by the specific radioactivity of [^{3}H]tyrosine (d.m.p./nmol) in the perfusate at the midpoint of the perfusion. The usual perfusion time was 60min.

Protein degradation was determined by measuring the dilution of [^{3}H]tyrosine by unlabelled tyrosine released from muscle protein. The specific radioactivity in the perfusate was determined after addition of 4.5ml of 10% trichloroacetic acid to 0.5ml of the perfusate and removal of the precipitate by centrifugation (500*g* for 10min). A sample of the acid supernatant was counted for radioactivity in a liquid-scintillation counter and the amount of tyrosine was determined in another portion by the method of Waalkes & Udenfriend (1957). The rate of protein degradation was calculated by the method described by Jefferson (1975).

Muscle protein synthesis *in vitro* was assayed by using isolated polyribosomes, cytosol, L-[U-^{14}C]-leucine, known concentrations of the common amino acids, and required cofactors by a modification (Bjercke, 1971) of the methods of Huston *et al.* (1970).

To determine the amount of free tyrosine in muscle, sulphosalicylic acid extracts were prepared and quantified by the methods described by Beecher (1978).

Assay of muscle metabolites

Muscles were quick frozen between liquid-N_2-cooled tongs and pulverized in a percussion mortar. $HClO_4$ extracts were prepared and metabolites assayed enzymically: ATP, ADP and AMP were assayed by the methods of Lamprecht & Trautschold (1974) and Jaworek *et al.* (1974), lactate was measured as described by Gutmann & Wahlefeld (1974), and glycogen was determined by the method of Lo *et al.* (1970).

Proteinase assays

For the assay of free (unbound) cathepsin D activity, a 20000*g* (10min) supernatant was prepared from a muscle homogenate (1:10, w/v, in 0.25 M-sucrose/0.001 M-Tris/HCl, pH 7.4). The total cathepsin D was assayed in a supernatant like that used for assay of free cathepsin D, except that Triton X-100 was added to the homogenization medium (0.2%, v/v, final concentration) to release the enzyme contained in the lysosome. Cathepsin D activity was measured by a modification of the procedure of Anson (1936). Muscle supernatant (0.05ml) was mixed with 0.05ml of a solution containing 0.15 M-sodium citrate, pH 3.0, and 2mg of methaemoglobin/ml (0.2μCi of [^{14}C]methylated methaemoglobin; New England Nuclear). The reaction was stopped by adding 0.075ml of 13% trichloroacetic acid, the precipitate was removed by centrifugation at 80000*g* for 30s in a Beckman Airfuge, and 0.1ml of the supernatant was counted for radioactivity in a liquid-scintillation counter (Beckman LS 233).

Alkaline proteinase activity was assayed in the myofibrillar fraction by the method of Mayer *et al.* (1974). Ca^{2+}-activated neutral proteinase activity was assayed by a modification of the method of Kar & Pearson (1976). A 0.5ml sample of the homogenate (1:10, w/v, in 0.25 M-sucrose/0.001 M-Tris/HCl, pH 7.4) was added to 1.0ml of a solution that contained 0.15 M-Tris/HCl (pH 7.5), 1.5 mM-$CaCl_2$, 1.5 mM-NaN_3, 15 mM-mercaptoethanol and Casein Yellow (3mg). After an 18h incubation at 37°C the reaction was stopped by adding 1.5ml of 10% trichloroacetic acid, the precipitate was removed by centrifugation at 2000*g* for 10min, 2.0ml of the supernatant was mixed with 3ml of 7% (w/v) KOH and the A_{428} of the clear solution was measured. A time course showed the assay to be linear with time for at least 18h, and proteinase activity was increased 4-fold by Ca^{2+} (with a medium containing EDTA instead of $CaCl_2$ as a control).

Results

Although perfused hemicorpus systems have been used for studies on protein synthesis and deg-

radation (Jefferson *et al.*, 1977), it was necessary to characterize the system in our laboratory. The results in Table 1 demonstrate that energy metabolite contents in perfused muscles were quite similar to those found in normal control muscles. Fig. 1 shows that the specific radioactivity of [^{3}H]tyrosine in the perfusate decreases during the perfusion. The decline in perfusate [^{3}H]tyrosine specific radioactivity is faster during the first 20 min of the perfusion, and this is probably due to the efflux of unlabelled free tyrosine from the muscle. After 20 min the rate of change is constant, and this part of the curve is used to calculate the rate of protein degradation.

Table 1. *Metabolite concentrations in muscle from intact rats and perfused muscle*

Values are means ± S.E.M.

	Control muscles ($n = 8$)	Perfused muscles ($n = 4$)
ATP (μmol/g dry wt. of muscle)	20.1 ± 0.6	19.0 ± 0.3
ADP (μmol/g dry wt. of muscle)	3.8 ± 0.2	4.9 ± 0.1
AMP (μmol/g dry wt. of muscle)	0.37 ± 0.07	0.55 ± 0.06
Glycogen (mg/g dry wt. of muscle)	22.2 ± 1.3	22.7 ± 1.8
Lactate (μmol/g dry wt. of muscle)	18.8 ± 1.6	20.6 ± 1.3

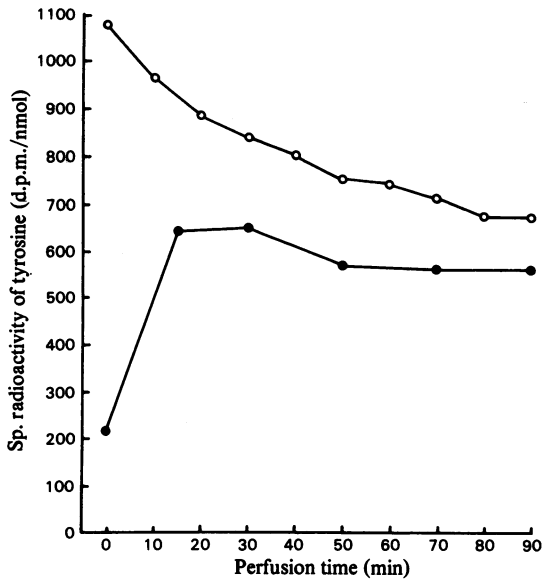

Fig. 1. *Specific radioactivity of* [^{3}*H*]*tyrosine in the perfusate* (○) *and muscle* (●) *during a perfusion*

The radioactivity and the amount of tyrosine were analysed as described in the Experimental section. Muscle specific radioactivity was not corrected for the extracellular space. Each point represents the mean of five observations.

The specific radioactivity of [^{3}H]tyrosine in muscle (Fig. 1) equilibrates and remains rather constant after 15 min. The specific radioactivities of [^{3}H]tyrosine in muscle and perfusate appear to be converging after 90 min of perfusion (Fig. 1), but the two pools are not completely equilibrated. In perfused heart, McKee *et al.* (1978) found that the specific radioactivity of [^{14}C]phenylalanine bound to tRNA (the precursor pool for protein synthesis) was intermediate between the specific radioactivities in the intracellular and extracellular pools at low perfusate phenylalanine concentrations. Jefferson *et al.* (1977) were able to equilibrate the intracellular and extracellular specific radioactivities of [^{3}H]-phenylalanine by using 5 times normal plasma concentrations of phenylalanine, and thus the need to measure the specific radioactivity of phenylalanine bound to tRNA was eliminated. In our perfusion system the intracellular and extracellular specific radioactivities of [^{3}H]tyrosine did not equilibrate completely even at 15 times the normal plasma concentration. Thus, in order to calculate the rate of protein synthesis we could either measure the specific radioactivity of tyrosine bound to tRNA or use the specific radioactivity in the perfusate or the muscle to estimate the specific radioactivity of the precursor pool. Since our purpose was to investigate the relative change (compared with a control group) in the rates of protein synthesis, we did not consider it necessary to determine the absolute protein-synthesis rate, and thus we chose to use the specific radioactivity of [^{3}H]tyrosine in the perfusate at the midpoint of the perfusion. In preliminary studies, we calculated the synthesis rate from the [^{3}H]tyrosine specific radioactivity in the perfusate and from the specific radioactivity in muscle and obtained values of 0.24 ± 0.02 and 0.39 ± 0.03 nmol/h per mg of protein respectively. The true synthesis rate probably lies between these two values, and thus our estimate (obtained by using the perfusate specific radioactivity) probably underestimates the true synthesis rate.

The rate of protein degradation is calculated in our system from the change in specific radioactivity of [^{3}H]tyrosine in the perfusate. Since the intracellular and extracellular specific radioactivities are not equal, our reported values may not be the absolute degradation rates. However, since the specific radioactivity in muscle remains relatively constant, changes in specific radioactivity in the perfusate should reflect the rate of release of unlabelled tyrosine. Since our rates compare favourably with those reported by other investigators, we consider that the values that we obtain are representative of the rates of protein synthesis and degradation. Jefferson *et al.* (1977), using a perfused

hemicorpus preparation, reported rates for protein synthesis (84 ± 8 nmol/h per g of muscle) and protein degradation (88 ± 4 nmol/h per g of hemicorpus) in the absence of insulin. If we express our results in compatible units they are very comparable with those published by Jefferson *et al.* (1977) (72 ± 4 nmol/h per g of muscle for the synthesis rate and 79 ± 5 nmol/h per g of hemicorpus for the degradation rate).

Incorporation of [^{3}H]tyrosine into total muscle protein (Fig. 2) is linear with time during the first 60 min of the perfusion, but decreases after 60 min. This fall in the rate of protein synthesis may be caused by a lack of insulin in the perfusion media, as shown by Jefferson *et al.* (1977). The finding that more than 1 h of perfusion was required to 'wash out' the insulin stimulation of protein synthesis suggested that results obtained during the first 1 h of perfusion may demonstrate an effect *in vivo* of a physiological treatment, such as exercise.

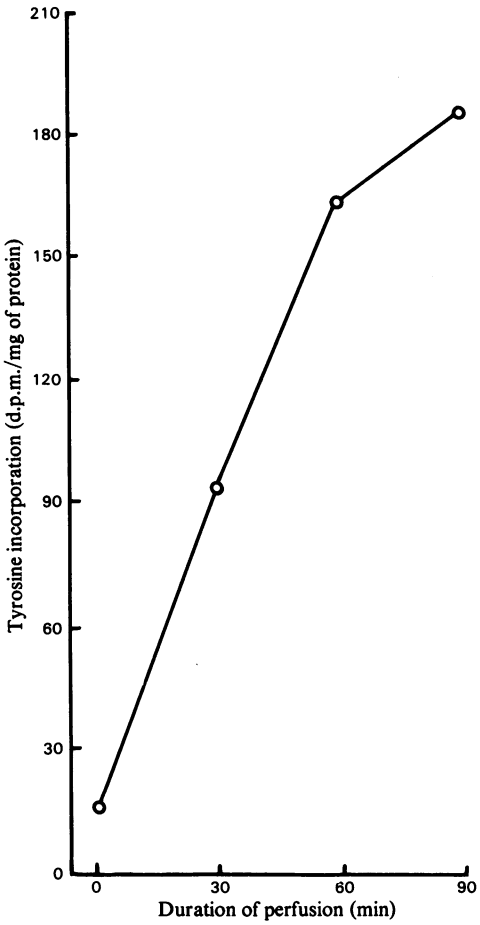

Fig. 2. *Incorporation of [^{3}H]tyrosine into total muscle protein during the perfusion*

Each point represents the mean of five perfusions. The data point at zero time was taken after the 5 min washout.

The effect of an acute bout of exercise (1 h swim) on protein synthesis in perfused muscle is shown in Table 2. Incorporation of [^{3}H]tyrosine was decreased in all three protein fractions by the exercise bout. We also investigated the effect of exercise on amino acid incorporation by muscle polyribosomes (Table 3) and found, in agreement with the preceding experiment, that exercise decreased protein synthesis. The extent of the change was greater when the rats were run to exhaustion than when they were made to swim for 1 h, but this probably reflects the severity of the stress.

An acute bout of exercise (1 h swim) also altered the rate of protein degradation in perfused muscle, with the exercised rats having a significant increase of 55% in the rate of protein breakdown (from 8.9 ± 0.6 to 13.8 ± 1.4 μmol of tyrosine released/h per hemicorpus; means ± S.E.M. of at least eight observations; $P < 0.05$). Since the dilution of [^{3}H]-tyrosine was being used as a measure of protein degradation, we also determined the effect of exercise on the amount of free tyrosine in the muscle.

Table 2. *Effect of an acute bout of exercise (1 h swim) on protein synthesis in perfused muscle*

Values are means ± S.E.M. for at least eight observations. A statistically significant difference ($P < 0.05$) between rested and exercised animals is shown by an asterisk (*).

	[^{3}H]Tyrosine incorporation (nmol/h per mg of protein)		
	Soluble fraction	Myofibrillar fraction	Insoluble fraction
Rested	0.36 ± 0.02	0.32 ± 0.01	0.31 ± 0.02
Exercised	0.30 ± 0.02*	0.26 ± 0.02*	0.26 ± 0.01*

Table 3. *Effect of an acute bout of exercise on [^{14}C]leucine incorporation into muscle protein by isolated polyribosomes*

Values are means ± S.E.M. for the numbers of observations in parentheses. A statistically significant difference ($P < 0.05$) between rested and exercised animals is shown by an asterisk (*).

	[^{14}C]Leucine incorporated (pmol/min per mg of RNA)	
	Rested	Exercised
Expt. 1 (run to exhaustion)	5.8 ± 0.5 (9)	3.2 ± 0.4 (4)*
Expt. 2 (swimming for 1 h)	3.4 ± 0.6 (5)	2.5 ± 0.3 (5)

The results shown in Table 4 demonstrate that exercise (either swimming for 1 h or running to exhaustion) caused an increase in tyrosine. The observed increase in muscle tyrosine in exercised rats raised the possibility that the free muscle tyrosine might not equilibrate with perfusate [^{3}H]-tyrosine as rapidly in the exercised rats as in rested animals (Fig. 1). To investigate this point, an experiment was performed to determine the specific radioactivity of [^{3}H]tyrosine in muscle of rested and exercised rats. The results (Table 5) demonstrate that perfusate [^{3}H]tyrosine equilibrates with muscle tyrosine before 15 min of perfusion in both the exercised and the rested rats. Since the muscle tyrosine pool is rapidly equilibrated, the dilution of [^{3}H]tyrosine (see above) should provide an accurate reflection of protein degradation.

Table 4. *Free tyrosine contents in gastrocnemius muscles from control and exercised rats*

Values are means ± S.E.M. for at least six observations. A statistically significant difference ($P < 0.05$) between rested and exercised rats is shown by an asterisk (*).

	Tyrosine (μmol/g dry wt. of muscle)	
	Control (rested)	Exercised
Swimming exercise (1 h)	0.36 ± 0.07	0.66 ± 0.07*
Treadmill running to exhaustion	0.61 ± 0.03	0.94 ± 0.12*

Since an increase in muscle protein degradation could be caused by an increase in the activity of one of the known muscle proteinases, we have measured the activities of three enzymes that are reported to be important in muscle protein turnover. Neither alkaline proteinase nor Ca^{2+}-activated proteinase activities were altered by exercise (Table 6). However, 'free' (or unbound) cathepsin D was increased in muscle of exercised rats, whereas the total activity of cathepsin D was unchanged.

Discussion

The results of the present study demonstrate that protein synthesis is decreased and protein degradation is increased in muscle of exercised compared with sedentary rats. These results are in agreement with findings of Zimmer & Gerlach (1973), who found decreased muscle protein synthesis in exercised rats, and those of Haralambie & Berg (1976), who report elevated tyrosine concentrations in plasma of exercised human subjects. The report of Décombaz *et al.* (1979) that urinary excretion of 3-methylhistidine was not increased by a 100 km race seems to be in conflict with the present results. Urinary 3-methylhistidine is accepted by many investigators as an index of muscle contractile protein breakdown. However, under conditions where large sweat loss occurs, it seems likely that 3-methylhistidine would be excreted along with other nitrogenous products that are known to be excreted in sweat; thus excretion in both urine and sweat should be measured to account for the

Table 5. *Effect of an acute bout of exercise (1 h swim) on specific radioactivity of free [^{3}H]tyrosine in gastrocnemius muscle after perfusion for various periods of time*

Values are means ± S.E.M. for six observations.

	Specific radioactivity of [^{3}H]tyrosine (d.p.m./nmol) after various perfusion times			
	15 min	30 min	45 min	55 min
Rested animals	1141 ± 20	Not determined	Not determined	1045 ± 36
Exercised animals (1 h swim before perfusion)	1015 ± 61	1050 ± 45	1017 ± 28	993 ± 23

Table 6. *Effect of exercise (run to exhaustion) on muscle proteinase activity*

Values are means ± S.E.M. for at least eight observations. A statistically significant difference ($P < 0.05$) between rested and exercised animals is shown by an asterisk (*).

	Rested	Exercised
Alkaline proteinase (μg of tyrosine/h per g of muscle)	268 ± 42	234 ± 32
Ca^{2+}-activated proteinase (0.001 ΔA/h per g of muscle)	33.0 ± 1.6	30.9 ± 1.7
Cathepsin D (total activity) (μg of haemoglobin degraded/min per g of muscle)	18.2 ± 0.8	15.8 ± 0.8
Cathepsin D ('free') (μg of haemoglobin degraded/min per g of muscle)	2.58 ± 0.28	4.76 ± 0.35*
'Free' Cathepsin D (% of total)	16.2 ± 1.7	29.9 ± 2.2*

total 3-methylhistidine excretion. Further research is necessary to investigate the apparent discrepancy between our results and those of Décombaz *et al.* (1979).

The increased breakdown of muscle protein during exercise is a somewhat surprising finding, since the loss of protein could be a factor in fatigue and exhaustion. Therefore, these results raise the teleological question of why an animal degrades muscle protein during exercise. One possible fate of the amino acids released by protein breakdown would be to serve as oxidizable substrates to cover the increased energy expenditure. Since the calculated protein breakdown rate was 20mg/h, this would be equivalent to 0.33kJ (0.08kcal), or 16.7kJ (4kcal)/g of protein. Since an exercising rat expends approx. 21kJ (5kcal)/h (net energy expenditure was calculated for a 300g rat running at 28.5m/min as previously described by Dohm *et al.*, 1977*a*), the energy value of the protein mobilized seems very small.

Another possible function of the amino acids that result from muscle protein degradation during exercise would be as a source of amino groups for the removal of pyruvate via the alanine cycle. In conjunction with the alanine cycle, the glucogenic amino acids could serve as gluconeogenic precursors to maintain blood glucose concentrations as the exercise progressed.

In previous studies we found that exhaustively exercising trained rats resulted in the loss of activity of several enzymes (Huston *et al.*, 1975; Dohm *et al.*, 1973). In the light of the present results, it seems possible that such a loss of enzyme activity might be the result of increased proteolytic activity during exercise.

We were particularly interested in the mechanism that caused the increased muscle protein breakdown. We therefore measured the activities of several muscle proteinases and found that the proportion of free (unbound) cathepsin D was increased in the exercised rats. This increase in the proportion of free lysosomal enzyme activity resembles the alteration found by Wildenthal (1978) in ischaemic heart, where lysosomal enzymes were found to leak out of the lysosomes into the cytoplasm. Thus leakage could play a role in the increased degradation during exercise.

Vihko *et al.* (1978) and Pilström *et al.* (1978) have reported that training or a single bout of exhaustive exercise increases the total (free plus bound) activity of cathepsin D and several other lysosomal enzymes. Vihko *et al.* (1978) found that these changes closely resembled those caused by ischaemia in rabbit muscle. They concluded that the two treatments, i.e. exhaustive exercise and temporary ischaemia, cause similar cell injuries, and that the lysosomal system seems to function in the post-stress recovery of the fibres from these injuries. We consider that the cell injury suggested by Vihko *et al.* (1978) may be caused by the increased protein breakdown that we observe during the exercise bout. The elevated activities of the lysosomal enzymes during the period of 1–5 days after exercise (Vihko *et al.*, 1978) may represent an adaptation of the muscle to 'clean up' damaged or partially degraded proteins as part of the recovery process.

The research was supported by grant 1 R01 AM19116 from the U.S. Public Health Service. We acknowledge the technical assistance of Mr. R. Q. Warren, Mr. T. Powell and Mr. A. Edge. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

References

Anson, M. L. (1936) *J. Gen. Physiol.* **20**, 565–574
Askew, E. W., Klain, G. J., Lowder, J. F., Jr. & Wise, W. R. (1979) *Med. Sci. Sports* **11**, 73
Åstrand, P. O. & Rodahl, K. (1970) *Textbook of Work Physiology*, McGraw-Hill, New York
Beecher, G. R. (1978) *Adv. Exp. Med. Biol.* **105**, 827–840
Beecher, G. R., Puente, F. R. & Dohm, G. L. (1979) *Biochem. Med.* **21**, 196–201
Bjercke, R. J. (1971) M.Sc. Thesis, Kansas State University
Chang, T. W. & Goldberg, A. L. (1978) *J. Biol. Chem.* **253**, 3677–3684
Décombaz, J., Reinhardt, P., Ananthanaman, K., Von Glutz, G. & Poortmans, J. R. (1979) *Eur. J. Appl. Physiol.* **36**, 61–72
Dohm, G. L., Huston, R. L., Askew, E. W. & Fleshood, H. L. (1973) *Can. J. Biochem.* **51**, 849–854
Dohm, G. L., Beecher, G. R., Stephenson, J. P. & Womack, M. (1977*a*) *J. Appl. Physiol.* **42**, 753–757
Dohm, G. L., Hecker, A. L., Brown, W. E., Klain, G. J., Puente, F. R., Askew, E. W. & Beecher, G. R. (1977*b*) *Biochem. J.* **164**, 705–708
Dohm, G. L., Puente, F. R., Smith, C. P. & Edge, A. (1978) *Life Sci.* **23**, 845–850
Felig, P. & Wahren, J. (1971) in *Muscle Metabolism During Exercise* (Pernow, B. & Saltin, B., eds.), pp. 205–214, Plenum Press, New York
Gontzea, I., Sutzescu, R. & Dumitrache, S. (1974) *Nutr. Rep. Int.* **10**, 35–43
Gontzea, I., Sutzescu, R. & Dumitrache, S. (1975) *Nutr. Rep. Int.* **11**, 231–236
Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–776
Gutmann, I. & Wahlefeld, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 4, pp. 1464–1466, Academic Press, New York
Haralambie, G. & Berg, A. (1976) *Eur. J. Appl. Physiol.* **36**, 39–48
Helander, E. (1957) *Acta Physiol. Scand.* **41**, *Suppl.* **141**, 1–99

Huston, R. L., Schrader, L. E., Honold, G. R., Beecher, G. R. & Sauberlich, H. E. (1970) *Biochim. Biophys. Acta* **209**, 220–239

Huston, R. L., Weiser, P. C., Dohm, G. L., Askew, E. W. & Boyd, J. B. (1975) *Life Sci.* **14**, 369–376

Jaworek, P., Gruber, W. & Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 4, pp. 2127–2129, Academic Press, New York

Jefferson, L. S. (1975) *Methods Enzymol.* **39**, 73–82

Jefferson, L. S., Li, J. B. & Rannels, S. R. (1977) *J. Biol. Chem.* **252**, 1476–1483

Kar, N. C. & Pearson, C. M. (1976) *Clin. Chim. Acta* **73**, 293–297

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lamprecht, W. & Trautschold, I. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 4, pp. 2101–2109, Academic Press, New York

Lo, S., Taylor, J. C. & Taylor, A. W. (1970) *J. Appl. Physiol.* **28**, 234–236

Mayer, M., Amin, R. & Shafrir, E. (1974) *Arch. Biochem. Biophys.* **161**, 20–25

McKee, E. E., Cheung, J. Y., Rannels, D. E. & Morgan, H. E. (1978) *J. Biol. Chem.* **253**, 1030–1040

Munoz-Clares, R., Garcia-Ruiz, J. P., Vargas, A. & Sanchez-Medina, F. (1979) *FEBS Lett.* **99**, 340–342

Pilström, L., Vihko, V., Åström, E. & Arstila, A. U. (1978) *Acta Physiol. Scand.* **104**, 217–224

Rennie, M. J. & Holloszy, J. O. (1977) *Biochem. J.* **168**, 161–170

Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971) *Biochem. J.* **124**, 639–651

Valeri, C. R. (1974) in *The Red Blood Cell* (Surgener, D. M., ed.), pp. 528–532, Academic Press, New York

Vihko, V., Salminen, A. & Rantamäki, J. (1978) *Pflügers Arch.* **378**, 99–106

Waalkes, T. P. & Udenfriend, S. (1957) *J. Lab. Clin. Med.* **50**, 733–736

White, T. P. & Brooks, G. A. (1977) *Med. Sci. Sports* **9**, 67

Wildenthal, K. (1978) *J. Mol. Cell. Cardiol.* **10**, 595–603

Zimmer, H.-G. & Gerlach, E. (1973) in *Limiting Factors of Physical Performance* (Keul, J., ed.), pp. 102–109, Thieme, Stuttgart