The Effects of Long-Term Aerobic Exercise and Energy Restriction on Protein Synthesis

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Long-term aerobic exercise and energy intake regulate body composition in a complex manner. To study the combined effects of exercise and energy restriction on muscle mass, we measured skeletal and cardiac muscle protein synthesis after 28 days of two levels of energy restriction with or without daily running-wheel exercise in female rats. Protein synthesis was measured as ^{3}H-Phe incorporation 10 minutes' postbolus of a flooding pulse injection. The two exercise plus energy-restriction groups had greater skeletal muscle and cardiac muscle mass compared with their food-matched groups. Cardiac, gastrocnemius, and soleus muscle protein synthetic rates were proportional to their muscle masses. Exercise-induced energy deficits preserved cardiac and soleus mass to a greater extent than gastrocnemius mass, whereas the effects of energy restriction were similar in all three muscles. These findings suggest that energy intake and exercise have independent effects on the regulation of muscle mass and protein synthesis.

THE GOALS OF obesity treatment protocols are to maximize the loss of fat mass and to minimize the loss of muscle mass. Energy restriction alone is associated with the loss of both fat and lean body mass and is ineffective as a long-term treatment program.[1] Daily aerobic exercise programs have been advocated as adjunctive treatment for two reasons: first is the increased calorie expenditure and second is the decreased loss of lean body mass than can occur compared with energy restriction.[2] However, the best combination of exercise and energy restriction to maintain muscle and decrease fat has not been agreed upon.

Rodent studies indicate that long-term aerobic exercise programs induce a greater loss of adipose tissue and reduce the loss of lean body mass compared with the energy deficit produced by energy restriction alone.[3,4] In man, the ability of daily aerobic exercise regimens to maintain lean body mass and induce loss of fat mass is most effective when exercise is combined with maintenance of caloric intake.[2,5,6] However, the combination of daily aerobic exercise and energy restriction of 50% or greater may not preserve lean body mass or increase energy expenditure versus energy restriction alone.[7] The mechanisms by which daily aerobic exercise maintains lean body mass during energy deficits are unclear. The skeletal muscle protein synthetic rate responses to a single bout of aerobic exercise are dependent on both the severity and duration of exercise and the time postexercise at which the measurements are taken.[8-13] However, the effects of daily aerobic exercise on skeletal muscle mass and protein synthetic rates under conditions that maintain skeletal and cardiac muscle mass during caloric restriction are unknown.

This investigation was undertaken to study further the effects of long-term intermittent daily aerobic exercise and energy restriction on the regulation of muscle mass and protein synthetic rates. In previous studies, we reported that daily voluntary running-wheel exercise in rodents pair-fed to sedentary animals produced a weight loss equivalent to a 25% energy restriction.[4,14] In this study, skeletal and cardiac muscle fractional protein synthetic rates and muscle mass were measured after 28 days of daily running-wheel exercise and two levels of energy restriction and compared with those in weight- and energy-matched sedentary animals. The exercise groups were either pair-fed to sedentary ad libitum–fed animals or restricted to 75% of normal food intake and were compared with two groups of energy-restricted weight-matched rodents (75% and 50%).

MATERIALS AND METHODS

After protocols were approved by the Institutional Animal Care and Use Committee, Sprague–Dawley female rats (200 to 225 g) were obtained from Charles River Laboratories (Wilmington, MA). The animals were housed individually in Plexiglas cages (Rohm & Haas, Philadelphia, PA) cages and placed into five groups each with six animals: group 1, ad libitum–fed sedentary (control); group 2, pair-fed to group 1 + exercise (EX); group 3, energy-restricted 25% to group 1 + sedentary (25% ER); group 4, energy-restricted 25% to group 1 + exercise (EX + 25% ER); group 5, energy-restricted 50% to group 1 + sedentary (50% ER).

The food intake of the ad libitum–fed group was measured daily (Purina Rodent Chow, Ralston Purina, St Louis, MO), and appropriate quantities of pellets were administered to groups 2 through 5 daily for a period of 28 days. The two EX groups had exercise wheels (circumference, 1.09 m) with electronic tachometers within their Plexiglas cages to allow daily measurements of wheel revolutions. All measurements of protein synthesis were taken 12 hours after food was administered to the animals, and the animals were removed to separate cages without exercise wheels 8 hours before killing. All animals were injected with 50 μCi/100 g body weight (BW) ^{3}H-Phe plus 150 mmol Phe in a total volume of 1 mL into a superficial femoral vein. Ten minutes' postinjection, the heart, gastrocnemius, and soleus muscles were rapidly dissected and frozen in liquid N_2. Serum for determinations of thyroid hormone levels and specific activity of ^{3}H-Phe was stored at −20°C. On day 28, the remaining animals underwent similar studies.

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Technical Methodology

Serum concentrations of triiodothyronine and thyroxine were measured by sensitive and specific radioimmunoassays using thyroid hormone–depleted rat sera for standards.[15,16]

Protein synthetic rates. An adaptation of the method of Garlick et al[17,18] that uses a flooding-pulse dose of ^{3}H-Phe was used. This method of measuring the protein synthetic rate assumes that a "flooding" rapid intravenous injection of radiolabeled plus nonradioactive precursor amino acid establishes specific radioactivities that are equal in blood, the intracellular space, and the aminoacyl tRNA pools. The pulse injection containing 50 μCi/100 g BW 1-4-^{3}H-Phe (New England Nuclear, Boston, MA) and 150 mmol/L Phe (Sigma, St Louis, MO) in 1 mL 150-mmol/L NaCl was injected into a superficial femoral vein. Specific muscles were rapidly dissected after 10 minutes and frozen in liquid N_2. The specific activity of the unbound or free Phe in tissue was determined by first treating 0.5 g tissue with 2 mol/L cold perchloric acid. The pellet was centrifuged, and the supernatant (free fraction) was saved for determination of ^{3}H-Phe disintegrations per minute by liquid scintillation counting and for measurement of total Phe concentration by spectrophotometric analysis after separation by high-performance liquid chromatography. The pellet was then resuspended and hydrolyzed into single amino acids after overnight incubation with 6N HCL. Determination of bound ^{3}H-Phe and total bound Phe concentrations was performed after separation by high-performance liquid chromatography. Fractional protein synthetic rates of the heart and skeletal muscles were calculated from the formula of McNurlan and Garlick and expressed as K_S, percent fractional turnover per day[18]: K_S = (specific activity of ^{3}H-Phe_{bound})/(Σspecific activity of ^{3}H-Phe_{free} between 0 and 10 minutes).

Statistical analyses. Muscle weight and protein synthesis rate differences between groups were analyzed by a 2-way ANOVA using the level of caloric intake and daily exercise distance as the independent variables.[19] Linear regression analysis using the least mean squares technique was also performed.[19]

RESULTS

The time course for BW change over the 28-day experimental period is shown as the line graph in Fig 1. The EX group had a BW similar to that of the 25% ER group. The EX + 25% ER group had a lower BW than the EX or 25% ER groups alone, but had a greater BW than the 50% ER group. The two EX groups had similar mean daily exercise distances (meters per day), and the daily running-wheel distance for group EX is depicted as the bar graph at the bottom of Fig 1. The absolute change in BW versus mean daily running-wheel exercise distance for each exercising animal in groups 2 and 4 is depicted in Fig 2. There is a direct inverse correlation between the amount of total running distance and weight loss in these animals (r = .71, P < .001).

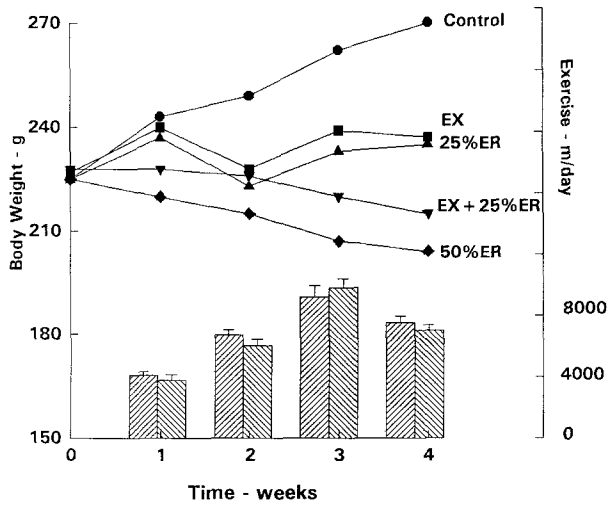

Fig 1. Line graph depicts the change in BW *v* time for the control, EX, 25% ER, EX + 25% ER, and 50% ER groups. Bar graph depicts the mean daily running-wheel distance achieved in m/d at weeks 1 through 4 for EX (▨) and EX + 25% ER (▧) groups.

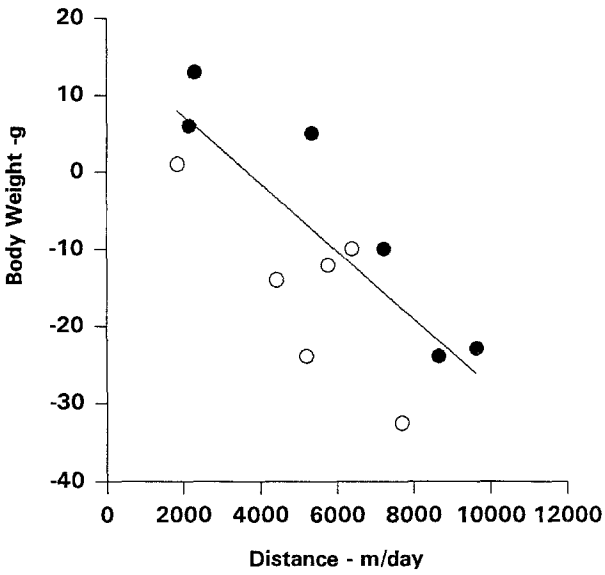

Fig 2. Line graph depicting the inverse linear relationship (r = .71, P < .001) for the EX (●) and EX + 25% ER (○) groups for the change in BW after 28 days *v* mean daily distance achieved on the running wheel.

Muscle weights and muscle weight to BW ratios are shown in Table 1. The absolute masses of the heart and skeletal muscles were decreased in a graded fashion in the ER groups (P < .02), but the heart and soleus muscle masses were maintained in the EX and EX + 25% ER groups. Gastrocnemius muscle masses were lower in the EX and EX + 25% ER groups versus controls, but were greater than those for the 25% and 50% ER groups (P < .02). There was a decrease in muscle mass in the EX + 25% ER versus EX groups for only the gastrocnemius muscle (P < .05). The muscle weight to BW ratios for all muscles were increased in both EX groups versus controls and were similar to control values in both ER groups.

Protein synthetic rates at 4 weeks are reported in Table 2. For the gastrocnemius muscle, K_S values were decreased more than 40% (P < .02) in both the EX and ER groups. For the soleus and cardiac tissues, there were minimal decreases in K_S values for the EX and 25% ER groups. In the two groups for which there were greater energy deficits (EX + 25% EA and 50% ER), K_S values for all three tissues were decreased markedly as compared with the control group. Individual values for protein synthesis rates

Table 1. Muscle Mass During Exercise and Energy Restriction

	Muscle Weights (mg)			Ratios of Muscle Weight (mg) to BW (g)		
	Gastrocnemius	Soleus	Heart	Gastrocnemius	Soleus	Heart
Control	1,801 ± 48	138 ± 23	863 ± 23	6.71 ± 0.14	0.51 ± 0.04	3.19 ± 0.21
EX	1,697 ± 29	138 ± 11	852 ± 16	7.25 ± 0.21†	0.59 ± 0.03*	3.64 ± 0.31*
25% ER	1,597 ± 28*	124 ± 7*	751 ± 33†	6.68 ± 0.18	0.52 ± 0.04	3.14 ± 0.15‡
EX + 25% ER	1,518 ± 132†§	128 ± 11	843 ± 45†	6.61 ± 0.39	0.56 ± 0.03	3.67 ± 0.21
50% ER	1,243 ± 112†	96 ± 8†	625 ± 18†	6.22 ± 0.24†‡	0.48 ± 0.04‡	3.13 ± 0.08‡

NOTE. Results are the mean ± SEM; n = 6 per group.
*$P < .05$ *v* control.
†$P < .02$ *v* control.
‡$P < .02$ ER *v* EX (ANOVA).
§$P < .05$ EX *v* EX + 25% ER.

(K_S) are linearly correlated with the absolute gastrocnemius ($r = .69$, $P < .001$) and heart ($r = .45$, $P < .01$) muscle weights and are depicted in Fig 3.

DISCUSSION

These studies demonstrate that daily aerobic exercise can preserve the mass of specific muscles despite a mild to moderate energy restriction, and that the fractional protein synthetic rate of each muscle is proportional to its mass. Daily aerobic exercise and energy intake appear to be independent factors that regulate both protein synthesis and muscle mass, with energy intake being the more dominant factor of the two. Despite the similar losses of total BW induced by exercise and energy restriction, daily intermittent aerobic exercise preserved muscle mass and protein synthesis to a greater extent than energy deficits induced by energy restriction alone. The preservation of muscle mass in response to the combination of exercise and energy restriction was variable: the slow-twitch soleus and heart muscles were preserved to a greater extent than the fast-twitch gastrocnemius.

Human studies of the effects of aerobic exercise and/or energy restriction have produced variable results with respect to the protein-sparing effects of exercise. Studies measuring body composition during daily aerobic exercise and ad libitum feeding report increased lean body mass with maintenance of fat mass.[2] The combination of daily exercise and pair-feeding to sedentary male adults produces a significant loss of body fat with maintenance of lean body mass.[2,5] However, in obese individuals who underwent 40% or 70% energy restriction with daily aerobic exercise, weight loss and body compositional changes appear similar to those induced by energy restriction alone, suggesting that daily aerobic exercise is not protein-sparing under these conditions.[6,7,20-22] Unfortunately, the precision of the methods used to measure body composition is low and cannot differentiate between alterations in muscle mass and visceral protein stores. Thus, it is unclear under what conditions of energy deprivation aerobic exercise preserves muscle mass in humans.

Table 2. Protein Synthetic Rates in Exercise and Calorie Restriction

	K_S (% turnover/d)		
	Gastrocnemius	Soleus	Heart
Control	10.6 ± 1.0	14.1 ± 0.8	18.6 ± 1.1
EX	7.1 ± 1.0†	12.9 ± 1.2	19.9 ± 2.5
25% ER	5.9 ± 0.4†	13.2 ± 0.6	16.1 ± 1.2*
EX + 25% ER	5.5 ± 0.2†	7.9 ± 1.5†	12.0 ± 1.0†
50% ER	6.0 ± 1.0†	3.0 ± 0.7†	8.1 ± 0.7†

NOTE. Results are the mean ± SEM for measurements taken at the 28-day time point; n = 6 per group.
*$P < .05$ *v* control.
†$P < .02$ *v* control.

There have been numerous studies measuring protein synthetic rates following various forms of exercise, but it has been difficult to compare the results due to differences in the type, duration, and intensity of exercise.[8,10,11,13] Aerobic exercise, unlike resistive exercise, normally does not induce muscle hypertrophy, but increases muscle oxygen consumption and mitochondrial enzyme activity.[23] The consensus of the majority of studies measuring the acute protein synthetic response to aerobic exercise (ie, treadmill or swimming) is that there is a depression in muscle protein synthesis during and immediately after a single bout of exercise.[8,10,11] The suppression of protein synthesis appears to be related to the intensity of the exercise: the greater the intensity of the exercise, the greater the transient depression of protein synthesis. In this study, protein synthesis was measured in the postabsorptive period, making comparisons difficult.

There are presently two methods for measuring protein synthetic rates in vivo. There is a constant-infusion technique, whereby the intracellular and extracellular pool of precursor amino acids reach equilibration after several hours, and the flooding-bolus technique popularized by Garlick et al.[17] The bolus of both a high concentration of "cold," or nonradioactive, amino acid and radiolabeled amino acid expands and equilibrates the intracellular and extracellular amino acid pools. However, Barnes et al report that both methods appear to have a similar problem in that the specific activity of the immediate protein precursor, the aminoacyl tRNA, does not equilibrate with the intracellular amino acid pool within the time frame of the measurements, suggesting that these methods underestimate the true fractional turnover rate.[24] Samarel suggests that the flooding technique may also favor measurement of the rapidly synthesized proteins within skeletal muscle.[25] Nevertheless, both methods for fractional synthesis rate determination should be able to compare differences between groups that are analyzed in a similar fashion.

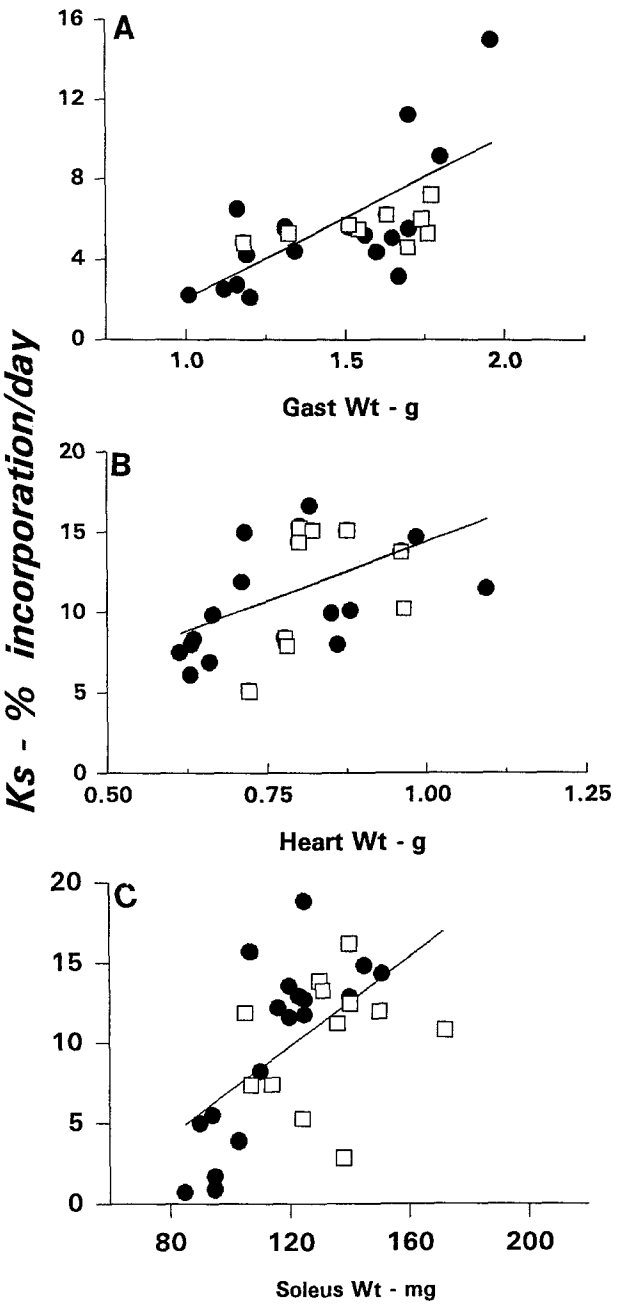

Fig 3. (A) Linear relationship ($r = .69$, $P < .001$) between the fractional protein synthetic rate (K_S, % incorporation/d) and gastrocnemius muscle weight (Gast Wt) after 4 weeks of daily running-wheel exercise (□) or energy restriction (●). (B) K_S for heart ($r = .45$, $P < .01$) v muscle weight (Wt). (C) K_S v muscle Wt or the soleus muscle ($r = .58$, $P < .001$).

Studies of acute starvation have consistently shown a depression in skeletal muscle and liver protein synthetic rates.[26,27] The suppression of protein synthesis appears to be at the translational level, with inhibition occurring at the pre-elongation phase, resulting in an increase in RNA unbound to ribosomes.[27] In this study, mild (25%) energy restriction also produced a decline in protein synthesis. Recent studies indicate that a deficiency in protein intake may independently suppress protein synthesis and may be more important than deficiencies in total energy intake.[28] Since rodents have a relatively high protein requirement, the restriction of pellet intake in this experiment produced both protein deficiency and energy deficiency and may be responsible for the suppression of protein synthesis.

Diet- and exercised-induced alterations in muscle mass are associated with alterations in protein catabolism, as well as in protein synthesis.[10,29] However, it is difficult to measure the rate of protein catabolism directly in vivo. Studies using urinary 3-methylhistidine excretion as a marker of muscle protein breakdown are potentially inaccurate due to the high turnover pool of this compound in the smooth muscle of the gastrointestinal tract. Urinary nitrogen excretion may be misleading, since the nitrogen loss may not be in the exercising muscles. Studies measuring the arteriovenous difference in serum tyrosine and 3-methylhistidine levels from an extremity suggest that exercise increases protein degradation during both the exercise and immediate postexercise period.[30] Our data suggest that under the conditions of this study running-wheel exercise probably does not alter protein catabolism, since the muscle mass at 28 days was proportional to its synthetic rate.

The loss in total BW was linearly related to both the level of energy intake and running-wheel distance achieved by the experimental animals. After correction for energy intake, running-wheel exercise produced a mean weight loss of approximately 40 g as compared with the energy intake–matched controls, and this loss of weight was directly related to the distance ran. Although we did not perform complete body composition analysis on the animals, we can assume that the weight loss contained an equal if not greater proportion of fat mass during underfeeding-induced weight loss. We estimate that running 6,000 m/d was equivalent to an energy restriction of approximately 25% for these animals.

In summary, skeletal and cardiac muscle protein synthetic rates are proportional to their muscle masses during exercise and/or energy restriction. Exercise-induced energy deficits can maintain muscle mass or increase the muscle to BW ratio. These findings suggest that energy balance and exercise have independent and interactive effects on the regulation of muscle protein synthesis. Further studies of the interaction of diet, dietary constituents, and exercise will be required to understand the regulation of muscle mass during energy deficits.

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