Enhanced protein breakdown after eccentric exercise in young and older men

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ECCENTRIC EXERCISE causes prolonged skeletal muscle damage (24), which is associated with delayed muscle soreness (23) and elevated plasma levels of creatine kinase (CK) (9). Activities known to have a large eccentric component are associated with alterations in muscle structural protein such as Z-band streaming and loss of myofibrillar contractile proteins. An example of eccentric exercise is the action of the quadriceps muscle during descent of a flight of stairs. Our laboratory has shown that, in untrained young men, high-intensity eccentric exercise resulted in elevated levels of urinary 3-methylhistidine (3 MEH) per gram of creatinine (12). 3-methylhistidine is eliminated in the urine at a metabolic rate that is 10–12% of the protein turnover rate of the muscle (9). Prolonged endurance training or previous performance of a single bout of eccentric exercise has been shown to reduce the eccentric exercise-induced increase in plasma CK levels, muscle soreness, and loss of dynamic strength (3).

Older individuals have a reduced maximal aerobic capacity (1) and a decreased muscle mass (31) and strength (13), at least in part due to a more sedentary lifestyle. This may make them more susceptible to exercise-induced muscle damage and its metabolic consequences (9). The purpose of this study was to compare the immediate and delayed effects of eccentric exercise on protein metabolism in young and older men by measuring rates of leucine turnover and oxidation. In addition, urinary nitrogen and 3 MEH excretion and plasma concentrations of CK were also determined.

MATERIALS AND METHODS

Subjects. Five untrained healthy young (22–30 yr) and five untrained older men (59–63 yr) participated in this study after giving informed consent. The research protocol was approved by the New England Medical Center—Tufts University Human Investigation Review Committee. Maximal O2 uptake (VO2 max) was determined before the study by means of a continuous incremental protocol on an electronically braked concentric cycle ergometer. Body fat, calculated from body density, was determined by hydrostatic weighing (2). Muscle mass was calculated from 24-h urinary creatinine excretion after adaptation to a meat-free diet, assuming that each gram of creatinine in a 24-h urine collection is equivalent to 18.5 kg of skeletal muscle (12).

Experimental design. All subjects lived in the metabolic ward at the US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for at least 16 days during the study and maintained their usual sedentary activity patterns. Complete 24-h urine collections were obtained during each day of the study. Body weight varied <1.0 kg during the entire residency.

Diet. All meals were provided by the metabolic kitchen and remained constant throughout the study. Subjects consumed the diet for 5 days before the exercise/infusion protocol. The diet was eucaloric and consisted of a liquid commercially available formula that supplied adequate intakes of all nutrients (Ensure HN, Ross Laboratories, Columbus, OH). Total energy intake (55% carbohydrate, 32% fat) was 2,650 ± 85 kcal/day in the young men and 2,739 ± 118 kcal/day in the older men. Protein intake was 1.5 g·kg⁻¹·day⁻¹ for all subjects.
Eccentric exercise protocol. On day 6 of the study, each subject participated in the eccentric exercise protocol. Eccentric muscle actions were performed by resisting the backward motion of motor-driven pedals on a specially designed cycle ergometer (15). Each subject exercised for three 15-min periods at an average power output equivalent to 80% of his VO₂max. Power on the eccentric cycle was calculated from the torque produced by the subject onto the pedals. For each 15-min work bout the power output was set at 90, 80, and 70% of VO₂max. Subjects rested for 5 min between each 15-min exercise period.

Tracer infusion procedure. On the day of exercise, a primed continuous infusion of L-[1-13C]leucine was administered for 7 h to determine leucine metabolism at rest and immediately after exercise. A second 3-h infusion was performed 10 days after the eccentric exercise bout (day 16).

On the morning of the infusion, each subject reported to the laboratory and rested in bed. Two 21-cm (8-in.) Teflon catheters (17 gauge Intracath) were inserted into the left and right antecubital veins. One catheter was used for blood sampling and the other for isotope infusion. Subjects were studied in the fed state, receiving one-twelfth of their daily energy intake at hourly intervals from 7 A.M. to the end of the infusion.

Before each infusion, priming doses of NaH13CO3 (100 µmol) and L-[1,13C]leucine (510 µmol) were administered. The L-[1,13C]leucine was diluted in sterile saline and infused using a calibrated syringe pump (Harvard Apparatus, Natick, MA) at a rate of 0.341 ml/min (0.115 µmol·kg⁻¹·min⁻¹).

Blood and expired air samples. Blood samples were obtained before each infusion began and at 15 min intervals during metabolic and isotopic steady state. Preexercise (Pre) samples were obtained 2-3 h after the infusion was begun. Immediately post-exercise (I-post) samples were obtained 2-3 h after the end of exercise. Ten-day postexercise (T-D post) samples were taken 2-3 h after the second infusion was begun. Plasma was separated and immediately frozen (−20°C) for subsequent analysis of isotopic enrichments. Samples were obtained for CK activity before exercise and 1, 2, 3, 5, 6, and 10 days after exercise.

Expired air samples were collected for measurement of 13CO2 enrichment before and at 15 min intervals during the last 60 min of Pre, I-post, and T-D post. Samples were collected with an anesthesia bag and transferred to 20-ml evacuated collection tubes (Venoject, Terumo, Elkton, MD).

The rate of CO2 production and O2 consumption was measured from expired air collected every hour during infusions and used to calculate energy expenditure (33). Expired air was collected in Douglas bags, and volume was measured in a Tissot spirometer (Collins, Braintree, MA); concentrations of O2 (Applied Electrochemistry, Sunnyvale, CA) and CO2 (Beckman Instruments, Anaheim, CA) were also determined. During the eccentric exercise protocol, expired air was collected during the final 5 min of each 15-min exercise bout.

Biochemical analyses. Daily 24-h urine samples were analyzed for creatinine, total nitrogen, and 3-MEH. Creatinine was determined colorimetrically (Roche Diagnostic Systems, kit 44905). Total nitrogen was measured using an automated Berthelot procedure (21), and urinary 3-MEH was analyzed using high-performance liquid chromatography (32).

CK activity was measured enzymatically on a centrifugal automated analyzer (Cobas, Switzerland) using a kit (Roche Diagnostic Systems kit 4402).

The silylquinoxalinol derivative of α-ketoisocaproic acid (KIC) was prepared from plasma aliquots deproteinized with sulfosalicylic acid (30). Analysis of isotopic enrichment of the derivatives of α-KIC was performed on a quadrupole gas chromatograph mass spectrometer (Hewlett-Packard 5985B, Palo Alto, CA). The 13C enrichment of expired CO2 was measured by isotope ratio mass spectrometry (SIRA-10, VG Isogas, Middlewich, Cheshire, UK).

Leucine metabolism was analyzed using a stochastic model (33). Leucine flux was calculated from the plasma enrichment of KIC to reflect the intracellular leucine pool (19) and to eliminate sampling site inconsistencies (16). Leucine oxidation was calculated from 14CO2 production and the enrichment of KIC in plasma. Enrichment of 13CO2 was corrected for changes in background 13CO2 enrichment that occurred as a result of the feeding schedule and exercise. The correction was determined in four young untrained male volunteers who consumed a formula diet identical to that consumed by the experimental subjects and performed a similar bout of exercise. A bicarbonate retention factor of 0.81 was used to calculate absolute rates of leucine oxidation. Devlin et al. (7) established previously that bicarbonate retention in a small number of subjects is increased slightly in the postexercise period. However, because of the number of subjects on which this has been determined, we have chosen to use 0.81. In any event, we would be underestimating the changes in leucine oxidation according to Devlin et al. Rate of leucine appearance from protein breakdown was calculated from total leucine flux minus dietary leucine intake. The rate at which leucine was incorporated into protein was calculated as the total leucine flux minus the oxidation.

Statistical analysis. Data are reported as means ± SE and were analyzed using multivariate analysis of variance or Students’t tests where appropriate. Significance was set at P < 0.05.

RESULTS

Subjects. There were no differences in body weight or body mass index between young and old subjects, but percent body fat was higher and muscle mass and VO2max were lower in the older subjects (Table 1). All the young subjects completed the 21-day diet period. However, all the older subjects were willing to be studied only through the final infusion period (day 16) because of the dietary restrictions imposed by this study.

Eccentric exercise. The subjects reported that eccentric exercise produced extreme muscle soreness. The relative metabolic cost of the exercise (%VO2max) was not different between groups, averaging 46% of VO2max in the young subjects and 53% of VO2max in the older subjects (Table 2). The mean power output during exercise aver-
eccentric exercise. Leucine incorporation into protein was not affected by Post = 109.3 ± 7.3 pmol

0.01), pmol

tern (Table 3). per kilogram of fat-free mass and followed a similar pattern (Table 3), increasing

0.0001). The rate of leucine oxidation followed a similar pattern (Table 3), increasing immediately after exercise (P < 0.028). The rate of leucine oxidation followed a similar pattern (Table 3), increasing 19% immediately after exercise (P < 0.0007) and remaining elevated 10 days after exercise with respect to preexercise (P < 0.04). The rates of leucine flux and oxidation were also expressed per kilogram of fat-free mass and followed a similar pattern (Table 3).

Leucine release from protein breakdown increased 17% immediately after exercise (Pre = 90.6 ± 8.0 μmol·kg⁻¹·h⁻¹, Post = 105.9 ± 9.6 μmol·kg⁻¹·h⁻¹; P < 0.01), and remained elevated 10 days after exercise (10-D Post = 109.3 ± 7.3 μmol·kg⁻¹·h⁻¹; P < 0.04). The rate of leucine incorporation into protein was not affected by eccentric exercise.

3 MEH excretion. Urinary 3-MEH per gram of creatinine did not differ between the young and older subjects before eccentric exercise. In the young subjects, 3-MEH excretion did not increase after eccentric exercise until 10 days postexercise (day 16) (P < 0.05). In the older subjects, however, 3-MEH excretion was elevated 5 days after exercise and remained elevated through day 16 (10 days postexercise) (P < 0.05) (Fig. 1). Urinary 3-MEH per gram of creatinine averaged 38% higher in the older subjects 7, 8, 9, and 10 days after exercise than in the young subjects (P < 0.05). Urinary nitrogen excretion. Total urinary nitrogen excretion was averaged for days 1-5, 6-10, and 11-15 of the study to examine both the effects of eccentric exercise and any long-term adaptation to the protein intake in this study. Urinary nitrogen excretion averaged 190 ± 6, 194 ± 6, and 213 ± 5 mg·kg⁻¹·day⁻¹ for days 1-5, 6-10, and 11-15, respectively. Urinary nitrogen excretion was not different from days 1 to 10, suggesting no real long-term adaptation to the diet, but increased significantly on days 11-15 (P < 0.01) as a result of the eccentric exercise.

Indirect calorimetry. There was no difference in resting metabolic rate (RMR) in the fed state between the young and older men (Pre: young = 1.17 ± 0.03, older = 1.12 ± 0.02; Post: young = 1.24 ± 0.06, older = 1.27 ± 0.02; 10-D post: young = 1.20 ± 0.05, older = 1.07 ± 0.02 kcal·kg⁻¹·h⁻¹). RMR values averaged 1.14 ± 0.03 kcal·kg⁻¹·h⁻¹ before exercise and were elevated ~10% 3
activity in skeletal muscle to a greater extent in older than trained mice, suggesting a prolonged increase in the rate been seen up to 5 days after exhaustive exercise in un-
trained men. In the present study, the subcellular activity has previously been shown to increase for up to 5 days after eccentric exercise in young untrained men

Increased skeletal muscle acid hydrolase activity has been seen up to 5 days after exhaustive exercise in untrained mice, suggesting a prolonged increase in the rate of protein turnover (29). Pilstrom et al. (25) demonstrated that acid hydrolase activity is higher in muscle from senescent than from young mice and that both acute exercise and training increased acid hydrolase activity in skeletal muscle to a greater extent in older than in young mice. In the present study, the subcellular disruption of organelles associated with eccentric exercise may increase intracellular protein degradative enzyme activities and be responsible for the increased leucine release from protein breakdown and increased 3-MEH per gram of creatinine observed up to 10 days after eccentric exercise. Skeletal muscle interleukin 1 (IL-1) immunohistochemical reactivity has previously been shown to increase for up to 5 days after eccentric exercise in young untrained men (5) and may be related to the prolonged increased muscle proteolysis following exercise, suggesting that increased skeletal muscle IL-1 levels may contribute to the prolonged increase in the rate of leucine flux and oxidation in response to eccentric exercise. Recently, Cannon et al. (6) reported a significant relationship between the secretion of interleukin 1β by isolated mononuclear cells and urinary 3-MEH excretion 12 days after 45 min of downhill running.

Several investigators have reported increases in 3-MEH excretion in response to a single bout of exercise (8, 9). Plante et al. (26) reported no change in urinary 3-MEH per gram of creatinine in young subjects after 60 min of eccentric exercise at an intensity similar to that in the present study. However, measurements of 3-MEH were carried out for only 7 days after the exercise. Our present findings and previous studies have shown that the increase in urinary 3-MEH per gram of creatinine in young men occurs beyond 7 days postexercise (9). Although 3-MEH per gram of creatinine was elevated only on day 16 in our young subjects, the pattern of delayed 3-MEH excretion was similar to that reported previously in young men (9). In contrast, the older subjects in the present study displayed a more rapid and sustained increase in urinary 3-MEH per gram of creatinine, which was significantly increased at 5 days postexercise and remained elevated 7–10 days after exercise. These results suggest that a greater degree of actomyosin breakdown occurred in the older men. It is possible that, because of a lower muscle mass, the forces applied by the eccentric ergometer during exercise were absorbed by a smaller amount of active muscle, causing increased disruption of myofibrillar proteins. The suggestion of greater muscle cell damage in the older subjects is not supported by the similar increase in plasma CK activity in both groups; however, in the present study we have observed a four-fold greater number of muscle fibers with ultrastructural damage in the older than in the young subjects (17).

Despite the 30–40% delayed increase in 3-MEH per gram of creatinine after exercise, total urinary nitrogen excretion tended to be higher after exercise from days 11 to 15. Previous studies have reported increased urinary urea excretion up to 48 h after prolonged submaximal exercise (27). No increase in urinary urea excretion has been found immediately after 90 min of concentric cycling at 45% of VO₂ max (3) or 48 h after 60 min of eccentric exercise at a similar intensity (26). It is likely that, with the generous dietary protein provided in this study (1.5 g · kg⁻¹ · day⁻¹), subtle alterations in protein utilization could not be detected.

Another consideration is that the increased urinary nitrogen and increased leucine oxidation are simply adaptations to this level of dietary protein. However, urinary nitrogen excretion was stable between days 0 and 10, suggesting adequate time for dietary adaptation. In addition, the protein intake employed in the present study is not unlike the protein level in the average American diet (18). Also, immediately postexercise, an increase in leucine flux and oxidation cannot be explained by long-term dietary adaptation, and the urinary nitrogen excretion pattern showed considerable variability, but the trend
toward greater nitrogen loss was consistent with the increased rate of leucine release from protein breakdown.

The data presented in this study stand in contrast to a recent report by Devlin et al. (7), who observed no change in leucine flux and a decreased leucine oxidation after cycle ergometer exercise to exhaustion at 75% VO\(_2\text{max}\). However, the predominantly concentric nature of their cycling protocol may not induce the same increases in leucine flux and oxidation observed with eccentric exercise protocol employed in this study.

In conclusion, a single bout of eccentric exercise elevated leucine oxidation for up to 10 days, causing a prolonged increase in the rate of leucine release from protein breakdown that was similar in young and older men. On the other hand, eccentric exercise led to a greater increase in urinary 3-MEH per gram of creatinine in the older than in the young men, suggesting that myofibrillar protein breakdown accounted for a greater proportion of whole body protein breakdown. The reasons for this are not clear but may be related to the smaller muscle mass and decreased physical fitness of the older men. Further studies are needed to confirm our observations on a relatively small group of subjects and to identify the mechanisms by which these changes occur.

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