Leucine Supplementation Enhances Skeletal Muscle Recovery in Rats Following Exercise

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ABSTRACT This study was designed to determine the ability of leucine to enhance muscle recovery after exercise. Male rats (200 g) were divided into five groups: sedentary, food-deprived (SF); exercised, food-deprived (EF); exercised, fed a carbohydrate meal (EC); exercised, fed a leucine meal (EL); and exercised, fed a combination of carbohydrate and leucine (ECL). All meals were administered by oral gavage immediately following exercise. EC and ECL meals were isocaloric and provided 15% of daily energy intake. EL and ECL meals each provided 270 mg leucine. Rats ran on a motor-driven treadmill for 2 h at 36 m/min and were killed 1 h postexercise. Plasma glucose and insulin were measured, and the gastrocnemius and plantaris muscles were excised as a unit to determine glycogen levels and the fractional rate of skeletal muscle protein synthesis ($K_s$). Exercise did not alter plasma glucose or insulin. In contrast, prolonged exercise reduced muscle glycogen (-51%) and $K_s$ (-18%). Refeeding a combination of carbohydrate and leucine increased plasma insulin relative to the EF and SF groups and produced complete recovery of muscle $K_s$ and glycogen to values not different from those in SF rats. Feeding leucine alone restored $K_s$ to that in the SF group without affecting plasma glucose or insulin concentrations. Feeding carbohydrate alone enhanced the rate of glycogen repletion compared to the EF group, concomitant with increases in plasma glucose and insulin. The degree of glycogen recovery correlated with plasma insulin concentrations ($r = 0.58, P < 0.05$). These data suggest that leucine stimulates muscle protein synthesis following exercise, independent of increased plasma insulin. This is the first demonstration that orally administered leucine stimulates recovery of skeletal muscle protein synthesis after exercise. J. Nutr. 129: 1102–1106, 1999.

KEY WORDS: • leucine • protein synthesis • glycogen • exercise • rats

Prolonged exercise induces physiological changes, including muscle glycogen depletion (Ivy 1991) and inhibition of skeletal muscle protein synthesis (Davis and Karl 1986, Dohm et al. 1980). Strategies to achieve recovery after strenuous exercise have focused on repletion of carbohydrates with little attention directed towards protein or amino acids. Further, potential synergistic effects of carbohydrate and amino acids have not been explored.

Consumption of carbohydrate following exercise results in rapid increases in the rate of muscle glycogen synthesis and recovery of muscle glycogen stores (Blom et al. 1987, Ivy et al. 1988). These responses to carbohydrate intake are believed to require increased plasma insulin. Elevated insulin levels increase the rate of skeletal muscle glucose transport, providing adequate substrate for glycogen synthesis. Additionally, insulin activates glycogen synthase, the rate-limiting enzyme in glycogen synthesis (Ivy 1991, James et al. 1985).

Whereas glucose is the primary stimulant for insulin secretion, certain amino acids, such as leucine, arginine, and methionine are insulin secretagogues (Malaisse 1984). Furthermore, intravenous infusion of glucose in combination with leucine or arginine has a synergistic effect on insulin secretion in humans (Floyd et al. 1967). These studies suggest that carbohydrate in combination with certain amino acids could enhance muscle glycogen recovery by further stimulating insulin secretion.

Postexercise nutrition also plays an important role in the stimulation of protein synthesis following exercise. We recently demonstrated that postexercise food intake improves recovery and that meal composition affects the rate of recovery (Gautsch et al. 1998). Rats administered a purely carbohydrate meal by oral gavage immediately following 2-h treadmill running demonstrated no increase in muscle protein synthesis 1 h after exercise compared to food-deprived controls. Conversely, feeding a macronutrient-mixed meal increased skeletal muscle protein synthesis rates by 30% to levels equivalent to nonexercised controls. Plasma insulin concentrations of rats fed either the carbohydrate meal or the mixed meal were similar; hence, the enhanced rate of recovery cannot be attributed to a differential insulin response. These data suggest that rapid recovery of protein synthesis following exercise requires both elevated plasma insulin and dietary protein or amino acids.

Previous studies suggest that the anabolic effect of dietary
protein may be attributable to specific amino acids. Garlick and Grant (1988) reported that infusion of glucose plus branched-chain amino acids (BCAA)\(^4\) stimulates protein synthesis in rats following 12-h food deprivation. Further, several studies report that the branched-chain amino acid leucine independently stimulates skeletal muscle protein synthesis (Buse and Reid 1975, Hong and Layman 1984, Li and Jefferson 1978). These data suggest that leucine has a unique anabolic potential; however, prior studies have not shown leucine to be effective in stimulating skeletal muscle protein synthesis in vivo (Funabiki et al. 1992, McNurlan et al. 1982). This result may be due to either an insufficient dose of leucine or lack of stimulation of plasma insulin concentrations.

The objective of this study was to determine the ability of leucine to enhance muscle recovery after exercise. Specifically, we examined the ability of leucine, alone or in combination with carbohydrate, to stimulate muscle glycogen restoration and skeletal muscle protein synthesis following a single bout of treadmill running in male rats.

**MATERIALS AND METHODS**

**Animals.** The animal facilities and protocol were reviewed and approved by the Institutional Animal Care Review Board of the University of Illinois. Male Sprague-Dawley rats weighing 140–150 g were purchased from Harlan-Sprague Dawley, Indianapolis, IN. Upon arrival, rats were individually housed in wire-bottom cages in a room maintained at 23–25°C with a 12:12 h light-dark cycle. All animals had free access to tap water and a commercial pelleted diet (Harlan-Teklad Rodent Chow, Madison, WI) until the day of the experiment.

The day after arrival, all rats began an 8-d treadmill acclimation schedule that gradually increased in either speed or duration up to 30 m/min for 10 min. Rats that refused to run during the acclimation period were eliminated from the study. All exercise sessions began at the onset of the light period after the recording of body weight and were performed on a motor-driven treadmill set at a 1.5% grade.

**Experimental design.** On the day of the experiment, all rats were deprived of food for 7 h and then randomly assigned to one of five treatment groups (n = 6 per group): SF, sedentary controls deprived of food for a total of 10 h (serving as the baseline); EF, exercised for 2 h and deprived of food for 1 h after the experimental run; EC, exercised and fed a 100% carbohydrate meal; EL, exercised and fed a 100% leucine meal; ECL, exercised and fed a combination of carbohydrate and leucine.

The carbohydrate meal provided 2.63 g of carbohydrate and consisted of 262.5 g glucose/L and 262.5 g sucrose/L in distilled water. The leucine meal provided 0.27 g of leucine prepared as 54.0 g leucine/L in distilled water and was equivalent to the amount of leucine consumed by rats of this age and strain during 24 h of free access toAIN-93 powdered diet (Harlan-Teklad) (Gautsch et al. 1998). The carbohydrate plus leucine meal was isocaloric with the carbohydrate meal and isonitrogenous with the leucine meal (235.5 g glucose/L, 235.5 g sucrose/L, and 54.0 g leucine/L in distilled water). The carbohydrate meal and the carbohydrate plus leucine meal supplied -15% of daily energy intake for this age and strain of rat (Gautsch et al. 1998). The dosage for all experimental meals was 5 mL administered by oral gavage immediately following the experimental run. SF and EF rats that were not fed following exercise were administered 5 mL saline (0.155 mol NaCl/L). All rats were allowed free access to water following the treadmill bout, but no food was available following exercise beyond defined postexercise meals.

The experimental run consisted of 2 h of treadmill running at 36 m/min (1.5% grade). Exactly 1 h after the termination of the experimental run, rats were anesthetized with a carbon dioxide overdose and killed by decapitation. Trunk blood was collected in chilled heparinized tubes and centrifuged at 1,800 g for 10 min to obtain plasma.

To investigate muscle recovery 1 h after exercise was based on previous work in rodents, which showed re-alimentation following food deprivation (Garlick et al. 1983) or exercise (Gautsch et al. 1998) to increase muscle protein synthesis within 1 h after refeeding.

**Administration of metabolic tracer.** A bolus dose (0.7 mL per 100 g body weight) of L-[4,5-\(^3\)H]-isoleucine (200 mmol/L, containing 4.81 GBq/L) was injected via the tail vein 45 min after the end of exercise for the measurement of skeletal muscle protein synthesis (Garlick et al. 1980, Gautsch et al. 1998). The right gastrocnemius and plantaris were excised as a unit 15 min after injection and quickly frozen in liquid nitrogen before storage in a -80°C freezer. The elapsed time from injection until freezing was recorded as the actual time for incorporation of the labeled amino acid into protein.

**Plasma measurements.** Plasma glucose was analyzed by a glucose oxidase-peroxidase automated method (YSI Model 2300 analyzer, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a commercial RIA kit for rat insulin (Linco Research, St. Charles, MO).

**Measurement of skeletal muscle glycogen.** Muscle glycogen was measured by a phenol-sulfuric acid colorimetric assay as described by Lo et al. (1970). Briefly, frozen muscle was powdered under liquid nitrogen with a mortar and pestle. A 50 mg sample was weighed and combined with 0.5 mL 5.35 mol/L potassium hydroxide saturated with sodium sulfate. Samples were boiled for 15 min until a homogenous solution was obtained, then cooled on ice for 30 min. To precipitate glycogen, 0.6 mL of 95% ethanol was added, and samples were chilled on ice for 30 min. Samples were centrifuged at 840 g for 25 min, and the supernatant was carefully aspirated. The resulting pellets were resuspended in 3 mL of distilled water. A standard curve ranging from 0 to 100 mg/L was prepared using a glycogen stock solution [100 mg Bovine Liver Glycogen/L (Sigma, St. Louis, MO) in distilled water]. Next, 1 mL of 50 g phenol/L was added to a 1-mL aliquots of the resuspended muscle glycogen and to each standard. Next 5 mL of 18.0 mol sulfuric acid/L was added to each tube, and the samples were allowed to stand at room temperature for 10 min. When samples had reached room temperature, they were vortexed, and the absorbance was read at 490 nm.

**Measurement of skeletal muscle protein synthesis.** Intracellular bound and free isoleucine was isolated from skeletal muscle using the method described by Gautsch et al. (1998). Free and protein-bound amino acid samples were derivatized with phenylisothiocyanate (Paul et al. 1996). The amount of isoleucine in each was determined using HPLC (PICO-TAG method, Millipore; Waters Chromatography, Milford, MA). The radioactivity of isoleucine in free and protein-bound amino acid samples was determined by liquid scintillation spectrometry (Model LS9000 Liquid Scintillation Counter, Beckman Instruments, Palo Alto, CA). Specific activity (SA) was calculated as described previously (Paul et al. 1996).

**Calculation of the fractional rate of protein synthesis.** Protein synthesis was calculated as a fractional rate of synthesis (Ks, %/d) according to McNurlan et al. (1979): Ks = (Sinc x 100)/(S free xt ) where Sinc represents isoleucine SA incorporated into muscle protein; S free represents the intracellular free pool isoleucine SA, and t represents the infusion time in days.

**Statistics.** All data were analyzed by the STATISTICA\(^\circ\) statistical software package for the Macintosh, volume II (StatSoft\(^\circ\), Tulsa, OK). A one-way ANOVA was performed to assess main effects with recovery group (exercise condition + meal) as the independent variable. When a significant overall effect was detected, differences among individual means were assessed with Duncan’s Multiple Range post-hoc test. The level of significance was set at P < 0.05 for all statistical tests.

**RESULTS**

Prolonged exercise at 36 m/min did not alter plasma glucose (Fig. 1) or insulin (Fig. 2) below nonexercised controls (EF vs.
SF) measured 1 h after exercise. Feeding a carbohydrate meal (EC) resulted in plasma glucose and insulin concentrations that were two- and threefold greater than in EF rats, respectively. In contrast, rats fed only leucine (EL) did not differ from EF rats in plasma glucose or insulin. Feeding a combination of carbohydrate and leucine (ECL) resulted in a fivefold stimulation of plasma insulin, but plasma glucose was not significantly different than the EF group. The ECL group had plasma glucose levels that were significantly greater than those of EL rats.

Two hours of treadmill running (EF) caused a 51% depression in skeletal muscle glycogen concentration compared to nonexercised SF controls (Fig. 3). Recovery of glycogen was enhanced by postexercise feeding and the degree of glycogen restoration correlated with plasma insulin concentrations ($r = 0.58, P < 0.05$). Refeeding carbohydrate (EC) or carbohydrate plus leucine (ECL) stimulated recovery of skeletal muscle glycogen 1 h after exercise, with values 76 and 88% of nonexercised controls, respectively. In contrast, rats administered leucine alone (EL) did not demonstrate significant improvement in glycogen concentration relative to EF rats.

Prolonged exercise (EF) reduced skeletal muscle protein synthesis 18% ($P < 0.05$) compared to nonexercised SF controls (Fig. 4). Feeding carbohydrate alone (EC) did not promote recovery of muscle protein synthesis. In contrast, supplements containing the branched-chain amino acid leucine (EL and ECL) reversed the depression in protein synthesis, resulting in protein synthesis values that were not different from nonexercised controls (SF) 1 h after exercise.

**DISCUSSION**

In this study, we examined the potential for oral supplements of leucine and carbohydrates to stimulate muscle recovery in rats after exercise. We exercised rats using a strenuous 2-h treadmill run to decrease muscle glycogen content and depress muscle protein synthesis. Repletion of muscle glycogen responded primarily to carbohydrate intake and was positively correlated with plasma insulin concentrations. Recovery of muscle protein synthesis was stimulated by leucine supplementation and was not dependent on plasma insulin levels. These data suggest that leucine in combination with carbohydrate can enhance recovery after exercise.

Efficient restoration of muscle glycogen following exercise
is a key component to muscle recovery. Numerous studies have established that adequate carbohydrate intake following exercise is essential to promote muscle glycogen recovery (reviewed by Ivy, 1991). Although the direct effect of carbohydrate intake on skeletal muscle glycogen after exercise is well documented, few studies have explored the effects of meal composition on glycogen restoration following prolonged exercise. Zawadzki et al. (1992) reported that a combination of carbohydrate and protein improves the rate of glycogen synthesis over either nutrient alone in men recovering after exercise. These results were attributed to an increased plasma insulin response; however, the study was not designed to control for total energy intake. The combination carbohydrate and protein supplement was an additive mixture of the carbohydrate supplement plus the protein supplement. In contrast, Tarnopolsky et al. (1997) examined the effects of isoenergetic supplements of carbohydrate and protein versus carbohydrate alone on skeletal muscle glycogen recovery following prolonged exercise. Although both supplements resulted in increased muscle glycogen compared to placebo after exercise, the combination of carbohydrate and protein did not stimulate glycogen resynthesis beyond carbohydrate alone. Thus, the efficacy of amino acid supplementation for glycogen repletion remains equivocal.

In the present study, rats administered carbohydrate plus leucine demonstrated greater plasma insulin (+34%) and muscle glycogen (+14%) values compared to those administered carbohydrate alone. These differences were observed despite 10% less total carbohydrate in the combination meal. These results suggest that the rate of recovery of muscle glycogen stores appears to be determined to a greater extent by the magnitude of the plasma insulin response than by the absolute amount of carbohydrate in the postexercise meal. This conclusion is supported by our observation that a significant positive correlation ($r = 0.58$, $P < 0.05$) existed between plasma insulin and muscle glycogen concentrations in exercised animals.

Prolonged exercise also induces acute catabolic changes in protein synthesis (Dohm et al. 1980); however, exercise is associated with maintenance or hypertrophy of skeletal muscle and not atrophy (Davis and Karl 1986). Clearly at some point during recovery, rates of protein synthesis must increase. This suggests that efficient recovery of protein synthesis is essential to drive the anabolic processes associated with regular exercise programs and maintenance or development of muscle mass.

Postexercise meal composition can have a profound effect on muscle recovery. We recently reported that a macronutrient-mixed meal administered immediately following exercise enhances the rate of skeletal muscle protein synthesis during recovery (Gautsch et al. 1998). In contrast, feeding carbohydrate alone did not stimulate protein synthesis rates above those of rats fed carbohydrate alone. Plasma insulin levels were elevated to a similar degree in rats fed either the macronutrient-mixed meal or the carbohydrate meal, suggesting that insulin acts in concert with some other component of the mixed meal to stimulate protein synthesis.

Other investigators hypothesize that insulin and amino acids act in concert to stimulate protein synthesis (Preedy and Garlick 1986, Yoshizawa et al. 1995). Preedy and Garlick (1986) reported that infusion of glucose and amino acids stimulated protein synthesis in rats following 12 h food deprivation. Conversely, infusion of amino acids and glucose plus insulin antiserum did not stimulate muscle protein synthesis rates despite a significant rise in plasma glucose concentrations. Similarly, Yoshizawa et al. (1995) reported that feeding mice a nutritionally complete meal, but not a protein-free meal, stimulated muscle protein synthesis following overnight food deprivation. These experiments suggest that amino acids and insulin act together to stimulate protein synthesis following a fast.

A study by Biolo et al. (1997) also suggests that the anabolic effect of amino acids on protein synthesis following exercise is also concomitant with elevations in plasma insulin. The authors reported that amino acids enhance the rate of skeletal muscle protein synthesis during exercise recovery. Normal male subjects were studied during a 3-h intravenous infusion of a complete amino acid mixture at rest or following a bout of leg resistance exercise. Muscle protein synthesis was significantly increased after exercise when amino acids were provided. The amino acid infusion produced elevated levels of plasma amino acids and insulin and increased levels of amino acid transport into muscle. These data suggest that amino acids ingested after exercise may promote rapid increases in skeletal muscle protein synthesis.

Previously, the effects of oral administration of specific amino acids on recovery of protein synthesis after exercise have not been studied. This is the first report that an oral dose of leucine enhances protein synthesis following exercise. Furthermore, leucine independently stimulated protein synthesis almost as effectively as a combination of carbohydrate and leucine, despite plasma insulin concentrations that were not statistically different than sedentary or exercised controls. These results indicate that leucine is capable of stimulating recovery of muscle protein synthesis after exhaustive exercise and that this effect is not dependent on elevated levels of plasma insulin.

While this study demonstrates that increased rates of skeletal muscle protein synthesis are independent of changes in plasma insulin concentration, these results do not address the role of basal levels of insulin in modulating protein synthesis in these rats. Prior studies demonstrate that either exercise (Davis and Karl 1986) or BCAA (Garlick and Grant 1988) may independently increase the sensitivity of skeletal muscle...
protein synthesis to insulin. Therefore, the combination of leucine with exercise may stimulate protein synthesis by enhancing muscle sensitivity to available insulin.

The inability of other investigators to demonstrate that leucine independently stimulates skeletal muscle protein synthesis in vivo may be attributable to an insufficient quantity of leucine administered to the animal. Previously, investigators used leucine doses in the range of 100–360 μmol/100 g body weight (Funabiki et al. 1992, McNurlan et al. 1982). In the present study, both the leucine meal and the combination of leucine plus carbohydrate contained ~1,030 μmol/100 g body weight. This amount is equivalent to the amount of leucine this age and strain of rat consumes over 24 h with free access to AIN-93 powdered diet (Harlan-Teklad) (Gautsch et al. 1998). This dose was chosen to maximize effects of leucine. Taken together, these findings suggest that leucine stimulation of skeletal muscle protein synthesis is dose dependent.

The mechanism for stimulation of muscle protein synthesis by leucine remains unclear. Buse and Reid (1975) suggest that leucine exerts its effects at a posttranscriptional level and most likely during initiation. This conclusion is based on in vitro data demonstrating that pretreatment of rat diaphragms with actinomycin D does not inhibit the stimulatory effects of leucine on protein synthesis. This is further supported by Li and Jefferson (1978) who reported that the stimulatory effect of leucine on protein synthesis in perfused rats is associated with a decrease in the level of free ribosomal subunits. Recently, Kimball et al. (1998) demonstrated that leucine stimulates the activity and increases the availability of specific proteins required for translation initiation in L6 myoblasts. Further, we found that rates of protein synthesis following exercise are capable of rapid changes and that these alterations are associated with changes in translation initiation factors (Gautsch et al. 1998). Specifically, we demonstrated that recovery of muscle protein synthesis after exercise correlates to the availability of eukaryotic initiation factor 4E (eIF4E) for 48S pre-initiation ribosomal complex formation. Moreover, recent papers by Hara et al. (1998) and Xu et al. (1998) report that amino acids, and specifically leucine, have a direct action on the phosphatidylinositol 3-kinase signal pathway in activating the eIF4E initiation complex and that this effect is independent of insulin (Patti et al. 1998). The physiological impact of leucine on this signal pathway remains to be further elucidated.

**LITERATURE CITED**


