Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle

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Morrison PJ, Hara D, Ding Z, Ivy JL. Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle. J Appl Physiol 104: 1029–1036, 2008. First published January 31, 2008; doi:10.1152/japplphysiol.01173.2007.—To examine the role of both endurance exercise and nutrient supplementation on the activation of mRNA translation signaling pathways postexercise, rats were subjected to a 3-h swimming protocol. Immediately following exercise, the rats were provided with a solution containing either 23.7% wt/vol carbohydrates (CHO), 7.9% wt/vol protein (Pro), 31.6% wt/vol CHO + 7.9% wt/vol Pro carbohydrates and Pro (CP), or a placebo (EX). The rats were then killed at 0, 30, and 90 min postexercise, and phosphorylation states of mammalian target of rapamycin (mTOR), ribosomal S6 kinase (p70S6K), ribosomal protein S6 (rpS6), and 4E-binding protein 1 (4E-BP1), were analyzed by immunoblot analysis in the red and white quadriceps muscle. Results demonstrated that rat groups provided with any of the three nutritional supplements (CHO, Pro, CP) transiently increased the phosphorylation states of mTOR, 4E-BP1, rpS6, and p70S6K compared with EX rats. Although CHO, Pro, and CP supplements phosphorylated mTOR and p70S6K after exercise, only CP elevated the phosphorylation of rpS6 above all other supplements 30 min postexercise and 4E-BP1 30 and 90 min postexercise. Furthermore, the phosphorylation states of 4E-BP1 (r² = 0.7942) and rpS6 (r² = 0.760) were highly correlated to insulin concentrations in each group. These results suggest that CP supplementation may be most effective in activating the mTOR-dependent signaling pathway in the postprandial state postexercise, and that there is a strong relationship between the insulin concentration and the activation of enzymes critical for mRNA translation.

insulin; amino acids; carbohydrates; signal transduction; mammalian target of rapamycin

PROLONGED ENDURANCE EXERCISE is a potent physiological stimulus that alters many components of skeletal muscle homeostasis. Some of the stimulating factors during endurance exercise include calcium release, redox state, mechanical stretch, ATP turnover, oxygen tension, growth factors, and cytokines (23, 36). Nutrition is also a major stimulator of protein translation after exercise (6, 8). The role of amino acids and carbohydrates as activators of protein synthesis has been well documented; however, only recently have the roles of each and both in modulating intracellular signal transduction pathways been described (27). Whole body protein synthesis rates have been shown to increase by >200% with the infusion of amino acids after resistance-type exercise (4), much greater than the 100% increase found without amino acid supplementation (3). Muscle protein degradation is also increased after exercise (2), and, in the absence of a supply of free amino acids to the body, this protein breakdown can continue long after exercise has ceased (2, 9).

Although the anabolic nature of insulin has been well defined, the role of insulin in activating protein synthesis after exercise remains unclear. Although most studies have not demonstrated the infusion of insulin to stimulate muscle protein synthesis after exercise (5, 8, 40), these same studies have suggested that insulin may play a role in enhancing net protein balance by attenuating protein degradation (5, 8, 40). Furthermore, there is evidence that a critical concentration of plasma insulin is required for protein synthesis to occur (15, 18, 25).

The failure of insulin to stimulate protein synthesis may be due to a lack of amino acids, where an interactive effect is required to stimulate the mRNA translation mechanism. Individual branched-chain amino acids, such as leucine, may act to promote insulin-dependent signaling by directly stimulating intracellular mRNA translation pathways (10, 38). These studies clearly demonstrate that carbohydrates and amino acids together play an important role in stimulating protein synthesis. In fact, there is evidence that supplementation with carbohydrates and amino acids after-exercise can have an additive effect on muscle protein synthesis (35), although there is evidence to the contrary (28). In any case, the role of either amino acids or carbohydrates in stimulating mRNA translation, or any synergistic relationship between the two, remains unclear.

The signal-transduction pathways involved in mRNA translation are controlled and activated by a number of mechanisms activated during endurance exercise. One critical signaling cascade involves the mammalian target of rapamycin (mTOR). mTOR is a serine/threonine kinase that plays a major role in integrating environmental signals from nutrients, growth factors, and exercise, to control cell growth (17). The activation of the mTOR-dependent signaling pathway leads to the phosphorylation of mTOR at Ser2448, which subsequently phosphorylates two downstream targets involved in the initiation of mRNA translation, (eIF)4E binding protein-1 (4E-BP1), and ribosomal protein S6 kinase-1 (p70S6K) (7, 37). The phosphorylation of 4E-BP1 converts the protein to its hyperphosphorylated gamma isoform, which has a lower binding affinity to eIF4E. This allows eIF4E to release from 4E-BP1 and bind to eIF4G, forming the eIF4F preinitiation complex (21). The active eIF4F complex then associates with the 5′-cap structure of the mRNA before binding to the 40S preinitiation complex for the initiation of mRNA translation (26). The phosphorylation-
tion of p70S6K by mTOR at Thr-389 allows the activation of the kinase, which then phosphorylates at least three proteins involved in mRNA translation, including ribosomal protein S6 (rpS6) (16). This leads to the preferential translation of a subset of mRNAs containing a 5′ TOP (tract of pyrimidine) sequence immediately downstream of the 5′-cap (12, 27, 37).

The purpose of this study was to examine the effect of both endurance exercise and nutrition on the phosphorylation states of mTOR and three of its dependent signaling proteins: p70S6K, rpS6, and 4E-BP1. Rats were subjected to an exhaustive endurance swim and provided with a liquid supplement immediately after exercise that consisted of either carbohydrate, protein, or a mixture of carbohydrate and protein. We hypothesized that the carbohydrate + protein mixture would result in a phosphorylation pattern more conducive to activation of mRNA translation than carbohydrate or protein alone. The enzyme phosphorylation states, along with glucose and insulin, were assessed at three distinct time-points post exercise, including immediately postexercise, and 30 and 90 min postexercise.

MATERIALS AND METHODS

Animal care and housing. Sixty male Sprague-Dawley rats (~250 g) were obtained from the Animal Resource Center at the University of Texas at Austin. This strain was chosen for their availability, ease of handling, and calmness, and is a widely accepted and dependable research model that is approved by the National Institute of Health. All rats were housed in a room kept at a temperature of 21°C with a 12-h light-dark cycle. The animals were provided free access to rat chow (Purina chow, Ralston Purina, St. Louis, MO) and water, except when indicated. All research and handling procedures were approved by the Institutional Animal Care and Use Committee. The animals were cared for according to Institutional Animal Care and Use Committee guidelines.

Experimental protocol. All rats were familiarized with the swimming protocol for 10 min/day for 2 days. On the experimental day, food was withdrawn 12 h before the start of the exercise protocol. The rats were then randomly assigned to five experimental groups: sedentary control (SED), exercise control (EX), exercise-carbohydrate (CHO), exercise-carbohydrate + protein (CP), and exercise-protein (Pro). The CHO, CP, and Pro groups were further subdivided into two different time points (30 and 90 min after supplementation), whereas the EX group was subdivided into three different groups (0, 30, and 90 min). There were a total of 10 experimental groups. All of the rats, except for the six rats in the sedentary control group, were subjected to a 3-h swimming exercise with a weight of 3% attached to their tails. The exercise protocol was performed in a barrel filled with water to a depth of 50 cm, and two rats could swim at the same time. The water temperature was kept between 33 and 34°C. If any rats fatigued during the swim, they were allowed a 5-min rest period before resuming. Immediately after exercise, the rats were either killed or intubated with a 50% vol/wt liquid supplement. The CHO group received 0.9 g carbohydrate (dextrose)/kg body wt. The Pro group received 0.3 g protein (whey isolate)/kg body wt. The CP group received 0.9 g carbohydrate/kg body wt + 0.3 g protein/kg body wt. The concentrations of the liquid supplements were 23.7% wt/vol, 7.9% wt/vol, and 31.6% wt/vol for CHO, Pro, and CP, respectively. The CHO, Pro, and CP supplements amounted to ~0.5, 2, and 2.5% of average daily caloric expenditure, respectively. The SED and EX groups (0 min group) were immediately killed after a 3-h rest or 3-h swim, respectively. Animals were considered exhausted based on the fact that they could not right themselves when placed on their sides. The four other treatment groups were killed either 30 or 90 min postexercise. All animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (65.0 mg/kg body wt). One milliliter of blood was withdrawn from the tail vein of the animals immediately before surgery. The deep red portion (RQ) and superficial white portion (WQ) of the quadriceps were rapidly excised from the anesthetized animals and immediately frozen in liquid nitrogen. Anesthetized animals were euthanized by cardiac injection of pentobarbital sodium.

Blood glucose. Blood samples (1 ml) were drawn from the tail vein in 1.5-ml microcentrifuge tubes containing 20 μl of EDTA (24 mg/ml, pH 7.4). Blood glucose was measured with the One Touch Basic glucose analyzer (Johnson & Johnson, Milpitas, CA). This instrument required a small drop of whole blood on a test strip, which was then read by the analyzer. Each glucose measurement was run in duplicate. The microcentrifuge tubes were then centrifuged at 14,000 g for 10 min, and the plasma was stored at −80°C for later analysis.

Plasma insulin. Plasma insulin was measured by 125I-RIA (MP Biomedicals, LLC, Orangeburg, NY). The 125I-labeled insulin was added to tubes containing the plasma samples, standards, and controls. Antisera to rat insulin was then added, and the tubes were incubated overnight at 4°C. Each tube was rinsed with 4 ml of distilled water and was assayed by a 1470 Automatic Gamma Counter (Packard Instrument, Boston, MA). Standards were used to construct a standard curve, and the samples were compared with the standard curve. Each insulin measurement was run in duplicate.

Tissue processing. Muscle biopsy specimens were homogenized in ice-cold buffer (in mM: 20 Hepes, 2 EGTA, 50 NaF, 100 KCl, 0.2 EDTA, 50 glycerophosphate, 1 DTT, 0.1 PMSF, 1 Benzamidine, 0.5 Na Vanadate) at a dilution of 900 μl/mg (1:10) of wet weight muscle with a glass tissue grinder pestle (Corning Life Sciences, Acton, MA). The homogenates were then centrifuged for 10 min at 14,000 g. The homogenate protein concentration was measured using the Lowry method (33). All homogenized muscle aliquots were then stored at −80°C.

Muscle analysis. Aliquots of homogenized muscle samples and standards were slowly thawed over ice and diluted 1:1 with Laemmli sample buffer (125 mM Tris, 20% glycerol, 2% SDS, 0.008% bromophenol blue, pH 6.8) (31). Samples containing 40 μg (4E-BP1), 60 μg (mTOR and rpS6), and 100 μg (p70S6K) of total protein (red and white quadriceps) were separated on 8–15% polyacrylamide gels by SDS-PAGE (Bio-Rad Laboratories, Richmond, CA) for 50 min (rpS6 and p70S6K), 120 min (mTOR), and 180 min (4E-BP1) at either 100 V (4E-BP1) or 200 V (mTOR, rpS6, and p70S6K) (Bio-Rad Laboratories). After electrophoresis, the gels were electrophotographically transferred for 15 min to 0.4-μm polyvinylidene fluoride membranes (Millipore, Bedford, MA) (mTOR, rpS6, p70S6K, and 4E-BP1). The membranes were then washed in TBS (50 mM Tris, 150 mM NaCl) containing 0.06% Tween 20 (TTBS), and 5% nonfat dry milk. The membranes were then incubated overnight at 4°C with antibodies directed against mTOR (Ser2448, no. 2971S), rpS6 (Ser235/236, no. 9252), 4E-BP1 (polyclonal, no. 92542), and p70S6K (Thr389, no. 9205S) (Cell Signaling Technology, Danvers, MA). The antibodies were diluted 1:1,000 (mTOR, rpS6, 4E-BP1, and p70S6K) in TTBS containing 1% nonfat dry milk. The membranes were then washed twice with TTBS and incubated for 2 h with a secondary antibody (anti-rabbit, HRP-linked IgG, no. 7074) diluted 1:2,000 in TTBS containing 1% nonfat dry milk. The protein immunoblots were visualized by enhanced chemiluminescence (Perkin Elmer, Boston, MA), and the mean density of each band was calculated. For the 4E-BP1 blot analysis, only red quadriceps muscle was analyzed. As well, the band representing the 4E-BP1 γ-isoform was compared with the total protein in the blot, and represented as the percent of γ-isoform.

Experimental design and statistics. A two-factor (treatment × time) ANOVA procedure was used to test for significant interaction effects of each of the four treatments (CHO, Pro, CP, EX) across two time points (30 and 90 min) postexercise. Post hoc tests were per-
formed using Fisher’s protected least significant difference to identify significant differences between group means. A t-test was performed to test for significant difference between the sedentary (SED) group and the exercise (EX) group at 0 min postexercise. A Spearman correlation regression analysis was run for insulin and the phosphorylation status of signaling proteins. Statistical significance was set at $P < 0.05$. All values are expressed as means ± SE. The statistical analysis of the data was performed using SPSS software version 14.0 (SPSS, Chicago, IL).

**RESULTS**

**Blood glucose.** Swimming to exhaustion resulted in a significant reduction in blood glucose ($P < 0.05$) (Fig. 1). Main effects for time and supplement were observed. Although, at 30 min postexercise, the CHO-30, Pro-30, and CP-30 groups were significantly elevated compared with the EX-30 group, the CHO-30 group had the highest concentration of blood glucose, ~163% above the EX-30 group ($P < 0.05$) (Fig. 1). The CHO-30 group was also ~32% elevated above the Pro-30 group, which was a significant difference ($P < 0.05$). At 90 min, there were no differences between treatments, but the Pro-90, CP-90, and EX-90 groups showed an elevation in blood glucose concentration compared with their respective 30-min levels ($P < 0.05$) (Fig. 1), leading to a significant time effect where blood glucose in all groups was greater at 90 min than at 30 min postexercise. There was also a significant nutrition effect for all treatment groups, irrespective of time, where the CHO and CP treatments were significantly elevated compared with the EX treatment.

**Plasma insulin.** Immediately postexercise, plasma insulin was reduced ~57% compared with the SED control group ($P < 0.05$) (Fig. 1). Main effects for supplement were observed. At 30 min postexercise, plasma insulin concentrations for the CHO-30 ($P = 0.07$) and CP-30 groups were significantly elevated compared with the EX-30 group (Fig. 1). At 90 min postexercise, there were no differences between any of the treatment groups. From 30 to 90 min postexercise, the EX-90 group plasma insulin increased significantly (79%) above the EX-30 group (Fig. 1). There were no time effects among treatment groups between 30 and 90 min, but irrespective of time there was a significant treatment effect observed, where the CP treatment was greater than EX.

**Analysis of mTOR phosphorylation at Ser2448.** Exhaustive endurance swimming was used as a means to maximize stress on intracellular signaling pathways, specifically the mTOR-dependent pathways. The added stress of glycogen depletion (data not shown) did not have a significant effect on mTOR phosphorylation at Ser2448 immediately postexercise in both RQ and WQ tissue (Fig. 2). Main effects for supplement were observed. Thirty minutes postexercise, the CHO-30, Pro-30, and CP-30 groups demonstrated a significantly elevated phosphorylation state over the EX-30 group ($P < 0.05$) (Fig. 2). However, by 90 min postexercise, these differences were no longer significant, and no overall time effect was observed. In WQ tissue, there was a reduction of ~45% in mTOR phosphorylation in the Pro fed group from 30 to 90 min, a significant decline ($P < 0.05$) (Fig. 2).

**Phosphorylation of p70S6K at Thr389.** As shown in Fig. 3, immediately postexercise, there was no change in the phosphorylation state of p70S6K at Thr389 in both RQ and WQ tissue. Main effects for supplement and time were observed. At 30 min postexercise in RQ, the CHO-30 and CP-30 treatment groups showed an elevation in phosphorylation states over the EX-30 group of ~143 and 170%, respectively ($P < 0.05$) (Fig. 3). The WQ demonstrated a very similar result at 30 min, except that all three of the supplement types (CHO-30, Pro-30, and CP-30) were significantly elevated above the EX-30 group (Fig. 3). In the RQ and WQ, the phosphorylation state of the EX group from 30 to 90 min was significantly elevated 105 and 170%, respectively (Fig. 3), where at 90 min in WQ, the EX-90 group was 61% above the Pro-90 group, which approached significance ($P = 0.06$) (Fig. 3). Also in the WQ, there was a significant time effect, where a significant reduction in the phosphorylation of the CHO, Pro, and CP groups was observed from 30 to 90 min postexercise ($P < 0.05$) (Fig. 3). This trend was also demonstrated in the RQ tissue, but only the Pro-30 group was significantly elevated above the Pro-90 group, and
no time effect was observed between 30 and 90 min postexercise (Fig. 3).

Phosphorylation of rpS6 at Ser235/236. Immediately after swimming exercise, there was no change in the phosphorylation state of rpS6 at Ser235/236 in both RQ and WQ tissue (Fig. 4). Main effects for supplement and time were observed. In the RQ at 30 min postexercise, the CP-30 group showed an elevation in rpS6 phosphorylation of \( \sim 150\% \) above the EX-30 group and of \( \sim 49\% \) above the Pro-30 group \( (P < 0.05) \) (Fig. 4). In the WQ at 30 min postexercise, the CP-30 group was elevated...
~134% above the EX-30 group, which approached significance \((P = 0.07)\) (Fig. 4). In both RQ and WQ, from 30 to 90 min in the EX groups, there were significant elevations in rpS6 phosphorylation of ~82 and 159%, respectively (Fig. 4). At 90 min postexercise, there were no differences between treatments in either RQ or WQ tissue (Fig. 4), but in the RQ a significant treatment effect was observed, irrespective of time, where the CP treatment was elevated compared with the EX treatment (Fig. 4). A significant positive Spearman correlation \((r^2 = 0.7606, P = 0.001)\) was observed between the group means of rpS6 in the RQ and insulin (see Fig. 6).

Analysis of 4E-BP1 hyperphosphorylation state. The hyperphosphorylation state of 4E-BP1 was assessed by calculating the percentage of the total 4E-BP1 protein in the hyperphosphorylated γ-isof orm. Only RQ tissue could be measured, since the signal was too low to calculate in WQ (data not shown). Immediately postexercise, the hyperphosphorylation state of 4E-BP1 in the EX group was ~55% lower than the SED control group, which was a significant decline \((P < 0.05)\) (Fig. 5). Main effects for supplement were observed. At 30 min postexercise, the CP-30 group was significantly more hyperphosphorylated compared with the CHO-30, Pro-30, and EX-30 groups (Fig. 5). At 90 min postexercise, the CP-90 group was still significantly elevated above the CHO-90 and Pro-90 groups \((P < 0.05)\), but the EX group from 30 to 90 min increased ~57% \((P < 0.05)\), eliminating the differences between the EX and CP groups that were observed at 30 min postexercise (Fig. 5). This led to a significant nutrient effect of CP, irrespective of time, where the CP treatment was significantly more hyperphosphorylated compared with the Pro and EX treatments, and approached significance compared with the CHO treatment \((P = 0.07)\). No time effect was observed between 30 and 90 min postexercise. A significant positive Spearman correlation \((r^2 = 0.7942, P < 0.001)\) was observed between the group means of 4E-BP1 and insulin (Fig. 6).

DISCUSSION

The present study was pursued with the goal of characterizing the postprandial response of the mTOR-dependent signaling pathway of rats that swam to exhaustion. The experimental design of this study allowed for two separate comparisons between the experimental groups: 1) immediately postexercise between an exercise and sedentary group and 2) at 30 and 90 min postexercise between supplemented and non-supplemented exercise groups. To our knowledge, no study has compared CHO, Pro, and CP supplementation on the mTOR-dependent signaling pathway at multiple time points postexercise.

The primary finding of this study is that exhausted exercised rat groups provided with either a CHO, Pro, or CP supplement demonstrated a transient increase in the phosphorylation states of enzymes located downstream of mTOR in the mTOR-dependent signaling pathway at 30 min postexercise. When no supplement was provided, this response was delayed until 90 min postexercise and appeared related to the return of blood glucose and insulin to preexercise levels as a result of gluconeogenesis. It was noted that, although all three supplements demonstrated significant phosphorylation activation above the EX group at 30 min, only the CP group showed a consistently significant activation in all enzymes measured in both RQ and WQ tissue. This result was most obvious in the phosphorylation states of 4E-BP1 and rpS6, where the CP group is significantly elevated above almost all of the groups at 30 min. Overall, these results clearly demonstrate that supplementation with either CHO or Pro alone stimulate both insulin and the
phosphorylation of mTOR-dependent targets above EX-only treatment groups, but not to the extent of carbohydrate and protein provided together.

The increased activation of the CP groups, over groups supplemented with CHO or Pro alone, may be explained from the differences in the insulin concentrations observed between each group. Only the CP group demonstrated a significantly elevated insulin concentration above the EX group at 30 min postexercise, which was concomitant with significant increases in the phosphorylation states of all mTOR-dependent targets measured.

In general, the phosphorylation responses of the enzymes associated with the activation of protein synthesis reflected the insulin response of each rat group. There was in fact a significant correlation between the insulin concentration and the phosphorylation states of all mTOR-dependent targets measured.

The present study reveals that endurance exercise leads to a dephosphorylation of 4E-BP1, suggesting that eIF4E, along phosphorylation of mTOR-dependent targets above EX-only treatment groups, but not to the extent of carbohydrate and protein provided together.

The phosphorylation states of mTOR-dependent targets measured in the present study are generally in line with the current literature, although there are some points of divergence, particularly due to the model employed, since the current protocol used both endurance exercise and nutrition as means of studying the phosphorylation states of mTOR-dependent targets. This study first examined the effect of exercise on mTOR targets immediately after swimming. Even though insulin was suppressed immediately postexercise, mTOR phosphorylation remained unchanged. This result is consistent with resistance-type exercise studies that have investigated phosphorylation of mTOR at Ser2448 and have not detected any changes immediately postexercise (7, 13, 14) but is inconsistent with Williamson et al. (41), who used an endurance-exercise model in rats and measured an increase in mTOR activation by calculating the percentage of Raptor bound to mTOR. In a human model, Mascher et al. (34) demonstrated clear increases in mTOR phosphorylation with 1-h cycling exercise. The results of the present study suggest that phosphorylation of Ser2448 on mTOR is more sensitive to nutritional stimuli and less correlated to cell stimuli associated with endurance exercise. The nutritional stimulus of mTOR at Ser2448 has recently been confirmed in humans (19).

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Fig. 5. 4E-BP1 phosphorylation as expressed as a percentage of the gamma isofrom ± SE in rat RQ tissue. Phosphorylation states were examined for SED, and EX killed 0, 30, and 90 min postexercise. *Significantly different from SED (P < 0.05). †Significantly different from EX-30 (P < 0.05). ‡Significantly different from CHO-30 (P = 0.078). §Significantly different from CHO-90 (P = 0.084). ‡Significantly different from Pro-90 (P < 0.05).

Fig. 6. Relationship between the increase in insulin concentration associated with the phosphorylation states of rpS6 (A) and 4E-BP1 (B) in rat RQ tissue.
with mRNA translation, is inactivated immediately after a long bout of endurance exercise. The present data also suggest that, in the absence of supplementation, 4E-BP1 hyperphosphorylation is rescued from 30 to 90 min, along with p70SGK and rpS6. This increase in phosphorylation is correlated to both the glucose response, pointing to gluconeogenesis occurring in the rats, and the corresponding insulin response in the EX groups. The fact that a rescued insulin response in the EX group from 30 to 90 min can be correlated to increases in 4E-BP1 and rpS6 underscores the role of insulin as a key player in the activation of the mTOR pathway. The observation that 4E-BP1 is suppressed immediately postexercise is consistent with previous studies involving both endurance- and resistance-type exercise, where 4E-BP1 is suppressed immediately postexercise but is rescued as the recovery period extends (1, 13, 30).

The phosphorylation of p70SGK at Thr389 by the Raptor-mTOR-G8L complex subsequently leads to the phosphorylation of rpS6 at Ser235/236, and, although the phosphorylation state of rpS6 is usually used as a gauge for p70SGK activation, the present study examined both targets simultaneously to observe the effects of nutrients and exercise on the activation of the mTOR-dependent signaling cascade. The results of this study clearly demonstrated that any suppression of 4E-BP1 phosphorylation observed immediately after exercise was eliminated after 30 min with nutrition. Both p70SGK and rpS6 demonstrated a nutrient effect at 30 min postexercise, in support of the literature pointing to p70SGK as strikingly sensitive to nutritional stimuli (1, 11, 22, 32), especially branched chain amino acids (6, 19, 24).

Both p70SGK and rpS6 failed to show any exercise-induced effect immediately postexercise. Williamson et al. (41) also found no increase in p70SGK following up to 30 min of endurance exercise in rats but showed an increase in rpS6 phosphorylation at Ser235/236. Mascher et al. (34) also failed to show an increase in p70SGK phosphorylation at Thr389 after endurance exercise. A number of resistance-type exercise studies have found both rpS6 and p70SGK phosphorylation elevated immediately postexercise (13, 14, 29), although Bolster et al. (7) have found no change in either 4E-BP1 or p70SGK in response. Although the present study did not observe an exercise effect having a positive effect on the phosphorylation status of mTOR, p70SGK, 4EBP1, or rpS6, the wide ranges of responses cited in the literature are most likely due to the differing exercise stimuli employed in each study. One explanation may be that the intensity of the current exercise protocol may not have activated the MAP kinase pathway to the extent observed during intense resistance exercise. In the cited studies, resistance exercise may have activated the ERK1/2 MAP kinase pathway, where the phosphorylation of rpS6 occurs independently of mTOR, by p90RSK (39). Differences may also be due to the transient nature of these signal transduction pathways, where a signal response may be very fast acting. It seems clear, however, that the exercise-type model employed is an important variable controlling mTOR and its downstream targets. Furthermore, the insulin response from a nutritional supplement acts to overcome any suppression caused by the exercise stimulus.

In summary, the results of this study demonstrate that nutrition provided immediately after exhaustive exercise will lead to a transient increase in the phosphorylation activation of signaling proteins involved in the initiation of mRNA translation. The results also suggest that supplementing with a mixture of carbohydrates and protein immediately after exercise may best promote the activation of protein synthesis. The nutrition effect observed is closely correlated to the insulin concentrations reached with each of the nutrient types provided, which may explain the transient nature of the observed enzyme phosphorylation states.

Further studies employing human models of both endurance exercise and multiple bolus supplementations are needed to observe whether any nutrient effect can be sustained to 90 min or longer postexercise.

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