Intake of branched-chain or essential amino acids attenuates the elevation in muscle levels of PGC-1α4 mRNA caused by resistance exercise

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Samuelsson H, Moberg M, Apró W, Ekblom B, Blomstrand E. Intake of branched-chain or essential amino acids attenuates the elevation in muscle levels of PGC-1α4 mRNA caused by resistance exercise. Am J Physiol Endocrinol Metab 311: E246–E251, 2016.—The transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC)-1α is recognized as the master regulator of mitochondrial biogenesis. However, recently a novel isoform, PGC-1α4, that specifically regulates muscle hypertrophy was discovered. Because stimulation of mechanistic target of rapamycin complex 1 (mTORC1) activity is tightly coupled to hypertrophy, we hypothesized that activation of this pathway would upregulate PGC-1α4. Eight male subjects performed heavy resistance exercise (10 × 8–12 repetitions at ∼75% of 1 repetition maximum in leg press) on four different occasions, ingesting in random order a solution containing essential amino acids (EAA), branched-chain amino acids (BCAA), leucine, or flavored water (placebo) during and after the exercise. Biopsies were taken from the vastus lateralis muscle before and immediately after exercise, as well as following 90 and 180 min of recovery. Signaling through mTORC1, as reflected in p70S6 kinase phosphorylation, was stimulated to a greater extent by the EAA and BCAA than the leucine or placebo supplements. Unexpectedly, intake of EAA or BCAA attenuated the stimulatory effect of exercise on PGC-1α4 expression by ∼50% (from a 10- to 5-fold increase with BCAA and EAA, P < 0.05) 3 h after exercise, whereas intake of leucine alone did not reduce this response. The 60% increase (P < 0.05) in the level of PGC-1α1 mRNA 90 min after exercise was unaltered by amino acid intake. Muscle glycogen levels were reduced and AMP-activated protein kinase α2 activity and phosphorylation of p38 mitogen-activated protein kinase enhanced to the same extent with all four supplements. In conclusion, induction of PGC-1α4 does not appear to regulate the nutritional (BCAA or EAA)-mediated activation of mTORC1 in human muscle.

mitochondrial biogenesis; mechanistic target of rapamycin; peroxisome proliferator-activated receptor-γ coactivator-1α isoforms; skeletal muscle

THE TRAINING-INDUCED ADAPTATIONS in skeletal muscle depend to a large extent on the type of exercise involved. The most well-known effect of endurance exercise is an increase in mitochondrial content, while resistance exercise enhances the content of contractile proteins. The master regulator of mitochondrial biogenesis has been recognized to be the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (16, 26, 32). PGC-1α is upregulated by endurance exercise (3, 24) and is associated with many of the changes related to endurance training, including an increased capacity to oxidize fat, improved glucose transport, and antioxidant defense (22). Interestingly, Ruas and colleagues (28) found that a novel isoform of PGC-1α (PGC-1α4), transcribed from the alternative promoter, is involved in regulating muscle hypertrophy in experimental animals, possibly through induction of the insulin-like growth factor 1 and downregulation of myostatin. However, follow-up studies have revealed that PGC-1α4 is induced not only by resistance exercise, but also by endurance training (17, 29, 33), emphasizing the need for further investigations of the cellular mechanisms underlying the potential involvement of PGC-1α4 in regulating skeletal muscle growth in humans.

Resistance exercise and nutritional supplementation in the form of protein or essential amino acids (EAA) independently activate the mechanistic target of rapamycin complex 1 (mTORC1) and thereby stimulate synthesis of contractile proteins (11, 21). Moreover, a combination of the two enhances the effect (21). The stimulatory effect of nutrition can be largely attributed to branched-chain amino acids (BCAA), in particular leucine, which has previously been reported to stimulate mTORC1 and protein synthesis in human skeletal muscle (2, 30). Importantly, stimulation of mTORC1 activity by leucine, as reflected in activation of p70S6 kinase (S6K1), is amplified when other EAA are also present in the supplement (2). Of interest in this context is the interaction between mTORC1 signaling and PGC-1α observed by Cunningham and coworkers (8). Inhibition of mTORC1 with rapamycin reduced the expression of PGC-1α in C2C12 myotubes and mouse skeletal muscle, resulting in downregulation of mitochondrial genes (8). Most likely this effect involves PGC-1α1, since PGC-1α4 does not induce mitochondrial biogenesis (28), but it is not yet known whether there is a link between mTORC1 signaling and PGC-1α4. In an attempt to investigate this issue further, we employed an experimental model that has previously been demonstrated to stimulate anabolic signaling.

In the present study, we examined the influence of ingesting EAA, BCAA, leucine, or flavored water during heavy resistance exercise on the expression of PGC-1α1 and PGC-1α4. Muscle biopsies were taken from the vastus lateralis muscle after 8 repetitions of 80% of 1 repetition maximum in leg press and immediately after exercise, as well as following 90 and 180 min of recovery. Signaling through mTORC1, as reflected in p70S6 kinase phosphorylation, was stimulated to a greater extent by the EAA and BCAA than the leucine or placebo supplements. Unexpectedly, intake of EAA or BCAA attenuated the stimulatory effect of exercise on PGC-1α4 expression by ∼50%, from a 10- to 5-fold increase with BCAA and EAA, P < 0.05) 3 h after exercise, whereas intake of leucine alone did not reduce this response. The 60% increase (P < 0.05) in the level of PGC-1α1 mRNA 90 min after exercise was unaltered by amino acid intake. Muscle glycogen levels were reduced and AMP-activated protein kinase α2 activity and phosphorylation of p38 mitogen-activated protein kinase enhanced to the same extent with all four supplements. In conclusion, induction of PGC-1α4 does not appear to regulate the nutritional (BCAA or EAA)-mediated activation of mTORC1 in human muscle.
MATERIALS AND METHODS

Subjects. Eight healthy male subjects, who had carried out weight training regularly for three to four times a week for longer than one year, participated in this study. Their mean age (± SE) was 27 ± 2 yr, height 181 ± 3 cm, weight 84 ± 3 kg, and maximal leg strength [1 repetition maximum (RM)] 430 ± 13 kg. The subjects gave their written consent after being fully informed about the purpose of the study and associated risks. The subjects also took part in a previous study (20). The study was approved by the Regional Ethical Review Board in Stockholm.

Pretests. The subjects took part in four pretest sessions. On the first occasion, 1RM (at a knee angle of 90–180°) on a leg press machine (243 Leg Press 45°; Gymleco, Stockholm, Sweden) was determined by gradually increasing the load until the subject was no longer able to perform. In the following three sessions each subject carried out the entire protocol (see below) for familiarization. These pretests were separated by 1 wk, with the last performed 7–10 days before the first experimental trial.

Experimental trials. The subjects were instructed to refrain from vigorous physical activity for 2 days before the experimental days and to document their food intake during the 2 days before the first experiment and repeat it before each of the following experiments.

On experimental days, the subjects reported to the laboratory at 6:00 AM after fasting since 9:00 PM the evening before. They were placed in supine position, and a catheter was inserted in the antecubital vein to enable repeated blood sampling. A biopsy was taken from the vastus lateralis muscle of one leg using a Weil-Blakesley conchotome (AB Wisex, Mölndal, Sweden) as described by Henriksson (13).

After warming up (three sets of 10 repetitions at 0, 30, and 60% of 1RM), the subjects performed 10 sets with 8–12 repetitions to fatigue with 3 min rest between sets. The initial workload (1st set) was 85% of 1RM followed by a gradual reduction to 65% of 1RM at the 10th set. The exercise protocol lasted for a total of 50 min. Immediately following completion of the final set, the subjects moved to supine position, and a second biopsy was taken from the opposite leg. Two additional biopsies were taken following 90 and 180 min of recovery, respectively. These biopsies were taken from different legs and through a new incision made at least 2.5 cm proximal to the previous one. The tissue samples were immediately blotted free of blood and frozen in liquid nitrogen. Blood samples were taken at rest (before warm up and before any drink was supplied), after warm up, after the fourth and seventh set, and at the end of exercise as well as during recovery at 15, 30, 60, 90, 120, 150, and 180 min after resistance exercise. Samples were centrifuged, and the plasma was collected. Both muscle and plasma samples were stored at −80°C.

During the experiment each subject ingested a solution of either leucine (50 mg/kg body wt), BCAA (110 mg/kg body wt), EAA (290 mg/kg body wt), or flavored water (placebo), in a double-blind counterbalanced fashion. The amount of leucine in the three solutions containing amino acids was the same as in previous studies (2, 14); the BCAA mixture containing amino acids was the same, as was the amount of BCAA in counterbalanced fashion. The amount of leucine in the three solutions (50 mg/kg body wt), or flavored water (placebo), in a double-blind (NextAdvance). The homogenate was then rotated for 30 min at 4°C and subsequently centrifuged for 10 min at 10,000 g at 4°C. After dilution of part of the supernatant (1:10 in distilled water), its protein concentration was determined using the Pierce 660-nm protein assay (Thermo Scientific), and the remainder of the supernatant was then stored at −80°C until further analysis.

The immunoblot analysis employed has been described in detail earlier (2). Briefly, aliquots of muscle homogenates were diluted in Laemmli sample buffer (Bio-Rad Laboratories, Richmond, CA), and the proteins were separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and incubated with the following antibodies: primary antibodies against 56K1 (Thr180, no. 9234; total, no. 2708), p38 (Thr180/Tyr182, no. 4511; total, no. 9212), and CamKII (Thr286, no. 12716; total, no. 4436) were purchased from Cell Signaling Technology (Beverly, MA). All primary antibodies were diluted 1:1,000. Secondary antirabbit (no. 7074) antibody (1:10,000) was purchased from Cell Signaling Technology. Proteins were visualized by applying Super Signal West Femto Chemiluminescent Substrate (Thermo Scientific), followed by detection on a Molecular Imager ChemiDoc XRS system and quantified using the contour tool in the Quantity One version 4.6.3 software (Bio-Rad Laboratories). Subsequently, the membranes were stripped using Restore Western Blot Stripping Buffer (Thermo Scientific) for 30 min at 37°C and then reprobed with primary antibodies for each respective total protein. The levels of all phosphoproteins were normalized to the total level of the same protein.

For analysis of AMPK α1 and α2 activities, muscle homogenates containing 250 μg protein were incubated with 3 μg goat anti-AMPK α1 or anti-AMPK α2 antibody (nos. sc-19128 and sc-19131, respectively; Santa Cruz Biotechnology, Heidelberg, Germany) together with 10 μl protein G-Sepharose beads (GE Healthcare, Uppsala, Sweden) overnight on a rotating platform at 4°C and then centrifuged and washed. The activities of AMPK α1 and α2 were assayed as described previously (19) in a reaction mixture containing 200 μM ATP, 200 μM AMP, 5 mM MgCl2, 32γ-ATP (specific radioactivity ~0.4 × 106 counts·min−1·nmol−1), and 200 μM synthetic substrate (AMARA: AMARRAASAAALARRR).

RNA extraction and quantitative real-time RT-PCR. From ~3 mg freeze-dried muscle tissue, total RNA was extracted by homogenization in PureZOL RNA isolation reagent (Bio-Rad Laboratories) employing the BulletBlender and RNase-free ZrO beads (0.5 mm). The percentage of purity and quality of the RNA were determined using a NanoDrop Lite (Thermo Scientific), and 2 μg RNA were used for reverse transcription of 40 μl cDNA with an iScript cDNA Synthesis Kit (Bio-Rad Laboratories). The mean 260/280 nm ratio of the RNA was 1.87 in purified water and the mean concentration 183 μg/ml. Quantitative RT-PCR was performed as described previously (5, 25) with the housekeeping GAPDH mRNA as a reference. The qRT-PCR amplification mixture (25 μl) contained 12.5 μl 2× SYBR Green Supermix (Bio-Rad Laboratories), 0.5 μl 10 μM forward and reverse primers, respectively, and 11.5 μl template cDNA in RNase-free water. Samples were run in triplicate, and all samples from each subject were run on the same plate to allow direct relative compari-
Ingestion of BCAA and EAA markedly suppressed the elevation of PGC-1α mRNA, whereas recovery after exercise, PGC-1α mRNA expression, intracellular signaling, kinase activity, and levels of glycolgen and amino acids. The area under the insulin-time curve in the four conditions was compared using a one-way ANOVA. Whenever a significant main or interaction effect was observed, a Fisher’s least-significant difference post hoc test was performed. All statistical analyses were performed with the STATISTICA software (version 12.0; StatSoft), and P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Exercise performance. Seven subjects performed exactly the same amount of work in the four trials, whereas one subject performed one repetition less in two of the trials. The subjects performed 103 ± 6 repetitions in the 10 sets.

Effects of exercise and intake of amino acids on the levels of PGC-1α mRNA in muscle. Resistance exercise induced a pronounced increase in the level of PGC-1α mRNA but only a minor elevation of PGC-1α1 mRNA (Fig. 1). This is in agreement with some previous reports (27, 29), but in contrast to another (33). Moreover, both isoforms were elevated 90 min after exercise, PGC-1α1 by ~60% and PGC-1α4 by three-to-fivefold (P < 0.05 in both cases), but only the expression of PGC-1α4 was further elevated (10-fold; P < 0.05) at 180 min of recovery.

Intake of leucine alone did not influence the exercise-induced changes in expression of PGC-1α4 mRNA, whereas ingestion of BCAA and EAA markedly suppressed the elevation after 180 min of recovery (P < 0.05 for both EAA and BCAA compared with both placebo and leucine) (Fig. 1B). Moreover, the three-to-fivefold enhancement in the level of PGC-1α4 mRNA 90 min after exercise was unaffected by amino acid intake (Fig. 1B). At the same time, intake of BCAA and EAA did not influence the small but significant elevation in PGC-1α1 mRNA (Fig. 1A). These divergent responses to resistance exercise, together with the differing sensitivities of the isoforms to intake of BCAA and EAA, indicate that they are regulated differently and play different roles in adaptation to training.

Interaction between mTORC1 signaling and level of PGC-1α mRNA. In agreement with previous reports, mTORC1 signaling, evaluated as phosphorylation of S6K1, was elevated to a larger extent by EAA (2) and BCAA (14) than by leucine or placebo, respectively. Although this effect of EAA was larger than with BCAA following 90 min of recovery, the increases in phosphorylation were similar (7- to 8-fold) 180 min after exercise (Fig. 2A). In disagreement with our hypothesis, this stimulation of mTORC1 by amino acids was not associated with an elevated level of PGC-1α mRNA; instead, the PGC-1α4 response was reduced by 50% and that of PGC-1α1 unaffected by ingestion of BCAA and EAA.

In light of previous indications of a positive relationship between mTORC1 and PGC-1α in mice skeletal muscle (8), this latter observation was unexpected. However, in a recent study, administration of rapamycin to mice was found to increase the PubMed ID in PGC-1α mRNA caused by 1 h of running, suggesting an inverse relationship between mTORC1 activity and PGC-1α (23). Nonetheless, the lack of any effect of leucine alone on the PGC-1α4 response, despite its stimulatory effect on mTORC1 signaling (Fig. 2A), suggests that there is no interaction between these two processes in human muscle. Alternatively, there may be a threshold level above which mTORC1 activation suppresses PGC-1α4 expression, which in the leucine trial was not attained.

Alterations in upstream kinases capable of activating PGC-1α. Exercise activates a number of signaling pathways, including those involving AMPK and p38 MAPK that contrib-

Fig. 1. Levels of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α1 (A) and PGC-1α4 (B) mRNA before and 90 and 180 min after resistance exercise. The subjects ingested (in random order) a solution containing either leucine, branched-chain amino acids (BCAA), essential amino acids (EAA), or placebo (flavored water) during the experiment. The values presented have been normalized to the level of GAPDH mRNA and presented as means ± SE for 8 subjects. The ANOVA revealed a main effect of time with respect to changes in the expression of PGC-1α1 and PGC-1α4 mRNA and a significant interaction (time × supplement) for mRNA expression of PGC-1α4. P < 0.05 vs. preexercise (*), placebo (#), and leucine ($\frac{\text{SE for GAPDH}}{\text{ME}}$).
ute to the acute induction of PGC-1α (1, 12). Here, the activity of AMPKα1 was not influenced by exercise, whereas AMPKα2 activity was elevated an average of 135% (*P* < 0.05) (Fig. 2, B and D), in agreement with a previous finding (10). Phosphorylation of p70S6 kinase (S6K1, A) and p38 mitogen-activated protein kinase (MAPK, C) and activity of AMP-activated protein kinase (AMPK) α1 (B) and AMPKα2 (D) before and immediately, 90 min, and 180 min after resistance exercise. Phosphorylation levels of S6K1 and p38 MAPK are normalized to corresponding total levels of each protein. Representative bands are shown above the graphs (rearranged to match the order of trials in the graph). The values presented are means ± SE for 8 subjects. The ANOVA revealed a main effect of time with respect to the changes in S6K1, AMPKα2, and p38 MAPK and a significant interaction (time × supplement) for S6K1.

The plasma level of leucine was increased to a similar extent (~30%) during exercise by ingestion of leucine alone, BCAA, and EAA.

**Changes in muscle glycogen, plasma amino acids, and insulin.** Availability of carbohydrates has been reported to have an impact on the induction of PGC-1α (4, 7, 25). Here, the initial levels of glycogen were similar in all four trials (average 536 mmol/kg dry muscle), as was the decrease during exercise (22–30%; *P* < 0.05) (Fig. 3). This would appear to rule out this factor as a potential mediator of the attenuated elevation of PGC-1α4 mRNA caused by intake of amino acids (Fig. 1A).

The plasma level of leucine was increased to a similar extent (~30%) during exercise by ingestion of leucine alone, BCAA, and EAA.

![Fig. 2. Phosphorylation of p70S6 kinase (S6K1, A) and p38 mitogen-activated protein kinase (MAPK, C) and activity of AMP-activated protein kinase (AMPK) α1 (B) and AMPKα2 (D) before and immediately, 90 min, and 180 min after resistance exercise. Phosphorylation levels of S6K1 and p38 MAPK are normalized to corresponding total levels of each protein. Representative bands are shown above the graphs (rearranged to match the order of trials in the graph). The values presented are means ± SE for 8 subjects. The ANOVA revealed a main effect of time with respect to the changes in S6K1, AMPKα2, and p38 MAPK and a significant interaction (time × supplement) for S6K1.](https://doi.org/10.220.33.2)"�"�

![Fig. 3. Changes in the muscle level of glycogen during resistance exercise and 3 h recovery. The values presented are means ± SE for 8 subjects. The ANOVA revealed a main effect of time, *P* < 0.05 vs. preexercise (*) and postexercise (†).](https://doi.org/10.220.33.2)"�"�
or EAA. The level increased even further and remained elevated throughout the recovery period (Fig. 4A). The levels of isoleucine and valine were elevated by the BCAA and EAA supplements but fell in the placebo and leucine trials (Fig. 4, B and C). The level of BCAA rose to the same extent when BCAA and EAA were ingested, but fell 25% in the placebo and leucine trials (Fig. 2D). Changes in plasma levels of these amino acids largely reflect changes in muscle levels (20). Hence, these observations suggest that the muscle level of BCAA may be related to the attenuated exercise-induced stimulation of PGC-1α expression, but, the potential underlying mechanism remains unclear.

The insulin release, evaluated as the area under the concentration-time curve for the period preexercise to 90 min postexercise, was larger when EAA was ingested compared with both placebo and leucine, whereas the release following BCAA intake differed significantly compared with placebo but not with leucine (Fig. 5). Although there was no difference between the EAA and BCAA trials, the lack of difference between the BCAA and leucine trials despite a 50% reduction of PGC-1α mRNA expression with the former supplement, does not support an involvement of insulin in the attenuated response of PGC-1α to resistance exercise.

In summary, intake of a mixture of BCAA or EAA attenuated the elevation in the level of PGC-1α mRNA caused by resistance exercise but had no effect on PGC-1α1 expression. Although the significance of this attenuation is not yet known, this difference does reveal differential regulation of the two isoforms. The attenuation of the PGC-1α response was not related to activation of AMPK, p38 MAPK, or to glycogen availability, since these factors changed to the same extent regardless of supplement. These observations raise the possibility that these factors induce PGC-1α1 rather than PGC-1α mRNA. Moreover, based on the differential response to ingestion of leucine alone compared with BCAA or EAA, induction of PGC-1α does not appear to influence the stimulation of mTORC1.

Fig. 4. Alterations in plasma levels of amino acids during and after resistance exercise. The values presented are means ± SE for 8 subjects. The ANOVA revealed a main effect of time as well as an interaction (time × supplement) for the individual amino acids and for BCAA. P < 0.05 vs. preexercise (*), placebo (#), and leucine (§).

Fig. 5. Changes in plasma concentration of insulin during and after resistance exercise as well as area under the curve (AUC) for the period preexercise to 90 min postexercise. At this time point the level had returned to basal. The values presented are means ± SE for 8 subjects. P < 0.05 vs. placebo (#) and leucine (§).
signaling. It is therefore possible that PGC-1α regulates muscle growth through interaction with other signaling pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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