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Intake of branched-chain amino acids influences the levels of MAFbx mRNA and MuRF-1 total protein in resting and exercising human muscle

Marcus Borgenvik,[1] William Apró,[1,2] and Eva Blomstrand[1,3]

[1]*The Åstrand Laboratory, Swedish School of Sport and Health Sciences; and* [2]*Departments of Clinical Sciences, Intervention and Technology and* [3]*Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*

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Borgenvik M, Apró W, Blomstrand E. Intake of branched-chain amino acids influences the levels of MAFbx mRNA and MuRF-1 total protein in resting and exercising human muscle. *Am J Physiol Endocrinol Metab* 302: E510–E521, 2012. First published November 29, 2011; doi:10.1152/ajpendo.00353.2011.—Resistance exercise and amino acids are two major factors that influence muscle protein turnover. Here, we examined the effects of resistance exercise and branched-chain amino acids (BCAA), individually and in combination, on the expression of anabolic and catabolic genes in human skeletal muscle. Seven subjects performed two sessions of unilateral leg press exercise with randomized supplementation with BCAA or flavored water. Biopsies were collected from the vastus lateralis muscle of both the resting and exercising legs before and repeatedly after exercise to determine levels of mRNA, protein phosphorylation, and amino acid concentrations. Intake of BCAA reduced ($P < 0.05$) MAFbx mRNA by 30 and 50% in the resting and exercising legs, respectively. The level of MuRF-1 mRNA was elevated ($P < 0.05$) in the exercising leg two- and threefold under the placebo and BCAA conditions, respectively, whereas MuRF-1 total protein increased by 20% ($P < 0.05$) only in the placebo condition. Phosphorylation of $p70^{S6k}$ increased to a larger extent (~2-fold; $P < 0.05$) in the early recovery period with BCAA supplementation, whereas the expression of genes regulating mTOR activity was not influenced by BCAA. Muscle levels of phenylalanine and tyrosine were reduced (13–17%) throughout recovery ($P < 0.05$) in the placebo condition and to a greater extent (32–43%; $P < 0.05$) following BCAA supplementation in both resting and exercising muscle. In conclusion, BCAA ingestion reduced MAFbx mRNA and prevented the exercise-induced increase in MuRF-1 total protein in both resting and exercising leg. Furthermore, resistance exercise differently influenced MAFbx and MuRF-1 mRNA expression, suggesting both common and divergent regulation of these two ubiquitin ligases.

muscle RING-finger 1; muscle atrophy F-box; phenylalanine; resistance exercise; Rheb; ubiquitin ligases

RESISTANCE EXERCISE AND AMINO ACIDS are each capable of stimulating human muscle protein synthesis, and when combined the effect is enlarged (6, 33, 46, 52). Because resistance exercise also increases the rate of muscle protein breakdown (52), ingestion of amino acids is essential to achieve a positive net muscle protein balance (33, 57). With respect to the amino acids, the branched-chain amino acids (BCAA) and, in particular leucine, appear to be the most potent based on data from experimental animals (31). Furthermore, both resistance exercise and amino acids are thought to mediate their effects on protein synthesis through activation of the pathway involving the mammalian target of rapamycin (mTOR) (17, 23).

Address for reprint requests and other correspondence: Address for correspondence: E. Blomstrand, Swedish School of Sport and Health Sciences, Box 5626, SE-114 86 Stockholm, Sweden (e-mail: eva.blomstrand@gih.se).

Activation of mTOR by phosphorylation exerts well-characterized effects on several downstream proteins, including activation of p70 S6 kinase ($p70^{S6k}$) and the eukaryotic elongation factor-2 (eEF2) as well as inactivation of eIF4E-binding protein-1 (4E-BP1), thereby enhancing translational initiation and elongation (58). In contrast, the upstream regulation of mTOR has been less extensively investigated, although this regulation associated with exercise and nutritional stimuli appears to differ. Muscle contraction (17) as well as insulin (32) are capable of activating protein kinase B/Akt, thereby leading to mTOR activation either directly (49) or via stimulation of GTP binding to the small G protein Rheb (28). Moreover, muscle contraction has been proposed to activate mTOR through the second messenger phosphatidic acid (27) and by downregulating the expression of REDD1 and -2, two negative regulators of mTOR (15). The corresponding activation by amino acids is suggested to involve not only Rheb but also stimulation of human vacuolar protein sorting-34 (hVps34), a class III phosphoinositide 3-kinase (4, 50). Even though hVps34 activity has been shown to be regulated by both Ca^{2+}/CaM (25) and hVps15 (61) in vitro, the mechanisms by which amino acids augment protein synthesis through mTOR signaling in vivo are unidentified.

The molecular mechanisms underlying protein breakdown involve tagging of proteins with ubiquitin molecules and subsequent degradation by the 26S proteasome (36). MuRF-1 (muscle RING-finger 1) and MAFbx (muscle atrophy F-box), two muscle-specific ubiquitin ligases, are upregulated in connection with muscle atrophy (10, 29) by the FOXO family of transcription factors. These factors are, in turn, regulated upstream by Akt in such way that activation of Akt downregulates the expression of MuRF-1 and MAFbx and thereby counteracts atrophy (53, 55).

Although extensive evidence for the stimulatory effect on protein synthesis of amino acids, either alone or in combination with exercise, has been reported (33), their effect on protein breakdown is elusive. Most previous investigations involving ingestion of essential amino acids (EAA) in connection with resistance exercise have revealed no attenuating effect on protein breakdown (33, 57). In contrast, when BCAA or leucine alone is administered, results indicate that protein degradation in subjects at rest (39, 48) or performing eccentric endurance exercise is reduced (41). Moreover, in rats, supplementation of BCAA/leucine appears to attenuate the expression of MAFbx and MuRF-1 mRNA (5), suppress protein degradation and attenuate muscle atrophy (47, 56).

In light of our limited knowledge of the upstream regulation of mTOR evoked by exercise and nutritional stimuli, as well as emerging indications that BCAA help attenuate muscle proteolysis, the present study was designed to examine the indepen-

dent and combined effects of resistance exercise and BCAA on the expression of mRNA-encoding proteins involved in the upstream regulation of mTOR, as well as the proteolytic factors MuRF-1 and MAFbx. In addition, phosphorylation of proteins in the mTOR pathway and levels of amino acids in resting and exercising muscle were determined. Our hypothesis was that BCAA would alter the mRNA expression of proteins that regulate mTOR in such manner that its activity would be elevated, while at the same time reducing expression of the ubiquitin ligases.

MATERIALS AND METHODS

Participants. Seven healthy participants, five men and two women, were included in the study. They were recreationally active once or twice per week but did not perform resistance exercise on a regular basis. Their mean (±SE) age was 27 (±2) yr; height 175 (±5) cm; weight 67 (±7) kg; and maximal oxygen uptake 46 (±1) $ml \cdot min^{-1} \cdot kg^{-1}$. Each subject was fully informed about the purpose and procedures of the study and their right to withdraw at any point. This study was approved by the Regional Ethics Review Board in Stockholm and performed in accordance with the principles outlined in the Declaration of Helsinki. Five of these same subjects (3 men and 2 women) also took part in one of our earlier investigations (2).

Pretest. Prior to initiation of the actual experiments, each participant underwent three preparatory sessions on a leg press machine (243 Leg Press 45°; Gymleco, Stockholm, Sweden) following a 10-min warm-up on a cycle ergometer. The first test was designed to determine each participant's one-repetition maximum (1-RM) performed with one leg that was chosen randomly. The 1-RM was assessed by gradually increasing the load until the participant was unable to perform more than one single repetition (90–180° knee angle). The purpose of the second and third sessions was to familiarize our subjects with the intensity and frequency of repetitions involved in the actual experimental protocol. These sessions began with a warm-up set of 10 repetitions with no load, followed by two sets consisting of five repetitions at 25% and 50% of 1-RM. Following these warm-ups, each participant performed the actual protocol of resistance exercise (see below). These two sessions of familiarization were conducted 1 wk apart, ~10 days prior to the actual experiment.

Utilizing continuous monitoring with an on-line system (Amis 2001; Innovision, Odense, Denmark), maximal oxygen uptake was determined while subjects exercised on a mechanically braked cycle ergometer (Monark Ergomedic 839E, Vansbro, Sweden) with a gradually increasing work rate until volitional exhaustion, as described earlier by Åstrand and Rodahl (3).

Experimental protocol. The experimental set-up involved a randomized, double-blinded, cross-over design. Participants had been instructed to refrain from any type of intense physical activity and to consume a standardized diet during the 2 days preceding the experiment. This diet consisted of 17 energy percent (E%) protein, 26 E% fat, and 57 E% carbohydrate and contained ~2,100 kcal for the women and 2,700 kcal for the men (values based on the subjects' reported levels of activity).

On the day of the experiment, the men and women arrived at the laboratory at 7:30 AM after having fasted since 9:00 PM the evening before. Upon arrival, the participants were asked to lie down, and a catheter was inserted into the antecubital vein for repeated sampling of blood, the first of which was drawn after 30 min of rest. Subsequently, local anesthesia was administered, and resting biopsies (Pre-Ex) taken from the vastus lateralis muscle of both legs using a Weil-Blakesley conchotome (Ab Wisex, Mölndal, Sweden).

Prior to performing the resistance exercise, each participant warmed up for 10 min on a cycle ergometer (Monark Ergomedic 839E) at a work rate of 100 W for the men and 60 W for the women. Thereafter, they were seated in the leg press machine, where they performed three warm-up sets of five repetitions at 0, 25, and 50% of 1-RM followed by four sets of 10 repetitions at 80% of 1-RM and, finally, four sets of 15 repetitions at 65% of 1-RM. All of the resistance exercise was performed with one leg only, with 5 min of rest after each set. The entire session lasted ~40 min. This experimental protocol was carried out twice by each subject, with 4 wk between the two occasions.

Blood samples were collected in heparinized tubes during the rest period prior to warm-up (see above), immediately before beginning the resistance exercise, after the fifth set (i.e., after ~25 min of exercise), directly after termination of the exercise, and following 15, 30, 60, 120, and 180 min of recovery. These blood samples were centrifuged at 9,000 *g* for 3 min, and the plasma thus obtained was stored at −80°C for future analyses.

Muscle biopsies from both legs (starting with the exercising leg) were taken immediately after termination of exercise (Post-Ex) and following 1 and 3 h of recovery. However, in two of the participants, biopsies were taken only prior to the exercise and following 3 h of recovery. The first biopsy was taken ~8–9 cm above the mid-patella and the following biopsies 3–4 cm proximal to the previous one. Biopsy samples were immediately frozen in liquid nitrogen and stored at −80°C for later analysis.

Each participant ingested 150 ml of either a mixture of the three BCAA (45% leucine, 30% valine, and 25% isoleucine; Ajinomoto, Kanagawa, Japan) in flavored water or flavored water alone at rest prior to the warm-up, directly before beginning the resistance exercise, during and immediately after exercise, and following 15 and 45 min of recovery, resulting in a total intake of 85 mg BCAA/kg body wt in 900 ml of flavored water. Both drinks were lemon flavored, contained salts and artificial sweetener, and were indistinguishable in taste. The amount and timing of BCAA ingestion were similar, and the composition of the BCAA mixture was the same as in a previous study that had led to marked increases in mTOR signaling (30).

Plasma analyses. For determination of amino acids, plasma samples were first deproteinized by precipitation with ice-cold 5% trichloroacetic acid (1:5), maintained on ice for 20 min, and centrifuged at 10,000 *g* for 3 min, and the supernatant obtained was stored at −80°C for later analysis. The concentrations of free amino acids in

Table 1. *Details of primers employed for RT-qPCR*

Gene	Function of the Product	Forward Primer	Reverse Primer	No. in GenBank
MuRF-1	Regulator of proteolysis	GCCACCTTCCTCTTGACTG	ATTCTTCCTCTTCATCTGTC	NM_032588
MAFbx	Regulator of proteolysis	GAGCGACCTCAGCAGTTAC	GGCAGTTGAGAAGTCCAGTC	NM_148177
Rheb	Positive regulator of mTOR	TTTTTGGAATCTTCTGCTAAAGAAA	AAGACTTGCCTTGTGAAGCTG	NM_005614
hVps34	Nutrient regulator of mTOR	GAAGCAGATGGATCAGAACC	CCAGCCAATCTACTTTCACC	NM_002647
REDD1	Negative regulator of mTOR	CTGGAGAGCTCGGACTGC	TCCAGGTAAGCCGTGTCTTC	NM_019058
REDD2	Negative regulator of mTOR	CCCAGAGAGCCTGCTGCTAAGTG	TTGCTTTGATTTGGACAGACA	NM_145244
GAPDH	Housekeeping (control)	AACCTGCCAAATATGATGAC	TCATACCAGGAAATGAGCTT	NM_002046

MuRF-1, muscle RING finger 1; MAFbx, muscle atrophy F-box; Rheb, ras-homolog enriched in brain; hVps34, human vacuolar protein sorting-34; REDD1 and -2, regulated in development and DNA damage response-1 and 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

the supernatants were measured by reversed-phase high-performance liquid chromatography (HPLC; Waters, Milford, MA) employing orthophtaldehyde (OPA) as the derivatizing agent as previously described (9). Plasma glucose and lactate were analyzed as described by Bergmeyer (7).

Muscle amino acid analyses. The muscle biopsies were lyophilized and blood and connective tissue subsequently removed by dissection under a light microscope (Carl Zeiss, Germany). Following transfer of 2–4 mg of muscle tissue to Eppendorf tubes, the amino acids were extracted in ice-cold 5% trichloroacetic acid (40 μl/mg), and the tubes were then maintained on ice for 30 min and centrifuged at 10,000 *g* for 3 min, and the resulting supernatant was removed and stored at −80°C. The concentrations of amino acids in the supernatants were later analyzed as described above.

Immunoblot analysis. Freeze-dried and cleaned muscle samples were homogenized as previously described (2). Homogenates were centrifuged, and the resulting supernatant was stored at −80°C. Protein concentrations were determined, after which samples were diluted in Laemmli sample buffer (Bio-Rad Laboratories, Richmond, CA) and homogenizing buffer to obtain a final protein concentration of 1.5 μg/μl. Following dilution, the samples were heated at 95°C for 5 min and then kept at −20°C until further analysis.

Details of the Western blotting procedures have been described elsewhere (2). Briefly, and with minor modifications, samples were separated by SDS-PAGE on Criterion cell gradient gels (4–20% acrylamide, Bio-Rad Laboratories) following which the gels were equilibrated in transfer buffer (25 mM Tris base, 192 mM glycine, and 10% methanol) for 30 min. The proteins were then transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories), which in turn were stained with MemCode Reversible Protein Stain Kit (Pierce Biotechnology) to confirm successful transfer of proteins. All samples from each subject were run on the same gel.

Membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS; 20 mM Tris base, 137 mM NaCl, pH 7.6) containing 5% nonfat dry milk. Thereafter, the membranes were incubated overnight with commercially available primary phospho-specific antibodies diluted in TBS supplemented with 0.1% Tween-20 containing 2.5% nonfat dry milk (TBS-TM). After incubation with primary antibodies, membranes were washed with TBS-TM and then incubated for 1 h at room temperature with appropriate secondary antibodies. Membranes were then washed serially prior to visualization of the phosphorylated proteins by chemiluminescent detection on a Molecular Imager ChemiDoc XRS system. All bands were analyzed using the contour tool in the Quantity One version 4.6.3 software (Bio-Rad Laboratories), and phosphorylated proteins were expressed as arbitrary units relative to α-tubulin.

Antibodies. Primary antibodies raised against phospho-mTOR (Ser^{2448}, 1:500), phospho-$p70^{S6k}$ (Thr^{389}, 1:1,000), phospho-Akt (Ser^{473}, 1:1,000) and phospho-4E-BP1 ($Thr^{36/37}$, 1:1,000) were purchased from Cell Signaling Technology (Beverly, MA). Primary antibody against MAFbx (1:1,000) was purchased from Abcam (Cambridge, UK), for MuRF-1(1:1,000) from Santa Cruz Biotechnology (Santa Cruz, CA), and for α-tubulin (1:5,000) from Sigma-Aldrich (St. Louis, MO). Secondary rabbit and mouse antibodies (1:10,000) were purchased from Cell Signaling Technology and secondary goat antibodies (1:5,000) from Abcam.

RNA extraction and real-time PCR. Total RNA was extracted from ~3 mg of muscle that had been freeze-dried and cleaned (see above) by homogenization with a Polytron (Kinematica, Lucerne, Switzerland) in PureZOL RNA isolation reagent (Bio-Rad Laboratories, Sundbyberg, Sweden) in accordance with the manufacturer's instruction. The final amount and purity of the RNA were determined by spectrophotometry.

Two micrograms of the isolated RNA was subjected to reverse transcription utilizing the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Sweden) to produce 20 μl of cDNA, with thermal cycling

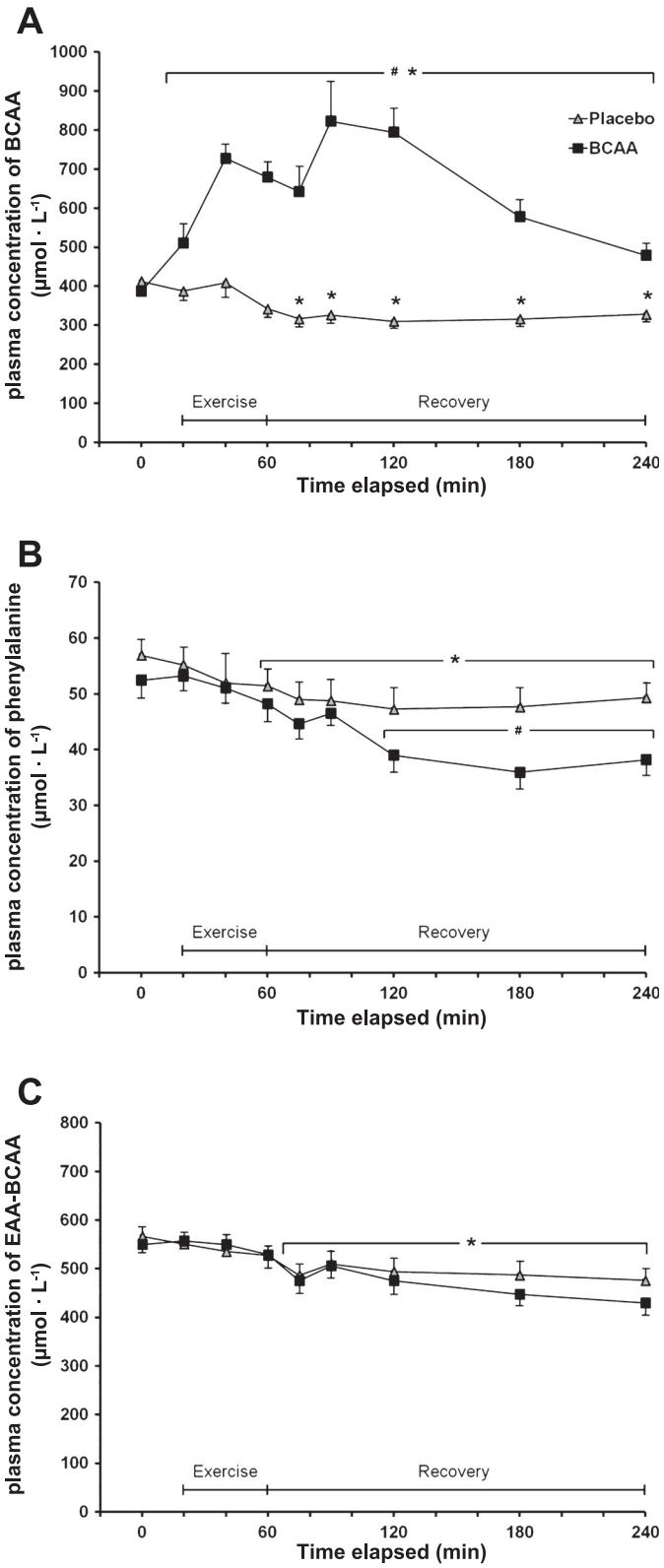

Fig. 1. The plasma concentration of branched-chain amino acids (BCAA; *A*), phenylalanine (*B*), and essential amino acids (EAA)-BCAA (*C*) under the placebo session (triangles) and the BCAA session (boxes). Values presented are means ± SE ($n = 7$). *Different from Rest, $P < 0.05$; #different from placebo, $P < 0.05$.

being performed in a Bio-Rad iCycler (Bio-Rad Laboratories, Sweden).

The mRNA was quantified using real-time quantitative PCR (RT-qPCR), with the housekeeping GAPDH mRNA as an internal control. To determine a suitable cDNA concentration, annealing temperature, and PCR cycling protocol for each primer, the pooled cDNA obtained from all of the participants was diluted (1:5, 1:25, 1:125, 1:625, and 1:3,125), and RT-qPCR was performed on a Bio-Rad iCycler. The standard curves for all of the primers exhibited high efficiency and an r^2 value of >0.99. In addition, single melting peaks were observed during melt curve analysis, confirming the presence of only a single product. The dilutions found to be suitable were 1:30 in the case of REDD1 and -2 and hVps34, 1:40 for Rheb, 1:200 for MuRF-1 and MAFbx, and 1:600 for GAPDH.

The reliability of GAPDH mRNA as an internal control was validated by the $2^{-\Delta C'_T}$ method, where $\Delta C'_T = C_{T\ time\ \times} - C_{T\ time\ 0}$, as described by Livak and Schmittgen (37). The mean $2^{-\Delta C'_T}$ values for the exercising leg in the placebo and BCAA condition were 0.84 ± 0.16 and 1.09 ± 0.24, respectively. The corresponding values in the resting leg were 0.94 ± 0.19 and 1.05 ± 0.19. A two-way repeated-measures ANOVA (leg × supplement) revealed no main effect or interaction ($P > 0.43$); i.e., the level of GAPDH mRNA was not influenced by either exercise or supplementation and was therefore suitable as an internal control.

All analyses were performed in triplicate, and all samples from each subject were analyzed on a single 96-well plate for direct relative comparisons. The 25-μl mixtures for RT-qPCR amplification contained 2× SYBR Green Supermix (Bio-Rad Laboratories, Sweden), 11.5 μl of template cDNA diluted in RNase-free water and 0.5 μl of 10 μmol/l forward and reverse primers (Cybergene, Stockholm, Sweden). The details concerning these primers are documented in Table 1. The RT-qPCR reactions were carried out in a Bio-Rad iCycler, initially at 95°C for 3 min, followed by thermal cycling, 40× at 95°C and 58°C and 72°C for 30 s each. All of the C_T values obtained ranged between 18 and 29, and the same fixed threshold was employed for all genes of interest. Relative changes in mRNA expression for all genes of interest were quantified by the $2^{-\Delta\Delta C_T}$ method, as described by Schmittgen and Livak (54), and the results are expressed as fold changes compared with baseline (3 h vs. Pre-Ex) for all conditions, i.e., resting and exercising leg with placebo or BCAA supplementation.

Statistical analyses. All data are expressed as means ± SE and were checked for normal distribution before performing parametric statistical analyses. For variables exhibiting a positively skewed distribution, log-transformation was performed prior to analysis. A two-way repeated-measures ANOVA (leg and supplement) was employed to compare fold changes (from Pre-Ex) in the levels of mRNA expression in the resting and exercising legs and with BCAA and placebo supplementation. In the case of MAFbx mRNA, an outlier value higher than six standard deviations above the mean was excluded from the analysis.

To detect changes in plasma concentrations of glucose, lactate, and amino acids, a two-way repeated-measures ANOVA was employed, with time and type of supplementation as factors. A three-way repeated-measures ANOVA was used to evaluate changes in muscle amino acid concentrations, in phosphorylation of enzymes in the mTOR pathway, and in protein content of MAFbx and MuRF-1 with time (Pre-Ex, Post-Ex, and 1 and 3 h of recovery), supplementation and leg as factors. In this latter analysis, the Post-Ex and 1-h values for two participants were missing; however, exclusion of these same participants from the analysis had no significant impact on the mean values or P values obtained. When a significant main effect and/or interaction was observed, Fisher's LSD post hoc test was employed to pinpoint where the differences occurred. All statistical analyses were performed with the Statistica 8.0 software (StatSoft, Tulsa, OK), and a P value < 0.05 was considered statistically significant.

RESULTS

Resistance exercise. With either placebo or BCAA supplementation, all subjects were able to perform the first four sets of 10 repetitions at 80% 1-RM. During the final four sets of 15 repetitions at 65% 1-RM, one subject could perform only 14

Table 2. *Plasma concentrations of amino acids following BCAA or placebo ingestion*

Amino acid	Condition	Rest	PreEx	Mid -Ex	PostEx	15 min	30 min	60 min	120 min	180 min
Histidine	Placebo	90 ± 2	88 ± 4	90 ± 4	88 ± 3	80 ± 4*	84 ± 2*	83 ± 1*	84 ± 2*	82 ± 2*
	BCAA	91 ± 4	92 ± 4	94 ± 4	90 ± 4	82 ± 4*	84 ± 4*	85 ± 3*	84 ± 4*	83 ± 3*
Isoleucine	Placebo	65 ± 6	60 ± 5	65 ± 8	51 ± 5	47 ± 4	47 ± 4	45 ± 3*	47 ± 3	51 ± 3
	BCAA	57 ± 4	87 ± 12*†	135 ± 7*†	121 ± 9*†	114 ± 15*†	158 ± 23*†	146 ± 15*†	90 ± 7*†	70 ± 5†
Leucine	Placebo	124 ± 10	117 ± 10	122 ± 14	99 ± 8	92 ± 8*	93 ± 8*	89 ± 7*	94 ± 7	102 ± 7
	BCAA	118 ± 8	168 ± 19*†	250 ± 13*†	227 ± 15*†	217 ± 26*†	292 ± 40*†	275 ± 25*†	185 ± 14*†	151 ± 11*†
Lysine	Placebo	187 ± 15	186 ± 14	176 ± 15	172 ± 12	157 ± 12*	165 ± 16*	161 ± 15*	164 ± 16*	160 ± 14*
	BCAA	182 ± 16	185 ± 14	183 ± 15	177 ± 18	151 ± 20*	167 ± 18*	158 ± 16*	149 ± 11*	140 ± 13*
Methionine	Placebo	27 ± 1	25 ± 2	25 ± 3	25 ± 2	23 ± 2*	23 ± 2*	22 ± 2*	22 ± 2*	22 ± 2*
	BCAA	27 ± 1	28 ± 1	28 ± 1	26 ± 2	23 ± 2*	25 ± 1*	22 ± 1*	20 ± 1*	18 ± 1*
Phenylalanine	Placebo	57 ± 3	55 ± 3	52 ± 5	51 ± 3*	49 ± 3*	49 ± 4*	47 ± 4*	48 ± 3*	49 ± 3*
	BCAA	52 ± 3	53 ± 3	51 ± 3	48 ± 3*	45 ± 3*	46 ± 2*	39 ± 3*†	36 ± 3*†	38 ± 3*†
Threonine	Placebo	147 ± 4	139 ± 5	139 ± 8	138 ± 5	128 ± 7*	137 ± 6*	129 ± 8*	122 ± 7*	116 ± 6*
	BCAA	143 ± 5	144 ± 5	143 ± 7	138 ± 9	129 ± 8*	136 ± 8*	127 ± 9*	119 ± 9*	111 ± 8*
Tryptophan	Placebo	58 ± 2	58 ± 3	54 ± 3	53 ± 2*	50 ± 3*	52 ± 1*	51 ± 2*	48 ± 2*	46 ± 2*
	BCAA	55 ± 3	55 ± 4	52 ± 3	49 ± 2*	46 ± 2*	48 ± 2*	45 ± 3*	39 ± 3*	40 ± 3*
Tyrosine	Placebo	63 ± 5	59 ± 5	56 ± 8	57 ± 5*	51 ± 5*	53 ± 5*	50 ± 4*	49 ± 4*	48 ± 4*
	BCAA	61 ± 7	61 ± 6	59 ± 7	55 ± 7*	49 ± 6*	51 ± 5*	43 ± 5*†	37 ± 6*†	33 ± 4*†
Valine	Placebo	224 ± 10	210 ± 10	222 ± 15	192 ± 8	178 ± 9*	185 ± 9*	176 ± 8*	175 ± 9*	175 ± 9*
	BCAA	212 ± 14	255 ± 21*†	343 ± 19*†	331 ± 26*†	311 ± 26*†	372 ± 41*†	373 ± 24*†	302 ± 24*†	259 ± 16*†
BCAA	Placebo	413 ± 24	387 ± 24	409 ± 37	342 ± 20	316 ± 21*	326 ± 21*	310 ± 18*	316 ± 18*	328 ± 18*
	BCAA	387 ± 25	510 ± 51*†	728 ± 36*†	679 ± 40*†	642 ± 65*†	822 ± 99*†	795 ± 62*†	578 ± 44*†	479 ± 32*†
EAA-BCAA	Placebo	567 ± 19	551 ± 24	536 ± 35	528 ± 19	486 ± 24*	510 ± 27*	494 ± 29*	487 ± 29*	476 ± 25*
	BCAA	550 ± 17	557 ± 11	550 ± 21	529 ± 27	475 ± 25*	506 ± 25*	476 ± 28*	447 ± 23*	430 ± 24*

Values presented are means ± SE (μmol/l) for 7 subjects. BCAA, branched-chain amino acids; EAA-BCAA, essential amino acids excluding BCAA. Blood samples were collected at rest prior to warm-up immediately before resistance exercise (Pre-Ex), after the 5th set (Mid-Ex), directly after termination of the exercise (Post-Ex), and following 15, 30, 60, 120, and 180 min of recovery. *Different from Rest, $P < 0.05$; †different from placebo, $P < 0.05$.

Table 3. *Levels of amino acids in vastus lateralis muscle of resting leg following supplementation with BCAA or placebo*

Amino Acid	Condition	Pre-Ex	Post-Ex	1 h	3 h
Histidine	Placebo	1,220 ± 70	1,580 ± 110	1,430 ± 120	1,370 ± 120
	BCAA	1,290 ± 130	1,330 ± 240	1,400 ± 180	1,160 ± 130
Isoleucine	Placebo	260 ± 30	210 ± 30	190 ± 10	220 ± 20
	BCAA	230 ± 20	410 ± 40*†	450 ± 40*†	280 ± 40*†
Leucine	Placebo	500 ± 50	380 ± 50	350 ± 30	430 ± 40
	BCAA	470 ± 40	810 ± 90*†	910 ± 70*†	600 ± 90*†
Lysine	Placebo	1,990 ± 250	2,190 ± 370	1,980 ± 410	1,950 ± 240
	BCAA	1,900 ± 220	1,730 ± 280	1,610 ± 210	1,300 ± 200
Methionine	Placebo	110 ± 20	100 ± 10	90 ± 10	110 ± 10
	BCAA	100 ± 10	110 ± 10	70 ± 10*†	70 ± 10*†
Phenylalanine	Placebo	210 ± 20	190 ± 20	170 ± 20*	180 ± 10*
	BCAA	190 ± 10	180 ± 20	120 ± 20*†	130 ± 10*†
Threonine	Placebo	1,990 ± 250	2,190 ± 370	1,980 ± 410	1,950 ± 240
	BCAA	1,900 ± 220	1,730 ± 280	1,610 ± 210	1,300 ± 200
Tryptophan	Placebo	60 ± 10	60 ± 5	60 ± 10	55 ± 5*
	BCAA	60 ± 10	60 ± 10	50 ± 10	40 ± 5*
Tyrosine	Placebo	250 ± 30	240 ± 40	230 ± 20	210 ± 20*
	BCAA	260 ± 30	240 ± 40	190 ± 50*†	150 ± 20*†
Valine	Placebo	660 ± 50	560 ± 50	540 ± 40	560 ± 40
	BCAA	620 ± 30	830 ± 70*†	970 ± 100*†	720 ± 70*†
BCAA	Placebo	1,430 ± 120	1,140 ± 110	1,080 ± 70	1,210 ± 90
	BCAA	1,330 ± 80	2,060 ± 200*†	2,330 ± 210*†	1,600 ± 200*†
EAA-BCAA	Placebo	5,670 ± 400	6,190 ± 490	5,910 ± 490	5,690 ± 260
	BCAA	5,650 ± 460	5,610 ± 530	5,320 ± 480*†	4,580 ± 310*†

Values presented are means ± SE (μmol/kg dry muscle tissue) for 7 subjects, except for Post-Ex and 1 h, where $n = 5$. Muscle biopsies were collected at rest prior to warm-up (Pre-Ex), directly after termination of exercise (Post-Ex), and following 1 and 3 h of recovery. *Different from Pre-Ex, $P < 0.05$; †different from placebo, $P < 0.05$.

and 13 repetitions in the last two sets, respectively, in both conditions. The average workload in the first and final four sets was 130 ± 15 and 105 ± 12 kg, respectively.

Plasma concentrations. The plasma concentrations of glucose with BCAA or placebo supplementation did not differ, but were reduced under both conditions ($P < 0.05$) following 30 min of recovery and were attenuated throughout the remaining period of recovery. The initial glucose concentrations were 5.4 ± 0.2 and 5.1 ± 0.2 mmol/l under the placebo and BCAA conditions, respectively, and did not fall below 4.5 mmol/l at any time point. Plasma levels of lactate were significantly elevated ($P < 0.05$) during resistance exercise, reaching a

Table 4. *Levels of amino acids in vastus lateralis muscle of exercising leg following supplementation with BCAA or placebo*

Amino Acid	Condition	Pre-Ex	Post-Ex	1 h	3 h
Histidine	Placebo	1,280 ± 100	1,570 ± 70	1,390 ± 50	1,240 ± 170
	BCAA	1,120 ± 120	1,260 ± 100	1,170 ± 110	1,140 ± 140
Isoleucine	Placebo	250 ± 30	220 ± 20	190 ± 10	210 ± 20
	BCAA	260 ± 10	440 ± 70*†	400 ± 50*†	290 ± 40*†
Leucine	Placebo	470 ± 50	390 ± 40	350 ± 30	400 ± 60
	BCAA	530 ± 30	850 ± 140*†	790 ± 80*†	630 ± 80*†
Lysine	Placebo	1,970 ± 250	2,180 ± 360	2,000 ± 230	1,820 ± 300
	BCAA	1,680 ± 160	1,790 ± 150	1,430 ± 120	1,290 ± 200
Methionine	Placebo	100 ± 10	110 ± 10	100 ± 10	100 ± 20
	BCAA	110 ± 10	110 ± 10	70 ± 10*†	80 ± 10*†
Phenylalanine	Placebo	200 ± 20	200 ± 10	170 ± 10*	170 ± 20*
	BCAA	220 ± 10	200 ± 20	120 ± 20*†	130 ± 10*†
Threonine	Placebo	1,920 ± 90	2,190 ± 140	2,320 ± 120	2190 ± 70
	BCAA	2,150 ± 130	2,400 ± 150	1,900 ± 60	2,180 ± 240
Tryptophan	Placebo	60 ± 5	60 ± 5	60 ± 5	50 ± 5*
	BCAA	60 ± 5	70 ± 10	50 ± 5	40 ± 5*
Tyrosine	Placebo	240 ± 20	240 ± 40	210 ± 30	200 ± 20*
	BCAA	270 ± 30	260 ± 50	180 ± 40*†	160 ± 20*†
Valine	Placebo	610 ± 50	590 ± 40	550 ± 30	540 ± 40
	BCAA	660 ± 40	870 ± 100*†	830 ± 80*†	760 ± 50*†
BCAA	Placebo	1,330 ± 120	1,200 ± 100	1,080 ± 50	1,140 ± 110
	BCAA	1,460 ± 70	2,150 ± 320*†	2,010 ± 200*†	1,680 ± 160*†
EAA-BCAA	Placebo	5,510 ± 400	6,300 ± 510	6,000 ± 330	5,550 ± 400
	BCAA	5,330 ± 300	5,810 ± 100	4,730 ± 240*†	4,830 ± 320*†

Values presented are means ± SE (μmol/kg dry muscle tissue) for 7 subjects, except for Post-Ex and 1 h, where $n = 5$. Muscle biopsies were collected at rest prior to warm-up (Pre-Ex), directly after termination of exercise (Post-Ex), and following 1 and 3 h of recovery. *Different from Pre-Ex, $P < 0.05$; †different from placebo, $P < 0.05$.

maximum of 8 ± 1.5 mmol/l and returning to basal levels following 60 min of recovery, with no differences between placebo and BCAA supplementation.

As expected, and as illustrated in Fig. 1*A* and Table 2, supplementation with BCAA resulted in a rapid elevation of the plasma levels of these amino acids, with a 113% increase following 30 min of recovery, and this value remained higher than the corresponding values at rest and with placebo supplementation throughout the rest of the trial ($P < 0.05$). Following supplementation, the changes in plasma for the individual BCAA differed. The plasma concentration of valine peaked following 30 min of recovery at a value 76% higher than at rest and remained significantly elevated during the entire recovery period. The corresponding values for leucine and isoleucine at the same time point were 150 and 175%, respectively, and the isoleucine concentration returned to basal levels after 120 min of recovery, whereas the concentration of leucine was still significantly elevated following 180 min of recovery. In the placebo condition, the plasma concentration of BCAA declined slightly 15 min after exercise and was attenuated during the remaining 180 min of recovery ($P < 0.05$).

With both kinds of supplementation, the plasma levels of phenylalanine fell ($P < 0.05$) below baseline values directly after resistance exercise (Fig. 1*B*). In the case of BCAA supplementation, this level was further reduced following 60 min of recovery and was lower than the corresponding placebo value for the remainder of the recovery period ($P < 0.05$). As also depicted in Fig. 1*C*, the plasma levels of EAA other than BCAA (EAA-BCAA), were lower ($P < 0.05$) than at rest compared with the levels at rest following 15 min of recovery and remained attenuated thereafter, with no difference between the placebo and BCAA conditions.

Muscle amino acids. Ingestion of BCAA also resulted in a significant enhancement of these amino acids in the vastus lateralis muscle of both the resting and exercising legs (Tables 3 and 4 and Fig. 2*A*). Analysis by three-way ANOVA revealed main effects of time ($P < 0.05$) and the type of supplementation ($P < 0.01$), as well as an interaction between these two factors ($P < 0.01$). In the exercising and resting legs, the highest levels of BCAA were observed immediately after exercise and following 1 h of recovery, respectively. The magnitude of the changes in muscle concentrations of valine, leucine, and isoleucine (Tables 3 and 4) paralleled the corresponding changes in the plasma.

The muscle level of phenylalanine was not altered immediately after exercise with either supplementation or in either leg. However, following 1 h of recovery, this level was reduced ($P < 0.05$) by 15 and 20% in the exercising and resting legs, respectively, in the placebo condition. This was also the case with BCAA supplementation, however, to a significantly greater extent, 37 and 45% ($P < 0.05$), in the resting and exercising leg, respectively (Fig. 2*B*). Thereafter, the phenylalanine concentrations remained reduced in both legs under both conditions during the entire recovery period. Muscle levels of tyrosine exhibited a similar pattern, but slightly delayed.

Muscle levels of EAA-BCAA were unaltered immediately after exercise under both conditions but were reduced in both legs ($P < 0.05$) following 1 and 3 h of recovery with BCAA supplementation. At these same time points, these latter concentrations were also lower ($P < 0.05$) than in the correspond-

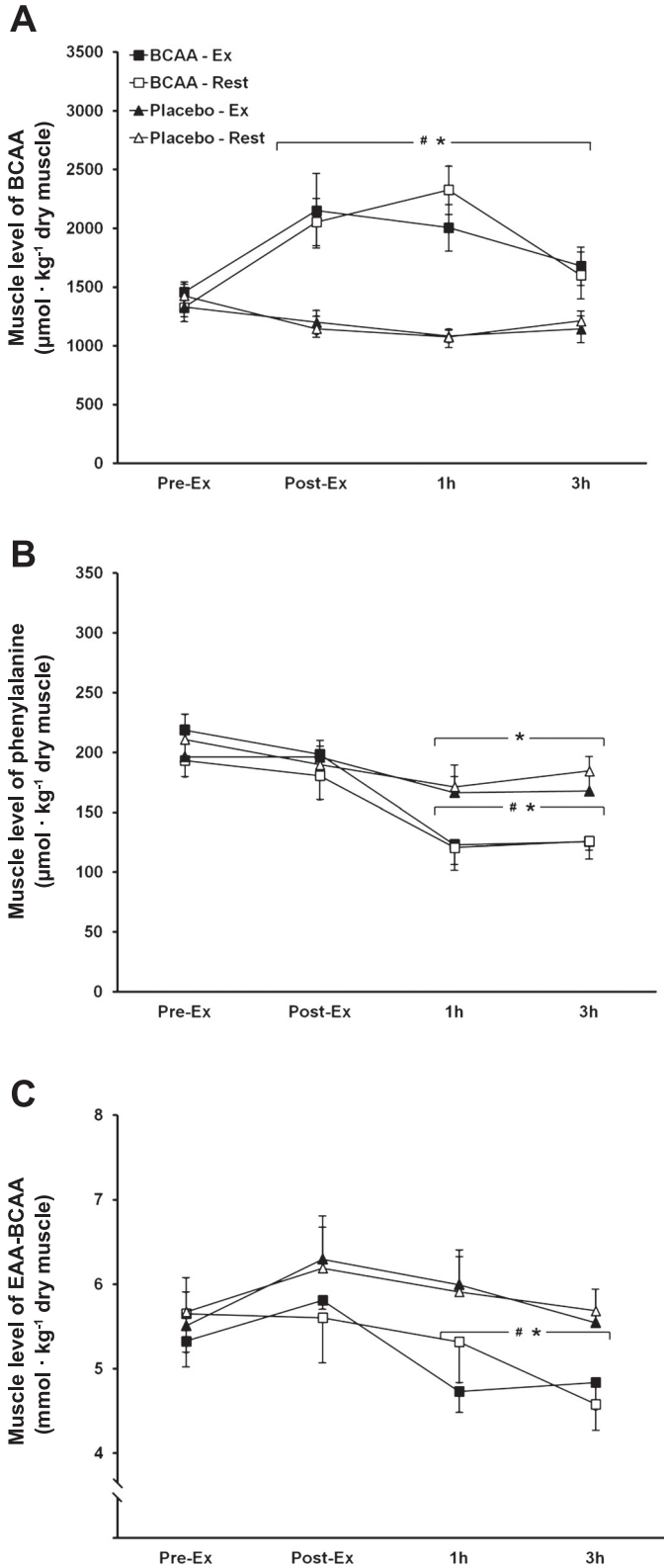

Fig. 2. Levels of BCAA (*A*), phenylalanine (*B*), and EAA-BCAA (*C*) in vastus lateralis muscle of the exercising leg (filled boxes or triangles) and the resting leg (open boxes or triangles) following supplementation with BCAA or placebo. Values are means ± SE ($n = 7$ for Pre-Ex and 3 h, $n = 5$ for Post-Ex and 1 h). *Different from Pre-Ex, $P < 0.05$; #different from placebo, $P < 0.05$.

ing leg in the placebo condition. At all time points in the placebo condition, values were unaltered compared with Pre-Ex values (Fig. 2*C*).

Protein signaling. The phosphorylation status of Akt at Ser^{473} was unaltered with both supplements and in both legs following 1 and 3 h of recovery from resistance exercise (data not shown), whereas the phosphorylation of mTOR at Ser^{2448} was increased 1.5- to 3-fold ($P < 0.05$) in both legs 1 and 3 h following exercise in both the placebo and the BCAA conditions (Fig. 3, *A* and *B*). Phosphorylation of p70^{S6k} at Thr^{389} was also enhanced in both legs 1 and 3 h after exercise ($P < 0.05$) and in addition, the level was 2.1- and 2.4-fold higher in the resting and exercising leg, respectively, in the BCAA condition at 1 h of recovery ($P < 0.05$; Fig. 3, *C* and D). The phosphorylation of 4E-BP1 at Thr$^{36/37}$, a downstream target of mTOR, remained unaltered in both legs during both conditions (data not shown).

The total protein level of MAFbx did not change significantly over time in any leg or in any condition, although there was a tendency for interaction between supplement and time ($P = 0.14$; Fig. 4, *A* and *B*). The protein content of MuRF-1 was increased 20–40% 3 h following exercise in both legs in the placebo condition ($P < 0.05$), whereas the level remained unchanged with the BCAA supplement (Fig. 4, *C* and *D*). In addition, there was a strong trend ($P = 0.052$) for a lower expression of MuRF-1 total protein in both legs 3 h after exercise during BCAA supplementation compared with placebo (Fig. 4, *C* and *D*).

Levels of mRNA expression. All of these data are expressed as fold changes following 3 h of recovery compared with Pre-Ex levels.

Proteolytic factors. Supplementation with BCAA resulted in levels of MAFbx mRNA that were 50 and 70% of those observed at Pre-Ex in the exercising and resting legs, respectively, whereas with placebo the levels were 1.4-fold of initial values in both legs (Fig. 4*E*). The levels of MAFbx mRNA were significantly lower ($P < 0.05$) with BCAA supplementation than with placebo. The expression of MuRF-1 mRNA was significantly higher in the exercising compared with the resting leg ($P < 0.05$), threefold in the case of the placebo and twofold with BCAA (Fig. 4*F*), with no significant difference between these supplements, mainly due to one subject who deviated from the others and showed a considerably larger increase in the BCAA trial.

Proposed negative regulators of mTOR. Neither exercise nor any form of supplementation had any significant impact on the expression of REDD1 mRNA (Fig. 5*A*). In contrast, the expression of REDD2 mRNA in the exercising leg tended to be reduced ($P = 0.07$) 32 and 24% under the placebo and BCAA conditions, respectively (Fig. 5*B*).

Proposed positive regulators of mTOR. The levels of Rheb mRNA tended to be lower in both legs after BCAA supplementation compared with placebo ($P = 0.05$; Fig. 5*C*). The fold increases in the BCAA condition were 1.2- and 1.5-fold compared with 1.6- and 2.4-fold in the placebo condition in the resting and exercising legs, respectively. The content of hVps34 mRNA was not significantly different in any condition (Fig. 5*D*).

There were no obvious differences between male and female subjects regarding the response to BCAA supplementation with regard to the analyzed variables; however, the number of

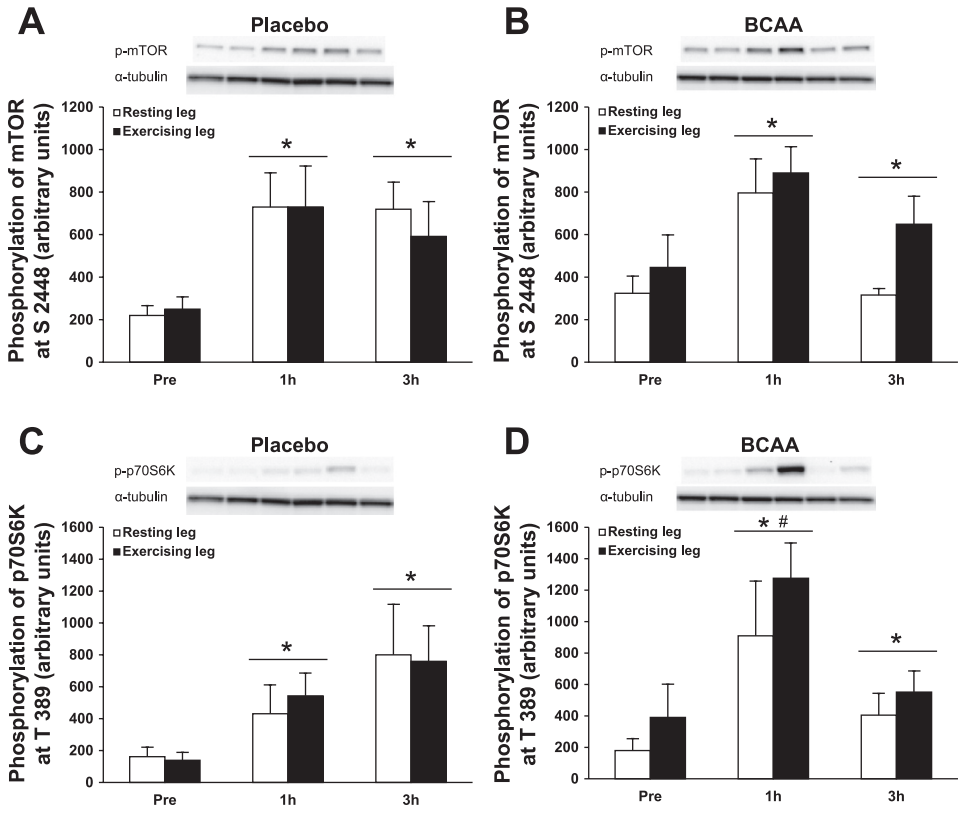

Fig. 3. Phosphorylation of mTOR at Ser^{2448} (*A* and *B*) and p70^{S6k} at Thr^{389} (*C* and *D*) in resting and exercising leg with placebo and BCAA supplementation. Data are expressed relative to total protein content of α-tubulin. Representative immunoblots from one subject are shown above each graph. Values are arbitrary units and presented as means ± SE ($n = 7$ for Pre-Ex and 3 h, $n = 5$ for Post-Ex and 1 h). *Different from Pre-Ex, $P < 0.05$; #different from placebo, $P < 0.05$.

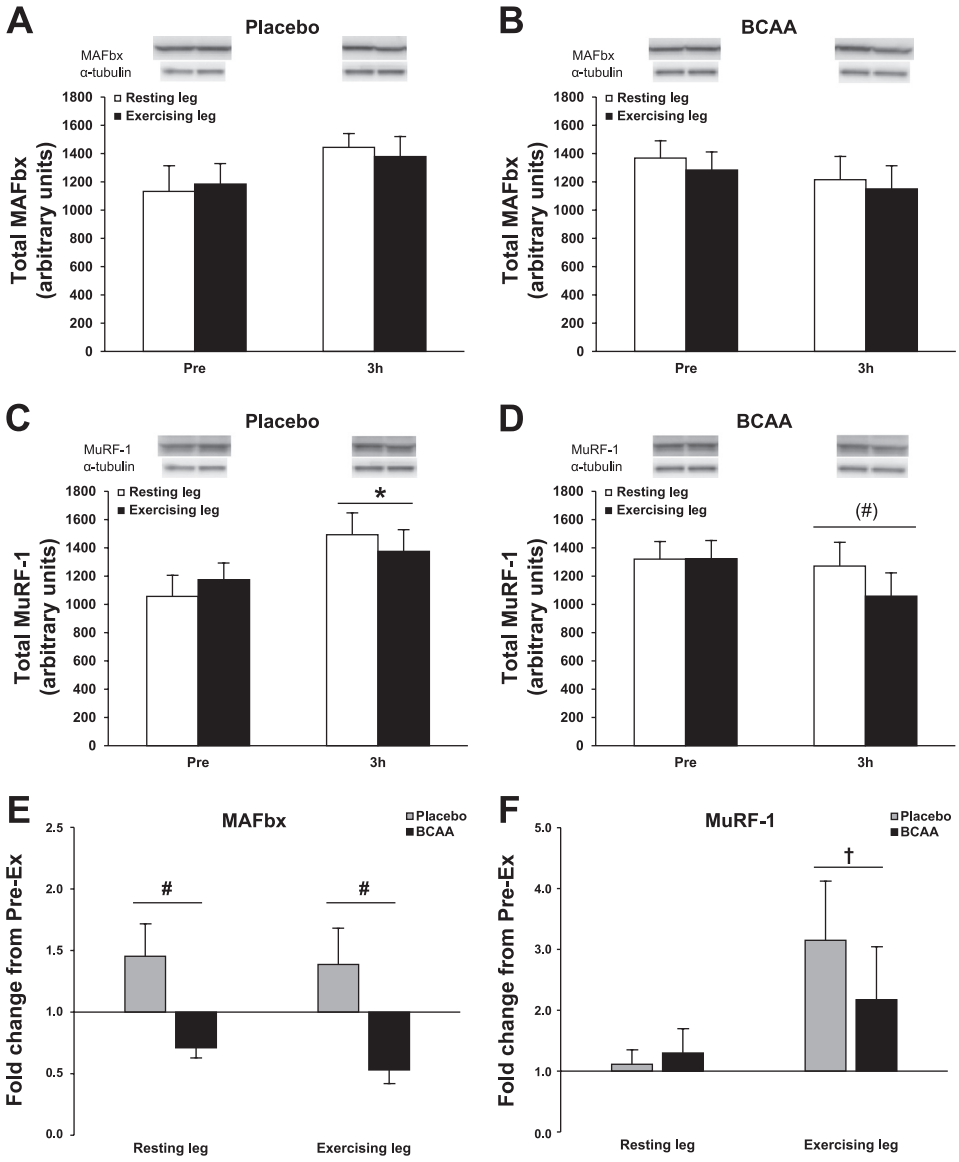

Fig. 4. Total protein content of muscle atrophy F-box (MAFbx; *A* and *B*) and muscle RING-finger 1 (MuRF-1; *C* and *D*) and levels of mRNA encoding MAFbx (*E*) and MuRF-1 (*F*) in vastus lateralis muscle of exercising and resting legs with placebo and BCAA supplementation after 3 h of recovery. mRNA values are fold changes from baseline (Pre-Ex) and presented as means ± SE ($n = 7$ or 6 for MAFbx; see *Statistical analyses*). Protein data are expressed relative to total protein content of α-tubulin. Representative immunoblots from one subject are shown above graphs *A–D*. *Different from Pre-Ex, $P < 0.05$; #different from placebo, $P < 0.05$; (#)different from placebo, $P = 0.05$; †different from resting leg, $P < 0.05$.

subjects was too small to allow any comparative analyses between men and women.

DISCUSSION

The present investigation was designed to evaluate the influence of resistance exercise and BCAA alone or in combination on selective markers for muscle protein synthesis and breakdown. The major novel findings are that supplementation with BCAA reduced the expression of MAFbx mRNA in both resting and exercising muscle and prevented the increase in total protein expression of the other ubiquitin ligase, MuRF-1, which, in contrast to MAFbx, exhibited an increase in both mRNA and protein expression after resistance exercise. Furthermore, muscle levels of phenylalanine and tyrosine were reduced during recovery from resistance exercise, most potently after BCAA supplementation, which also attenuated muscle levels of EAA (BCAA excluded). Phosphorylation of $p70^{S6k}$ increased to a larger extent in the early recovery period when BCAA were ingested, and the level was still elevated in both legs three h after exercise, although at that time the level was similarly elevated in the placebo condition.

This reduction in the level of MAFbx mRNA by BCAA has not been reported previously in human muscle. However, no significant effect on total protein content was observed, suggesting that the 3-h time span was too short to detect changes in MAFbx protein. In contrast, the levels of both MuRF-1 mRNA and total protein were increased in the exercised leg (Fig. 4), which is in accord with results from Glynn et al. (24). Furthermore, intake of BCAA prevented the increase in total protein and attenuated (insignificantly) the increase in mRNA level. This concordance between mRNA expression and total protein of MuRF-1 might reflect the relatively large changes in the mRNA expression of MuRF-1 reported here and by others (24, 40, 43). Administration of BCAA or leucine alone was earlier reported to reduce the levels of both MAFbx and MuRF-1 mRNA in starved C_2C_{12} myotubes (26) and in atrophied rat muscle (5). Of interest in this context are the observations by Herningtyas et al. (26) that although BCAA reduces

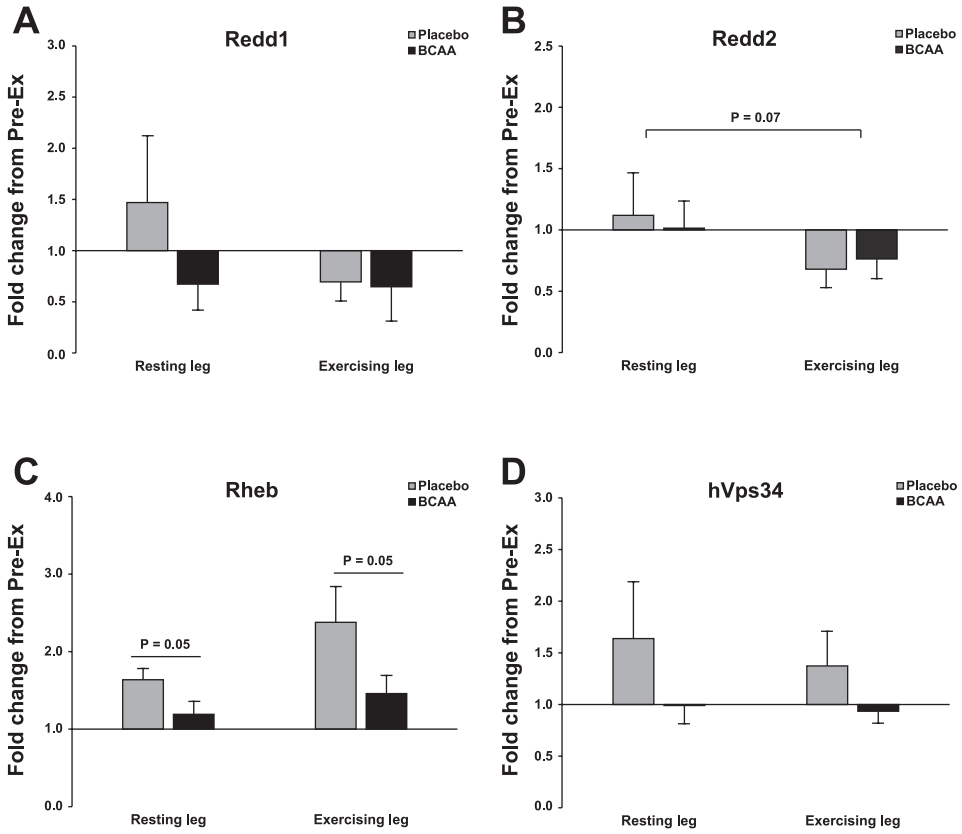

Fig. 5. Levels of mRNA encoding regulated in development and DNA damage response-1 (REDD1; *A*), REDD2 (*B*), ras homolog enriched in brain (Rheb; *C*), and human vacuolar protein sorting-34 (hVps34; *D*) in vastus lateralis muscle of exercising and resting legs with BCAA or placebo supplementation after 3 h of recovery. Values are fold changes from baseline (Pre-Ex) and presented as means ± SE ($n = 7$).

the expression of both of these genes in myotubes the effect on MAFbx mRNA was dependent on activation of mTOR, whereas the effect on MuRF-1 was not, indicating differential regulation of these two ubiquitin ligases.

The divergent response to exercise with respect to the levels of MAFbx and MuRF-1 mRNA observed here is in agreement with most previous studies (12, 13, 19, 40, 43). This may reflect the different functions of these two proteins; MAFbx targets the regulatory protein transcription factor MyoD (34) and eukaryotic initiation factor 3F (eIF-3F) (35), which is of importance in mTOR-$p70^{S6k}$ signaling, whereas MuRF-1 appears to interact with structural proteins like titin and myosin light-chains 1 and 2 (14, 22). Our current observation that the level of MAFbx mRNA decreases following BCAA supplementation suggests that the corresponding protein attenuates the breakdown of regulatory proteins involved in hypertrophic signaling mediated by mTOR. The acute increase in MuRF-1 expression following resistance exercise is likely to reflect enhanced degradation of contractile proteins. The similar response to BCAA but not to exercise suggests the presence of both common and divergent regulation of these ubiquitin ligases; however, we were not able to detect involvement of Akt activation in either case. The absence of effect on Akt phosphorylation directly after resistance exercise (2) as well as 1 and 3 h later despite intake of BCAA may be explained by the minor elevation in insulin (2), which probably was insufficient to activate Akt. Although phosphorylation of both mTOR and $p70^{S6k}$ was elevated after exercise, the latter to a larger extent with BCAA supplementation (Fig. 3), phosphorylation of 4E-BP1 remained unchanged in both conditions. This finding is in agreement with some (17, 59) but not all (18, 45) previous studies. The divergent effects seen on $p70^{S6k}$ and 4E-BP1 herein and the discrepancy in the literature suggest that these two downstream targets of mTOR may be differentially regulated under certain conditions (11).

Ingestion of BCAA led to a more pronounced reduction in the concentration of the aromatic amino acids tyrosine and phenylalanine, in both plasma and muscle, as well as muscle EAA (BCAA excluded) during the recovery period (Tables 3 and 4), an effect that is likely to be caused by leucine, as demonstrated by Eriksson et al. (21). The results here are in agreement with previous studies showing a reduction in plasma and muscle levels of tyrosine and phenylalanine at rest (1) or after endurance exercise (9) following administration of leucine or BCAA. The novel finding in the present study is that similar reductions were seen in resting and exercising muscle. Since tyrosine and phenylalanine are neither synthesized nor degraded in skeletal muscle, reduction in the levels of these amino acids could be indicative of an improved net muscle protein balance, i.e., an enhanced rate of synthesis and/or decreased rate of breakdown. In the study by Alvestrand et al. (1), the exchange of amino acids from the muscle was calculated from measurements of blood flow and arterial-venous concentration differences, and since no change in the net release of tyrosine and phenylalanine was detected, the authors concluded that part of the observed fall in intracellular amino acids could be explained by incorporation into protein. These observations, together with our findings that ingestion of BCAA led to a more pronounced increase in $p70^{S6k}$ phosphorylation, attenuated MAFbx expression, and prevented the exercise-induced increase in MuRF-1 protein provide additional

support to the conclusion that administration of BCAA has anabolic effects on human skeletal muscle (9, 39, 48).

The similar reductions in the levels of phenylalanine and EAA-BCAA during the recovery phase in both resting and exercising legs following BCAA supplementation (Fig. 2) was unexpected. Supplementation with amino acids influences $p70^{S6k}$ phosphorylation and the rate of protein synthesis more potently when combined with resistance exercise (2, 8, 46). However, Moore et al. (46) reported that the rates of protein synthesis in resting and exercising human muscle in response to amino acid ingestion were similar during the initial 1–3 h of recovery. Thus, changes in muscle levels of phenylalanine and EAA-BCAA might have occurred later than the 3-h recovery period monitored here. Other possible explanations for the similar effect of BCAA on resting and exercising muscle could be that resistance exercise also increases the rate of protein breakdown, thus partially counterbalancing the enhanced synthesis (52) or that because our subjects were fasting the availability of the remaining EAA may have been inadequate to further increase the rate of synthesis in the exercised muscle (60).

In the present investigation, we observed a tendency for BCAA supplementation to attenuate the elevation in the level of Rheb mRNA in both resting (1.7-fold under placebo vs. 1.2-fold in the BCAA condition) and exercising muscle (2.4-fold vs. 1.5-fold). Although we did not asses statistical differences from baseline (Pre-Ex), the 2.4-fold elevation (individual values ranging from 1.6 to 5.0) with placebo supplementation clearly indicates an enhancement of this expression following exercise, which was not observed in the case of BCAA supplementation (Fig. 5*C*). Drummond et al. (20) demonstrated a 1.5-fold increase in the level of Rheb mRNA above baseline following combined resistance exercise and amino acid ingestion, but they did not examine the effect of exercise alone. Rheb is a low-molecular-weight GTPase located immediately upstream of mTOR and is involved in its activation (42). The mechanisms through which various stimuli influence Rheb-mTOR signaling have not yet been fully investigated, but present evidence indicates that growth factors stimulate binding of GTP to Rheb, whereas amino acids may affect the formation of a Rheb-mTOR complex (38). This interaction is rapidly inhibited in cultured cells during withdrawal of leucine from the incubation medium (38); however, future studies are needed to clarify whether a change in Rheb mRNA expression influences the binding of Rheb to mTOR.

hVps34, which seems to play a pivotal role in the control of macroautophagy (51), has also been suggested to play a role in the activation of mTOR by amino acids (25). However, in agreement with others (20), here the mRNA expression of this protein was unaltered by either resistance exercise or amino acid supplementation, suggesting that amino acids do not promote hVps34 signaling by elevating the level of this protein.

The stress/hypoxia-induced proteins REDD1 and -2 act as negative regulators of mTOR (15) by modulating the tuberous sclerosis tumor suppressor protein-2 (TSC2) (16, 44), located upstream of mTOR. In the present investigation, the level of REDD2 mRNA in the exercising leg tended to be lower ($P = 0.07$) with both types of supplementation, with no effect on REDD1. This dissimilar effect of resistance exercise on REDD1 and -2 is in agreement with the findings of Drummond et al. (20). Moreover, our findings suggest that exercise rather than amino acids is responsible for the lower expression observed here.

In conclusion, BCAA supplementation reduced the expression of MAFbx mRNA and prevented the increase in MuRF-1 total protein in both resting and exercising muscle. Resistance exercise caused an elevation in the level of MuRF-1 mRNA and total protein without significantly influencing MAFbx, suggesting both common and divergent regulation of these two ubiquitin ligases. Furthermore, the reduction in the levels of phenylalanine and tyrosine, as well as the sum of EAA (BCAA excluded) in both resting and exercising muscle during the 3-h recovery period was more pronounced with BCAA supplementation. These observations, together with the BCAA-induced enlargement in $p70^{S6k}$ phosphorylation, provide additional support for the view that BCAA has an anabolic effect on human skeletal muscle, an effect that appears to be similar in resting and exercising human muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.B. and W.A. analyzed data; M.B., W.A., and E.B. interpreted results of experiments; M.B. prepared figures; M.B. drafted manuscript; M.B., W.A., and E.B. edited and revised manuscript; M.B., W.A., and E.B. approved final version of manuscript; W.A. and E.B. conception and design of research; W.A. and E.B. performed experiments.

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