

## Branched-Chain Amino Acid Supplementation Before Squat Exercise and Delayed-Onset Muscle Soreness

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The authors examined the effect of branched-chain amino acid (BCAA) supplementation on squat-exercise-induced delayed-onset muscle soreness (DOMS) using 12 young, healthy, untrained female participants. The experiment was conducted with a crossover double-blind design. In the morning on the exercise-session day, the participants ingested either BCAA (isoleucine:leucine:valine = 1:2.3:1.2) or dextrin at 100 mg/kg body weight before the squat exercise, which consisted of 7 sets of 20 squats/set with 3-min intervals between sets. DOMS showed a peak on Days 2 and 3 in both trials, but the level of soreness was significantly lower in the BCAA trial than in the placebo. Leg-muscle force during maximal voluntary isometric contractions was measured 2 d after exercise (Day 3), and the BCAA supplementation suppressed the muscle-force decrease (to ~80% of the value recorded under the control conditions) observed in the placebo trial. Plasma BCAA concentrations, which decreased after exercise in the placebo trial, were markedly elevated during the 2 hr postexercise in the BCAA trial. Serum myoglobin concentration was increased by exercise in the placebo but not in the BCAA trial. The concentration of plasma elastase as an index of neutrophil activation appeared to increase after the squat exercise in both trials, but the change in the elastase level was significant only in the placebo trial. These results suggest that muscle damage may be suppressed by BCAA supplementation.

**Keywords:** muscle damage, BCAA, myoglobin, supplement

Branched-chain amino acids (BCAAs) are abundant in muscle proteins, accounting for 14–18% of the total amino acids and ~35% of the essential amino acids in the proteins (Harper, Miller, & Block, 1984; Layman & Baum, 2004; Riazzi, Wykes, Ball, & Pencharz, 2003). It is known that BCAAs are primarily catabolized in muscles, in contrast to other essential amino acids, which are catabolized mainly in the liver (Harper et al., 1984). It has been demonstrated that BCAA catabolism is promoted by exercise (Shimomura et al., 1995; Wagenmakers, Brookes, Coakley, Reilly, & Edwards, 1989; Xu et al., 2001).

BCAAs, especially leucine, have functions beyond the role of essential amino acids. They regulate protein metabolism, promoting protein synthesis by stimulating mRNA translation and suppressing protein degradation

by inhibiting the autophagic system via mechanisms involving the mammalian target of rapamycin (Bolster, Jefferson, & Kimball, 2004; Garlick, 2005; Kadowaki & Kanazawa, 2003). The other protein-degradation system involving the proteasome might also be influenced by dietary leucine (Combarret et al., 2005). These findings suggest that BCAA supplementation before or after exercise may improve recovery of damaged muscles. This hypothesis is in line with the findings that (a) an oral BCAA supplement (77 mg/kg body weight) before exercise decreased the release of essential amino acids from exercising muscles, suggesting suppression of endogenous muscle-protein breakdown during exercise (MacLean, Graham, & Saltin, 1994); (b) oral BCAA administration (12 g/day for 2 weeks in a preexercise period and 20 g both before and after the exercise test) reduced the rise in serum creatine kinase activity for several days after exercise (Coombes & McNaughton, 2000); and (c) supplementation with a mixture of 6 g essential amino acids (40% BCAAs) and 35 g carbohydrate before and after eccentric exercise increased protein synthesis in skeletal muscle (Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000; Tipton et al., 2001), with greater effect when the supplement was taken just before exercise (Tipton et al., 2001).

Based on this background, we had conducted a preliminary study to examine the effect of BCAA

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supplementation on the delayed-onset muscle soreness (DOMS) induced by squat exercise and found that ingestion of 5 g BCAA per participant before squat exercise reduced DOMS (Shimomura et al., 2006). This effect of BCAA supplementation was more pronounced in female than in male participants, although the underlying reasons for the gender difference remained unresolved. In the current study, we confirmed the effect of BCAA supplementation on DOMS and examined the mechanisms responsible for the effect in female untrained participants.

## Methods

### Participants

Twelve women age 20–25 years were recruited from the Nagoya Institute of Technology (Nagoya, Japan) and Sugiyama Jogakuen University (Nagoya, Japan). Healthy and untrained sedentary individuals were included in this study. Those with diabetes, obesity, or cardiovascular diseases were excluded, as well as those who exercised regularly. All 12 participants were undergraduate or graduate students age  $22.2 \pm 1.6$  years, height  $158 \pm 4$  cm, weight  $48.5 \pm 5.2$  kg, body-mass index  $19.4 \pm 1.7$  kg/m<sup>2</sup>, and body fat  $22.7\% \pm 3.5\%$  measured with a TBF-501 body-composition analyzer (Tanita, Tokyo). Serum progesterone concentration was measured on the first day of the experiment in both placebo and BCAA trials, and the concentrations for all participants were within a normal, nonpregnant range (data not shown). All participants were instructed to refrain from vigorous physical exercise for ~2 months before and during the experiments. They were also strongly requested to keep their regular dietary habits, especially the intake of proteins, and to avoid ingesting soft drinks supplemented with free amino acids during the experiments. Three of the 12 participants participated in both the current study and the preliminary study (Shimomura et al., 2006). The study design, purpose, and possible risks were explained to each participant before written consent was obtained. The study protocol was approved by the human research review committee of the Nagoya University School of Medicine.

### Drink Composition

The compositions of test and placebo drinks used in this study were as follows: (a) a BCAA drink composed of 5.5 g of a BCAA mixture (Ile:Leu:Val = 1:2.3:1.2), 1 g of instant green-tea powder (Ajinomoto General Foods, Inc., Tokyo), and 1.2 g of artificial sweetener containing aspartame (Pal Sweet, Ajinomoto Co. Ltd., Tokyo) dissolved in 200 ml of de-ionized distilled water and (b) a placebo drink (200 ml) containing the same ingredients as the BCAA drink but substituting dextrin (Sandec #70, Sanwa Cornstarch Co., Ltd., Kashihara, Nara, Japan) for BCAAs. The three individual BCAAs were obtained from Ajinomoto Co., and the ratio of the BCAA mixture used

was based on the amino acid composition reported by the Food and Agricultural Organization of the World Health Organization ("Energy and Protein Requirements," 1985). The two drinks were designed to taste similar by using the instant green-tea powder to mask the bitter taste of BCAAs. The texture of the two drinks was almost the same, although the BCAAs were not completely dissolved. Thus, the drinks were served to the participants in lidded paper cups, and the participants ingested the drinks through straws. The volume of the drink was adjusted on the basis of body weight at 100 mg BCAAs or dextrin per kilogram body weight. It was confirmed that each participant consumed almost all the BCAAs or dextrin in the drink.

### Experimental Design

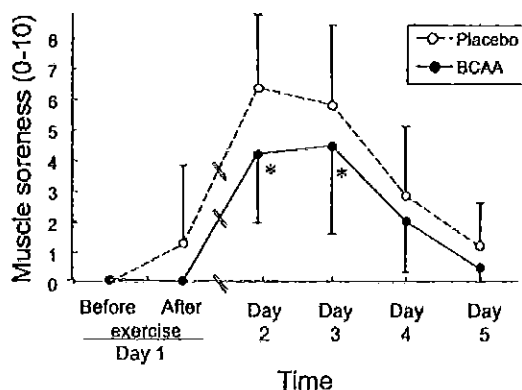
The experimental design was essentially the same as that in the preliminary study (Shimomura et al., 2006), except the participants ingested 100 mg of the BCAA mixture or dextrin per kilogram body weight and blood samples were collected at several time points during the experiment.

Squat exercise, which was used as resistance exercise to induce DOMS, was performed simply with body weight in the same manner as in the preliminary study (Shimomura et al., 2006). The exercise session consisted of seven sets of 20 squats per set (total 140 squats), with squats performed rhythmically every 2 s during the sets and 3-min intervals between sets. The experiment was conducted with a crossover design, so that each participant was tested with both BCAA and placebo drinks, separated by an 11-week interval, and carried out exactly the same squat exercise in the two trials. During each trial, the participants were randomly divided into two groups, with half of them taking BCAAs and half placebo. At the end of the first trial, the participants were again instructed to refrain from vigorous physical exercise for 11 weeks, and at the beginning of the second trial their physical condition was confirmed by interview to be the same as at the first trial.

On the first day of the experiment (Day 1), participants who had fasted overnight, reported to the laboratory at 8:30 a.m., when the first blood collection was carried out. They were given a jelly-type food (200 g in weight containing 100 kcal of sugar; Otsuka Pharmaceutical Co., Ltd., Tokyo) at 9 a.m. to minimize the effect of starvation on BCAA catabolism (Shimomura et al., 1995). At 9:30 a.m., the BCAA or placebo drink was provided in a double-blind fashion. The squat-exercise session commenced ~15 min after ingestion of the test drink. BCAAs (or placebo) were ingested before the exercise session because it has been reported that (a) BCAA supplementation before exercise may attenuate muscle-protein breakdown (MacLean et al., 1994); (b) postexercise muscle-protein synthesis is greater when the essential amino acid-carbohydrate mixture is consumed before exercise, rather than after (Tipton et al., 2001); and (c) dietary BCAAs may affect energy metabolism

during exercise (Shimomura et al., 2000). In addition, we have found that the plasma BCAA concentrations were elevated within 15 min and reached a peak 30 min after ingestion of a 5-g BCAA mixture (Shimomura et al., 2006). Blood was collected again immediately after exercise (0-hr time point) and 1 and 2 hr after exercise. At the end of the experiment on the first day, the participants were given two rice balls as lunch. The rice balls (~200 kcal/ball) were chosen for the food after the experiment because they are a very popular food in Japan and their major component is carbohydrate (~92% of total energy). Blood was also collected at about 8:30 a.m. on the next day (Day 2) and Day 3 under the same conditions as Day 1. On Day 3, as a test of muscle function, the force generated by maximal voluntary isometric contractions (knee extension) with both legs was measured as reported elsewhere (Balnave & Thompson, 1993) using a dynamometer especially designed for this purpose (T.K.K.5002/5710m, Takei Scientific Instruments Co., Ltd., Niigata, Japan). The muscle-function test was performed at about 9:30 a.m., which was approximately 20 min after the participants had breakfast (two rice balls). The muscle force under control (no-soreness) conditions for each participant was measured ~2 months before the first experiment, and the muscle force measured on Day 3 is expressed as a percentage of the value obtained under control conditions.

Blood samples obtained as described were separated into two tubes for plasma and serum preparations. The tube for plasma preparation contained 20  $\mu$ mol of neutralized EDTA and was cooled in ice before centrifugation. The tube for serum preparation was kept at room temperature for 1–2 hr before centrifugation. Both plasma and serum were prepared by centrifugation at ~1,000 g for 15 min. The centrifugation for plasma preparation was carried out at 4 °C.



**Figure 1** — Muscle-soreness sensation in the lower limbs for the branched-chain amino acid (BCAA) and placebo trials while squatting down,  $M \pm SD$  ( $n = 12$ ). \*Significantly different from placebo at the corresponding time point ( $p < .05$ ).

Muscle soreness of the lower limbs before and after exercise and in the morning of the following 4 days (from Day 2 through Day 5) was evaluated while participants squatted down slowly (taking ~3 s), using a visual analog scale consisting of a 10-cm line with *no pain* (0 cm) printed at one end and *extremely sore* (10 cm) at the other (Nosaka, Newton, & Sacco, 2002). Participants were instructed to make a mark on the line indicating the degree of muscle soreness they felt. The area under the curve (AUC) of muscle soreness was calculated as reported previously (Wolever, Jenkins, Jenkins, & Josse, 1991).

The muscle soreness induced by eccentric exercise often shows relatively large individual variability. Therefore, the coefficient of variation (CV) for the muscle-soreness data (Figure 1) was calculated using the following equation and was compared between placebo and BCAA trials:  $CV (\%) = (SD/M) \cdot 100$ .

### Analyses of Blood Components

Concentrations of blood components were measured as reported previously: plasma glucose (Banauch et al., 1975) and elastase (Hafner et al., 1991); serum free fatty acids (Sugo, Matsumoto, Yamaoka, & Sakurabayashi, 1990), insulin (Morgan & Lazarow, 1963), progesterone (Kanazawa, 1999), myoglobin (Haraoka, Yamanari, & Abe, 1995), and creatine kinase activity (Shoji, 1995); and whole-blood lactate (Polani, 1995) and ammonia (Okuda & Fujii, 1966). Elastase was measured by immunoassay using the immunoactivation method for rapid and specific determination of human plasma granulocyte elastase described by Hafner et al. (1991, 1997). In this assay, a polyclonal sheep antibody raised against human elastase, conjugated to latex particles, was used (Hafner et al., 1997). The analyses of these blood components were carried out by Special Reference Laboratories Inc. (Tokyo), and all values were within reliable analyzing ranges. Plasma BCAA concentrations were evaluated using an automated JLC-500/V amino acid analyzer (JEOL, Tokyo).

### Statistics

Data are expressed as  $M \pm SD$ . Wilcoxon's signed-rank test was used to compare muscle soreness at each time point, because some parts of the data were not normally distributed. A paired  $t$  test was used to test differences in muscle-force and AUC data for muscle soreness between trials. To compare the variation of the blood components over time and between trials, two-way repeated-measures ANOVA and Tukey's post hoc test were used. Changes of blood-component levels over time within each trial were tested by one-way repeated-measures ANOVA. The two-way repeated-measures ANOVA was performed using Stat View 5.0 software (SAS Institute, Cary, NC), and the other calculations were performed using Stat Mate III 3.14 software (ATMS, Co., Ltd., Tokyo). Differences were significant at  $p < .05$  in two-tailed testing.

## Results

### Muscle Soreness and Muscle Force During Maximal Voluntary Contractions

We have reported that peak muscle soreness in the placebo trial appears on Days 2 and 3 after squat exercise (Shimomura et al., 2006). In the current study, the same squat-exercise procedure was used, and, as expected, peak muscle soreness while squatting down was observed in the placebo trial at the same time points (Figure 1); average levels of soreness in the placebo trial while squatting on Days 2 and 3 were  $6.4 \pm 2.4$  and  $5.8 \pm 2.7$ , respectively. On the other hand, although muscle soreness also occurred in participants during the BCAA trial, the levels of soreness on the same days ( $4.2 \pm 2.2$  and  $4.5 \pm 2.7$ , respectively) were significantly lower than those experienced during the placebo trial (Figure 1). The respective CVs for these data (38% and 46% for the placebo trial and 53% and 61% for the BCAA trial) indicated similar variability of these data in both trials. The AUCs of muscle soreness over the 5-day postexercise period were also significantly lower in the BCAA trial than with placebo ( $10.8 \pm 6.1$  vs.  $16.2 \pm 7.0$  cm  $\times$  day). The CVs for the AUCs (56% and 43%, respectively) were also similar in both trials.

In the placebo trial, the muscle force during maximal voluntary isometric contractions measured on Day 3 was decreased to ~80% of the value recorded under control (no-pain) conditions (Figure 2). In contrast, muscle force in the BCAA trial was almost the same as during control conditions and was significantly higher than in the placebo trial (Figure 2), indicating that BCAA ingestion suppressed a muscle-function decrease induced by unaccustomed squat exercise.

### Blood, Plasma, Serum Metabolites, and Insulin

Plasma glucose concentration did not significantly change throughout the experiment in the two trials (Table 1).

The glucose level right after exercise (0-hr time point) in the placebo trial tended to be elevated, because the participants ingested the test drink containing dextrin 15 min before exercise. Serum free-fatty-acid concentration was decreased at the time points right after exercise and 1 hr postexercise in both trials, probably as a result of ingestion of the jelly-type food containing sugar before exercise, but there was no significant difference between trials at any time point (Table 1). Blood lactate concentration was elevated by the squat exercise, but no difference between the two trials was detected at any time point (Table 1). A small but significant increase in blood ammonia concentration after exercise was observed only in the BCAA trial (Table 1), suggesting that BCAA ingestion before exercise augments ammonia metabolism, as reported by MacLean et al. (1994).

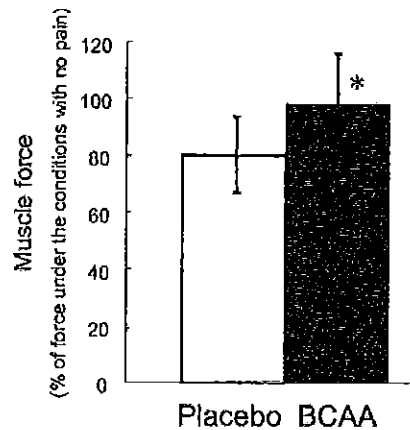


Figure 2 — Muscle force generated by maximal voluntary isometric contractions (knee extension) with both legs measured on Day 3 in the branched-chain amino acid (BCAA) and placebo trials,  $M \pm SD$  ( $n = 12$ ). The muscle force is expressed as a percentage of the value obtained under control (no-pain) conditions. \*Significantly different from placebo ( $p < .05$ ).

Table 1 Concentrations of Metabolites and Insulin in Blood,  $M \pm SD$

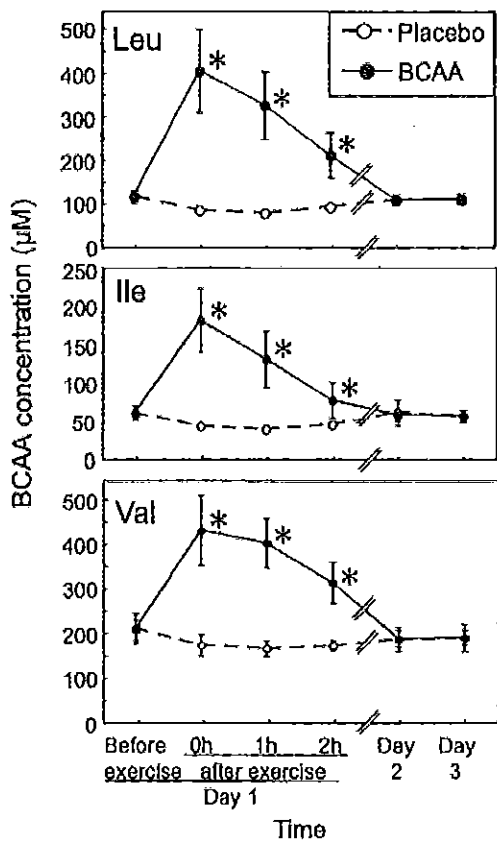
Blood component	Trial	Preexercise	Day 1			Day 2	Day 3	<i>p</i>
			0 hr after exercise	1 hr after exercise	2 hr after exercise			
Glucose, mmol/L	P	$5.0 \pm 0.1$	$5.6 \pm 1.6$	$5.0 \pm 0.6$	$4.7 \pm 0.3$	$4.9 \pm 0.3$	$4.8 \pm 0.3$	.052
	B	$4.9 \pm 0.3$	$4.5 \pm 1.3$	$4.7 \pm 0.4$	$4.7 \pm 0.3$	$4.9 \pm 0.2$	$4.8 \pm 0.2$	>.1
Free fatty acids, mmol/L	P	$0.54 \pm 0.26$	$0.12 \pm 0.06$	$0.21 \pm 0.09$	$0.58 \pm 0.15$	$0.39 \pm 0.20$	$0.54 \pm 0.33$	<.001
	B	$0.45 \pm 0.25$	$0.09 \pm 0.03$	$0.22 \pm 0.13$	$0.45 \pm 0.25$	$0.39 \pm 0.14$	$0.49 \pm 0.21$	<.001
Lactate, mmol/L	P	$0.85 \pm 0.35$	$3.51 \pm 1.18$	$1.09 \pm 0.54$	$0.87 \pm 0.58$	$1.21 \pm 0.85$	$0.99 \pm 0.65$	<.001
	B	$0.86 \pm 0.43$	$3.16 \pm 1.21$	$1.02 \pm 0.56$	$0.77 \pm 0.32$	$0.90 \pm 0.48$	$1.06 \pm 0.55$	<.001
Ammonia, $\mu$ mol/L	P	$28.0 \pm 4.5$	$25.3 \pm 4.7$	$24.6 \pm 5.0$	$25.1 \pm 5.2$	$29.6 \pm 13.6$	$25.4 \pm 4.3$	>.1
	B	$25.2 \pm 3.9$	$26.9 \pm 5.1$	$31.1 \pm 8.4$	$27.5 \pm 9.3$	$24.6 \pm 3.8$	$25.5 \pm 4.5$	.043
Insulin, $\mu$ mol/L	P	$8.9 \pm 2.5$	$26.1 \pm 9.8$	$12.6 \pm 7.0$	$6.0 \pm 2.0$	$7.3 \pm 2.2$	$7.4 \pm 2.3$	<.001
	B	$8.3 \pm 2.9$	$32.1 \pm 22.0$	$11.0 \pm 8.0$	$5.5 \pm 2.1$	$7.4 \pm 3.2$	$7.9 \pm 2.9$	<.001

Note. P = placebo; B = branched-chain amino acids. *p* values were calculated for changes over time within each trial using one-way repeated-measures ANOVA.

Increases in serum insulin concentration right after exercise were observed in both trials, but no difference between trials was identified (Table 1).

**Plasma Free-BCAA Concentrations**

Plasma concentrations of the three BCAAs in the placebo trial were decreased by the squat exercise, and these changes were significant (Figure 3). On the other hand, plasma concentrations of the BCAAs were markedly elevated in the BCAA trial, with a peak right after exercise. The peak levels were 2 to 3.5-fold higher than the concentrations before exercise (Figure 3). The BCAA concentrations from the time point right after exercise through 2 hr postexercise were significantly higher in the

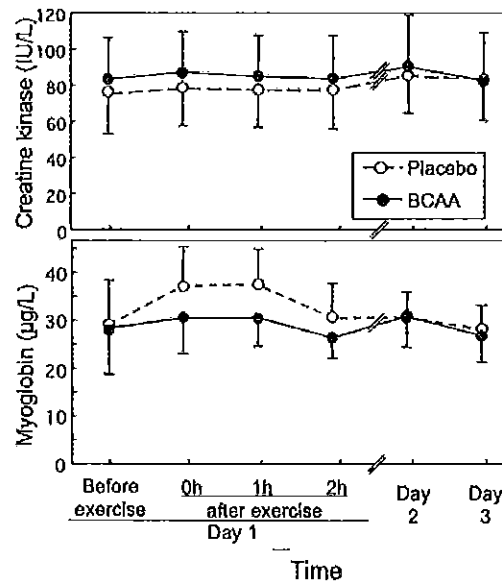


**Figure 3** — Plasma leucine (Leu), isoleucine (Ile), and valine (Val) concentrations in the branched-chain amino acid (BCAA) and placebo trials,  $M \pm SD$  ( $n = 12$ ). Changes in individual amino acid concentrations over time were significant ( $p < .001$ ) for both trials when they were tested using one-way repeated-measures ANOVA. \*Significantly different from placebo at the corresponding time point ( $p < .05$ ).

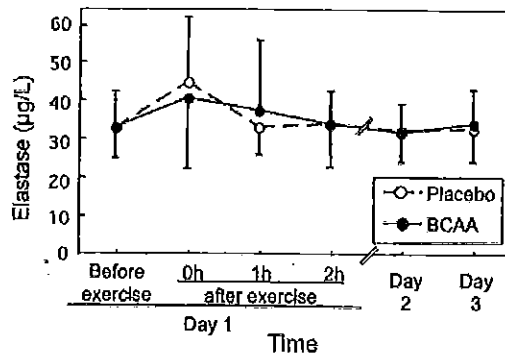
BCAA trial than those at the corresponding time points in the placebo trial.

**Serum Markers of Muscle Damage**

Serum creatine kinase activity is generally used as an indicator of muscle damage induced by exercise, although it is known that the creatine kinase response to exercise shows marked individual differences (Totsuka, Nakaji, Suzuki, Sugawara, & Sato, 2002). In the current study, although a detectable change in enzyme activity was significant only in the placebo trial, the change was quite small (Figure 4), indicating that the intensity of the squat-exercise task used was low. It has been reported that myoglobin is rapidly released after muscle damage and that its serum concentration appears to be more sensitive to muscle damage than serum creatine kinase activity (Sorichter, Puschendorf, & Mair, 1999). In the current study, a significant change in serum myoglobin concentration was observed in the placebo trial but not in the BCAA trial (Figure 4). These results suggest that muscle damage was less in the BCAA trial than in the placebo trial, although the squat exercise performed in the current study appeared to cause minor muscle damage.



**Figure 4** — Serum creatine kinase activity and myoglobin concentration in the branched-chain amino acid (BCAA) and placebo trials,  $M \pm SD$  ( $n = 12$ ). Changes in both serum creatine kinase activity and myoglobin concentration over time were significant in the placebo trial ( $p = .043$  and  $p = .008$ , respectively), but not in the BCAA trial, when they were tested using one-way repeated-measures ANOVA.



**Figure 5** — Plasma elastase concentration in the branched-chain amino acid (BCAA) and placebo trials,  $M \pm SD$  ( $n = 12$ ). Changes in elastase concentration over time were significant in the placebo trial ( $p = .007$ ), but not in the BCAA trial, when they were tested using one-way repeated-measures ANOVA.

### Plasma Elastase Concentration

The release of elastase into the circulation is used as an index of neutrophil activation, and it has been demonstrated that moderate exercise significantly increases plasma elastase level (Smith et al., 1996). In the current study, the concentration of plasma elastase tended to be increased by the squat exercise in both trials, but the change in the elastase level was significant only in the placebo trial (Figure 5).

### Discussion

DOMS is commonly caused by unaccustomed eccentric exercise in humans (Sorichter et al., 1999). It is generally an unpleasant sensation and can adversely affect muscle performance from voluntary reduction of effort, as well as from the muscles' inherent loss of capacity to produce force (Proske & Morgan, 2001). Therefore, it is desirable to reduce exercise-induced DOMS not only in athletes but also in untrained individuals. We used the squat exercise as resistance exercise to induce DOMS in untrained female participants because a relatively large number of untrained participants can perform the exercise safely and at the same time. In the current study, participants reported peak muscle pain on Days 2 and 3 after exercise, clearly indicating that DOMS occurred. In our preliminary study (Shimomura et al., 2006), DOMS induced by the squat exercise was significantly reduced by preexercise BCAA supplementation at  $92 \pm 2$  mg/kg body weight in female participants. A similar result was obtained in the current study, in which the participants ingested a dose of BCAAs adjusted to body weight (100 mg/kg) before exercise. The results of these studies suggest that BCAAs have an anti-DOMS effect, at least when they are ingested as a supplement with a dose range of

92–100 mg/kg body weight before exercise. The muscle force measured on Day 3, during peak muscle soreness, was decreased by ~20% in the placebo trial but not in the BCAA trial, suggesting that a decrease in the contractile capacity of skeletal muscles, probably because of the pain (Armstrong, 1984), may be suppressed by BCAA supplementation. During the study period, none of the participants reported any side effects such as intestinal dysfunction.

It is known that DOMS after a period of exercise becomes much less when the exercise is repeated a week later (Armstrong, 1984; Proske & Morgan, 2001). In this case, decreased DOMS has been attributed to an adaptation of skeletal muscles, but the underlying mechanism is not known. Thus, to avoid any possible influences of muscle adaptation in this study, the two trials of the crossover design were separated by 11 weeks. In both trials, participants were randomly divided into two groups, with half taking BCAAs and half placebo. When the DOMS data were calculated in terms of the first and the second experiments of the crossover design, there were no significant differences in the degree of DOMS at all time points and AUC between the first and the second experiments. Therefore, it may be concluded that the effect of BCAA supplementation on DOMS observed in the current study was not affected by muscle adaptation to the squat exercise.

It has been demonstrated that DOMS is associated with muscle damage (Armstrong, 1984; Proske & Morgan, 2001; Sorichter et al., 1999). Muscle damage induced by exercise is commonly detected by measuring plasma (or serum) muscle-damage markers, for example, creatine kinase activity and myoglobin concentration (Sorichter et al., 1999). In the current study, the increase in serum creatine kinase activity was quite small in both trials. This may be a result of the relatively low intensity of the squat exercise (seven sets of 20 squats per set) performed; in this squat-exercise program, the total exercise time was only 4.7 min, because 20 squats in 1 set were performed for 40 s. Many studies reporting several-fold increases in creatine kinase activity used 20–60 min of eccentric exercise or downhill running (Balnave & Thompson, 1993; Cannon et al., 1990; Close et al., 2005; Maughan et al., 1989; Sorichter et al., 1999). As suggested by Sorichter et al., the intensity of the exercise task is one of the most important factors in inducing muscle damage.

It has been reported that increased serum myoglobin concentration can be used as a muscle-damage marker and is a more sensitive marker than creatine kinase (Sorichter et al., 1999). Based on the significant change in serum myoglobin concentration observed in the placebo trial, we may infer that the squat-exercise protocol used in the current study resulted in muscle damage, although its extent was small. Because preexercise BCAA supplementation suppressed changes in myoglobin circulating levels subsequent to exercise, it is possible that decreased muscle damage is involved in the mechanism responsible for the anti-DOMS effect of BCAAs. Recently, we found

that BCAA supplementation right after squat exercise does not affect the elevation of serum myoglobin concentration observed after exercise (unpublished results), suggesting that preexercise supplementation is critical to minimize muscle damage. It has been demonstrated that muscle injury can produce a stereotypic inflammatory response, in which muscle invasion of neutrophils, followed by macrophages, is stimulated (Tidball, 2005), and that the plasma elastase level is elevated immediately after moderate exercise (Smith et al., 1996). We also measured plasma elastase concentration as an index of neutrophil activation and found that it tended to increase after squat exercise in both trials. However, significant change was observed only in the placebo trial, suggesting that the activation of immune cells (inflammatory response) may have been greater in that trial.

Further possible mechanisms responsible for the protective effect of BCAAs against DOMS may be related to the findings that (a) BCAA supplementation at a dose of 77 mg/kg body weight before exercise decreased the release of essential amino acids from muscles during exercise (MacLean et al., 1994), (b) supplementation with a solution containing 6 g essential amino acid (40% BCAAs) and 35 g carbohydrate before eccentric exercise increased protein synthesis in skeletal muscle during the postexercise period (Tipton et al., 2001), and (c) dietary BCAAs may affect energy metabolism during exercise (Shimomura et al., 2000). In the current study, plasma BCAA concentration was significantly changed by the squat exercise in the placebo trial, suggesting that BCAA oxidation was promoted by the exercise. It has been reported that exercise training attenuates leucine oxidation during exercise in humans (McKenzie et al., 2000), suggesting that untrained individuals may have a higher dependency of the energy metabolism on BCAAs during exercise. Thus, circulating BCAA scarcity after exercise might result in delayed muscle recovery in participants during a placebo trial. It should be pointed out that, in the current study, the participants ingested 100 kcal of sugar 30 min before BCAA supplementation, because insulin enhances the leucine-induced stimulation of protein synthesis in muscles (Kimball & Jefferson, 2006). Actually, the serum insulin level was higher right after exercise (0-hr time point) than before exercise. Further studies are required to clarify the molecular mechanisms responsible for the effect of BCAAs against DOMS and muscle fatigue.

Potential protective effects of vitamin C (Bryer & Goldfarb, 2006; Thompson et al., 2001), leucine metabolites ( $\beta$ -hydroxy- $\beta$ -methylbutyrate and  $\alpha$ -ketoisocaproate; van Someren, Edwards, & Howatson, 2005), and creatine (Santos, Bassit, Caperuto, & Costa Rosa, 2004) against DOMS and muscle damage after eccentric exercise or running have been studied in humans, and some positive effects were observed. However, other studies have reported negative results (Close et al., 2006; Paddon-Jones, Keech, & Jenkins, 2001; Shafiq, Butler, Jensen, & Donnelly, 2004). In the reports in which supplementation reduced DOMS, participants ingested

the test supplements for 5–14 days before exercise. In the current study, however, a single dose of the BCAA supplement was provided to untrained participants before exercise, and an anti-DOMS effect was observed.

In conclusion, the current study showed an anti-DOMS effect of preexercise BCAA supplementation in untrained female participants, suggesting that the BCAA supplement may be beneficial to untrained individuals who exercise. The efficacy of BCAA supplementation in untrained males and athletes remains to be evaluated. However, a very recent study using athletes showed that the addition of amino acids (consisting of 80% BCAAs and 20% arginine) to a carbohydrate beverage reduced muscle damage, decreased fatigue, and maintained exercise performance after consecutive days of exercise (Skillen et al., 2008), suggesting that BCAA supplementation may be beneficial to athletes, as well.

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