International Journal of Sport Nutrition and Exercise Metabolism, 2006, **16**, 620-635

Effects of Amino Acid Supplementation on Muscle Soreness and Damage

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This study investigated the effect of a supplement containing 9 essential and 3 non-essential amino acids on muscle soreness and damage by comparing two endurance exercise bouts of the elbow flexors with amino acid or placebo supplementation in a double blind crossover design. The supplement was ingested 30 min before (10 h post-fasting) and immediately after exercise (Experiment 1), or 30 min before (2-3 h after breakfast), immediately post, and 8 more occasions over 4-day post-exercise (Experiment 2). Changes in muscle soreness and indicators of muscle damage for 4 days following exercise were compared between supplement conditions using two-way ANOVA. No significant differences between conditions were evident for Experiment 1; however, plasma creatine kinase, aldolase, myoglobin, and muscle soreness were significantly lower for the amino acid versus placebo condition in Experiment 2. These results suggest that amino acid supplementation attenuates DOMS and muscle damage when ingested in recovery days.

Key Words: BCAA, maximal isometric strength, creatine kinase, myoglobin, swelling

Following unaccustomed or prolonged exercise, muscle soreness, often referred to as delayed onset muscle soreness (DOMS), develops gradually and lasts for several days (2, 3). DOMS is a symptom of muscle damage and prevalent following activities involving a high component of lengthening muscle actions (2, 13). DOMS and the associated impairment of muscle function due to muscle damage can reduce the ability to perform both daily tasks and athletic activities (3) and has potentially negative effects on adherence to physical activity programs. Thus, it is important to find effective prophylactic or therapeutic interventions to prevent or reduce DOMS and/or enhance recovery of muscle function after exercise.

Many interventions, including various physical treatments and pharmacological therapies, have been investigated on the pretext of either preventing or reducing DOMS and muscle damage or enhancing recovery from exercise-induced muscle

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damage (2, 3). A number of nutritional supplements have been also investigated as potential prophylactic/therapeutic agents, including antioxidants (6), L-carnitine (9), creatine (19), β-hydroxy-β-methylbutyrate (10, 16), and branched-chain amino acids (4, 21) or amino acid combinations (11, 15, 22). Fielding et al. (8) reported that a bout of cycling that consisted of repeated lengthening muscle actions, increased whole body protein breakdown and leucine oxidation for 10 days after exercise. Evans (7) documented that an increase in dietary protein intake is necessary after exercise resulting in muscle damage. It has been documented that amino acid supplementation stimulates the transport of amino acids into skeletal muscles and administration of exogenous amino acids after exercise increases protein synthesis while reducing protein breakdown (1, 20, 24, 26). Thus, it is plausible that amino acid supplementation may benefit the recovery processes associated with muscle damage.

Several studies have implied an effect of amino acid supplement on reducing muscle damage induced by endurance or resistance exercise. Coombes and McNaughton (4) showed that increases in serum creatine kinase (CK) and lactate dehydrogenase activities after 120 min cycling were significantly lower for the group of subjects who took branched-chain amino acid (BCAA) supplement. Ohtani et al. (15) found that a mixed amino acid supplement attenuated increases in serum CK activity during a long-distance running training period compared to a placebo condition. Kraemer et al. (11) recently reported that amino acid supplement (0.4 g/kg per day) attenuated increases in plasma CK activity and decreases in 1RM squat and bench press after the first week of 4-week overreaching program.

A few studies have examined the effect of amino acid supplement on exercise mainly consisting of lengthening muscle actions, and only one study has examined the effect of amino acid supplement on DOMS. Sugita et al. (22) showed that an amino acid supplement containing 12 amino acids (5.6 g) taken twice daily from 1 to 10 days after elbow flexion/extension exercise enhanced recovery of elbow extensor strength. Shimomura et al. (21) recently reported that BCAA supplementation prior to squat exercise attenuated development of DOMS. It appears that there is evidence to support the efficacy of amino acid supplementation in attenuating muscle damage; however, systematic studies investigating the effect of amino acid supplementation on DOMS and markers of muscle damage is still scarce.

Therefore, the aim of this study was first to determine whether an amino acid supplement taken prior to and immediately after exercise would reduce muscle damage induced by a bout of exercise of the elbow flexors. Second, we wished to ascertain whether continuing the intake of amino acids over four days following the exercise would enhance any beneficial effects of supplementation.

Methods

Study Design

This study was conducted after obtaining approval from the local Institutional Ethics Committee. This study used an "arm-to-arm comparison model," since comparison of the two conditions in the same subjects is preferable given the wide variation in responses to exercise between individuals and the subjective nature of pain sensation. Both arms performed the same exercise protocol separated by

3-4 weeks, with amino acid supplementation for one arm test and placebo (maltitol) supplementation for exercise of the contralateral arm in a randomized order with double-blind design. The criterion measures of muscle damage included isometric maximal voluntary contraction (MVC), range of motion (ROM), upper arm circumference, plasma creatine kinase (CK) and aldolase (ALD) activities, myoglobin concentration (Mb), and muscle soreness. As shown in Figure 1, the criterion measures were assessed before, immediately after, and 1, 6, 24, 48, 72, and 96 hours post-exercise.

Two experiments were conducted in this study using subjects from the same population. In Experiment 1, supplements were ingested 30 min before and immediately after exercise, whereas in Experiment 2, supplements were also ingested on additional eight occasions over 4 days after exercise (Figure 1). For the exercise day, the first supplement was given immediately after the first blood sampling but before the pre-exercise criterion measurements, which required approximately 20 min. The second supplement was ingested immediately after taking the immediately-post blood sample but prior to other criterion measurements. Concentrations of 20 amino acids in plasma were measured six occasions (before supplementation, 30 min after supplementation, immediately, 1, 6, and 24 h after exercise) for Experiment 1. For Experiment 2, the amino acid concentrations were assessed before supplementation, and 6 and 24 h after exercise only.

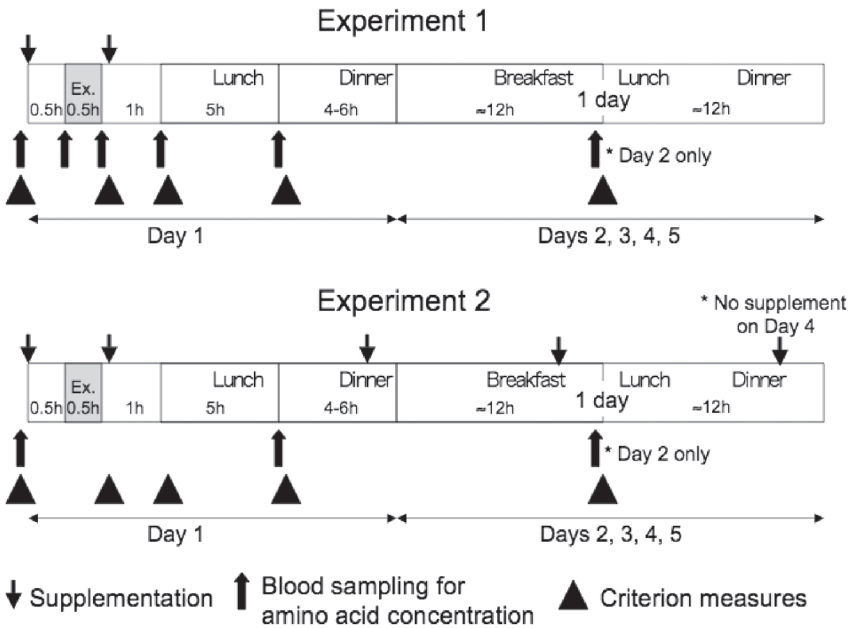

Figure 1—Experimental protocol of Experiment 1 and Experiment 2. The diagram shows the timing of the supplementation, blood sampling for plasma amino acid concentrations, and criterion measures of each experiment. Each experiment had the same experiment protocol with a difference in the supplement (amino acids or placebo). It should be noted that the Day 1 is the exercise day, and Days 2 to 5 are recovery days from exercise.

Subjects

Thirty-eight male students who were non-athletes, free from any musculo-skeletal disorders, and had not been involved in any regular resistance training for at least 1 year before this study were recruited. Their mean ± SD (range) age, height, body mass, and percent body fat was 21.1 ± 2.8 (18 to 31) years, 170.0 ± 5.5 (159 to 183) cm, 59.4 ± 5.8 (48 to 74) kg, and 15.3 ± 3.7 (8.0 to 24.4)%, respectively. Body fat was determined using a body composition analyzer (InBody 3.0, Biospace, Seoul, South Korea). The number of subjects was determined on the assumption that the amino acid supplementation could attenuate changes in the criterion measures at least 10% if it was effective. With 80% power and a one-tailed level of significance at $P = 0.05$, a sample size of 12 was obtained using the data of a previous study (14) in which a similar exercise to that used in the present study was performed. We recruited 14 subjects for Experiment 1 and 24 subjects for Experiment 2. No significant differences in the physical characteristics of subjects were evident between the experiments.

Subjects gave written informed consent in accordance with the ethical standards of the Declaration of Helsinki. Subjects were requested not to take any nutritional supplements other than those provided, refrain from use of medicines including anti-inflammatory drugs or any treatments (e.g., massage), and avoid any unaccustomed exercise or vigorous physical activities during the experimental period. Subjects were asked to record their food intake during the course of the trials. In order to match the food intake for one day prior to exercise, the exercise day, and four days following exercise for the first and second experimental periods, the subjects were asked to eat the same things that were listed on the record of the first experimental period. According to the diet records, it was confirmed that all of the subjects followed the instruction, and the overall diets were similar between subjects in Experiment 1 and Experiment 2.

In Experiment 1, subjects did not have breakfast and refrained from eating and drinking anything other than water for approximately 10 hours before the first supplementation. The reasons were that we had thought that the supplement effect would be clarified by this way, and we wanted to investigate changes in plasma amino acid concentrations after ingestion of the supplement by eliminating the influence of breakfast. In contrast, subjects in Experiment 2 had a light breakfast (1200 to 2000 kJ; carbohydrate 60 to 70%, fat: 20 to 25%, protein: 10 to 15%) at least 2 hours before coming to laboratory on the exercise day. This modification was made because the subjects in Experiment 1 had to refrain from food for a long time (> 12 h) until they ate lunch more than 2 h after exercise, which did not appear to be realistic before exercise in practical situation when people use supplement, and we assumed that the effect of supplement might have been eliminated by the fasting condition.

Supplements

This study used a commercially available amino acid mixture supplement, AminoVital®Pro (Ajinomoto CO. Inc., Tokyo, Japan). The supplement (AMINO) contained 12 amino acids (L-arginine: Arg, L-glutamine: Gln, L-histidine: His, L-isoleucine: Ile, L-leucine: Leu, L-lysine: Lys, L-methionine: Met, L-phenylalanine: Phe, L-proline: Pro, L-valine: Val, L-threonine: Thr, L-tryptophan Trp; total: 3.6

g), 10 vitamins (A, B_1, B_2, B_6, B_{12}, C, pantothenic acid, niacin, D, E), two minerals (Ca, Fe), carbohydrate (0.5 g), and a small amount of fat (0.045 g) per packet. Thus, one packet of the supplement weighed 4.5 g in total. Among the 12 amino acids, 60% were made up of essential amino acids, and approximately 60% of the essential amino acids consisted of branched-chain amino acids (Ile, Leu, Val). The placebo consisted of maltitol (3.6 g) and other ingredients of AMINO except the amino acids. Each supplement packet was ingested with 200 ml of water, and both AMINO and PLACEBO were presented in identical packages so that neither subjects nor experimenters could distinguish between them. However, since a taste difference between the supplements was discernible (one being more bitter and powdery than other), subjects were told that both were amino acids but of different kinds. Therefore, subjects took both supplements as "amino acids."

As shown in Figure 1, subjects ingested the supplement 30 min before and immediately after exercise only for Experiment 1; thus, the total amount of supplement ingested was 9 g, and the portion of amino acids was 7.2g. In Experiment 2, subjects took the supplements before, immediately after exercise, and at night (after dinner) for the exercise day and after breakfast and dinner for 3 days following exercise and after breakfast on day 4 after exercise (Figure 1). The increased supplementation timing was based on an assumption that amino acids would be also required in the recovery from muscle damage, and the amount given in Experiment 1 was not enough to see any beneficial effects on DOMS and other markers. This amounted to a total supplement intake of 10 packets (45 g) over the 5-day experimental period, including 36 g of amino acids.

Exercise

Subjects performed an arm curl exercise with a wristband weight (mean: 2.57 kg, range: 1.80 to 3.44 kg) that was set at 9% of their maximal isometric strength determined at an elbow joint of 90° (1.57 rad). This exercise protocol was modified from a previous study (14) and had been shown to induce muscle soreness and changes in all markers of muscle damage. During exercise, subjects were seated and the arm was positioned in front of the body on a padded support adjusted to 45° (0.79 rad) of shoulder flexion, and the forearm was kept supinated during exercise. Subjects were asked to flex (1 s) and extend (1 s) their elbow joint rhythmically for 30 min (900 actions) in time to a metronome. The range of motion was approximately 120° from a flexed (60°, 1.05 rad) to an extended elbow position (180°, 3.14 rad). During the elbow extending actions, subjects were asked to lower the dumbbell in a controlled manner using the elbow flexors. The first bout of exercise was performed with one arm, and following a 3 to 4 week exercise-free interval, a second exercise bout was performed using the contralateral arm. Arm dominance and order of condition (AMINO, PLACEBO) were counter-balanced among subjects. There was no significant difference in exercise workload between the AMINO (2.61 ± 0.43 kg) and PLACEBO (2.55 ± 0.36 kg) conditions.

Criterion Measures

Several indirect markers of muscle damage and muscle soreness, which have been widely used in previous studies (13,14,16,19), were assessed before and

immediately after, and 1, 6, 24, 48, 72, and 96 hours following exercise. To assess perceived exertion during the exercise, the Borg scale (6: no exertion at all – 20: maximal exertion) was used, and an investigator asked subjects for a rating of perceived exertion (RPE) at 5, 10, 15, 20, 25 and 30 min of exercise.

Maximal Voluntary Contraction (MVC). MVC was measured twice (1 min between the measurements) at an elbow joint of 90° (1.57 rad) for 3 s, by way of a transducer (Model 100, Takei Scientific Instrument Co. Ltd., Japan) connected to a computer (Macintosh Power Mac G4, Apple Computer, Cupertino, USA) via a Power Lab system with accompanying software program (PowerLab /8SP, ADInstruments, Castle Hill, Australia). Subjects were seated on a specially designed bench so that the arm was positioned in front of the body with the shoulder and elbow joints flexed at 1.57 rad (90º). A wristband was attached to the transducer via a metal cable. The subjects were asked to perform two maximal isometric contractions for 3 s each with an interval of 30 s, and the mean of two trials was used for further analyses.

Range of Motion (ROM). With the arm held by their side, subjects were asked to extend the elbow joint maximally (extended elbow joint angle), and flex maximally (flexed elbow joint angle). Each angle was measured twice by a goniometer, and the mean of the two values was used for analysis. The difference between the two angles (extended angle – flexed angle) was considered as ROM of the elbow joint.

Upper Arm Circumference. To assess muscle swelling, upper arm circumference was assessed at 3, 5, 7, 9, and 11 cm from the elbow joint by a tape measure with the arm hanging loosely by their side. Measurements were taken twice for each site, and the mean value of the two measurements was used for analysis, as well as the average of the five sites.

Blood Markers. Approximately 5 ml of blood was drawn from the antecubital vein by a standard venipuncture technique using heparin lithium coated tubes. After obtaining three 20-μl blood samples for lactate analysis, blood was centrifuged for 10 min to obtain plasma. The plasma samples were stored at -20 °C until they were analyzed for creatine kinase (CK) and aldolase (ALD) activities and myoglobin (Mb) concentration. Plasma CK and ALD activities were determined spectrophotometrically using an automatic blood analyzer (Model 7170; Hitachi, Tokyo, Japan) and standard test kit (Shikarikid CK; Kanto Chemical Co. Ltd, Tokyo, Japan). The normal reference ranges for male adults using this method for CK and ALD was 45-135 $IU \cdot L^{-1}$ and 1.7-5.7 $IU \cdot L^{-1}$, respectively. Plasma Mb concentration was measured using a γ-counter (ARC-950; Aloka Co. Ltd., Tokyo, Japan) with a commercially available kit (Daichi Radioisotope, Tokyo, Japan). The normal reference range for Mb in male adults using this method is $< 60\ ng \cdot ml^{-1}$. Blood lactate concentration was determined using a Biosen 5010 (EKF Industrie-Elektronik GmbH, Germany).

Muscle Soreness. Muscle soreness during palpation on the upper-arm (SOR-pal) as well as flexion (SOR-flx) and extension of the elbow joint (SOR-ext) were evaluated by a visual analog scale (VAS) that had a 50-mm line with “no pain” on one end and “extremely sore” on the other.

Concentration of Amino Acids in the Blood

Using the aliquot (200 μl) of the plasma samples described above, free amino acid concentrations were examined for selected time points (Figure 1). Plasma samples were deproteinized with 200 μl of 3% sulfosalicylic acid and after centrifugation, the supernatant was assayed with an amino acids analyzer (L-8500, Hitachi High-Technology Co., Japan) to determine the concentration of amino acids. The analysis included the nine essential amino acids (L-histidine: His, L-isoleucine: Ile, L-leucine: Leu, L-lysine: Lys, L-methionine: Met, L-phenylalanine: Phe, L-valine: Val, L-threonine: Thr, L-tryptophan: Trp) and 11 non-essential (L-alanine: Ala, L-arginine: Arg, L-asparate: Asp, L-asparagine: Asn, L-cycteine: Cys, L-glutamine: Gln, L-glutamate: Glu, L-glycine: Gly, L-proline: Pro, L-serine: Ser, L-tyrosine: Try). S-(2-aminoethyl)-L-cysteine was used as an internal standard.

Statistical Analyses

Changes in all criterion measures over time were compared between the conditions (AMINO vs PLACEBO) using two-way repeated-measures ANOVA. When the ANOVA found a significant main effect, a Sheffé's post hoc test was used to specify where the differences occurred. Changes in criterion measures from the pre-exercise baseline were analyzed by one-way repeated measures ANOVA. A matched-pairs signed-ranks test was also included to compare between conditions for peak soreness scores. For plasma CK and ALD activities and Mb concentration, comparisons between conditions were made for their peak values after exercise by Student t-test. Changes in plasma amino acid concentrations over time from pre-supplement value were analyzed by one-way repeated measures ANOVA for each condition to examine the changes from baseline. Significance was set at $P < 0.05$. The values of the criterion measures are shown as mean ± SEM, unless otherwise stated.

Results

Exercise

Although many of the subjects had difficulty keeping the tempo of the arm movement with metronome toward the end of exercise, all subjects completed the 30-min exercise. RPE increased significantly during exercise (5 min, 13.5 ± 0.2; 10 min, 14.2 ± 0.2; 15 min, 14.7 ± 0.2; 20 min, 15.4 ± 0.2; 25 min, 16.2 ± 0.2) and reached 17.2 ± 0.2 (very hard) at the end of the exercise; however, there was no significant difference between the conditions. A small but significant increase in blood lactate concentration was observed from before (2.5 ± 0.1 mmol·L^{-1}) to immediately after exercise (3.2 ± 0.2 mmol·L^{-1}), but the increase (approximately 30%) was not significantly different between conditions.

Experiment 1

Changes in Amino Acids Following Supplementation. Prior to supplementation and in both groups, approximately 35% of plasma amino acids were essential amino acids, of which 45% were BCAA. Following amino acid ingestion, differ-

ences between the conditions were evident (Figure 2). At 30 min after ingestion, significant increases in total amino acids, essential amino acids, non-essential amino acids, and BCAA occurred in the AMINO condition, with no significant changes observed in the PLACEBO condition at this time point. Immediately after exercise, plasma total amino acids, essential amino acids, non-essential amino acids, and BCAA concentrations decreased in the AMINO condition but were still significantly higher than baseline for essential amino acids and BCAA. In contrast, the PLACEBO condition showed significant decreases in essential amino acids and BCAA immediately after exercise.

At 1 hour after exercise, total amino acids, essential amino acids, non-essential amino acids, and BCAA were significantly lower than baseline in the PLACEBO condition. However, this was not the case for the AMINO condition, where increases (essential amino acids and BCAA) or no changes (total amino acids, non-essential amino acids) from baseline occurred. By 6 hours post-exercise, significant decreases in total amino acids, essential amino acids, non-essential amino acids, and BCAA from the baseline occurred in the AMINO condition, but not the PLACEBO condition. At 24 hours after exercise, the plasma amino acid concentrations of both conditions had returned to baseline values, with no significant differences between conditions. Changes in the individual essential and non-essential amino acids followed a similar pattern to the group changes for essential (Figure 2b), and non-essential amino acids (Figure 2c), respectively.

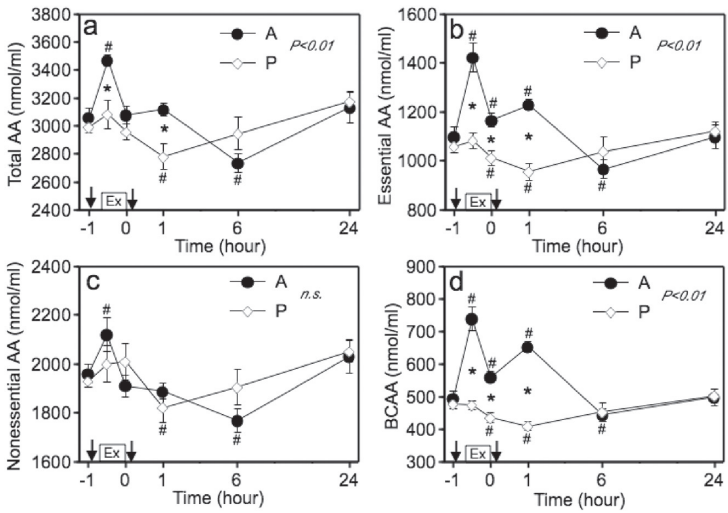

Figure 2—Experiment 1. Changes in total amino acid (a), essential amino acid (b), nonessential amino acid (c), and branched chain amino acid (d) concentration in the blood before supplementation (30-min before exercise; -1), 30-min after supplementation (immediately before exercise), immediately after (0), 1 and 6 h after exercise for amino acid (A) and placebo (P) conditions in Experiment 1. The supplementation point is indicated by ↓, and the second supplement was taken after the immediately post-exercise blood sampling. *: a significant difference between the conditions. #: a significant difference from the baseline (before supplementation).

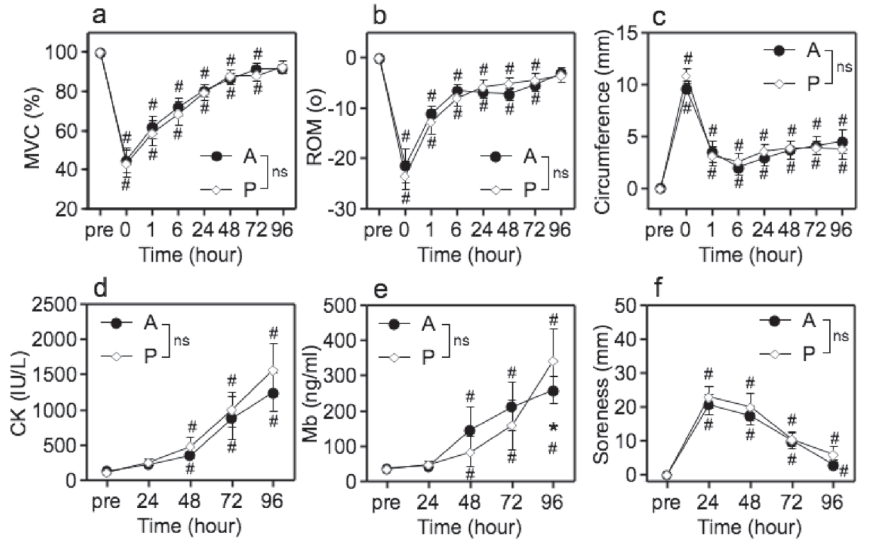

Figure 3—Experiment 1. Changes in maximal isometric strength (a), ROM (b), upper arm circumference (c), plasma CK activity (d), myoglobin concentration (e), and muscle soreness upon palpation (f) before (pre), immediately after (0), and 1-96 h after exercise for amino acid (A) and placebo (P) conditions. #: a significant difference from the pre value. ns: no significant interaction effect.

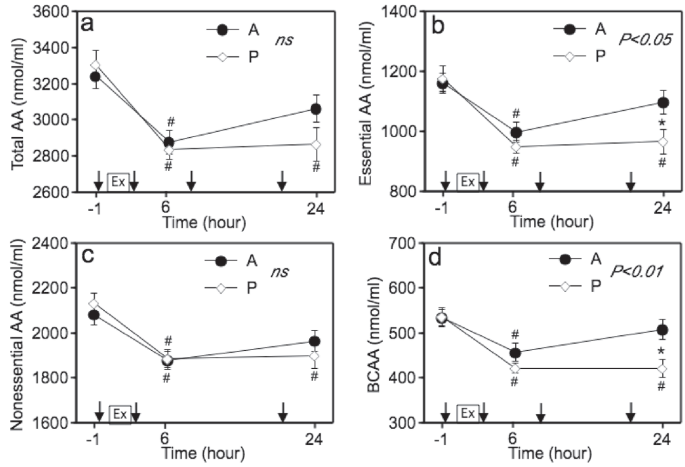

Figure 4—Experiment 2. Changes in total amino acid (a), essential amino acid (b), nonessential amino acid (c), and branched chain amino acid (d) concentration in the blood before supplementation (30-min before exercise; -1), 6 and 24 hours after exercise for amino acid (A) and placebo (P) conditions. The supplementation point is indicated by ↓, and "Ex" shows exercise. *: a significant difference between the conditions. #: a significant difference from the pre value.

Changes in Criterion Measures After Exercise. All measures changed significantly following exercise; however, no significant differences between conditions were evident for any of the measures. MVC (Figure 3a) decreased to approximately 50% of the pre-exercise value immediately after exercise, recovered to approximately 70% at 1 h, 80% at 6 h, and had returned to baseline by 96 hours post-exercise. ROM of the elbow joint (Figure 3b) decreased by more than 20° immediately after exercise and gradually recovered to the pre-exercise value by 96 hours after exercise. Upper arm circumference that indicated swelling (Figure 3c) increased by around 10-mm (approximately 5%) immediately after exercise, before decreasing to near pre-exercise values 1-6 hours after exercise for both conditions, with some degree of swelling still apparent 48 hours post-exercise. Plasma CK activity (Figure 3d) increased significantly after exercise and remained elevated 72-96 hours post-exercise, as did plasma ALD activity. Plasma Mb concentration (Figure 3e) increased significantly after exercise and peaked 72-96 hours later. All subjects reported some degree of muscle soreness (Figure 3f), which peaked 24-48 hours after exercise for palpation, extension, and flexion soreness.

Experiment 2

Changes in Amino Acids Following Supplementation. Figure 4 shows changes in amino acids (total amino acids, essential amino acids, non-essential amino acids, and BCAA) before supplementation and 1 and 6 hours after exercise. No significant differences between conditions were seen before supplementation, but changes in amino acids were significantly different for essential amino acids and BCAA, showing the same pattern seen in Experiment 1. Significant decreases in amino acids from baseline values occurred 6 hours after exercise in both groups. When amino acid concentrations were compared at 24 hours after exercise, levels remained significantly below baseline values in the placebo group, whereas those of the amino acid group had returned to baseline.

Changes in Criterion Measures After Exercise. The time course of changes in the criterion measures were similar to those seen in Experiment 1 (Figure 2); however, significant differences between the AMINO and PLACEBO conditions were evident for some measures. No significant differences between the two conditions were evident for the changes in MVC (Figure 5a) and ROM (Figure 5b). Increases in upper arm circumference after 72 hours post exercise were larger in the PLACEBO condition than in the AMINO condition, and the post hoc analysis showed that increases in circumference between 72 and 96 hours after exercise were significantly larger in the PLACEBO versus the AMINO condition (Figure 5c). Changes in plasma CK and ALD activities and Mb concentration were significantly different between the AMINO and PLACEBO conditions, and the post-hoc analysis showed a significant difference between conditions at 3 and/or 4 days after exercise (Figures 5d & 5e). Peak plasma CK and ALD activity, and Mb concentration were significantly lower in the AMINO condition (CK: 702 ± 270 $IU \cdot L^{-1}$, ALD: 9.4 ± 2.3 $IU \cdot L^{-1}$, Mb: 182 ± 50 $ng \cdot ml^{-1}$) than the PLACEBO condition (CK: 1,731 ± 380 $IU \cdot L^{-1}$, ALD: 16.9 ± 2.5 $IU \cdot L^{-1}$, Mb: 379 ± 86 $ng \cdot ml^{-1}$). The development of palpation and extension muscle soreness was significantly smaller for the AMINO condition compared to the PLACEBO condition, and significant differences between conditions were evident 1-3 days post-exercise (Figure 5f).

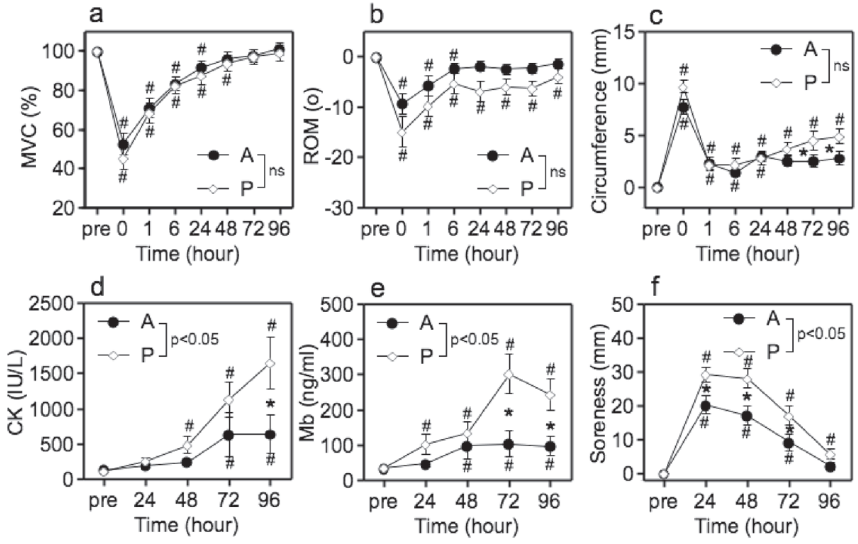

Figure 5—Experiment 2. Changes in maximal isometric strength (a), ROM (b), upper arm circumference (c), plasma CK activity (d), myoglobin concentration (e), and muscle soreness upon palpation (f) before (pre), immediately after (0), and 1-96 h after exercise for amino acid (A) and placebo (P) conditions. #: a significant difference from the pre value. *: a significant difference between conditions. ns: no significant interaction effect. $P < 0.05$: a significant interaction effect.

Subjects reported approximately 30% less peak soreness on palpation and extension of the arm for the AMINO condition (palpation: 23.8 ± 2.8, extension: 19.4 ± 2.9, flexion: 10.4 ± 2.0 mm) compared to PLACEBO condition (palpation: 33.1 ± 2.4, extension: 27.0 ± 3.0, flexion: 14.8 ± 2.4 mm), but the difference in the flexion measure was not statistically significant.

Discussion

The present study investigated whether supplementation with amino acids would reduce muscle damage and DOMS following an endurance exercise bout of the elbow flexors. The main findings of the study were that 1) both amino acid supplementation and exercise altered plasma amino acid concentrations, 2) acute amino acid supplementation 30 min before and immediately after exercise in a fasting condition (Experiment 1) did not affect muscle damage and DOMS, and 3) additional supplementation of amino acids for 4 days following exercise (Experiment 2) reduced some markers of muscle damage and DOMS compared to the placebo condition.

We are limited in our ability to discuss the amino acid balance in the muscle from the venous amino acid concentration alone, and we can only speculate about the possible significance and mechanism of the changes in plasma amino acid

concentrations. It is important to note that most of amino acids contained in the amino acid supplement increased significantly 30 min after initial ingestion and 1 hour after the second ingestion, but this did not occur in the placebo condition (Figures 2 & 4). Because of the limited blood sampling points, the exact time course of changes in plasma amino acid concentrations is not clear. However, if amino acid levels peaked at around 30 min after ingestion as shown in Figure 2, and considering the duration of exercise (30 min), it is questioned whether the timing of supplementation was the best. Further study is necessary to investigate the best timing of the amino acid supplementation before exercise. It may be dependent on type, intensity, and duration of exercise, and the method of amino acid supplementation and the amount of amino acids should be also taken into account.

The larger magnitude of decline in amino acid concentrations from pre to immediately post exercise observed in the AMINO condition (Figure 2) may reflect greater uptake into skeletal muscle in response to the exercise, but it might be simply due to increased splanchnic clearance or decreased release from the splanchinic bed (25). In Experiment 2, significant decreases in amino acids from baseline were evident at 6 h post-exercise in both conditions (Figure 4); however, in Experiment 1, this was observed only in the AMINO condition (Figure 2). This is likely due to the higher baseline plasma amino acid concentrations, possibly due to the effect of breakfast. It is also worthy of note that plasma amino acid concentrations returned to baseline by 24 hours post-exercise in the AMINO condition, but not the PLACEBO (Figure 4). Although the higher amino acid concentrations for the AMINO condition could be explained by the amino acid supplement taken at night of the exercise day and morning of the following day, it is difficult to explain why amino acid concentrations did not return to baseline for the PLACEBO condition, contradicting the findings of Experiment 1 (Figure 2).

In both experiments, we found that amino acid supplementation had no significant effects on RPE during exercise, and similar changes in MVC, ROM, and circumference immediately after exercise occurred in the AMINO and PLACEBO conditions (Figures 3 & 5). Ratamess et al. (18) reported that an amino acid supplement attenuated reductions of muscle strength and power in the first 1-2 weeks of moderate-intensity, high-volume resistance training. The present study was the first to examine the effects of amino acid supplement ingested 30 min prior to a local muscle exercise on muscle function. The results of the present study shows that the amino acid supplementation (3.6 g) ingested 30 min before exercise does not affect the exercise performance and muscle function.

Based on previous studies (4, 11, 15, 21, 22), we expected that amino acid supplementation would enhance the recovery of MVC and ROM, and reduce muscle swelling, soreness, plasma CK activity, and Mb concentration. However, no significant differences between the conditions were evident for these parameters in Experiment 1 (Figure 3). It is important to note that the supplement was given in a fasting state for the Experiment 1. In contrast to Experiment 1, Experiment 2 resulted in significant differences between conditions for some criterion measures following exercise. These included smaller increases in upper arm circumference, reduced plasma CK and ALD activities and Mb concentration, and attenuated DOMS for the AMINO versus PLACEBO condition (Figure 5). In Experiment 2, subjects had breakfast before exercise, and the supplement was given at the night of the exercise day and additional 7 occasions over 4 day recovery period. It

should be noted that the breakfast alone did not have effect on muscle damage as shown in the PLACEBO condition (Figure 5), and the amino acid supplement alone did not produce any beneficial effects as shown in Experiment 1 (Figure 3). It would appear that a combination of regular meal and amino acid supplement is important, probably the impact of greater energy intake or other nutrients provided in the breakfast is a factor. It is possible that the breakfast changed the insulin response, which might modify the amino acid intake into the exercised muscle. However, it has been reported that increases in muscle protein synthesis after oral essential amino acid ingestion are independent of changes in insulin (1). Since the difference between Experiments 1 and 2 was not only the breakfast, the influence of regular meal on the effect of amino acid supplement needs more investigation.

The timing and frequency of supplementation, the amount of supplement, and the inclusion of carbohydrate with the supplement are also important factors to be considered. Tipton et al. (23) documented that the exercise-induced anabolic response of muscle was greater when amino acid-carbohydrate supplement containing 6 g of essential amino acids and 35 g of sucrose was consumed prior to rather than after exercise. Levenhagen et al. (12) reported that the rate of skeletal muscle protein synthesis was higher when supplement included 10 g protein, 8 g carbohydrate, and 3 g fat was administered immediately after, compared with 3 h after exercise. These results suggested that pre-exercise supplementation is more effective than post-exercise supplementation for protein synthesis. Rennie (20) stated that amounts of dietary protein as little as 3-5 g would effectively double muscle protein synthesis rate for most adults. It can be assumed that the amount of amino acid supplement (3.6 g) was adequate as pre-exercise dose. Even if the pre-exercise amino acid supplement enhanced protein synthesis and reduced protein breakdown, it might be possible that the effect was not detected by the criterion measures used in the present study. In spite of the effect of amino acid supplement on blood markers of muscle damage and DOMS, the Experiment 2 did not find a significant prophylactic effect on muscle function (Figure 5). This result is in contrast to the findings by Kraemer et al. (11) showing that amino acid supplement (0.4 g/kg per day) attenuated decreases in 1RM squat and bench press after the first week of 4-week overreaching program, and Sugita et al. (22) reporting faster recovery of elbow extensor strength when subjects took an amino acid supplement versus placebo. The amount of amino acids ingested before exercise (3.6 g) in the present study was smaller than that given in the previous studies reporting the effect on muscle function (11, 22). It is interesting to examine if a larger amount of amino acid supplement can affect the changes in muscle function after exercise. Moreover, the exercise protocol we used was less intense than the aforementioned studies (11, 24). Therefore, any beneficial effects of amino acid supplementation on recovery of muscle function may not have been detectable. It would be interesting to investigate whether amino acid supplementation could be beneficial to recovery of muscle function following a more severe form of eccentric exercise resulting in more severe and prolonged changes in muscle function.

The total amount of amino acids ingested in Experiment 2 (36 g) was much larger than that of Experiment 1 (7.2 g), and this might be the reason for the beneficial effects found in Experiment 2. However, it is also possible that the additional supplements given in the recovery period contributed to the attenuation of muscle

damage shown in Experiment 2. Rasmussen et al. (18) showed that ingestion of 6 g essential amino acids with 35 g carbohydrate 1 or 3 h after resistance exercise promoted muscle anabolism. The lack of effect of amino acid supplement on muscle function (Figure 5a & 5b) might be associated with the small amount of carbohydrate (0.5 g) contained in the amino acid supplement. Bohé et al. (1) have shown that muscle protein synthesis is regulated by blood essential amino acid concentration, and the protein synthesis is increased when extracellular essential amino acids are high. It would appear that regeneration or remodeling of muscle and connective tissue after eccentric exercise is modulated by amino acid supplementation. However, it is possible that this effect was not directly represented in the recovery of muscle function. Clearly, more work needs to be carried out to determine the optimum timing, quantity of supplementation, and effect of carbohydrate.

We found that peak soreness scores on palpation and extension of the upper arm were significantly lower (approximately 30%) in the AMINO condition compared to the PLACEBO in Experiment 2 (Figure 5f). This supports the finding of Shimomura et al. (21) reporting that BCAA supplementation before squat exercise attenuated DOMS. Although DOMS is a consequence of eccentric exercise-induced muscle damage, it is not necessarily indicative of the extent of muscle damage (13), and changes in DOMS should be considered separately from other indicators of muscle damage. The underlying mechanisms of DOMS are not clearly understood but are probably related to the inflammatory response to injury (2). The mechanisms by which supplemented amino acids act to reduce DOMS are speculative. Dawson et al. (5) showed that supplementation with taurine or ß-alanine reduced lipid peroxidation after exercise in rats. Thus, it can be assumed that the amino acids used in the present study had a similar effect to taurine or ß-alanine on attenuating free-radical mediated damage. The present data provide tentative evidence that amino acid supplementation may reduce muscle swelling resulting from inflammation (Figure 5).

In conclusion, the present study showed that the amino acid supplement was effective in reducing DOMS and muscle protein efflux following endurance elbow flexor exercise when ingested 30 min prior to exercise, immediately after exercise, and over a 4-day recovery period under the condition that subjects ate breakfast before exercise. It appears that the effect of amino acid supplement on DOMS and muscle damage is not always produced. The importance of regular meal should not be discounted for the effects of amino acid supplement to be produced. Further studies are necessary to establish the amino acid supplementation protocol effective for DOMS and muscle damage, and the underlying mechanisms by which these compounds act to confer such effects remain to be elucidated.

Acknowledgment

The authors appreciate Dr. Jonathan Peake for his critical review and valuable suggestions to improve the manuscript.

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