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Effects of an amino acid mixture of arginine, valine, and serine on anaerobic performance, muscle strength, and biochemical parameters after aerobic exercise in recreationally active men: a randomized, double-blind, placebo-controlled crossover study

Yuichi Tsuda¹*, Ryoichi Tagawa¹, Keisuke Ueda², and Chiaki Sanbongi¹

¹: R&D Division, Meiji Co., Ltd.; 1-29-1 Nanakuni, Hachioji, Tokyo 192-0919, Japan
²: Food Product Development Division, Meiji Co., Ltd.; 2-2-1 Kyobashi, Chuo-ku, Tokyo 104-8306, Japan

* Corresponding author
E-mail: yuuichi.tsuda@meiji.com; Tel: +81-42-632-5847; Fax: +81-42-637-3022

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Running Title: Effects of an amino acid mixture on exercise performance.
Abstract
The aim of the present study was to examine the effect of an amino acid (AA) mixture of arginine, valine, and serine on exercise performance after prolonged exercise in humans. In a randomized, double-blinded, placebo-controlled crossover trial, nineteen recreationally active healthy males ingested an AA mixture of 1.8 g of arginine, 1.1 g of valine, and 0.1 g of serine or a placebo twice a day for 3 days and carried out a cycling exercise at 50% VO2max for 90 min with a 15-min rest at the midpoint. After the exercise, subjects performed a 30-s Wingate test. Their leg and grip strength, rating scale of perceived exertion (RPE), and blood biochemical parameters were also evaluated. There were no significant differences between the two conditions in the Wingate test performance (peak power: AA 650.9 ± 80.8 vs placebo 644.7 ± 78.0, p=0.585; mean power: AA 491.6 ± 58.8 vs placebo 490.8 ± 63.7, p=0.907), leg and grip strength, or RPE score during exercise. The plasma noradrenaline concentrations in the AA condition were significantly higher than those in the placebo condition during exercise (p<0.05). Moreover, strong correlations were found between the Wingate test performance and the level of plasma noradrenaline (p<0.001). These results indicated that the AA mixture supplement significantly elevated the plasma noradrenaline level during exercise, while sprint performance after prolonged exercise was not improved by the AA mixture supplement in the study.

Keywords: Amino acid, Exercise performance, Noradrenaline.
アルギニン・パリン・セリン混合物摂取が運動パフォーマンスおよび生化学パラメーターに及ぼす影響

一健常成人を対象としたランダム化二重盲検プラセボ対照クロスオーバー比較試験一

津田 悠一 1、田川 勇一 1、上田 啓輔 2、三本木 千秋 1

1：株式会社 明治 研究本部 192-0919 東京都八王子市七国 1-29-1
2：株式会社 明治 食品開発本部 104-8306 東京都中央区京橋 2-2-1

要旨
アルギニン・パリン・セリン混合物摂取が長時間運動後の運動パフォーマンスに及ぼす影響をヒトで評価することを目的に、健常成人男性に対してランダム化二重盲検プラセボ対照クロスオーバー比較試験を実施した。被験者にアルギニン 1.8 g、パリン 1.1 g、セリン 0.1 gを含むアミノ酸混合物あるいはプラセボを 1 日 2 回 3 日間摂取させ、50% VO2max の運動強度で 90 分間自転車運動を 15 分間の休憩を間隔に挟んで実施させた。運動後、被験者には 30 秒間 Wingate テストを実施させた。また、脚力、握力、自覚運動強度、および血中生化学マーカーを測定した。Wingate テストのパフォーマンス、脚力、握力、および自覚運動強度では、群間で有意差は認められなかった。一方、アルギニン・パリン・セリン混合物摂取条件において、運動時の血中ノルアドレナリン濃度がプラセボ条件と比較して有意に高値を示した。また、Wingate テストのパフォーマンスと血中ノルアドレナリン濃度は強い正の相関を示した。本研究の結果から、アルギニン・パリン・セリン混合物摂取は、本試験条件においては運動パフォーマンスを向上させなかったが、運動時の血中ノルアドレナリン濃度を顕著に上昇させることが示された。
Introduction

There are many nutritional strategies for improving exercise performance. For example, ingestion of carbohydrates during exercise can lead to an improvement in exercise performance. It was reported that carbohydrate feeding during prolonged exercise resulted in the maintenance of a sufficiently high rate of carbohydrate oxidation and the postponement of fatigue\(^1\). Sugiura et al. observed that ingestion of glucose polymer at the halfway point of a 90-min exercise could maintain carbohydrate utilization and improve sprint performance\(^2\). Many other studies and reviews have also demonstrated improvements in exercise performance with carbohydrates by sparing endogenous glycogen and maintaining blood glucose\(^3,4,5,6\).

There have also been several reports evaluating the effects of amino acid (AA) supplementation on exercise performance. It was indicated that β-alanine supplementation (approximately 2-7 g/day) for several days increased muscle carnosine synthesis and improved high-intensity exercise performance\(^7,8\). Bailey et al. suggested that short-term citrulline (6 g/day) supplementation could enhance endurance exercise performance in healthy adults\(^9\). However, there are few reports on the effects of combined amino acid mixtures except branched-chain amino acids on exercise performance, although a recent study showed that acute supplementation with 0.17 g/kg branched-chain amino acids, 0.05 g/kg arginine, and 0.05 g/kg citrulline could enhance time-trial performance over two consecutive days in endurance runners\(^10\).

In a previous study, we demonstrated that an AA mixture of 1.8 g of arginine, 1.1 g of valine, and 0.1 g of serine (6 g/day for 14 days) reduced the feeling of fatigue during 120 min of prolonged exercise in healthy men\(^11\). In this study, the AA mixture also changed the levels of serum ketone bodies and the plasma tryptophan/BCAA ratio, which might contribute to the antifatigue effect of the AA mixture. It was also reported that acute supplementation with an AA mixture of arginine, valine, and serine (3 g) suppressed the
cortisol response during an 80-min prolonged exercise in healthy men, which suggested that the AA mixture might contribute to stress reduction or improvement of physical condition during exercise. In addition, the acute supplementation of valine is reported to effectively decrease the level of glucocorticoid during swimming exercise by maintaining liver glycogen and blood glucose in a rodent study, which suggests that muscle or liver glycogen might be maintained by the AA mixture containing valine during exercise. The sense of fatigue is known to cause poor exercise performance, and significant correlations have been found between the cortisol response and the sense of fatigue during stressful exercise. Thus, we hypothesized that the above-described AA mixture of arginine, valine, and serine may improve exercise performance after prolonged exercise by reducing cortisol response and exercise-induced fatigue during prolonged exercise. The expected mechanism of actions by the AA mixture is following. First, the synthesis of nitric oxide (NO) by arginine, the maintenance of muscle or liver glycogen content or blood glucose by valine, or phosphatidylserine production by serine might contribute to suppress the cortisol response. It was revealed that NO, synthesized by arginine, and phosphatidylserine, produced by serine, modulates the adrenal response, and that the decrease in glycogen content or blood glucose stimulates the hypothalamic-pituitary-adrenal axis during exercise. Second, in addition to the suppression of cortisol response, the reduction of blood ammonia via increased ureagenesis by arginine or the attenuation of serotonin synthesis in brain via decrease in transport of tryptophan across the blood–brain barrier by valine might contribute to reduce the exercise-induced fatigue. It was reported that ammonia in blood caused the feeling of fatigue through neurotoxic effects and that serotonin in brain contributed to the development of central fatigue. Here, we conducted a randomized, double-blinded, placebo-controlled crossover study in recreationally active healthy men. The aim of the present study was to examine the effect...
of AA mixture supplementation on exercise performance after prolonged exercise in humans. We hypothesized that AA mixture supplementation would reduce cortisol response and exercise-induced fatigue during prolonged exercise, which could lead to improved exercise performance after prolonged exercise. We also investigated the effects of the AA mixture on several biochemical parameters related to exercise performance in the study. Improving exercise performance after prolonged exercise is expected to lead to improved sports performance such as soccer especially in the latter half of long-term exercise.
Materials and methods

Subjects

Thirty-two recreationally active healthy males aged 20 to 38 years old were recruited for this study. All subjects provided written informed consent prior to participation. The exclusion criteria were subjects with food allergies, subjects who had blood samples of more than 200 ml or 400 ml taken within 1 month or 3 months prior to the start of the study, or subjects who were judged as ineligible by a doctor for other reasons. Subjects who could not complete an exercise trial with the same intensity as the main trials were also excluded. A total of 20 subjects were randomly allocated into two groups in a 1:1 ratio using a computer-generated random number sequence by an investigator who had no contact with the subjects or researchers. The sequence allocation concealment and blinding of the subjects and researchers were maintained throughout the study. The sample size of subjects was selected to achieve 80% power at a 5% significance level with an effect size of 0.65. Before the statistical analysis, one participant was removed due to an abnormal value observed in the blood analysis not related to the supplementation. We therefore analyzed a final total of 19 subjects (Fig. 1). The basic characteristics of the study subjects are shown in Table 1. The subjects were instructed not to consume any supplements and not to change their usual exercise routine or diet during the study. They were also prohibited from consuming alcohol on the day before and on the day of the exercise test and instructed not to change their regular caffeine intake. They recorded their amount of exercise, dietary intake, and medication use every day during the study period.

Study design
A randomized, double-blinded, placebo-controlled crossover trial was conducted with two groups at the CPCC Company Limited (Tokyo, Japan).

First, the individual’s maximal oxygen consumption (VO_{2max}) was measured using an incremental cycle exercise test on a cycle ergometer (Aerobike 75XLIII, Konami Sports Life Co., Ltd., Tokyo, Japan). After the measurement of VO_{2max}, participants conducted an exercise trial on a cycle ergometer at 50% VO_{2max} for 45 min to determine whether the subjects could complete the exercise trial. After selection and allocation, the subjects in both groups had a 1-week washout period. They ingested one of the test samples (AA or placebo) for 3 days and carried out an exercise trial on the final day of supplementation. After the washout period, the participants repeated the same protocol with the other test sample.

These trials were conducted between January and March 2017. The study protocol was approved by the Institutional Review Board of Chiyoda Paramedical Care Clinic (Tokyo, Japan) (Approval No. 17011906) and the Meiji Institutional Review Board (Tokyo, Japan) (Approval No. 108) and was registered in the UMIN Clinical Trials Registry (UMIN000025850) on January 26, 2017. The study was conducted in accordance with the Declaration of Helsinki.

**Experimental procedure**

Subjects were provided cellulose capsules (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) containing 1.8 g of arginine, 1.1 g of valine, and 0.1 g of serine (Kyowa Hakko Bio Co., Ltd., Tokyo, Japan) or cellulose capsules containing 3 g of dextrin as the placebo in a randomized order in a double-blinded fashion. The two test samples were indistinguishable by appearance. Subjects ingested the test samples twice per day (at lunch and dinner) for 2 days. On the 2nd day, the participants had the same dinner. On the 3rd day, they ingested the test sample 30 min before the exercise trial. Then, they carried
out an exercise trial on a cycle ergometer at 50% VO$_2$max for 90 min with a 15-min rest at the midpoint. The additional test sample was ingested during the rest period. To prevent dehydration, subjects ingested equal amounts of water and sodium chloride tablets every 15 min during the exercise. The blending ratio of AAs followed previous studies$^{11,12}$. The dose and the intake period of the AA mixture were also set with reference to previous studies$^{11,12}$. First, the AA mixture of arginine, valine, and serine (6 g/day for 14 days) reduced the feeling of fatigue during prolonged exercise. Second, acute supplementation with the AA mixture of arginine, valine, and serine (3 g) suppressed the cortisol response during prolonged exercise. We hypothesized that the short-term supplementation with the AA mixture may reduce cortisol response and exercise-induced fatigue during prolonged exercise, which lead to improve exercise performance after prolonged exercise. The exercise protocol was determined by referring to a past study evaluating exercise performance after prolonged exercise in men$^{2,27,28}$. The experimental procedure is shown in Fig. 2. The exercise trial was conducted in a temperature- and humidity-controlled environment.

**Fig. 2**

**Wingate test**

Subjects performed a 30-s Wingate test on a cycle ergometer (POWERMAX-VIII, Konami Sports Life Co., Ltd.) immediately after the 90-min exercise. They were required to exert an all-out effort for 30 s at a pedal load equivalent to 7.5% of their body weight. Values for peak power and mean power were measured and recorded during the 30-s testing period. Peak power was defined as the highest mechanical power output recorded during the test. Mean power was defined as the average mechanical power output recorded during the test.
Leg strength and grip strength

Subjects measured their leg and grip strength before exercise and after the Wingate test. The leg strength was measured three times using a leg extension dynamometer (LEG POWERII, Takei Scientific Instruments Co., Ltd., Niigata, Japan), and the maximum score was recorded. The grip strength was measured once for each hand using a grip dynamometer (GRIP-D, Takei Scientific Instruments Co., Ltd.), and the average score was recorded.

Subjective measurement

Subjects were asked to subjectively rate their feeling of fatigue on a Borg’s 6- to 20-point rating scale of perceived exertion (RPE) at 5 min, 45 min, and 90 min after the exercise.

Blood analysis

Blood samples were collected from the brachial vein 30 min before exercise, after 45 min of exercise, after 90 min of exercise, and after the Wingate test. Whole blood in an EDTA-2Na-containing tube was centrifuged immediately at 1,700 × g for 10 min at 4°C, and the plasma was separated for analysis of adrenaline, noradrenaline, dopamine, and cortisol. Serum samples were prepared by collecting whole blood in a plain tube and centrifuging the blood at 1,700 × g for 10 min at 4°C for analysis of total ketone bodies and free fatty acids. Whole blood was deproteinized in a 1 N perchloric acid-containing tube for 15-60 min on ice and then centrifuged at 1,700 × g for 10 min at 4°C for lactate analysis. Whole blood collected in a NaF- and EDTA-2Na-containing tube was used for analysis of glucose. Assays of blood parameters were performed at LSI Medience Corporation (Tokyo, Japan).

Statistical analysis
In the study, the primary outcome was the peak power and mean power during the 30-s Wingate test, and secondary outcomes were the leg strength, grip strength, RPE score, levels of plasma adrenaline, plasma noradrenaline, plasma dopamine, plasma cortisol, plasma lactate, blood glucose, serum total ketone bodies, and serum free fatty acids. All data are expressed as the mean ± standard deviation of the mean (SD). Data were analyzed using a two-way repeated-measures analysis of variance (ANOVA) with treatment (placebo and AA) and time (leg strength and grip strength: before the exercise and after the Wingate test; RPE score: after 5-min exercise, after 45-min exercise, and after 90-min exercise; blood parameters: before exercise, after 45-min exercise, after 90-min exercise, and after the Wingate test). Significant main effects and interactions were explored with post hoc t-tests. The Wingate test performance was analyzed using Student’s t-test for the comparisons of pairs of conditions. The correlations between the Wingate test performance and plasma noradrenaline were analyzed using Pearson’s correlation analysis. The Benjamini-Hochberg false discovery rate (FDR) approach was used to correct for multiple comparisons. Analyses were performed with SPSS v. 22 (IBM Japan, Ltd., Tokyo). Differences with p-values <0.05 were considered significant.
Results

No subjects reported any side effects related to the supplementation, and there was no significant treatment order effect in the study. There were no differences between the AA condition and placebo condition in their amount of exercise, dietary intake, or medication use.

Wingate test

The effects of supplementation with the AA mixture on the 30-s Wingate test performance are shown in Table 2 and Fig. 3. The peak power and mean power during the Wingate test were not significantly different between the AA condition and placebo condition (peak power: 650.9 ± 80.8 vs 644.7 ± 78.0, p=0.585; mean power: 491.6 ± 58.8 vs 490.8 ± 63.7, p=0.907).

Table 2  

Fig. 3

Leg strength and grip strength

The effects of supplementation with the AA mixture on the leg strength and grip strength are shown in Table 3. A two-way repeated-measures ANOVA (treatment × time) for leg strength revealed a significant interaction between treatment and time (p=0.029). Post hoc t-tests showed that the leg strength after the Wingate test was significantly lower than that before the exercise in both conditions (p<0.001), while no significant differences were observed between the two conditions before the exercise and after the Wingate test (before the exercise: AA 822 ± 151 vs placebo 800 ± 158, p=0.199; after the Wingate test: AA 745 ± 115 vs placebo 762 ± 123, p=0.225). The change scores of leg strength between before the exercise and after the Wingate test in the AA condition were significantly higher than those in the placebo condition (p=0.029) (Fig. 4). ANOVA for grip strength
showed that there was a significant main effect of time (p<0.001), with post hoc t-tests revealing significant differences before the exercise and after the Wingate test in both conditions (AA: before 40.4 ± 5.7 vs after 42.1 ± 6.0, p<0.001; placebo: before 39.4 ± 5.7 vs after 41.9 ± 6.7, p=0.001). ANOVA for grip strength also showed that there was no significant main effect of treatment (p=0.085) or interaction between treatment and time (p=0.108).

Table 3

Fig. 4

Subjective measurement

The effects of supplementation with the AA mixture on the RPE score are shown in Table 4. A two-way repeated-measures ANOVA (treatment × time) for the RPE score revealed a significant main effect for time (p<0.001), while there was no significant main effect for treatment or interaction between treatment and time. Post hoc t-tests showed that the RPE scores after 45 min and 90 min of exercise were significantly higher than those before exercise in both conditions (p<0.001).

Table 4

Blood analysis

The effects of supplementation with the AA mixture on the biological parameters are shown in Table 5. A two-way repeated-measures ANOVA (treatment × time) for plasma noradrenaline revealed a significant main effect of treatment (p<0.001). Post hoc t-tests showed that the levels of plasma noradrenaline in the AA condition were significantly higher than those in the placebo condition after 45 min of exercise (p=0.023), after 90 min of exercise (p=0.048), and after the Wingate test (p=0.017). ANOVA for plasma dopamine revealed a significant interaction between treatment and time (p=0.038). Post
hoc t-tests showed that the levels of plasma dopamine after 45-min exercise, after 90-min exercise, and after the Wingate test were significantly higher than those before exercise in the AA conditions (before exercise vs after 45-min exercise, p=0.018; after 90-min exercise, p=0.015; after Wingate test, p=0.002, respectively), while in the placebo condition, the only difference observed was between plasma dopamine levels before the exercise and those after the Wingate test (before exercise vs after 45-min exercise, p=0.331; after 90-min exercise, p=0.360; after Wingate test, p=0.036, respectively). However, there were no significant differences between the two conditions. ANOVA for blood glucose, plasma lactate, serum total ketone bodies, serum free fatty acid, plasma adrenaline, and plasma cortisol revealed significant main effects for time (p<0.001), while there were no significant main effects for treatment or interactions between treatment and time.

Table 5

**Correlation between Wingate test performance and plasma noradrenaline**

The correlations between the Wingate test performance and plasma noradrenaline concentrations after the Wingate test are displayed in Fig. 5 and Fig. 6. Strong correlations were found between the peak power during the Wingate test and the level of plasma noradrenaline (r=0.59, p<0.001) and between the mean power during the Wingate test and the level of plasma noradrenaline (r=0.57, p<0.001). In addition, there was a significant correlation between the change scores of the mean power during the Wingate test and the change scores of the level of plasma noradrenaline between the two conditions (r=0.50, p=0.030).

Fig. 5

Fig. 6
Discussion

The present study aimed to investigate the effect of combined arginine, valine, and serine supplementation on exercise performance after prolonged exercise in recreationally active men. In the study, the peak power and mean power during the 30-s Wingate test after the 90-min prolonged exercise were not significantly different between the AA condition and placebo condition, which indicated that the AA mixture supplement did not improve the sprint performance in this study protocol.

Arslan et al. showed that there was a significant positive relationship between the 30-s Wingate test performance and leg strength in young subjects. In the present study, leg strength was significantly reduced by exercise in both conditions, which suggested that the exercise protocol in this study significantly induced fatigue on the subjects’ legs. It was also demonstrated that there is a significant correlation between grip strength and whole-body physical performance or muscle strength capacity. In the present study, grip strength was significantly upregulated by prolonged exercise and the Wingate test in both conditions, which suggested that the decrease in whole-body muscle strength was not caused by the exercise protocol of this study. No significant differences were observed between the two conditions before the exercise or after the Wingate test on the leg or grip strength, which demonstrated that the AA mixture supplement did not affect the leg or grip strength performance in the study.

The RPE score, which was used to evaluate the feeling of fatigue during exercise in the study, has been shown to be a valid instrument for the quantitative assessment of fatigue. In the study, AA mixture supplementation did not improve the RPE score before or during exercise. This result is inconsistent with a previous study, which demonstrated that AA mixture intake for 14 days improved the RPE score before exercise and the VAS score after 120 min of prolonged exercise. In the present study, the supplementation period was 3 days, which was considered to be one of the reasons why there was no difference in the
RPE score. As the improvement of the sense of fatigue could affect exercise performance, further studies examining the effects of longer-term use of the AA mixture on exercise performance should also be conducted. Blood analysis showed that the plasma noradrenaline concentrations in the AA condition were significantly higher than those in the placebo condition after 45 min of exercise, after 90 min of exercise, and after the Wingate test, which indicated that the AA mixture supplement enhanced the levels of plasma noradrenaline during exercise. Past studies suggested that some kinds of AAs affect the level of noradrenaline. It was reported that administration of a 1:1:1 mixture of branched-chain amino acids evoked hippocampal noradrenaline release in a concentration-dependent manner through activation of gamma aminobutyric acid receptors\textsuperscript{33}. Daniela et al. demonstrated that lysine and arginine treatment (3 g each/day for 10 days) resulted in enhanced levels of noradrenaline during psychosocial stress via modulating benzodiazepine receptors by lysine and nitric oxide release by arginine in healthy subjects\textsuperscript{34}. These reports suggest that the ingestion of valine and arginine in the AA mixture used in the study may be involved in the enhancing effect of noradrenaline. In addition, the AA mixture enhanced plasma noradrenaline concentrations not before exercise but during exercise, which indicated that the combination of the AA mixture supplement and exercise might be related to stimulating noradrenaline secretion. Blood analysis also showed that there was no significant difference between the two conditions in the levels of plasma cortisol. This result is inconsistent with a previous study, which demonstrated that acute supplementation with the AA mixture suppressed the cortisol response during prolonged exercise. The reason why no difference was observed between the two conditions in the levels of plasma cortisol in this study is considered to be that the subjects in this study did not have much increase in cortisol by exercise load and the effect of the AA mixture on the cortisol response could not be accurately evaluated.
It is necessary to conduct a future study to evaluate the effect of the AA mixture on exercise performance in subjects whose cortisol is clearly increased by exercise. Strong correlations were found between Wingate test performance and the level of plasma noradrenaline in the study. Kreisman et al. showed that noradrenaline infusion enhanced the ability to shift to higher levels of muscular carbohydrate use during high-intensity exercise\(^{35}\), which supports the result of the strong correlations between the Wingate test performance and the level of plasma noradrenaline in the study because elevated noradrenaline could activate glucose metabolism and improve the anaerobic sprint performance of the Wingate test. In addition, there was a significant correlation between the change scores of the mean power during the Wingate test and the change scores of the level of plasma noradrenaline between the two conditions in the study. These results suggested that the increase in plasma noradrenaline levels by the AA mixture supplement may in part have the potential to improve exercise performance. In this study, the AA mixture increased the levels of plasma noradrenaline after the Wingate test by approximately 1.2-fold compared to the placebo condition. It was reported that the intake of supplement such as caffeine affected high-intensity exercise performance or stress-induced anxiety by increasing blood noradrenaline concentration by about 1.5-2 times\(^{34,36}\). Horton et al. demonstrated that norepinephrine levels were 1.5 times greater during exercise in men than in women, which generates more power output\(^{37}\). Although the rate of increase in this study is smaller than these past reports, the significant increase in plasma noradrenaline by 1.2-fold could contribute to affect the exercise performance considering the result of the correlation between the Wingate test performance and plasma noradrenaline.

The AA mixture supplement did not affect the other blood parameters. This result is consistent with a previous study\(^{12}\), which demonstrated that there were no significant differences in the levels of blood glucose, plasma lactate, serum total ketone bodies, or
serum free fatty acids before and after exercise between the AA condition and the placebo condition. On the other hand, AA mixture supplementation for 14 days enhanced the production of ketone bodies and accelerated the utilization of ketone bodies during exercise, which might be related to the antifatigue effect during exercise\(^{(1)}\). Therefore, further studies examining the effects of longer-term use of the AA mixture on exercise performance should also be conducted.

This study has some limitations. Although we used the 30-s Wingate test to evaluate exercise performance in the study, which is a widely used method for measuring anaerobic sprint performance\(^{2,38,39}\), there are several other methods for evaluating exercise performance whose exercise intensity differs from that of the 30-s Wingate test. For example, a time trial performance\(^{40,41,42,43}\) or a time-to-exhaustion test\(^{1,44,45,46}\) is often used as the evaluation method for moderate-to-high intensity exercise performance. It is possible that different results regarding the effects of the AA mixture supplement may be obtained with different exercise intensity protocols. Further studies should be conducted to clarify whether the AA mixture supplement could improve moderate-to-high intensity exercise performance by measuring time trial performance or time to exhaustion. In addition, we did not measure the levels of amino acids in blood, which might allow us to consider the detailed mechanism of action. Further examinations measuring the levels of plasma amino acids will clarify the mechanism of the effects of the AA mixture on the level of noradrenaline. Moreover, the participants ingested the AA mixture for 2 days before the day of exercise and on the day of exercise in this study. This protocol cannot distinguish between the effect of 3-day supplement before exercise and acute effect on the day of exercise. Future studies should also be conducted to clarify the chronic or acute effects of the AA mixture.

In conclusion, short-term supplementation with the AA mixture of arginine, valine, and serine did not improve the 30-s Wingate test performance after prolonged exercise in the
present study protocol. However, the level of plasma noradrenaline during exercise was significantly increased by the AA mixture supplement, which suggested that the AA mixture supplement may affect the catecholamine response and have the potential to improve exercise performance under conditions different from those in the present study.

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27) Lim, K., Ryu, S., Nho, H.-S., Choi, S.-K., KWOW, T., Suh, H., So, J., Tomita, K., Okuhara,


Contributions

RT, KU and CS designed the study. All authors interpreted the data. YT drafted the manuscript. RT and CS revised the manuscript. All authors have critically reviewed, revised and approved the manuscript.

Conflict of interest

YT, RT, KU and CS are employees of Meiji Co., Ltd. The authors have declared that no competing interests exist.

Data availability

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval

The study protocol was approved by the Institutional Review Board of Chiyoda Paramedical Care Clinic (Tokyo, Japan) (Approval No. 17011906) and the Meiji Institutional Review Board (Tokyo, Japan) (Approval No. 108) and was registered in the UMIN Clinical Trials Registry (UMIN000025850) on January 26, 2017. The study was conducted in accordance with the Declaration of Helsinki.
Figure legends

**Fig. 1.** Flowchart of the study participants. AA: amino acid.

**Fig. 2.** Experimental procedure in the study. AA: amino acid; RPE: rating scale of perceived exertion.

**Fig. 3.** Effects of the AA mixture supplement on 30-s Wingate test performance. AA: amino acid.

**Fig. 4.** The change scores of leg strength between before the exercise and after the Wingate test AA: amino acid.

**Fig. 5.** Correlations between the 30-s Wingate test performance and plasma noradrenaline concentrations after the Wingate test. AA: amino acid.

**Fig. 6.** Correlations between the change scores of the 30-s Wingate test performance and the change scores of the level of plasma noradrenaline between the two conditions.
Fig. 1.

Assessed for eligibility (n=32)

Excluded (n=12)
- Not meeting inclusion criteria (n=5)
- Declined to participate (n=4)
- Protocol deviations (n=3)

Randomized (n=20)

Allocated to AA -> Placebo (n=10)
- Lost to follow-up (n=0)
- Discontinued intervention (n=0)
  Analyzed (n=10)
  - Excluded from analysis (Abnormal values in blood not related to supplementation) (n=1)

Allocated to Placebo -> AA (n=10)
- Lost to follow-up (n=0)
- Discontinued intervention (n=0)
  Analyzed (n=10)
  - Excluded from analysis (n=0)
Day 1

Day 2

Rest 30 min

Exercise (50%VO₂max) 45 min

Rest 15 min

Exercise (50%VO₂max) 45 min

30-s Wingate test

△ Blood sampling

▲ AA or placebo intake

▽ Leg and grip strength

△ RPE

Day 3 (exercise trial day)
Fig. 3.
Fig. 4.
Fig. 5.

- Peak power during Wingate test (W)
  - $r = 0.59$
  - $p < 0.001$

- Mean power during Wingate test (W)
  - $r = 0.57$
  - $p < 0.001$
Fig. 6.
## Table 1

Basic characteristics of the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>AA -&gt; Placebo</th>
<th>Placebo -&gt; AA</th>
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<td>n=10</td>
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<td>VO₂ max (mL/min/kg)</td>
<td>44.2 ± 3.6</td>
<td>41.7 ± 6.7</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SD.
Table 2. Effects of the AA mixture supplement on 30-s Wingate test performance.

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak power (W)</strong></td>
<td>650.9 ± 80.8</td>
<td>644.7 ± 78.0</td>
<td>0.585</td>
</tr>
<tr>
<td><strong>Mean power (W)</strong></td>
<td>491.6 ± 58.8</td>
<td>490.8 ± 63.7</td>
<td>0.907</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SD (n=19). AA: amino acid.
Table 3. Effects of the AA mixture supplement on leg strength and grip strength.

<table>
<thead>
<tr>
<th></th>
<th>Before exercise</th>
<th>After Wingate test</th>
<th>p-value for interaction</th>
<th>p-value for treatment</th>
<th>p-value for time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg strength (W)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>822 ± 151</td>
<td>745 ± 115 #</td>
<td>0.029</td>
<td>0.868</td>
<td>0.003</td>
</tr>
<tr>
<td>Placebo</td>
<td>800 ± 158</td>
<td>762 ± 123 #</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>40.4 ± 5.7</td>
<td>42.1 ± 6.0 #</td>
<td>0.108</td>
<td>0.085</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placebo</td>
<td>39.4 ± 5.7</td>
<td>41.9 ± 6.7 #</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SD (n=19). AA: amino acid.

# p<0.05 compared to before exercise.
Table 4. Effects of the AA mixture supplement on RPE score.

<table>
<thead>
<tr>
<th></th>
<th>After 5-min of exercise</th>
<th>After 45-min of exercise</th>
<th>After 90-min of exercise</th>
<th>p-value for interaction</th>
<th>p-value for treatment</th>
<th>p-value for time</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>10.1 ± 1.7</td>
<td>12.5 ± 2.5 #</td>
<td>14.2 ± 3.0 #</td>
<td>0.885</td>
<td>0.904</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placebo</td>
<td>10.3 ± 1.6</td>
<td>12.5 ± 2.1 #</td>
<td>14.2 ± 2.4 #</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SD (n=19). AA: amino acid.
# p<0.05 compared to before exercise.
Table 5. Effects of the AA mixture supplement on blood biological parameters.

<table>
<thead>
<tr>
<th></th>
<th>Before exercise</th>
<th>After 45-min exercise</th>
<th>After 90-min exercise</th>
<th>After Wingate test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>78.8 ± 5.1</td>
<td>62.9 ± 10.2</td>
<td>66.5 ± 8.5</td>
<td>73.1 ± 7.1</td>
</tr>
<tr>
<td>Placebo</td>
<td>80.4 ± 7.6</td>
<td>64.5 ± 5.9</td>
<td>68.3 ± 6.3</td>
<td>74.1 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>p-value for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>interaction</td>
<td></td>
<td></td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td>0.203</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Serum total ketone body (µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>73.6 ± 83.2</td>
<td>39.9 ± 24.3</td>
<td>246.6 ± 202.0</td>
<td>233.5 ± 172.1</td>
</tr>
<tr>
<td>Placebo</td>
<td>55.5 ± 49.6</td>
<td>50.3 ± 52.8</td>
<td>206.5 ± 196.3</td>
<td>193.4 ± 159.9</td>
</tr>
<tr>
<td></td>
<td>p-value for</td>
<td></td>
<td></td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>interaction</td>
<td></td>
<td></td>
<td>0.335</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>7.8 ± 3.6</td>
<td>18.4 ± 5.6</td>
<td>14.8 ± 4.6</td>
<td>65.1 ± 13.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>7.3 ± 3.5</td>
<td>17.3 ± 6.3</td>
<td>14.1 ± 6.5</td>
<td>63.7 ± 18.1</td>
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<td>p-value for</td>
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<td>interaction</td>
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<tr>
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<td></td>
<td>time</td>
<td></td>
<td></td>
<td>0.019</td>
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</tbody>
</table>

Values are presented as the mean ± SD (n=19). AA: amino acid.

* p<0.05 compared to the placebo group. # p<0.05 compared to before exercise.