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The effects of acute and chronic oral L-arginine supplementation on exercise-induced ammonia accumulation and exercise performance in healthy young men: A randomised, double-blind, cross-over, placebo-controlled trial

Ayano Hiratsu [a, 1], Yusei Tataka [a, 1], Saki Namura [a], Chihiro Nagayama [a], Yuka Hamada [b], Masashi Miyashita [c, *]

[a] Graduate School of Sport Sciences, Waseda University, Saitama, Japan
[b] Waseda Institute for Sport Sciences, Waseda University, Saitama, Japan
[c] Faculty of Sport Sciences, Waseda University, Saitama, Japan

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ABSTRACT

Objective: This study examined the effects of a single and chronic oral intake of L-arginine supplementation on blood ammonia concentration and exercise performance.
Methods: Sixteen healthy young men (mean ± standard deviation, 23 ± 3 years) participated in a randomised, double-blind, cross-over, placebo-controlled study. For the acute trials, the participants consumed 200 mL of water containing either L-arginine (5 g) or placebo (dextrin; 5.5 g) and performed cycling exercise at 75% of heart rate reserve for 60 min, followed by a 15-min cycling performance test. For the chronic trials, the participants continued to consume each designated supplement twice a day for another 13 days, and then repeated the same protocol as the acute trials at day 15. After a 14-day washout period, the participants changed the supplement and repeated the same protocol as above.
Results: Plasma ammonia concentrations were lower in the chronic arginine trial than those in both acute placebo (mean difference - 4.5 μmol/L) and acute arginine (mean difference - 5.1 μmol/L) trials ($p < 0.05$). There was no difference in plasma ammonia concentration between the chronic arginine and chronic placebo trials (mean difference - 1.2 μmol/L). No differences were found in mean power output during the performance test between the chronic arginine and chronic placebo trials (mean difference 0.5 W) or between the acute arginine and acute placebo trials (mean difference 0.0 W).
Conclusions: An acute and chronic oral intake of L-arginine supplementation did not attenuate exercise-induced increases in ammonia accumulation or had no significant impact on cycling performance.

1. Introduction

Arginine is a substrate for both nitric oxide synthase and arginase to produce nitric oxide and urea, respectively.[1] Regarding the physiological roles of arginine in response to exercise, acute arginine supplementation (arginine aspartate) and intravenous injection attenuates exercise-induced ammonia accumulation possibly by increasing ureagenesis.[2,3] Acute arginine intake also mitigates exercise-induced muscle damage possibly by augmenting antioxidant capacity via nitric oxide mediation.[4] Arginine is a precursor of nitric oxide that mediates smooth muscle relaxation, promotes vasodilation, and increased blood flow regulation.[5] Collectively, these potential roles of arginine in the alteration of metabolic pathways have been the focus of research on exercise performance.

The acute effect of arginine supplementation on exercise performance has been evaluated in previous efficacy studies using a

* Corresponding author. Waseda University, Faculty of Sport Sciences 2-579-15 Mikajima, Tokorozawa, Saitama, 359-1192, Japan.
E-mail addresses: a.hiratsu@ruri.waseda.jp (A. Hiratsu), y.tataka@ruri.waseda.jp (Y. Tataka), s_namu076@asagi.waseda.jp (S. Namura), c-nagayama@moegi.waseda.jp (C. Nagayama), y.hamada2@kurenai.waseda.jp (Y. Hamada), m.miyashita@waseda.jp (M. Miyashita).
[1] These authors contributed equally to this work.

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randomised, double-blind, cross-over, placebo-controlled design[6–12] (for a review of these, see Ref. 13). These studies reported that acute arginine supplementation (a few hours or 3 days prior to the study) does not alter anaerobic, aerobic or strength exercise performance,[6–9,11,12] except for one study.[10] In a randomised, double-blind, placebo-controlled design, the effect of chronic arginine supplementation (i.e., from 7 days to 45 days) on exercise performance was examined, and the findings were inconsistent.[13] Two studies showed that chronic arginine supplementation (i.e., 4 or 45 days) is effective for improving aerobic exercise performance lasting more than 5 min in duration.[14,15] Conversely, three studies did not observe favourable effects of chronic arginine supplementation on aerobic exercise performance.[16–18] The dose (amount of absolute or relative), the timing of the intake before the exercise (i.e., acute effect), and the duration of the intake (i.e., chronic effect) of the arginine supplementation are possible reasons for the inconsistent findings among studies. In addition, it has been recommended that more research is needed to examine the potential mechanisms underlying the effect of arginine on exercise performance.[13] For instance, to our knowledge, only one study using intravenous injection of arginine is available to examine circulating concentrations of ammonia in response to acute exercise.[3] Thus, further studies are warranted to examine the effects of chronic arginine supplementation on exercise performance and its potential mechanisms. Furthermore, a direct comparison (i.e., a cross-over study) of the effects of acute and chronic arginine supplementation on exercise performance has not been investigated to date. Although both a parallel study and a cross-over study are recommended as the “gold standard” for investigating the effect of supplements on sports performance,[19] the latter may have an advantage to minimise other variables that may influence the results (i.e., diet and exercise throughout the intervention period) as each individual acts as his/her control. This is important to address as such a study design allows us to understand the magnitude of arginine supplementation effect, if any, on exercise-induced ammonia accumulation and exercise performance.

Therefore, this study aimed to examine the effects of an acute and chronic oral intake of L-arginine supplementation on ammonia and subsequent cycling performance in healthy young men. We tested the hypothesis that compared to ingestion of dextrin, oral L-arginine supplementation would attenuate exercise-induced increases in ammonia accumulation and improve cycling performance.

2. Methods

Ethics approval

The present study was approved by the institutional ethics committee (approval number: 2018–202) and conducted in accordance with the Declaration of Helsinki. Participants of the present study were recruited between November 2018 and September 2019 through advertisements placed on the campus. Sixteen healthy men provided written informed consent to participate in the study.

2.1. Participants

A participant flow diagramme shows in Fig. 1. Participants were recruited if they met the following criteria: non-smoker, not overweight or obese, and not taking any supplementation or medication. After obtaining written informed consent for participation in the present study, 16 participants were initially enrolled, and the investigator generated the randomisation sequence using computer-generated random number. The physical characteristics of the participants (mean ± standard deviation) were as follows: age, 23 ± 3 years; height, 173.5 ± 6.4 cm; body mass, 69.6 ± 8.5 kg; body mass index, 23.0 ± 1.5 kg/m^2; and maximum oxygen uptake, 53.3 ± 11.0 ml/kg/min.

2.2. Anthropometry

Body mass was measured to the nearest 0.1 kg using a digital scale (Inner Scan 50; Tanita Corporation, Tokyo, Japan) and height to the nearest 0.1 cm using a stadiometer (YS-OA; As One Corporation, Osaka, Japan). Body mass index was calculated as weight in kilogrammes divided by the square of height in metres.

2.3. Preliminary tests

Participants participated in two preliminary exercise tests on a cycle ergometer (Monark 894E; Monark, Varberg, Sweden). A 16-min, four-stage, submaximal cycling test was conducted to determine the relationship between cycling workload and oxygen uptake. The initial cycling workload was set at 0.5 kg. The cadence of the cycle ergometer was set at 60 rpm throughout the test. The workload was increased by 0.5 kg every 4 min. After a 20-min rest (i.e., following completion of the submaximal cycling test), maximum oxygen uptake was measured directly with an incremental protocol until the participants reached volitional fatigue. The initial workload of the cycle ergometer was set between 2.0 and 3.5 kg depending on the fitness level of each participant obtained via interviews for this test. Thereafter, the workload was increased by 0.5 kg every 3 min. Criteria used to confirm a maximum value included (1) heart rate >95% of age-predicted maximum heart rate (HRmax) and (2) ratings of perceived exertion ≥19 using the Borg scale.[20] Oxygen uptake, carbon dioxide production and respiratory exchange ratio were measured breath-to-breath using a stationary gas analyser (Quark CPFT; COSMED, Rome, Italy). Heart rate (HR) was monitored throughout these tests using a short-range telemetry (Polar RCX3; Polar Electro, Kempele, Finland). Ratings of perceived exertion were assessed periodically during the tests using the Borg scale.[20] Data generated from these two tests were used to determine the cycling workload at 75% of each participant's HR reserve (75% of HR reserve 165 ± 8 beats per minute (bpm)), and this workload was used for the main trials.

2.4. Study design and protocol

A randomised, double-blind, cross-over, placebo-controlled design was used in the present study. Each participant underwent four, one-day laboratory-based trials in a random order: (1) acute placebo trial (Day 1), followed by another 13-day placebo supplementation and (2) chronic placebo trial (Day 15), and (3) acute arginine trial (Day 1), followed by 13-day L-arginine supplementation and (4) chronic arginine trial (Day 15). Trial order and randomisation were selected from one of the two possible sequences using computer-generated random numbers in a counterbalanced manner to avoid order effects. A schematic representation of the study protocol is shown in Fig. 2. Participants weighed and recorded all foods and drinks consumed the day before the first trial and replicated their dietary intake from the first trial in all subsequent trials to ensure that meals were standardised across trials. Additionally, participants refrained from drinking alcohol for two days prior to each trial. Participants were also requested to remain inactive the day before each trial. Participants reported to the laboratory at 0850 h after a 10-h overnight fast (except water). After a 10-min seated rest, a fasting venous blood sample was collected by venipuncture at 0900 h (−60 min) to measure circulating

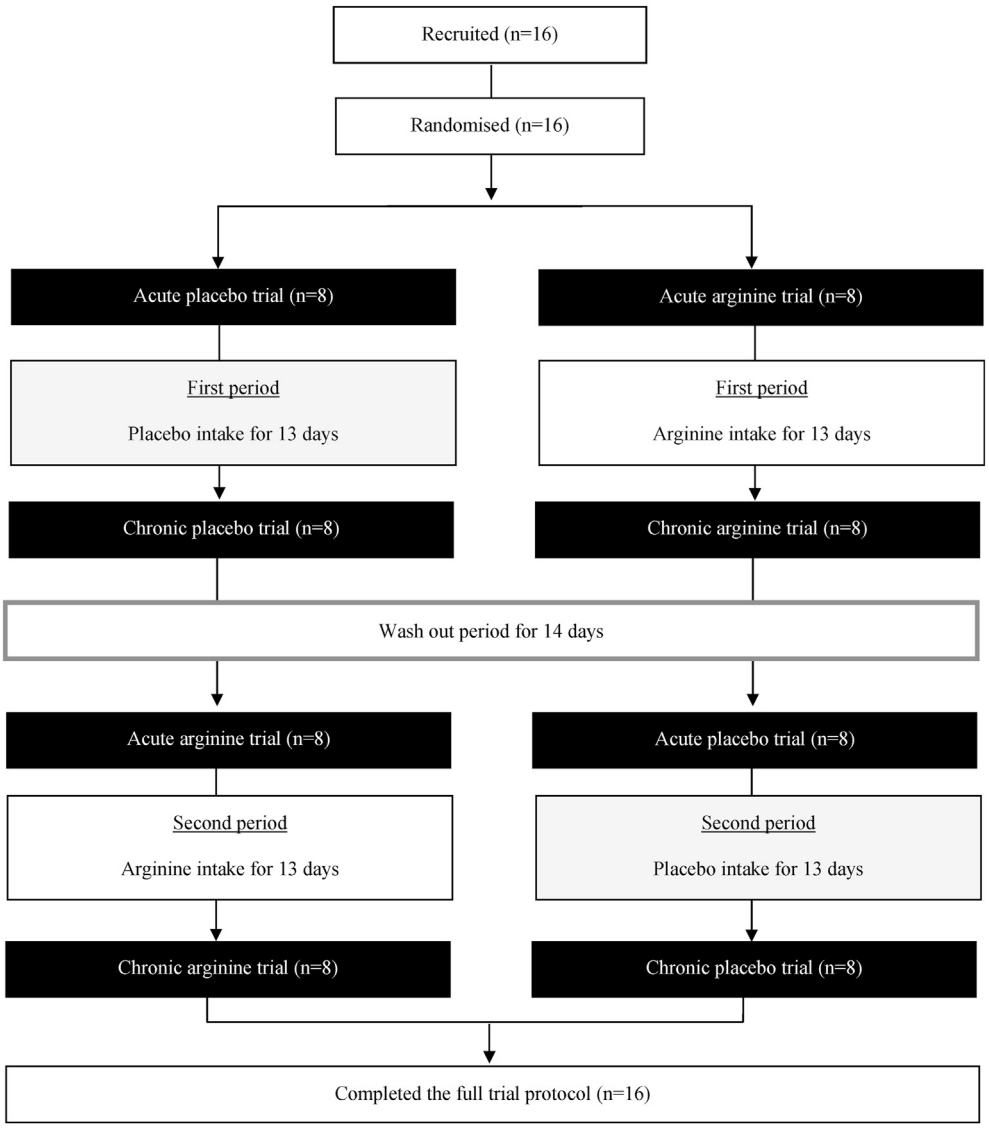

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) diagramme showing the participant flow.

concentrations of amino acids, ammonia, creatine kinase (CK), glucose, triglycerides (TG) and non-esterified fatty acids (NEFA). For the acute trials, participants consumed 200 mL of water containing either L-arginine (5 g) or placebo (arginine replaced with 5.5 g of dextrin). This L-arginine dose was chosen since previous studies have reported that 3 g of L-arginine hydrochloride intravenous infusion (i.e., equivalent to 4–5 g of oral L-arginine supplementation) attenuated exercise-induced ammonia accumulation[3] and observed no adverse effects.[21] After a 60-min rest, the participants performed cycling exercise at 75% of HR reserve for 60 min, followed by a 15-min cycling performance test.[22] In this performance test, the participants were instructed to pedal a cycle ergometer (Monark 874E; Monark, Varberg, Sweden), exerting as much effort as possible at a self-selected pace. The work for each exercise performance test was calculated as the mean power output multiplied by duration (i.e., 15 min) using the Anaerobic Test Software (Monark ATS Software, Monark, Varberg, Sweden). Heart rate was monitored continuously using a short-range telemetry (Polar RS400; Polar Electro Oy, Finland). Thereafter, participants were requested to sit in a chair (reading, writing or working on a computer) in the laboratory for 90 min. Further venous blood samples were collected immediately before cycling exercise (0 min), immediately post-cycling exercise (60 min), 30 min post-cycling performance test (105 min) and 90 min post-cycling performance test (165 min). Subjective fatigue was assessed using a visual analogue scale for the seven time points (at −60, 0, 30, 60, 75, 105 and 165 min). From the day after each acute trial, the participants continued to consume each designated supplement twice a day (i.e., 5 g L-arginine or placebo) for 13 days. The lead investigator asked each participant for their compliance in each main exercise day – all participants reported that they consumed all requested supplements during the supplementation period. For the chronic trials, the participants repeated the same protocol as the acute trials at day 15. After a 14-day washout period, the participants changed the supplement and repeated the same protocol as above. No serious adverse events were observed during the study. Also, none of participants dropped out from the study.

2.5. Analytical methods

For serum TG, NEFA, and CK measurements, venous blood samples were collected into tubes containing clotting activators for

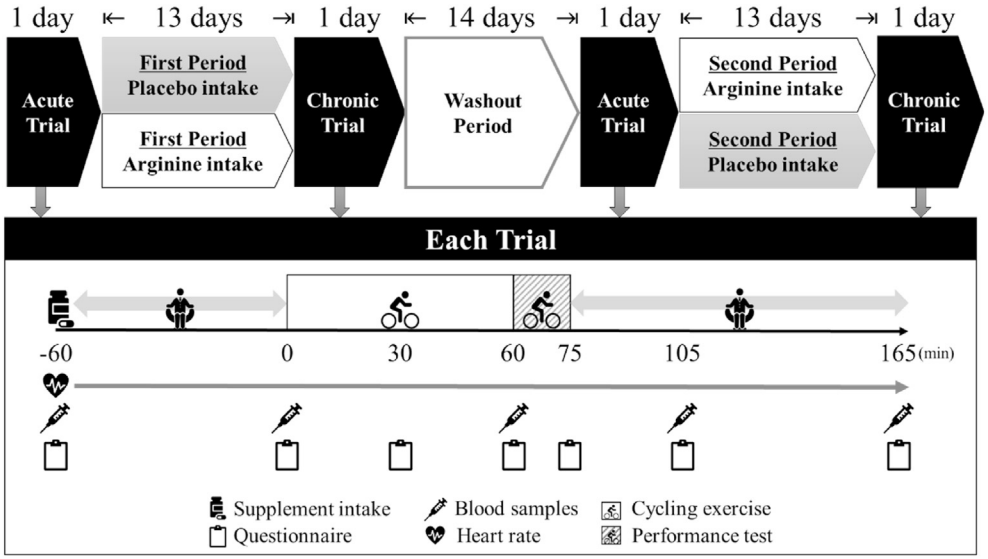

Fig. 2. A schematic representation of the study protocol.Each participant underwent four, one-day laboratory-based trials in a random order: (1) acute placebo trial (Day 1), followed by another 13-day placebo supplementation and (2) chronic placebo trial (Day 15), and (3) acute arginine trial (Day 1), followed by 13-day L-arginine supplementation and (4) chronic arginine trial (Day 15). Participants reported to the laboratory at 0850 h. After 10 min seated rest, a fasting venous blood sample was collected (−60 min). For the acute trials, the participants consumed 200 mL of water containing either L-arginine (5 g) or placebo (L-arginine replaced with dextrin). Further venous blood samples were before cycling exercise (0 min), immediately post-cycling exercise (60 min), immediately post-cycling performance test (75 min), 30 min post-cycling performance test (105 min) and 90 min post-cycling performance test (165 min). From the day after both acute trials, the participants continued to consume each designated supplement twice a day for 13 days. For the chronic trials, theparticipants repeated the same protocol as the acute trials at day 15. After a 14-day washout period, the participants changed the supplement and repeated the same protocol.

serum isolation. Samples were allowed to clot for 30 min at room temperature and then centrifuged at 1861×*g* for 10 min at 4 °C. Serum was removed, divided into aliquots, and stored at −80 °C for later analysis. For plasma glucose and ammonia measurements, venous blood samples were collected into tubes containing sodium fluoride-EDTA and dipotassium salt-EDTA. For plasma selected amino acid fraction measurements, venous blood samples were collected into tubes containing heparin-sodium EDTA. These tubes were then immediately centrifuged and treated as described above. Enzymatic colorimetric assays were used to measure serum TG (Pure Auto S TG-N; Sekisui Medical Co., Ltd., Tokyo, Japan), serum NEFA (NEFA-HR; Wako Pure Chemical Industries, Ltd., Osaka, Japan), serum CK (CK; FUJIFILM Wako Pure Chemical Co., Osaka, Japan), plasma glucose (GLU-HK(M); Shino-Test Corporation, Tokyo, Japan) and plasma ammonia (FUJI DRI-CHEM SLIDE NH3-PII; Fujifilm Co., Tokyo, Japan). Plasma arginine, ornithine and citrulline were measured using high-performance liquid chromatography (MassTrak AAA; Waters Co., Massachusetts, USA). All analyses for each participant were completed within the same run for each measure. The intra-assay coefficients of variation were 0.3% for TG, 0.5% for NEFA, 1.1% for CK, 0.4% for glucose, 3.6% for ammonia, 1.3% for arginine, 1.8% for ornithine and 3.1% for citrulline.

2.6. Calculations and statistical analysis

We calculated the required sample size based on data from a previous study.[3] The previous study reported the within subject effect (effect size, Cohen's d = 1.43 for the peak blood ammonia concentrations) using L-arginine versus placebo in response to a graded cycling exercise.[3] For two trials with an alpha level set at 0.05 and a correlation of 0.5, an estimated total sample size of 8 would provide 90% power to detect between trial differences. We doubled the required participants to 16 since our study design was both acute and chronic supplementation interventions (i.e., for a total of 4 trials) and potential withdrawals were considered. Data were analysed with Predictive Analytics Software version 22.0 for Windows (SPSS Japan Inc., Tokyo, Japan). The linear mixed model was used to examine between-trial differences over the 1-day or 2-week intervention for fasting serum or plasma concentrations, serum or plasma concentrations across five time points, visual analogue scale, mean power output and HR values. Where significant trial or trial-by-time interactions effects were found, the data were subsequently analysed using post-hoc analysis and were adjusted for multiple comparisons using the Bonferroni method. Statistical significance was accepted at the 5% level. The 95% confidence interval (95% CI) for the mean absolute pairwise differences between the trials was calculated using the t-distribution and degrees of freedom (n − 1). Absolute standardised effect sizes (ES) (Cohen's d) are provided to supplement the findings. An ES of 0.2 was considered a small difference in all outcome measurements, 0.5 moderate and 0.8 large.[23] Results are reported as the mean ± standard deviation.

3. Results

3.1. Amino acid concentrations

The plasma amino acid concentrations for each trial are shown in Table 1. Differences were found among trials in fasting (i.e., at −60 min) plasma arginine and ornithine concentrations (all for $p \leq 0.0005$). Post-hoc analyses of the main effect of trial revealed that fasting plasma arginine concentration was higher in the chronic arginine trial than in the acute arginine (mean difference 41.7 μmol/L, 95% CI 22.3–61.0 μmol/L; ES = 1.23) and in both acute placebo (mean difference 44.2 μmol/L, 95% CI 24.8–63.6 μmol/L; ES = 1.30) and chronic placebo (mean difference 44.1 μmol/L, 95% CI 24.7–63.5 μmol/L; ES = 1.28) trials (all for $p < 0.0005$). Post-hoc analyses of the main effect of trial showed that fasting plasma ornithine concentration was higher in the chronic arginine trial than in both acute placebo (mean difference 13.0 μmol/L, 95% CI

Table 1
Arginine, ornithine and citrulline concentrations measured at each time-point in the placebo and arginine trials.

			−60 min	0 min	60 min	105 min	165 min	Whole experimental period	
								Time averaged	95% CI (Effect size)
Arginine*(μmol/L)	Placebo	Acute	106.7 ± 13.8	95.5 ± 13.2	102.9 ± 12.7	92.3 ± 12.1	91.0 ± 12.3	97.7 ± 11.2	† 55.2 to 75.8 (2.81)
		Chronic	106.8 ± 12.3	98.8 ± 12.0	105.1 ± 11.0	95.6 ± 9.2	93.2 ± 11.6	99.9 ± 10.3	‡ 53.0 to 73.6 (2.70)
	Arginine	Acute	109.2 ± 24.7	211.5 ± 55.6	197.0 ± 36.6	159.6 ± 42.3	138.8 ± 30.3	163.2 ± 26.8	§ 87.2 to 107.8 (2.50)
		Chronic	150.9 ± 38.8	255.5 ± 54.4	223.0 ± 57.9	183.8 ± 45.2	162.9 ± 32.9	195.2 ± 43.3	‖ 85.0 to 105.6 (2.43)
									¶ 21.7 to 42.3 (0.85)
Ornithine*(μmol/L)	Placebo	Acute	46.9 ± 8.5	44.3 ± 8.4	42.3 ± 7.2	39.6 ± 8.0	40.0 ± 6.3	42.6 ± 7.2	† 27.8 to 36.5 (2.75)
		Chronic	45.1 ± 8.0	41.0 ± 6.9	41.1 ± 6.5	37.4 ± 6.8	38.9 ± 6.4	40.7 ± 6.7	‡ 29.7 to 38.4 (2.92)
	Arginine	Acute	50.8 ± 15.1	86.6 ± 25.8	91.7 ± 16.7	75.3 ± 13.0	69.4 ± 14.3	74.8 ± 13.5	§ 33.4 to 42.1 (2.92)
		Chronic	59.9 ± 16.8	97.3 ± 18.6	95.5 ± 17.0	79.4 ± 18.6	69.6 ± 15.6	80.3 ± 14.9	‖ 35.3 to 44.0 (3.06)
									¶ 1.2 to 9.9 (0.39)
Citrulline**(μmol/L)	Placebo	Acute	29.0 ± 4.1	26.2 ± 3.8	27.8 ± 5.5	27.4 ± 4.1	25.2 ± 4.1	27.1 ± 3.8	† 1.7 to 4.6 (0.80)
		Chronic	28.6 ± 5.1	25.5 ± 3.9	27.7 ± 5.3	27.1 ± 4.4	25.7 ± 3.4	26.9 ± 4.1	‡ 1.9 to 4.8 (0.82)
	Arginine	Acute	28.7 ± 3.2	24.7 ± 4.5	37.4 ± 6.0	33.6 ± 6.1	26.9 ± 4.7	30.3 ± 4.2	§ 2.4 to 5.2 (0.83)
		Chronic	30.2 ± 6.5	25.0 ± 4.4	37.9 ± 7.3	33.7 ± 5.8	27.8 ± 4.6	30.9 ± 5.1	‖ 2.6 to 5.4 (0.85)

CI: confidence intervals. Values are mean ± standard deviation for n = 16. Means were compared using the linear mixed model. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Post-hoc analysis of the main effect of trial: *p < 0.05 between both acute arginine and chronic arginine trials and both acute placebo and chronic placebo trials, and between the chronic arginine and the acute arginine trial. **p < 0.05 between both acute arginine and chronic arginine trials and both acute placebo and chronic placebo trials. † Acute arginine vs Acute placebo, ‡ Acute arginine vs Chronic placebo, § Chronic arginine vs Acute placebo, ‖ Chronic arginine vs Chronic placebo, ¶ Chronic arginine vs Acute arginine (whole experimental period).

3.6–22.5 μmol/L; ES = 0.89) and chronic placebo (mean difference 14.8 μmol/L, 95% CI 5.3–24.3 μmol/L; ES = 1.02) trials (all for ≤0.003).

Differences were found among trials in plasma arginine, ornithine and citrulline concentrations measured during the whole experimental period (all for p < 0.0005). Post-hoc analyses of the main effect of trial showed that plasma arginine and ornithine concentrations were higher in both acute arginine and chronic arginine trials than in both acute placebo and chronic placebo trials (all for p < 0.0005). The plasma arginine and ornithine concentrations were higher in the chronic arginine trial than those in the acute arginine trial (p ≤ 0.004). Post-hoc analyses of the main effect of trial revealed that plasma citrulline concentrations were higher in both acute arginine and chronic arginine trials than in both acute placebo and chronic placebo trials (all for p < 0.0005).

3.2. Ammonia and creatine kinase concentrations

Two participants who had fasting serum concentrations >5000 IU/L in the acute arginine trial and the chronic placebo trial were excluded from the CK analysis. Further interview with the participants revealed that they had been injured before the trial. The concentrations of plasma ammonia and serum CK for each trial are shown in Fig. 3. No differences were found among trials in fasting (i.e., at −60 min) plasma ammonia or serum CK concentrations (all for p > 0.05). Differences were observed in plasma ammonia concentrations measured during the whole experimental period (p = 0.003). Post-hoc analysis of the main effect of trial showed that plasma ammonia concentrations were lower in the chronic arginine trial (25.5 ± 5.8 μmol/L) than those in both acute placebo (30.0 ± 6.5 μmol/L) (mean difference - 4.5 μmol/L, 95% CI - 8.8 to - 0.2 μmol/L; ES = 0.73) and acute arginine (30.6 ± 9.2 μmol/L) (mean difference - 5.1 μmol/L, 95% CI - 9.3 to - 0.8 μmol/L; ES = 0.63) trials (all for p ≤ 0.035). There was no difference in plasma ammonia concentration between the chronic arginine and chronic placebo trials (26.7 ± 5.8 μmol/L) (mean difference - 1.2 μmol/L, 95% CI - 5.4 to 3.2; ES = 0.21, p > 0.05) or between the acute arginine and acute placebo trials (mean difference 0.6 μmol/L, 95% CI - 3.7 to 4.9; ES = 0.07, p > 0.05). Differences were observed among trials in serum CK concentrations measured during the whole experimental period (p < 0.0005). Post-hoc analysis of the main effect of trial revealed that serum CK concentrations were lower in the chronic

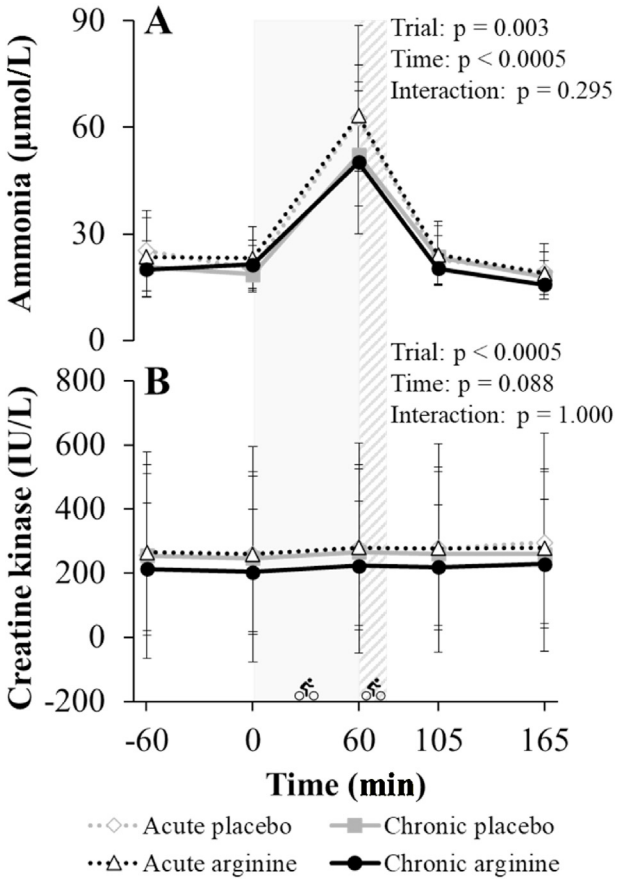

Fig. 3. Plasma ammonia (A) and serum creatine kinase (CK) (B) concentrations in the acute placebo, chronic placebo, acute arginine and chronic arginine trials. Data are means ± standard deviation for n = 16 (ammonia) and n = 14 (CK). Grey shaded area indicates a 60-min cycling period. Diagonally shaded area indicates a 15-min cycling performance test period. Data were analysed using the linear mixed model. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Plasma ammonia concentrations were lower in the chronic arginine trial versus both acute placebo and arginine trials (all for p ≤ 0.035). Serum CK concentrations were lower in the chronic arginine trial versus acute placebo, chronic placebo and acute arginine trials (all for p ≤ 0.004).

arginine trial (218.0 ± 199.2 IU/L) than those in the acute placebo (mean difference - 55.6 IU/L, 95% CI - 83.7 to - 29.8 IU/L; ES = 0.19), chronic placebo (mean difference - 40.2 IU/L, 95% CI - 63.5 to - 8.4 IU/L; ES = 0.16) and acute arginine (mean difference - 54.5 IU/L, 95% CI - 82.6 to - 27.6 IU/L; ES = 0.24) trials (all for $p \leq 0.004$). Differences were found among trials in subjective fatigue ($p = 0.004$). Post-hoc analysis of the main effect of trial showed that subjective fatigue was lower in the chronic placebo trial (50.4 ± 24.7 mm) than in the acute arginine trial (mean difference - 8.8 mm, 95% CI - 14.5 to - 2.1 mm, ES = 0.38; $p = 0.003$).

3.3. Exercise performance test

No differences were observed among trials in the mean HR during a 60-min cycling exercise (acute placebo, 159 ± 10 bpm; chronic placebo, 153 ± 12 bpm; acute arginine, 156 ± 11 bpm; chronic arginine, 154 ± 11 bpm: $p = 0.109$). The mean power output during a 15-min exercise performance test for each trial is shown in Fig. 4. Differences were found among trials in the mean power output ($p < 0.0005$). Post-hoc analysis of the main effect of trial showed that the mean power output was higher in the chronic placebo (168.8 ± 35.5 W) trial than that in both acute placebo (158.4 ± 37.7 W) (mean difference 10.4 W, 95% CI 5.2–15.7 W; ES = 0.28) and acute arginine (158.4 ± 47.4 W) (mean difference 10.4 W, 95% CI 5.1–15.6 W; ES = 0.24) trials (all for $p \leq 0.0005$). Additionally, the chronic arginine trial (169.3 ± 45.9 W) was higher than both acute placebo (mean difference 10.9 W, 95% CI 5.7 to 16.2; ES = 0.26) and acute arginine (mean difference 10.9 W, 95% CI 5.6 to 16.2; ES = 0.23) trials (all for $p \leq 0.0005$). There was no difference in exercise performance between the chronic arginine and chronic placebo trials (mean difference 0.5 W, 95% CI - 4.7 to 5.8; ES = 0.01, $p > 0.05$) or between the acute arginine and acute placebo trials (mean difference 0.0 W, 95% CI - 5.2 to 5.3; ES = 0.00, $p > 0.05$). We checked the order effect for the mean power output. Differences were observed among trials in the mean power output ($p < 0.0005$). Post-hoc analysis of the main effect of order revealed that the mean power output was higher in the 4th visit than the 1st and 3rd visits, and higher in the 2nd and 3rd visits than the 1st visit (all for $p \leq 0.001$). No differences were noted among trials in the mean HR during a 15-min exercise performance test (acute placebo, 164 ± 21 bpm; chronic placebo, 166 ± 16 bpm; acute arginine, 162 ± 24 bpm; chronic arginine, 166 ± 20 bpm: $p > 0.05$).

3.4. Glucose, triglycerides and non-esterified fatty acids concentrations

The concentrations of plasma glucose, serum TG and serum NEFA for each trial are shown in Table 2. No differences were found among trials in the concentrations of fasting (i.e., at −60 min) plasma glucose, serum TG or serum NEFA (all for $p > 0.05$). Differences were noted in plasma glucose concentrations measured during the whole experimental period ($p < 0.0005$). Post-hoc analysis of the main effect of trial revealed that plasma glucose concentrations were higher in the chronic arginine trial than those in both acute placebo and chronic placebo trials. Additionally, the acute arginine trial was higher than the acute placebo trial (all for $p \leq 0.005$). No differences were observed among trials in serum TG concentrations ($p > 0.05$). Differences were noted among trials in serum NEFA concentrations ($p = 0.010$). Post-hoc analysis of the main effect of trial revealed that serum NEFA concentrations were lower in the chronic arginine trial than those in the acute placebo trial ($p = 0.006$).

4. Discussion

The present study demonstrated that both acute (5 g for one day) and chronic (5 g/day for 14 days), oral intake of arginine supplementation does not attenuate exercise-induced increases in plasma ammonia concentration or improve exercise performance in healthy young men. Further studies on the effect of oral arginine intake on exercise performance are needed to examine over a longer supplemental period of time.[13]

The findings that increased plasma arginine concentrations measured 1 h after L-arginine intake were consistent with the finding of a previous study using a 10 g of L-arginine administration.[24] Additionally, our findings extend the previous study by demonstrating that these increased plasma arginine concentrations were further enhanced by chronic oral L-arginine intake (5 g/day, fasting (i.e., at −60 min) arginine concentration was increased by 40.1 ± 32.5% from acute arginine intake to chronic arginine

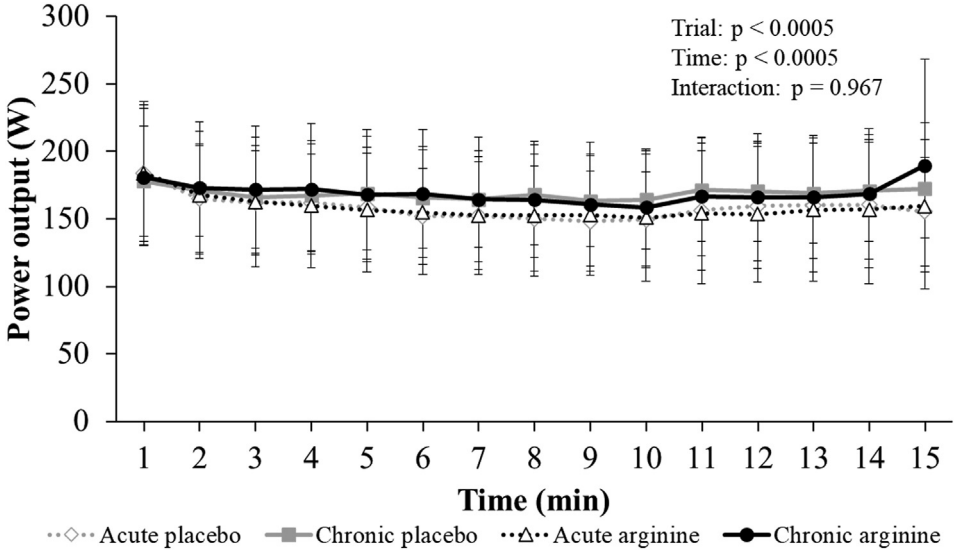

Fig. 4. The mean power output during a 15-min exercise performance test in the acute placebo, chronic placebo, acute arginine and chronic arginine trials. Data are means ± standard deviation for n = 16. Data were analysed using the linear mixed model. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Mean power outputs were higher in both the chronic placebo and arginine trials than that in both the acute placebo and arginine trials (all for $p \leq 0.0005$).

Table 2
Glucose, triglycerides (TG) and non-esterified fatty acids (NEFA) concentrations measured at each time-point in the placebo and arginine trials.

			−60 min	0 min	60 min	105 min	165 min	Whole experimental period	
								Time averaged	95% CI (Effect size)
Glucose*(mmol/L)	Placebo	Acute	4.85 ± 0.26	4.59 ± 0.67	4.36 ± 0.68	4.23 ± 0.66	4.24 ± 0.60	4.46 ± 0.44	† 0.05 to 0.39 (0.41)
		Chronic	4.78 ± 0.40	4.41 ± 0.76	4.53 ± 0.55	4.50 ± 0.55	4.41 ± 0.48	4.53 ± 0.45	§ 0.16 to 0.51 (0.85)
	Arginine	Acute	4.84 ± 0.31	4.89 ± 0.29	4.71 ± 0.48	4.53 ± 0.52	4.41 ± 0.57	4.67 ± 0.57	|| 0.09 to 0.44 (0.66)
		Chronic	4.92 ± 0.35	4.94 ± 0.44	4.80 ± 0.32	4.69 ± 0.39	4.62 ± 0.37	4.79 ± 0.30	
TG(mmol/L)	Placebo	Acute	1.05 ± 0.70	1.05 ± 0.65	0.90 ± 0.67	0.90 ± 0.58	0.90 ± 0.58	0.96 ± 0.63	
		Chronic	1.18 ± 0.66	1.14 ± 0.58	1.05 ± 0.60	1.00 ± 0.51	0.94 ± 0.45	1.06 ± 0.55	
	Arginine	Acute	1.20 ± 0.83	1.05 ± 0.62	0.95 ± 0.54	0.88 ± 0.44	0.81 ± 0.40	0.98 ± 0.53	
		Chronic	1.16 ± 0.50	1.08 ± 0.53	1.03 ± 0.63	0.92 ± 0.53	0.87 ± 0.50	1.01 ± 0.52	
NEFA**(mmol/L)	Placebo	Acute	0.40 ± 0.17	0.25 ± 0.13	1.01 ± 0.46	1.22 ± 0.60	1.38 ± 0.72	0.85 ± 0.36	§ - 0.30 to - 0.03 (0.50)
		Chronic	0.40 ± 0.17	0.23 ± 0.08	0.92 ± 0.33	1.09 ± 0.44	1.16 ± 0.55	0.76 ± 0.26	
	Arginine	Acute	0.37 ± 0.14	0.26 ± 0.11	0.99 ± 0.47	1.09 ± 0.52	1.23 ± 0.60	0.79 ± 0.31	
		Chronic	0.37 ± 0.17	0.30 ± 0.12	0.87 ± 0.33	0.96 ± 0.37	0.94 ± 0.40	0.69 ± 0.24	

CI: confidence intervals. Values are mean ± standard deviation for n = 16. Means were compared using the linear mixed model. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Post-hoc analysis of the main effect of trial: *$p < 0.05$ between the chronic arginine trial and both the acute placebo and chronic placebo trials, and between the acute arginine trial and the acute placebo trial. **$p < 0.05$ between the chronic arginine trial and the acute placebo trial. † Acute arginine vs Acute placebo, § Chronic arginine vs Acute placebo, || Chronic arginine vs Chronic placebo (whole experimental period).

intake). This was confirmed by the fasting concentrations in the present study. Arginine is hydrolysed to produce ornithine or improve urea in the urea cycle.[1] Arginine is also involved in the synthesis of nitric oxide by nitric oxide synthase and the formation of citrulline.[1] In the present study, acute L-arginine supplementation increased both plasma ornithine and citrulline concentrations. These findings were again consistent with the findings of a previous study[3] suggesting that exogenous arginine facilitates the urea cycle. Although urea and nitric oxide were not measured in the present study, these acute effects were also observed in the chronic arginine trial. Furthermore, this was partly explained by the increased ornithine concentration observed in the chronic arginine trial compared with that in the acute arginine trial.

Arginine enhances urea production by ornithine in vivo and enhances ammonia removal during and after exercise.[1] Furthermore, arginine improves blood flow through nitric oxide,[25] leading to attenuation of muscle-damage markers, including CK, by augmenting antioxidative capacity after performing acute resistance exercise.[4] Despite these favourable effects of arginine, previous studies examining the effects of oral L-arginine intake on ammonia and CK in response to exercise observed several discrepant findings. Some reported ammonia and/or CK attenuation,[4,26] whereas others reported no attenuation of ammonia and/or CK.[6,7,17,27,28] Moreover, our findings highlight the need for caution. The magnitude changes in plasma ammonia concentrations observed before and after exercise in the present study may not be sensitive enough to influence its attenuation in response to oral supplementation of L-arginine as the exercise load was not enough to increase plasma ammonia concentrations. Also, lowered CK concentrations observed in the chronic L-arginine supplementation trial in the present study were not reflected by attenuating the exercise-induced increase in CK since our blood sampling point was not long enough to observe increased CK concentrations typically seen a day after performing acute exercise.

In the present study, both acute and chronic L-arginine supplementation (consuming 200 mL of water containing 5 g of L-arginine) did not seem to improve self-paced cycling performance determined by work performed during a fixed time period in young active men. These findings are in line with those of previous studies,[9,12,17,18] but direct comparison may not be possible as different populations, performance testing protocols and performance outcomes were used among the studies. The most likely explanation for the lack of improvement in cycling performance may be related to the dose and duration of arginine supplementation.[13] A recent systematic review and meta-analysis found that 0.15 g/kg (≈10–11 g) and 1.5–2 g/day of arginine supplementation ingested between 60 and 90 min before exercise and for 4–7 weeks, respectively, enhances aerobic (i.e., exercise lasting more than 5 min) exercise performance.[13] Furthermore, although we have chosen 5 g of oral L-arginine supplementation based on a previous study with 3 g of L-arginine hydrochloride intravenous infusion, the bioavailability of oral L-arginine is low as discussed previously.[24]

In the present study, increased glucose concentrations were observed in the chronic L-arginine supplementation trial. However, since we were unable to assess glucose kinetics (i.e., rates of appearance and disappearance) during the entire trial, whether pre-exercise L-arginine ingestion maintained prolonged blood glucose availability in the present study is unknown. Notably, a lowered NEFA concentration was observed in the chronic arginine trial. This observation may be reflected by the nitric oxide-mediated suppression of lipolysis in adipose tissue via arginine supplementation.[29] Although arginine infusion was shown to increase glucose clearance during exercise in a previous study,[30] maintaining blood glucose during exercise may be beneficial for improving exercise performance. Nonetheless, it is not known to what extent oral arginine supplementation influences carbohydrate and fat metabolism in humans as its bioavailability is low (i.e., the absolute bioavailability of a single oral 10 g dose of L-arginine is approximately 20%).[24] Therefore, the effects of oral L-arginine intake on carbohydrate and fat metabolism during exercise need to be further investigated.

The present study has several strengths. The present study evaluated both acute and chronic intakes of L-arginine supplementation on blood ammonia and exercise performance. To date, the present study is the first to compare these effects in a cross-over study design. The unique nature of the present study design allows us to distinguish between a single and a chronic intake of L-arginine regarding the magnitude of L-arginine supplementation effect, if any, on these outcomes. However, reduced ammonia concentration was observed after a chronic intake of L-arginine supplementation and placebo trials in the present study. The present study also has considerable limitations. We did not conduct a familiarisation session for the cycling performance test and did not check the reliability/sensitivity of the cycling performance test protocol in the present study. Indeed, an order effect of the cycling performance test was observed in the present study. Again, this implies that a familiarisation session is needed to conduct for avoiding an order

effect. Furthermore, the cycling performance test used may not have been elicited well enough to maximise the participants' effort. None of the participants were highly trained cyclists. Therefore, pre-determining their maximum effort to cover the entire duration (i.e., 15 min) is difficult as the participants were asked to select a pedalling cadence freely at their own pace. Indeed, athletic status may be a factor that affects the exercise performance test - the coefficient of variation was larger for non-athletes than for athletes.[31] In addition, given the only ~1% of oral arginine is available as substrate for nitric oxide production, future efficacy study is required to evaluate exercise performance regarding the use of other oral supplementations for nitric oxide bioavailability.[32] Indeed, a previous study demonstrated that oral citrulline (6 g/day), but not arginine (6 g/day), intake for 7 days appears to be effective at improving oxygen uptake kinetics or cycling exercise performance.[18]

5. Conclusions

In conclusion, both acute (5 g) and chronic (5 g/day, 14-day) oral intake of L-arginine supplementation did not attenuate exercise-induced increases in ammonia accumulation or had no significant impact on cycling performance.

Author contributions

AH and YT contributed equally to this study. AH, YT, SN, CN and YH supervised the data collection, assisted with all aspects of the biochemistry and performed the data analysis. AH and YT drafted the manuscript. MM conceived the study, obtained the funding and took the lead in writing the manuscript. MM, SN, CN and YH commented and edited each section of the manuscript. All authors approved the final version of the manuscript.

Disclosure of interest

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