

# Chronic, But Not Acute, Oral L-Arginine Supplementation Attenuates Exercise-Induced Ammonia Accumulation in Healthy Young Men: A Randomised, Double-Blind, Cross-Over, Placebo-Controlled Trial

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## Research article

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## Abstract

## Background

This study examined the effects of a single and continuous oral intake of L-arginine supplementation on blood metabolites and exercise performance.

## Methods

Sixteen healthy young men (mean  $\pm$  standard deviation,  $23 \pm 3$  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 (L-arginine was replaced with dextrin) and performed cycling exercise at 75 % of heart rate reserve for 60 min, followed by a 15-min cycling performance test. The participants continued to consume each designated supplement twice a day for 13 days. 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. The linear mixed model was used to examine between-trial differences over the 1-day or 2-week intervention for outcome variables.

## Results

Plasma ammonia concentrations were lower in the chronic arginine ( $43.5 \pm 27.6$   $\mu\text{mol/L}$ ) trial than in both acute arginine ( $52.1 \pm 36.3$   $\mu\text{mol/L}$ , 95% confidence interval - 15.907 to - 1.318  $\mu\text{mol/L}$ , Effect size = 0.262) and placebo ( $51.1 \pm 32.7$   $\mu\text{mol/L}$ , 95% confidence interval - 14.932 to - 0.343  $\mu\text{mol/L}$ , Effect size = 0.249) trials ( $p < 0.05$ ). No differences were found in mean power output during the performance test between the chronic arginine ( $169.3 \pm 8.6$  W) and placebo ( $168.8 \pm 2.3$  W) trials ( $p > 0.05$ ).

## Conclusions

These results indicate that a continuous oral intake of L-arginine supplementation attenuated ammonia accumulation, but this did not influence cycling performance.

## Background

Arginine, a substrate for both nitric oxide synthase and arginase to produce nitric oxide and urea, respectively, plays an important role in numerous physiological and biological processes [1]. Regarding the physiological roles of arginine in response to exercise, acute arginine supplementation and intravenous injection attenuates exercise-induced ammonia accumulation [2, 3] possibly by increasing ureagenesis. Acute arginine intake also mitigates exercise-induced muscle damage possibly by augmenting antioxidant capacity via nitric oxide mediation [4]. Arginine is classified as a glycolytic amino acid, and therefore, it can be converted to glucose [5]. Collectively, these potential roles of arginine in the alteration of metabolic pathways have been the focus of research on exercise performance.

The effect of arginine supplementation on exercise performance has been evaluated in previous efficacy studies using a randomised, double-blind, cross-over, placebo-controlled design [6–12] (for a review of these, see Reference [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 minutes in duration [14, 15]. Conversely, three studies did not observe favourable effects of chronic arginine supplementation on aerobic exercise performance [16–18]. Thus, further studies are warranted to examine the effect of chronic arginine supplementation on exercise performance. 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. 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 a single and continuous 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.

## Methods

### Participants

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. Participants were recruited if they met the following criteria: non-smoker, not overweight or obese, and not taking any supplementation or medication. The physical characteristics of the participants (mean  $\pm$  standard deviation) were as follows: age,  $23 \pm 3$  years; height,  $173.5 \pm 6.4$  cm; body mass,  $69.6 \pm 8.5$  kg; body mass index,  $23.0 \pm 1.5$   $\text{kg/m}^2$ ; and maximum oxygen uptake,  $53.3 \pm 11.0$  ml/kg/min.

## 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.

## Preliminary tests

Participants participated in two preliminary exercise tests performed 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. Subsequently, 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. 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 [19]. 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  $165 \pm 8$  beats per minute (bpm)), and this workload was used for the main trials.

## 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) one-day placebo (acute placebo trial), followed by (2) 14-day placebo (chronic placebo trial) and (3) one-day L-arginine (acute arginine trial), followed by (4) 14-day L-arginine (chronic arginine trial) supplementation. 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 (performed the acute placebo trial, followed by the chronic placebo trial first or performed the acute arginine trial, followed by the chronic arginine trial first). A schematic representation of the study protocol is shown in Figure 1. 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 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 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 [20]. 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 [21]. 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. 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.

## Outcomes

The primary outcome was plasma ammonia. The secondary outcome was a 15-min exercise performance test.

## Analytical methods

For serum TG, NEFA, and CK measurements, venous blood samples were collected into tubes containing clotting activators for serum isolation. Samples were allowed to clot for 30 min at room temperature and then centrifuged at  $1861 \times g$  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.

## 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 (trial: L-arginine vs. placebo) (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) in order to consider for potential withdrawals. 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 a significant trial effect was 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) 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 [22]. Results are reported as the mean  $\pm$  standard deviation.

## Results

### Amino acid concentrations

The plasma amino acid concentrations for each trial are shown in Table 1. Differences were found among trials in fasting plasma arginine and ornithine concentrations (all for  $p \leq 0.008$ ). 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 trial (95% CI 18.055 to 65.295  $\mu\text{mol/L}$ , ES = 2.690) and in both acute (95% CI 20.568 to 67.807  $\mu\text{mol/L}$ , ES = 10.111) and chronic (95% CI 20.468 to 67.707  $\mu\text{mol/L}$ , ES = 10.149) placebo 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 (95% CI 0.801 to 25.312  $\mu\text{mol/L}$ , ES = 33.185) and chronic (95% CI 2.544 to 27.056  $\mu\text{mol/L}$ , ES = 33.387) placebo trials (all for  $\leq 0.031$ ).

Differences were found among trials in plasma arginine, ornithine and citrulline concentrations (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 and chronic arginine trials than in both acute 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 ( $p \leq 0.004$ ). Post-hoc analyses of the main effect of trial revealed that plasma citrulline concentrations were higher in both acute and chronic arginine trials than in both acute and chronic placebo trials (all for  $p < 0.0005$ ).

### Ammonia and creatine kinase concentrations

The concentrations of plasma ammonia and serum CK for each trial are shown in Figure 2. No differences were found among trials in fasting plasma ammonia ( $p = 0.366$ ) or serum CK ( $p = 0.956$ ) concentrations. Differences were observed in plasma ammonia concentrations ( $p = 0.003$ ). Post-hoc analysis of the main effect of trial showed that plasma ammonia concentrations were lower in the chronic arginine trial than those in both acute placebo (95% CI -14.932 to -0.343  $\mu\text{mol/L}$ , ES = 0.249) and arginine (95% CI -15.907 to -1.318  $\mu\text{mol/L}$ , ES = 0.262) trials (all for  $p \leq 0.035$ ). Differences were observed among trials in serum CK concentrations ( $p < 0.0005$ ). Post-hoc analysis of the main effect of trial revealed that serum CK concentrations were lower in the chronic arginine trial than those in the acute placebo (95% CI -83.738 to -29.756 IU/L, ES = 8.116), chronic placebo (95% CI -63.464 to -8.421 IU/L, ES = 6.167) and acute arginine (95% CI -82.621 to -27.647 IU/L, ES = 13.511) 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 than in the acute arginine trial (95% CI -14.493 to -2.097 mm, ES = 0.281,  $p = 0.003$ ).

### 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 plasma glucose ( $p = 0.745$ ), serum TG ( $p = 0.909$ ) or NEFA ( $p = 0.889$ ). Differences were noted in plasma glucose concentrations ( $p < 0.0005$ ). 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 and chronic placebo trials. Additionally, the acute arginine trial was higher than the acute placebo trial (all for  $p \leq 0.004$ ). 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.

### Exercise performance test

The mean power output during a 15-min exercise performance test for each trial is shown in Figure 3. 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 trial than that in both acute placebo (95% CI 3.107 to 13.893 W, ES = 1.593) and arginine (95% CI 4.984 to 15.769 W, ES = 2.450) trials (all for  $p \leq 0.0005$ ). Additionally, the chronic arginine trial was higher than both acute placebo (95% CI 3.627 to 14.413 W, ES = 1.175) and arginine (95% CI 5.504 to 16.289 W, ES = 1.459) trials (all for  $p \leq 0.0005$ ). No differences were noted among trials in the mean HR during a 15-min exercise performance test (acute placebo,  $164 \pm 21$  bpm; chronic placebo,  $166 \pm 17$  bpm; acute arginine,  $162 \pm 25$  bpm; chronic arginine,  $166 \pm 21$  bpm:  $p = 0.118$ ).

## Discussion

The present study demonstrated that continuous, but not acute, oral intake of arginine supplementation attenuated exercise-induced increases in plasma ammonia concentration in healthy young men. Despite the favourable effect on blood ammonia observed in the continuous intake of L-arginine for 14 days (including the acute trial day), self-paced cycling exercise performance did not differ between the arginine and placebo trials regardless of the duration of the supplementation period (acute versus chronic). These findings suggest that oral intake of L-arginine supplementation facilitates the removal of ammonia, which was partly confirmed by amino acid results observed in the present study. However, whether arginine intake affects exercise performance needs to be further examined in a future study with a longer period [13].

The findings that increased plasma arginine concentrations measured 1 h after L-arginine intake were consistent with the findings of a previous study using 3 g of L-arginine infusion (via a forearm vein) [3]. Additionally, our findings extend the previous study by demonstrating that these increased plasma arginine concentrations were further enhanced by continuous oral L-arginine intake (5 g/day). This was confirmed by the fasting concentrations in the present study. Arginine is hydrolysed to produce ornithine and 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. Collectively, chronic oral L-arginine intake conducted in the present study may lead to inhibition of ammonia production and/or facilitation of ammonia removal.

Higher arginine concentrations might be the most plausible reason the chronic, but not acute, L-arginine supplementation trial reduced the circulating concentrations of ammonia and CK. 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 [23], 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, 24], whereas others reported no attenuation of ammonia and/or CK [6, 7, 17, 25, 26]. Moreover, our findings highlight the need for caution. Lowered CK concentrations observed in the chronic L-arginine supplementation trial were not reflected by the exercise-induced increase in CK. Thus, given the inconsistent reports in the literature, the effects of arginine intake on exercise-induced increases in fatigue-related markers need to be examined further in future studies.

Given that arginine is a glucogenic amino acid and serves as a precursor in gluconeogenesis for glucose formation, increased glucose concentrations were observed in the chronic L-arginine supplementation trial of the present study. 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 [27]. Although arginine infusion was shown to increase glucose clearance during exercise in a previous study [28], maintaining blood glucose during and after exercise periods may be beneficial for exercise performance and recovery (*i.e.*, creating an anabolic environment). Despite these potential physiological benefits during and after exercise periods, L-arginine intake did not seem to improve cycling performance in the present study.

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 ( $\approx$  10–11 g) and 1.5–2 g/day of arginine supplementation ingested between 60–90 minutes before exercise and for 4–7 weeks, respectively, enhances aerobic (*i.e.*, exercise lasting more than 5 minutes) exercise performance [13]. Therefore, the dose and duration of L-arginine intake used in the present study were possibly insufficient to achieve a favourable effect on performance.

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 continuous intake of L-arginine regarding the magnitude of L-arginine supplementation effect, if any, on these outcomes. Reduced ammonia concentration was only observed after a continuous intake of L-arginine supplementation trial in the present study. However, no improvement in self-paced cycling performance was found in both single and continuous L-arginine supplementation trials, possibly due to a lower dose (*i.e.*, acute L-arginine supplementation) and a shorter duration (*i.e.*, chronic L-arginine supplementation) of the supplementation [13]. The present study also has considerable limitations. 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 minutes) 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 [29]. In addition, given the only  $\sim$  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 [30]. 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 and cycling exercise performance [18].

## Conclusions

In conclusion, oral intake of L-arginine supplementation for 14 days, but not acute L-arginine supplementation, attenuated exercise-induced plasma ammonia accumulation. However, this did not seem to improve cycling performance in healthy young men.

## Abbreviations

HR: Heart rate; bpm: beats per minute; CK: Creatine kinase; TG: Triglycerides; NEFA: Non-esterified fatty acids; 95% CI: 95% confidence interval; ES: Effect size

## Declarations

### Ethics approval and consent to participate

The present study was approved by the institutional ethics committee (approval number: 2018–202) and conducted in accordance with the Declaration of Helsinki.

### Consent for publication

Not applicable.

### Availability of data and materials

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

### Competing interests

M. Miyashita received a research grant from Emetore Co., Ltd. The funder has no role in the study design; collection, management, analysis and interpretation of data; writing of any reports; and the decision to submit any reports for publication, and will not have authority over any of these activities. For the remaining authors none were declared conflicts of interest.

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### Authors' 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.

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## Tables

**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	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	† 55.178 to 75.822 (7.716) ‡ 52.971 to 73.614 (7.693) § 87.188 to 107.832 (10.143)    84.981 to 105.624 (10.196) ¶ 21.688 to 42.332 (2.699)
		Chronic	106.8 ± 12.3	98.8 ± 12.0	105.1 ± 11.0	95.6 ± 9.2	93.2 ± 11.6	
	Arginine	Acute	109.2 ± 24.7	211.5 ± 55.6	197.0 ± 36.6	159.6 ± 42.3	138.8 ± 30.3	
		Chronic	150.9 ± 38.8	255.5 ± 54.4	223.0 ± 57.9	183.8 ± 45.2	162.9 ± 32.9	
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	† 27.791 to 36.494 (7.343) ‡ 29.719 to 38.421 (7.320) § 33.374 to 42.076 (34.206)    35.301 to 44.004 (34.005) ¶ 1.231 to 9.934 (0.908)
		Chronic	45.1 ± 8.0	41.0 ± 6.9	41.1 ± 6.5	37.4 ± 6.8	38.9 ± 6.4	
	Arginine	Acute	50.8 ± 15.1	86.6 ± 25.8	91.7 ± 16.7	75.3 ± 13.0	69.4 ± 14.3	
		Chronic	59.9 ± 16.8	97.3 ± 18.6	95.5 ± 17.0	79.4 ± 18.6	69.6 ± 15.6	
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	† 1.699 to 4.568 (4.922) ‡ 1.890 to 4.760 (4.873) § 2.365 to 5.235 (3.817)    2.557 to 5.426 (3.901)
		Chronic	28.6 ± 5.1	25.5 ± 3.9	27.7 ± 5.3	27.1 ± 4.4	25.7 ± 3.4	
	Arginine	Acute	28.7 ± 3.2	24.7 ± 4.5	37.4 ± 6.0	33.6 ± 6.1	26.9 ± 4.7	
		Chronic	30.2 ± 6.5	25.0 ± 4.4	37.9 ± 7.3	33.7 ± 5.8	27.8 ± 4.6	

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 and chronic arginine trials and both acute and chronic placebo trials, and between the chronic arginine and the acute arginine trial. \*\* p < 0.05 between both acute and chronic arginine trials and both acute 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.

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		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	† 0.890 to 7.010 (1.364) § 3.015 to 9.135 (2.080)    1.753 to 7.872 (2.268)
		Chronic	4.78	± 0.40	4.41	± 0.76	4.53	± 0.55	4.50	± 0.55	4.41	± 0.48	
	Arginine	Acute	4.84	± 0.31	4.89	± 0.29	4.71	± 0.48	4.53	± 0.52	4.41	± 0.57	
		Chronic	4.92	± 0.35	4.94	± 0.44	4.80	± 0.32	4.69	± 0.39	4.62	± 0.37	
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	
		Chronic	1.18	± 0.66	1.14	± 0.58	1.05	± 0.60	1.00	± 0.51	0.94	± 0.45	
	Arginine	Acute	1.20	± 0.83	1.05	± 0.62	0.95	± 0.54	0.88	± 0.44	0.81	± 0.40	
		Chronic	1.16	± 0.50	1.08	± 0.53	1.03	± 0.63	0.92	± 0.53	0.87	± 0.50	
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	§ -0.297 to -0.034 (0.710)
		Chronic	0.40	± 0.17	0.23	± 0.08	0.92	± 0.33	1.09	± 0.44	1.16	± 0.55	
	Arginine	Acute	0.37	± 0.14	0.26	± 0.11	0.99	± 0.47	1.09	± 0.52	1.23	± 0.60	
		Chronic	0.37	± 0.17	0.30	± 0.12	0.87	± 0.33	0.96	± 0.37	0.94	± 0.40	

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 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.

## Figures

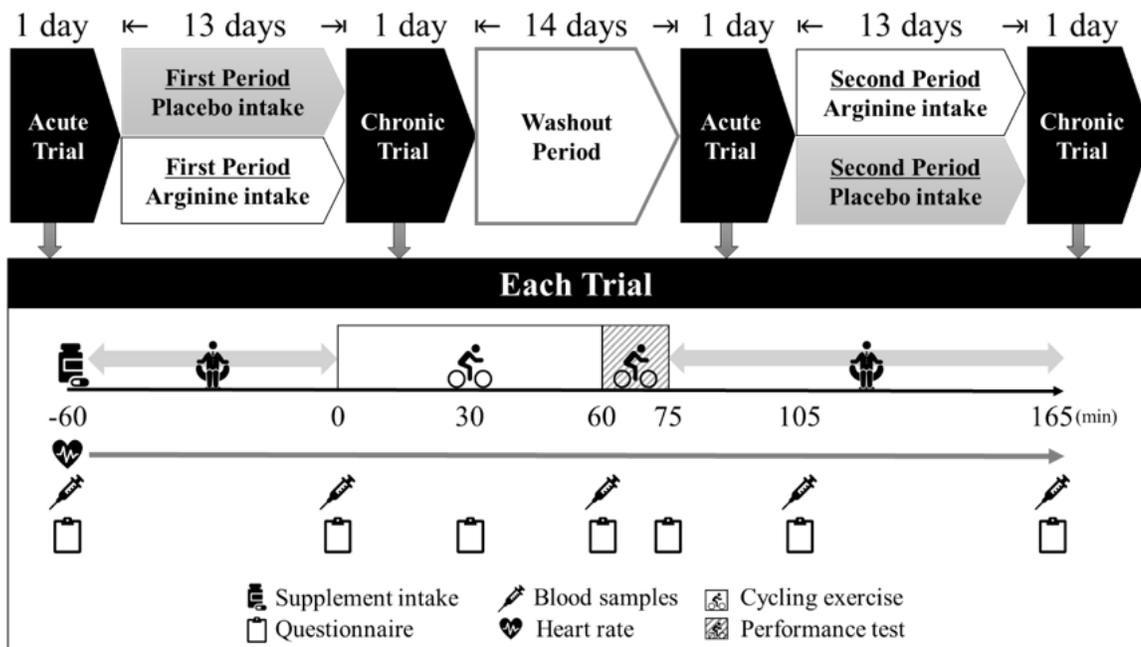


Figure 1

A schematic representation of the study protocol. Each participant underwent four, one-day laboratory-based trials in a random order: (1) one-day placebo (acute placebo trial) followed by (2) 14-day placebo (chronic placebo trial) and (3) one-day L-arginine (acute arginine trial) followed by (4) 14-day L-arginine (chronic arginine trial) supplementation. 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, 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.

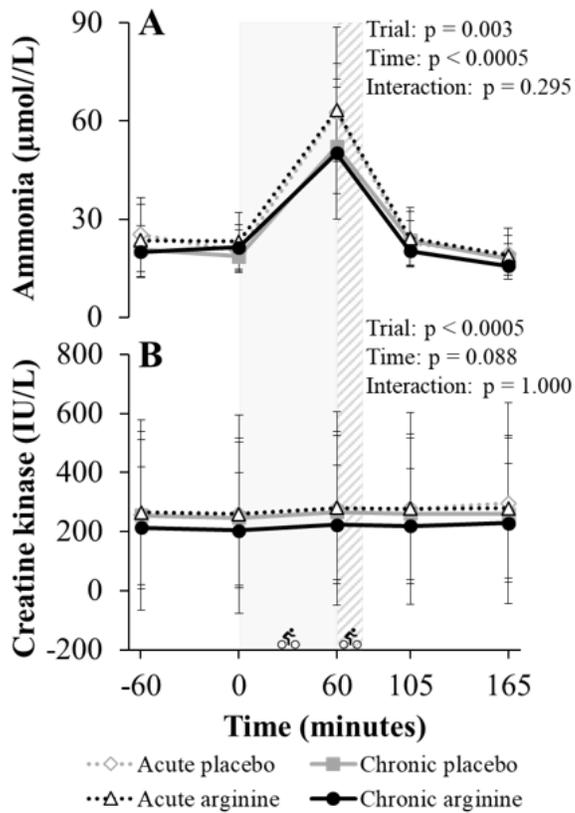
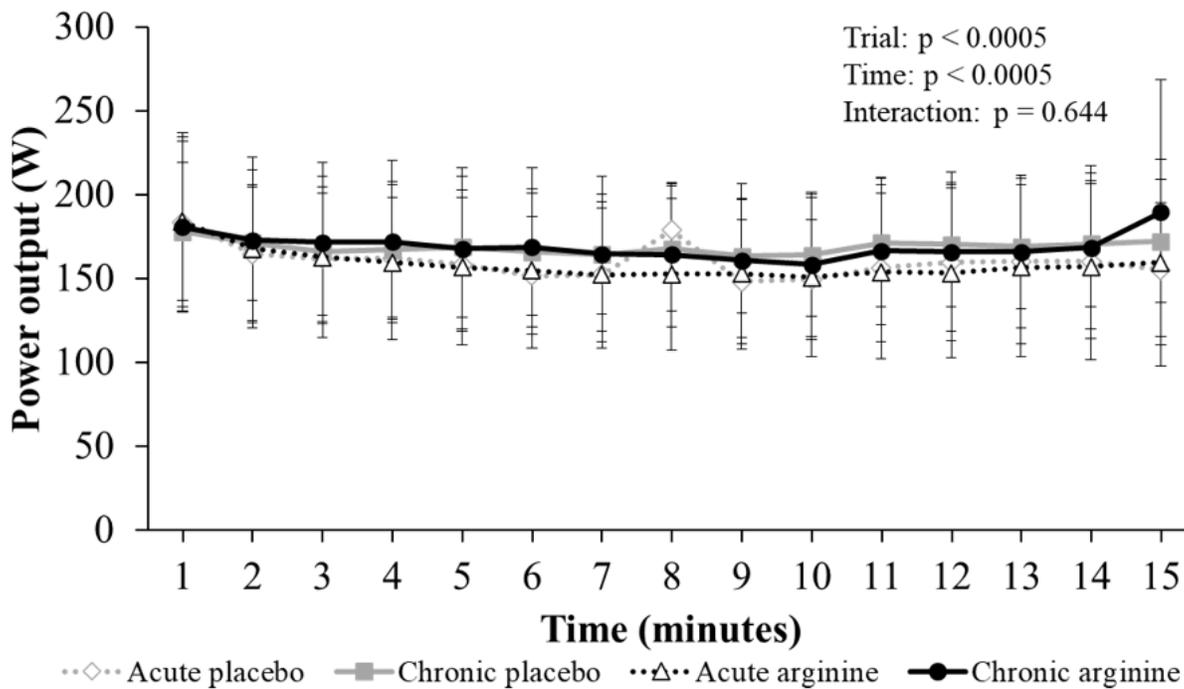


Figure 2

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  $\pm$  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  $p \leq 0.035$ ). 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. Serum CK concentrations were lower in the chronic placebo trial versus acute placebo, chronic placebo and acute arginine trials (all for  $p \leq 0.004$ ).



### Figure 3

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  $\pm$  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  $p \leq 0.0005$ ).