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One-Week L-Arginine Supplementation Had No Effect on 200m Freestyle Swimming Time Trial in Moderately-Trained Male Swimmers

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ABSTRACT

Research on the effect of L-arginine supplementation on exercise performance is still inconsistent and its influence on single-bout swimming performance has not been investigated. Therefore, the aim of this study was to assess if one-week L-arginine supplementation would enhance 200-m freestyle swimming performance in trained/developmental (regularly training ~3 times per week with a purpose to compete) male swimmers. In a randomized, cross-over, double-blind design, 8 trained/developmental male swimmers (age 25 ± 5 years; mean ± SD) completed 200-m freestyle swimming time-trial on 3 separate occasions: a control trial; and after 2 separate 7-d supplementation periods, with a daily dose of either 8 g/d of L-arginine or placebo trials. Blood lactate concentration was measured immediately post time-trial swimming. Completion time of the 200-m freestyle swimming time-trial did not differ significantly (F = 4.55; P = 0.060; ηp² = 0.394) between control (149.40 ± 9.88 s), L-arginine (146.02 ± 10.34 s) and placebo trials (147.58 ± 10.86 s). There was no statistically significant difference in post time-trial swimming blood lactate concentration between trials (control: 11.2 ± 2.7; L-arginine: 13.1 ± 1.8; Placebo: 12.2 ± 2.7, F = 3.52; P = 0.058; ηp² = 0.335). One-week of supplementation with 8 g/d of L-arginine, had no ergogenic effect on middle-distance (200-m), freestyle swimming performance in trained/developmental male swimmers.

INTRODUCTION

Supplements that contain nitric oxide (NO) precursors have emerged as popular ergogenic aids to enhance exercise performance in the last two decades (1, 2). The signaling molecule NO is generated through the oxidation of semi-essential amino acid L-arginine to NO and L-citrulline by the NO synthase (NOS) enzyme (3, 4), and plays a key role for the regulation of blood flow (BF) leading to enhanced oxygen (O₂) and nutrients delivery to contracting muscle during exercise (5, 6). This increased NO bioavailability via exogenous L-arginine supplementation has been reported to reduce O₂ cost...
of muscle force production (7, 8), attenuate blood lactate and ammonium accumulation (9), and therefore improve exercise performance (7, 10–12).

Ergogenic properties of L-arginine as a NO precursor are widely studied and regular supplementation occurs. However, findings from studies are often conflicting. While some studies reported that L-arginine supplementation enhanced high-intensity exercise tolerance (1, 7), repeated sprint performance (10) and muscle strength and power (11) in moderately trained individuals, others found no effects on moderate- and high-intensity exercise (13, 14) or fatigue resistance (15, 16). The reasons for the conflicting findings are unclear, however there may be some explanations that need considering. (I) Performance enhancing effect of L-arginine have been reported following high doses supplementation (7–11), but not after lower doses (13–15). Therefore, administered doses of L-arginine in different studies may be a plausible explanation for the observed variation of the ergogenic effects. It has been reported that the maximal concentration of plasma L-arginine is reached following the ingestion of 6 g of L-arginine (3). As such, evaluating the effect of L-arginine supplementation, at an appropriate dose, on exercise performance is important to improve the understanding of L-arginine supplementations as an ergogenic aid. (II) In some studies, L-arginine was combined with 2 g glycine and 3.2 g α-ketoisocaproic acid (10, 11), rather than with alpha-ketoglutarate (13–15). One study administered the supplement with no additional compounds (16). Thus, it is important to untangle the interaction of these other compounds with the L-arginine in the supplement to understand the exact causes of the difference observed in the various studies (10, 11). Some other variables that diverged amongst the studies, such as differences in exercise protocols, or/and training level of individuals might be also a reason for the inconsistent findings. Therefore, it is difficult to draw conclusions about whether L-arginine supplementation has an ergogenic effect on exercise performance. While the findings of acute supplementation are inconsistent (13, 15, 17), there is evidence that L-arginine supplementation was able to improve exercise performance following short-term and/or chronic supplementation (17, 18). Taken together, it may be possible that short-term supplementation with ≥ 6 g/d, might be a convenient regimen to provide ergogenic effects and therefore performance enhancements.

Effects of L-arginine supplementation have been studied predominantly in cycling (1, 10, 19, 20), running (7) and resistance exercise (13, 15). However, the potential ergogenic effect of L-arginine supplementation on swimming performance has yet to be determined. Due to unique physiological and technical demands of swimming, it is difficult to extrapolate the reported ergogenic effect of L-arginine supplementation from cycling or running. However, given L-arginine supplementation was reported to have positive effect in high metabolic demands exercise (10, 15, 19), it is possible that there might be an effective, at least in a similar form, in swimming. High blood lactate concentration has been reported post-200-m swimming competition (21), which indicates significant acidosis and increased anaerobic energy contribution to this swimming event. Further, the metabolic contributions to 200-m swimming distance were estimated as: 53–55% aerobic; 35–40% glycolytic; and 5–7% anaerobic alactic (22). Considering the metabolic demands of 200-m swimming and documented potential ergogenic effect of L-arginine in other exercise modes, it can be suggested that L-arginine supplementation may enhance 200-m freestyle swimming performance.
The aim of this study was to assess if one-week supplementation with L-arginine supplementation would enhance 200-m freestyle swimming performance in trained/developmental (23) male swimmers, doing regular and planned swimming training sessions ~3 days a week. Based on the physiological properties of L-arginine on BF, O₂ cost, blood lactate concentration, and performance (7, 9), we hypothesized that L-arginine supplementation would enhance 200-m freestyle swimming performance and reduce immediate post time-trial swimming blood lactate concentration.

**Materials and methods**

**Participants**

The sample size of this study was based on a priori calculation using G*Power software (Version 3.1). Based on a previous study (1) with a power of 0.80, one tailed α set at 0.05 (it was assumed the possibility of an effect in one, positive, direction) and Glass’s effect size of 1.04, a sample size of eight was determined sufficient. Although initially 10 participants were recruited for the present study, two participants withdrew due to illness during the second supplementation period, and thus a total of eight trained/developmental (23) male swimmers (age 25±5 years, body mass 75±8 kg, height 176±7 cm; mean±SD) completed this study. Participants in the present study were physically active, nonsmoking young adults, and did not currently, or in the previous 3 months, have a musculoskeletal injury. All participants were involved in a regular swimming training program (~3 days per week, average of 3×2–3h pool based) standardized by a qualified swimming coach with a purpose to compete. Training workload was kept similar during those three training days leading up to each arm. All participants had at least 10 years competitive swimming experience at club standard and at least five years of experience competing in regional and university-level competitions. Ethical approval for this study was obtained from the Faculty of Applied and Health Sciences Ethics Committee at the University of Chester (reference no: 1071/15/OE/CSN). All participants gave their written informed consent after the experimental procedures, associated risks, and potential benefits of participation had been explained. Participants were asked to record their dietary intake in the 24 h before the control arm and to repeat the same diet in the 24 h before subsequent arms. Participants were instructed to refrain from ingesting caffeine (6h before), alcohol and any anti-inflammatory drugs 24h before each arm. Participants were asked not to use nitric oxide precursors supplements (L-citrulline and inorganic nitrate) and sodium bicarbonate as these supplements would have ergogenic effect on performance (24). However, all participants were habitually using protein powder (20–30 g/day of whey), as a dietary supplement. Participants were also instructed not to swim or do any severe exercise 24h before each time-trial.

**Experimental design and supplementation procedure**

This study was performed in United Kingdom and participants were required to report to the test facility on three separate visits over a 22 to 24-day period. Participants were required to complete a maximal effort race simulated 200-m freestyle swim at each visit. The first visit was a control trial with no supplement use. Following the control
trial, in a randomized, counterbalanced, double-blind, crossover design, participants were assigned to consume either L-arginine or placebo for 7 days. A washout period of 7 days separated the supplementation periods (1). During the two 7-day supplementation periods, participants consumed 8 g/d of L-arginine (L-Arginine, Hydroxypropylmethyl Cellulose, Magnesium Stearate; Just Vitamins Ltd, Coventry, UK) or placebo (gluten free flour; Organ®, UK) in gelatin capsules (8 × 1 g/d capsules). Given 8 g/d of L-arginine has been reported safe and well tolerated when supplemented orally (25) and elevated plasma arginine concentration in healthy human has been reported following lower dose (6 g) supplementation (3), 8 g/d of L-arginine was used in the present study.

Participants were instructed to set up a reminder for supplementation twice daily, 12 h apart and asked to keep a record of any days when supplements were missed, in order to monitor compliance. Participants were also sent daily reminder email for the times that supplements were to be consumed on the 6 days prior to the trial and on the final day before the trial. Each treatment was prepared in masked, identical capsules by the laboratory team member who did not take part in data collection and analysis. The laboratory team member informed the research team of active and placebo supplements only after the completion of the trial and collection of all raw data. Participants ingested 4 capsules in the morning (~9 AM) and evening (~9 PM) over the first 6 days of the supplementation period. On the final day of supplementation, 8 × 1 g capsules were ingested together ~90 min before the 200-m time-trial. It has been reported that the peak concentration on plasma L-arginine is reached ~90 min after 6 g of L-arginine supplementation (3). Each time-trial was conducted following one day rest.

200-m Freestyle swimming time-trial

All swims took place in the same pool (depth 1 m to 3 m × length 25 m × width 12.5 m and water temperature 28 °C), with trials performed at the same time of day for each arm (~12 PM). The swimmers were all familiar with competing over the set distance. Participants completed a standardized low- to moderate-intensity warm-up (1000 m, ~25 min) before each trial. Then, 10 min rest was given by sitting during which heart rate was monitored via telemetry (Polar, FS1, Polar Electro Oy, Finland). Participants were permitted only water during this time. Ten minutes post warm-up, a 200-m freestyle time-trial was performed. Throughout the time-trial, each 100 m split and total 200 m time were recorded. Participants completed the time-trial individually (with no other competitors present). time-trials were commenced with a diving start from a diving block and were timed with a stopwatch by a qualified and experienced national head swimming coach. Capillary blood lactate concentration was measured using a lactate analyzer (Lactate Pro, Kyoto, Japan) from finger pinprick samples prior to the warm-up, and immediately post time-trial swimming.

Data analysis

All data were analyzed using SPSS 27.0 (IBM Corp., Armonk, NY), and presented as mean ± SD. Statistical significance was set at \( p \leq 0.05 \). All data were normally
distributed. One-way repeated measure ANOVA was used to determine whether there are differences in 200-m freestyle swimming time-trials and post time-trial swimming blood lactate concentration between trials. Two-way repeated measures ANOVAs were \textit{(supplementation \times time)} applied to determine whether differences existed between control, L-arginine and placebo trials for $2 \times 100$-m split times of 200 m freestyle swimming time-trial. Effect size was calculated as partial eta-squared ($\eta^2_p$) varying small ($<0.25$), medium (0.26–0.63) and large (>0.63) (26). Where the analysis of variance revealed a significant effect, Bonferroni corrected paired $t$-tests were applied as post-hoc paired comparisons. Cohen’s $d$ effect sizes were determined for each paired comparison, which varies from small ($\geq 0.01$) to moderate ($\geq 0.06$) and to a large effect ($\geq 0.14$) (26).

**Results**

All participants reported that they had fully complied with their supplement use requirement. The group mean values for time to completion of 200-m time-trial, the first and second 100-m split times, and post time-trial swimming blood lactate concentration in control, L-arginine and placebo trials were reported in Table 1. The mean group completion time of 200 m time-trial, the first and second 100-m split times in control, and post time-trial swimming blood lactate concentration following both L-arginine and placebo supplementation were shown in Figures 1–3 respectively. Completion time of the 200-m freestyle swimming time-trial was not significantly different between control, L-arginine and placebo trials (ANOVA: supplementation, $F=4.55; p=0.060; \eta^2_p = 0.394$).

There was no supplementation \times time interaction effect ($F=1.02; p=0.387; \eta^2_p = 0.127$) nor a main effect of supplementation ($F=4.53; p=0.060; \eta^2_p = 0.393$) on the split times across each 100-m distance during 200-m time-trial. There was a main effect of time on the split times across each 100-m distance during 200-m time-trial ($F=139.47; p<0.001; \eta^2_p = 0.952$). Post-hoc paired comparisons showed that the time of the first 100-m split (71.37 ± 1.68 s) was faster than the time of the second 100-m split (76.10 ± 1.95, $p<0.001$, $d=2.71$, 95% CI $[-5.92, -3.95]$).

There was no significant difference in post time-trial swimming blood lactate concentration between control, L-arginine and placebo trials (ANOVA: supplementation, $F=3.52; p=0.058; \eta^2_p = 0.335$).

| Table 1. Group mean (SD) of 200 m time-trial, the first and second 100-m split times, and post-swimming blood lactate concentration (BLa) following 7-day L-arginine or placebo supplementation. |
|-----------------|-----------------|-----------------|
| 200-m Time-trial (s) | Control | L-arginine | Placebo |
| First 100-m split (s) | 149.40 ± 9.88 | 146.02 ± 10.34 | 147.58 ± 10.86 |
| Second 100-m split (s) | 71.85 ± 5.08 | 71.01 ± 4.85 | 71.25 ± 4.90 |
| Post-swimming BLa (mmol/L$^{-1}$) | 77.55 ± 5.17 | 75.02 ± 5.75 | 76.34 ± 6.00 |
| Post-swimming BLa (mmol/L$^{-1}$) | 11.2 ± 2.7 | 13.1 ± 1.8 | 12.2 ± 2.7 |
Discussion

To our knowledge, this is the first study to investigate the effects of L-arginine supplementation on freestyle swimming time-trial performance. Based on potential physiological impacts of L-arginine and its potential benefits on BF, accumulation of lactate and ammonia, and exercise performance (7, 9), we hypothesized that L-arginine supplementation would enhance freestyle swimming time-trial performance and attenuate post time-trial swimming blood lactate concentration. The primary findings of this study showed that 7 days of
L-arginine supplementation did not significantly enhance 200-m freestyle swimming time-trial performance in trained/developmental male swimmers. L-arginine supplementation had no significant effect on the split times (the first and second 100-m) of 200-m swimming time-trial. The secondary finding of this study is that L-arginine supplementation did not reduce post time-trial swimming blood lactate concentration. Overall, these findings do not support 7 days of L-arginine supplementation as an ergogenic aid in trained/developmental male swimmers over 200-m. The chosen swimming time-trial distance in the present study is a unique swimming distance due to the high physiological, metabolic and technical demands (21) and therefore performance depends on swimmers’ skill (27). Considering trained swimmers would probably perform this distance in less than three minutes, as observed in the present study, the findings can be interpreted for trained swimmers. However, elite swimmers are significantly faster than lower standard competitors and given male world record is less than two minutes in this particular distance (01:42:00 min), the time-trial data in this study is not comparable to elite swimmers.

Evidence from previous studies that administered acute supplementation remains controversial. While some previous studies reported improvements in anaerobic performance (10, 17) and muscular endurance (18) after L-arginine supplementation, others reported no effect on exercise performance (13–16). The reasons for the disparate findings are unclear but may be explained by differences in the exercise protocols or/and by the supplementation loading period. Despite a longer duration (7-day vs. acute) and higher dose (~ 8 g/d vs. 3 g/d) of L-arginine supplementation in the present study, our findings are in line with previous performance studies, reporting no effect of L-arginine supplementation on maximal dynamic strength (16), muscle endurance (13, 14), or performance in intermittent anaerobic exercise (15).

Enhanced exercise performance was attributed to elevated NO bioavailability following L-arginine supplementation (7). Whilst it is known that increased NO bioavailability via NO precursor supplementation enhance vasodilation and BF, NO has also been reported: (I) to inhibit sympathetic vasoconstriction in resting and contracting skeletal muscle (sympatholysis) (28–30), and (II) to lower muscle sympathetic nerve activity

Figure 3. Group mean (SD) and individual post-swimming blood lactate concentrations after 7-day L-arginine or placebo supplementation are shown in the black and dashed lines, respectively. There was no significant difference in post-swimming blood lactate concentration between L-arginine and placebo trials.
during exercise (31). Overall, these findings suggest that a potential ergogenic effect of L-arginine likely depends on if it elevates NO bioavailability. Although 6 g/d of L-arginine has been reported to elevate plasma L-arginine level (19, 32), elevated plasma L-arginine level might not always stimulate further NO production given that L-arginine would largely be absorbed by the liver through digestive tract after oral consumption (33). Therefore, the lack of effect of L-arginine supplementation on 200-m freestyle swimming time-trial may be because L-arginine supplementation did not lead to an elevation in NO bioavailability due to its insufficient uptake into the systematic circulation.

In the present study, there was no effect of L-arginine supplementation on blood lactate concentration immediately after 200-m freestyle swimming time-trial. While this result is consistent with some previous studies which showed no effect of supplementation on blood lactate concentration (1, 19, 20) and performance (19, 34, 35), in contrast to some others (9, 36, 37). Although speculative, the disparate findings might be related to the administration of L-arginine with other components (e.g. arginine aspartate and glutamate arginine salt) or differences in exercise task (submaximal vs. maximal). Nevertheless, the finding of the present study does not support that L-arginine supplementation might reduce post time-trial swimming blood lactate concentration in trained/developmental male swimmers.

The present study has several limitations that should be addressed in future investigations. A major limitation of this study is that we did not measure arginine concentrations and/or any markers of NO capacity (e.g. plasma nitrite concentration). Although L-arginine supplementation was reported to increases plasma arginine concentration in previous studies that applied similar doses (9, 36, 37), increase in markers of NO production was rare (7). We cannot confirm if there is, or/and to what extent, any change L-arginine concentration or NO bioavailability in the present study. Another limitation in this study was that we were unable to control participants’ diet and relied instead on the participants recording their dietary intake in the 24h before the control arm and replicating this before subsequent arms. While the participants reported that they had complied with this requirement, future studies might control pretest diet more rigorously. Since it was self-reported, monitoring of supplement compliance can also be considered as a limitation despite participants reporting that they fully complied with their supplement use. It has been suggested that the hormonal effects of estrogen increase NOS activity, which may lower muscle sympathetic nerve activity and thus reduce vasoconstriction (38). Therefore, the possibility that L-arginine supplementation may be ergogenic for female athletes cannot be ruled out and further research is required in female athletes. Finally, the time-trials were hand timed in the present study, which might have caused missing small performance chances given the error of such a method is likely higher compared with electronic timing methods. Therefore, it is suggested the use of electronic timing pads to overcome this limitation for future studies

**Conclusions**

In conclusion, one-week of L-arginine supplementation with 8 g/d had no ergogenic effect on middle-distance (200-m), freestyle swimming performance in trained/developmental male swimmers. Therefore, it is premature to suggest L-arginine supplementation as an ergogenic aid to enhance freestyle swimming performance at least in
trained/developmental male swimmers. Further studies are required to assess the effect of L-arginine supplementation in other swimming distances (>200-m) and female swimmers with different training status (e.g. recreational people and/or master athletes), before making any recommendation about its use as an ergogenic aid.

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**Author contributions**

Conceptualization, O.E., R.K.; methodology, O.E., R.K.; validation, O.E.; formal analysis, O.E., R.K.; investigation, O.E.; data curation, O.E., R.K.; writing—original draft preparation, O.E., R.K.; writing—review and editing, O.E., R.K. All authors have read and agreed to the published version of the manuscript.

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**Data availability statement**

Data is available for research purpose upon reasonable request to the corresponding author.

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