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# L-Arginine Reduces Exercise-Induced Increase in Plasma Lactate and Ammonia



**Abstract**

To investigate the effect of L-arginine supplementation (L-ARG) on physiological and metabolic changes during exercise, we determined in a double-blind study the cardiorespiratory (heart rate, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) and the metabolic (lactate and ammonia) responses to maximal exercise after either an intravenous L-ARG hydrochloride salt or placebo load in 8 healthy subjects.
Exercise-induced increases in heart rate, $\dot{V}O_2$ and $\dot{V}CO_2$ were not significantly different after L-ARG or placebo. By contrast, peak plasma ammonia and lactate were significantly decreased after L-ARG load ($60.6 \pm 8.2$ vs. $73.1 \pm 9.1$ $\mu mol \times l^{-1}$, $p < 0.01$ and $7.1 \pm 0.7$ vs. $8.2 \pm 1.1$ $mmol \times l^{-1}$, $p < 0.01$, for ammonia and lactate, respectively). Plasma L-citrulline increased significantly during exercise only after L-ARG load, despite a concomitant decrease in plasma L-ARG. Furthermore, a significant inverse relationship was observed between changes in lactate and L-citrulline concentrations after L-ARG load ($r = -0.84$, $p = 0.009$).
These results demonstrate that intravenous L-ARG reduces significantly exercise-induced increase in plasma lactate and ammonia. Taken together, the specific L-citrulline increase and the inverse relationship observed between L-citrulline and plasma lactate after L-ARG might support that L-ARG supplementation enhances the L-arginine-nitric oxide (NO) pathway during exercise.

**Key words**
Muscle metabolism · NO · L-citrulline · L-ornithine



## Introduction

Metabolic changes going along with muscular activity are usually considered as important peripheral limiting factors for physical exercise. Beside energy substrate depletion or poor oxygen supply, ammonia and lactate, studied through their plasma accumulation during exhaustive exercise, are important factors involved in muscular fatigue mechanisms [6,15,22,24,25]. Indeed, lactate and ammonia accumulation increases muscular hydrogen ion concentration and acidity, depressing the force generating capacity of the working muscle [1,10,12].

Some attempts have therefore been made to reduce accumulation of these metabolites during exercise in man, using supplementation of amino acids known to induce beneficial metabolic changes. Among them, L-arginine (L-ARG), an intermediate of the urea cycle, was highlighted by the discovery of its precursor role in the formation of nitric oxide (NO) [18]. Considering this amino acid, chronic treatment (4 to 8 weeks) with oral L-arginine-aspartate salts has been shown to reduce blood lactate [11] or ammonia [8] after maximal or submaximal exercises. Similarly, Eto et al. [9] observed that acute oral administration of 20 grams of L-arginine-glutamate salt reduced the exercise-induced increase in plasma ammonia, as compared to placebo.

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These results suggested but did not clearly establish a beneficial effect of L-ARG supplementation on exercise-associated blood metabolite changes. The aim of the present study was to investigate whether L-ARG might reduce actually exercise-induced increase in blood lactate and ammonia and to uncover the implicated mechanism, by the simultaneous determination of the L-arginine metabolically related amino acids. We therefore determined cardiorespiratory and metabolic responses to maximal exercise after either an intravenous L-ARG hydrochloride salt or placebo load in healthy subjects. Furthermore, we determined simultaneously plasma L-ARG and the metabolically derived aminoacids, plasma L-ornithine, as a urea cycle marker [3] and plasma L-citrulline, the by-product of NO synthesis [22].



## Methods

### Subjects

Eight healthy men, after receiving informed consent, participated in this study which was approved by the University Review Board for Human Studies. None of them had prior history of cardiovascular disease or was taking any medication. They were active in recreational physical activities but not entered in an endurance training program.

### Study design and exercise protocol

The experiment had a double-blind, randomized, placebo controlled, cross-overdesign. All subjects underwent two tests, separated by one week, receiving either intravenous L-ARG or placebo.

After an overnight fast, an indwelling catheter was inserted into a forearm vein under local anesthesia, to allow for L-ARG or placebo load and blood sampling. The L-ARG load consisted of 3 grams of L-arginine hydrochloride (Veyron, Paris, France) dissolved in 30 ml of 0.9 % saline solution which was rapidly (30 seconds) infused. For the placebo load only the 30 ml of saline was infused.



Exercise was performed 90 minutes after L-ARG or placebo administration, in seated position on a cycle ergometer (Godart, Bilthoven, Netherlands). Pedalling frequency was held constant at 60 rpm. After an initial period of two minutes at 0 W, followed by a 2-minutes period at 50 W, the workload was thereafter increased by 25 W each two minutes in order to reach the maximal power output, previously determined for each subject. Each subject underwent exactly the same protocol for the two tests, the second exercise test (power and duration) being set as the first one.

### Hemodynamic and respiratory parameters

Electrocardiogram and respiratory parameters were monitored throughout the exercise and during the first 30 minutes of recovery. Heart rate was obtained from a continuous electrocardiogram recorder (Schiller ECG, Paris, France). Respiratory variables, $\dot{V}O_2$, $\dot{V}CO_2$ and minute ventilation ($\dot{V}$), were determined breath by breath using an original device $O_2$-$CO_2$ analyser (Medisoft, Dinant, Belgium).

### Blood sampling and analysis

A first blood sample was taken before L-ARG or placebo infusion. After 90 minutes, a second blood sample was collected and exercise began. This time delay was chosen to afford stable plasma L-ARG concentrations, as inferred from preliminary L-ARG kinetic patterns obtained in four subjects receiving the same intravenous load. Blood samples were then obtained at the end of exercise and 1, 2, 3, 4, 5, 8, 10, 15, 20, 30, 40 and 60 minutes after exercise. All the samples were obtained in seated position.

In all samples, blood lactate was assayed by an enzymatic method using lactate oxidase (GM7 Analox analyzer, London, UK). Plasma ammonia was obtained by an enzymatic method (Cobas Bio, Roche Diagnostics, Basel, Switzerland), in all samples at 2, 4, 10 and 30 minutes of recovery. Four plasma amino acid determinations were performed before L-ARG or placebo load, before beginning of exercise, at the end of exercise and after 60 minutes of recovery, using liquid chromatography separation and fluorimetric detection with orthophtaldialdehyde (Liquimat IV Kontron, Zurich, Switzerland). The mean variation coefficients were 5 %, 2.9 % and 6.5 % for lactate, ammonia and amino acids, respectively.

### Statistical analysis

All values are means ± SEM. The cardio-respiratory parameters obtained after placebo or L-ARG load were compared using Student's paired t-test. Changes in cardio-respiratory parameters and metabolites concentrations were assessed by a two-way ANOVA with repeated measures, with consideration of the effect of L-arginine and the effect of exercise. *A posteriori* Tukey's test was used after analysis of variance to evaluate when means of placebo and L-ARG loading subjects were significantly different from baselines and from each other. Relationships between two groups of variables were assessed by calculating Pearson correlation coefficient. Statistical significance required a $p < 0.05$.

## Results

Subjects were 29 ± 4 years old with a mean body weight of 73 ± 2 kg. Maximal workload (209 ± 11 W) and duration of exercise (17.2 ± 1.0 minutes) were held steady for the two exercise tests.

The effect of L-ARG administration on cardio-respiratory parameters are shown in Fig. **1**, with a significant increase observed during exercise ($p < 0.001$), heart rate and ventilatory air flow were not modified by L-ARG administration. Although not significantly different, exercise-induced oxygen uptake and carbon dioxide production tended to be lesser after L-ARG load, as compared to placebo. Recovery was similar in the two groups.

Time courses of blood lactate and ammonia are presented in Fig. **2**. As commonly observed, both lactate and ammonia concentrations increased during exercise and reached peak values in the first minutes of recovery (from 1.5 ± 0.1 to 8.2 ± 1.1 mmol × $l^{-1}$, $p < 0.001$ and from 25.2 ± 4.0 to 73.1 ± 9.1 µmol × $l^{-1}$, $p < 0.001$, for lactate and ammonia, respectively, in placebo ). L-ARG load reduced significantly peak plasma lactate (7.1 ± 0.7mmol × $l^{-1}$) and ammonia (60.6 ± 8.2 µmol × $l^{-1}$) concentrations. Significant differences ($p < 0.05$) were observed at the third and fifth minutes of recovery, for ammonia, and from the third to the tenth minute of recovery, for lactate. The

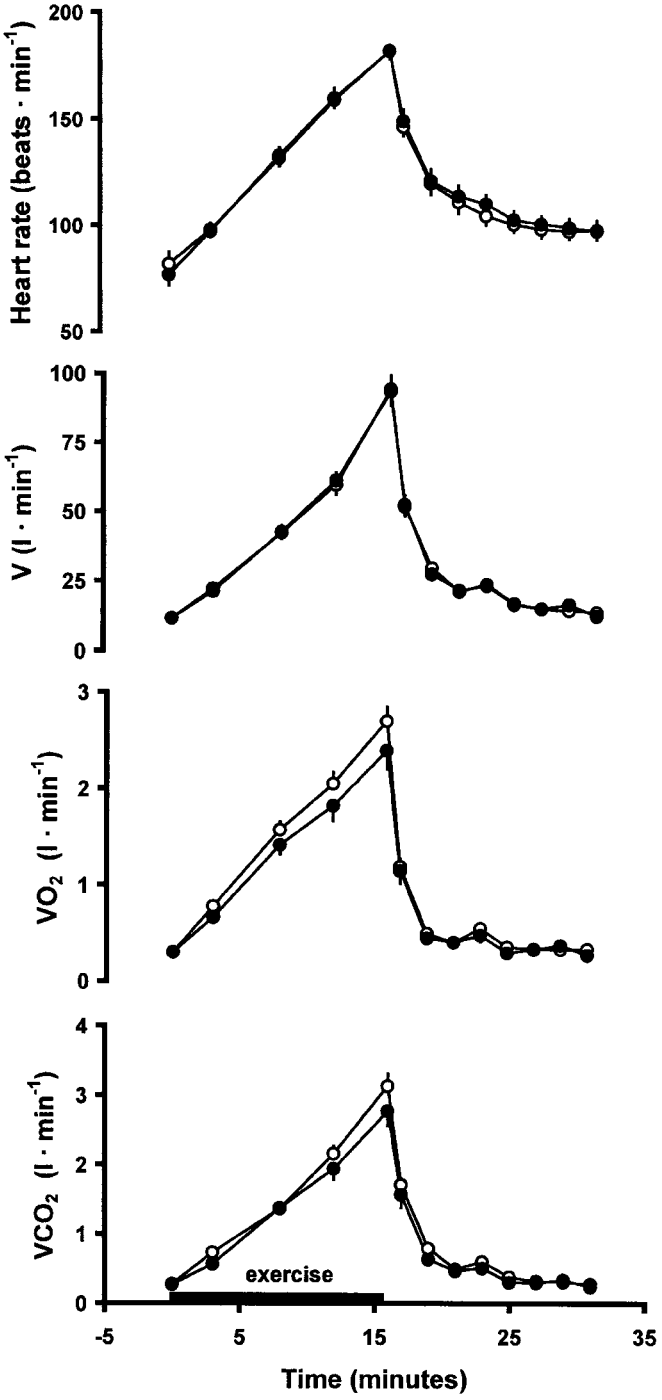


Fig. **1** Time course of heart rate, ventilatory air flow ($\dot{V}$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) during and after exercise after intravenous administration of L-ARG (●) or placebo (○).

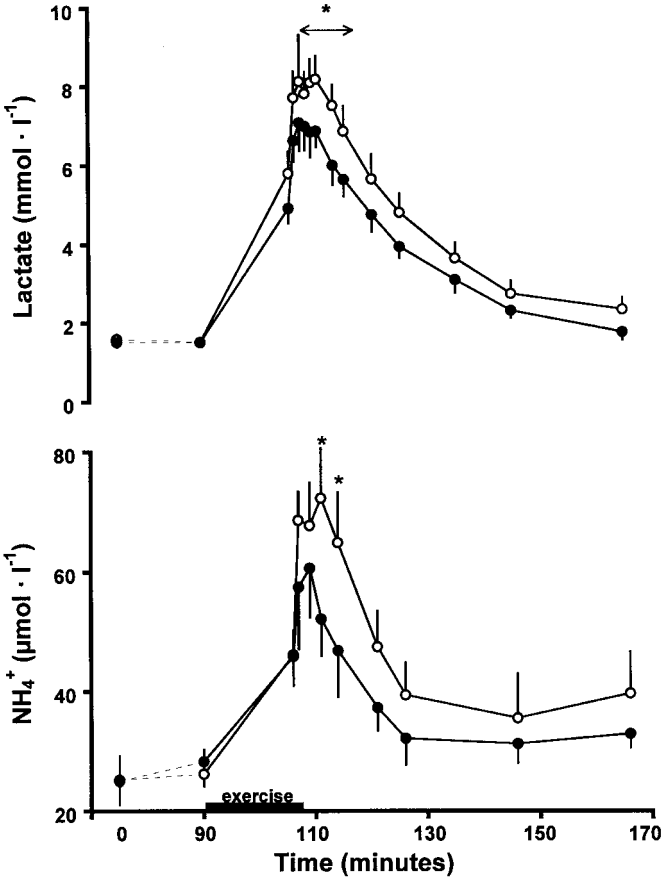


Fig. **2** Blood lactate and ammonia concentrations (means ± SEM) at rest, during maximal exercise and during recovery after L-arginine (●) or placebo (○) intravenous administration. Rest values are displayed on the graphs at time 0. Differences between L-arginine and placebo: *$p < 0.05$.

concentrations observed after placebo were always greater than those observed after L-ARG administration, for both lactate and ammonia.

Fig. **3** illustrates the time course of plasma L-arginine, L-ornithine and L-citrulline concentrations. No significant changes in the concentrations of these amino acids could be observed in placebo-treated subjects. However, significant differences were noted between L-ARG and placebo-treated subjects. L-ARG administration increased both L-arginine and L-ornithine plasma concentrations. During the exercise, plasma L-arginine decreased (from 250 ± 24 to 220 ± 20 µmol × l$^{-1}$, P = 0.03), plasma L-citrulline increased (from 42.8 ± 3.1 to 54.9 ± 5.7 µmol × l$^{-1}$, P = 0.01), in L-ARG administered subjects. Interestingly during exercise, a negative correlation was observed between lactate and L-citrulline changes after L-ARG load (r = –0.84, P = 0.009, Fig. **4**).

## Discussion

The main results of this study demonstrate that intravenous L-ARG reduced actually exercise-induced increases in plasma lactate and ammonia.

Interestingly, such marked effects on lactate and ammonia plasma levels were observed despite the relatively low dose of 3 grams intravenous L-arginine hydrochloride used. Thus, exercise was performed in this study when L-ARG residual concentrations were only twice the basal values. Since bioavailability of oral L-ARG is about 70% [4] as compared to intravenous load, it suggests that relatively small oral L-ARG doses (4 – 5 grams) could be needed to obtain similar results. Such a dose was lower than the 10 to 30 g oral L-ARG previously used during exercise in healthy humans [5,8,9,11]. This ability of L-ARG to lower the metabolic parameters involved in muscular fatigue is further supported by observations that long term low dose oral L-ARG

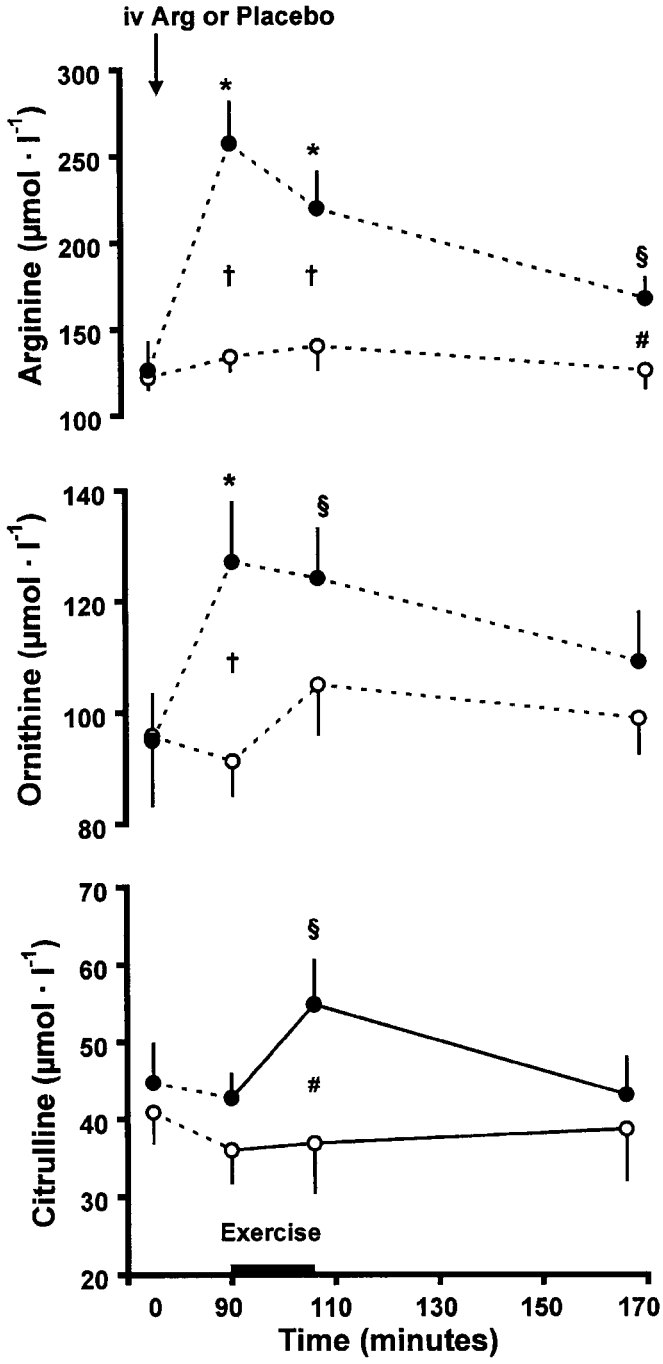


Fig. **3** Plasma L-arginine, L-ornithine and L-citrulline concentrations (means ± SEM) at rest, at the beginning and the end of an exhaustive exercise and at the end of recovery after L-ARG (●) or placebo (○) intravenous administration. Rest values are displayed on the graphs at time 0. Differences with rest values: § $p < 0.05$; * $p < 0.01$. Differences between L-arginine and placebo: # $p < 0.05$, † $p < 0.01$.

supplementation [5.6 or 12.6 g × day$^{-1}$] partly restores exercise capacity of chronic heart failure or angina pectoris patients [7,19]

In agreement with previous studies [5,9,11], cardiorespiratory parameters of our subjects were not significantly modified after L-ARG load, suggesting it could not account for the plasma ammonia and lactate decreases observed during exercise after L-ARG.

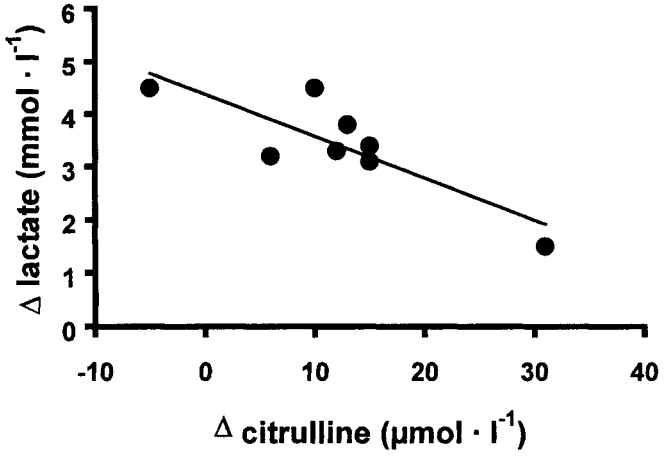


Fig. **4** Relationship between lactate and L-citrulline during exercise in L-ARG -infused subjects. $r = -0.84$, $p = 0.009$. Deltas are differences between values obtained before and after exercise.

Conversely, the changes observed in the L-ARG derived amino-acids L-ornithine and L-citrulline is particularly interesting, in view of the fate of the L-ARG load. L-ARG load increased plasma L-arginine and L-ornithine at rest, whereas L-citrulline concentrations were not affected [14]. This dose-dependent increase of L-ornithine after L-ARG load may be interpreted as the well-known enhancement of ureagenesis by L-ARG supplementation [2]. Such ureagenesis enhancement could explain the higher ammonia removal rate and the consequent lower plasma values during and after exercise. On the other hand, plasma L-citrulline, the by-product of NO synthesis [22], increased significantly and specifically during exercise after L-ARG whereas L-ornithine, the other intermediate of the urea cycle, did not increase. This suggests that a part of the L-ARG load was also directed toward L-citrulline and NO production during exercise. Consistently, increased NO synthase activity and increased NO production has been observed during exercise [13,17,20,21].

We observed a close inverse relationship between changes in lactate levels and L-citrulline concentrations during exercise after L-ARG administration. It is likely that the blunted lactate levels observed during exercise after L-ARG administration could be due, at least partly, to the L-ARG-NO pathway stimulation. Accordingly, it has been reported that NO synthase inhibition, enhanced plasma lactate concentrations during exercise [16] and reduced NO production goes along with reduced exercise capacity in heart transplant patients [23].

In these experiments we have not tested the effect of L-arginine supplementation on the exercise capacity of the subjects. The study was focused on the changes of the metabolites during exercise. The two double-blind randomized exercise tests used for both the placebo and L-ARG load were kept on an absolutely identical level. Thus, it was not possible to determine if L-ARG supplementation could enhance the physical muscular performance of the subjects. Nevertheless, the results of this study show that L-ARG reduces significantly lactate and ammonia release and shows a tendency to reduce oxygen consumption/carbone dioxide release, suggesting an increased exercise capacity. Further studies could be useful to investigate whether oral L-ARG might also improve exercise capacity in healthy humans.

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