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著者	Demura Shinichi, Yamada Takayoshi, Yamaji Shunsuke, Komatsu Miho, Morishita Koji
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Title

The effect of L-ornithine hydrochloride ingestion on performance during incremental exhaustive ergometer bicycle exercise and ammonia metabolism during and after exercise

Authors

Shinichi Demura Ph. D.[1], Takayoshi Yamada Ph. D.[2], Shunsuke Yamaji Ph. D.[3], Miho Komatsu[4], Koji Morishita[4]

1 Graduate school of Natural Science & Technology, Kanazawa University

2 General Education Center, Fukui National College of Technology

3 Faculty of Medical Science, University of Fukui

4 Healthcare Products Development Center, KYOWA HAKKO BIO CO., LTD.

Corresponding author

Takayoshi Yamada Ph. D.

Fukui National College of Technology

Mailing address: Geshi, Sabae, Fukui 916-8507 Japan

Telephone: +81-778-62-8229

Fax: +81-778-62-3417

e-mail: takay@fukui-nct.ac.jp

Running title

Effect of L-ornithine hydrochloride supplement

Abstract

Objectives: L-ornithine plays an important role in ammonia metabolism via the urea cycle. This study aimed to examine the effect of L-ornithine hydrochloride ingestion on performance during incremental exhaustive ergometer bicycle exercise and ammonia metabolism during and after exercise. **Subjects/Methods:** Fourteen healthy young adults (age: 22.2 +/- 1.0 yr, height: 173.5 +/- 4.6 cm, body-mass: 72.5 +/- 12.5 kg) who trained regularly conducted incremental exhaustive ergometer bicycle exercises after L-ornithine hydrochloride supplementation (0.1g/kg, body-mass) and placebo conditions with a cross-over design. The exercise time (sec) of the incremental ergometer exercise, exercise intensity at exhaustion (watt), maximal oxygen uptake (ml/kg/min), maximal heart rate (bpm) and the following serum parameters were measured before ingestion, one hour after ingestion, just after exhaustion and 15 min after exhaustion: ornithine, ammonia, urea, lactic acid and glutamate. All indices on maximal aerobic capacity showed insignificant differences between both conditions. **Results:** Plasma ammonia concentrations just after exhaustion and at 15 min after exhaustion were significantly more with placebo ingestion than with ornithine. Plasma glutamate concentrations were significantly higher after exhaustion with placebo ingestion than with ornithine. **Conclusions:** It was suggested that while the ingestion of L-ornithine hydrochloride before exercise cannot be expected to improve performance, it does increase the ability to buffer ammonia both during and after exercise.

Key words: L-ornithine hydrochloride, aerobic exercise, fatigue recovery, ammonia metabolism

Introduction

Skeletal muscle fatigue occurs as the result of physical exercise and leads to a marked decrease in performance. Depletion of energy resources required for muscle contraction, accumulation of metabolic products, an imbalance of the internal milieu have been pointed out as major factors of physical fatigue (Allen et al., 2008). Fatigue induced by an imbalance of the internal milieu occurs due to the accumulation of lactic acid and ammonia produced by muscle contraction (Wada & Matsunaga, 1997). The accumulation of lactic acid and ammonia is a limiting factor of performance in moderate to high intensity exercise. Namely, both agents induce force output decrease of skeletal muscles because of their marked inhibition of pH homeostasis in the sarcoplasma. In particular, because the ammonia production consumes substantial amounts of inosinic acid (IMP) (Stathis et al., 1994), the ability to produce adenosine triphosphate (ATP) via oxidative phosphorylation in the skeletal muscles decreases. Ultimately, the accumulation of ammonia in skeletal muscle induces muscle fatigue (Sahlin et al., 1990; MacLean et al., 1991) and is linked to a decrease in performance.

During anaerobic exercise, lactic acid conveyed to the liver by the blood is used for the generation of glucose via the Cori cycle. Moreover, during aerobic exercise, lactic acid is decomposed to H_2O and CO_2 after being used for the regeneration of ATP via the tricarboxylic acid (TCA) cycle. Meanwhile, ammonia is metabolized to urea via the urea cycle in hepatocytes (Hirai et al., 1995). Another organ for ammonia detoxification is the skeletal muscle itself. Skeletal muscle has a high capacity to buffer ammonia by alanine generated from pyruvate and by glutamine from glutamate and α-oxoglutarate generated via transamination reactions (Graham & MacLean., 1992).

L-ornithine is a free amino acid that is not coded by DNA or involved in protein synthesis. In addition, although many effects of its ingestion have not been clarified, the L-ornithine promotes growth hormone release by stimulating the pituitary gland (Evain-Brion et al., 1982; Demura et al., 2010). Additionally, L-ornithine is one product of arginase enzyme activity on L-arginine, and is also related to the creation of urea. In other words, L-ornithine is a central part of the urea cycle, which allows for the disposal of excess nitrogen

(Rodwell, 2000). From the above, the metabolism of ammonia produced by skeletal muscle contraction is expected to be accentuated by L-ornithine ingestion. Moreover, performances may be maintained or enhanced because the decrease in the ability to produce ATP via oxidative phosphorylation in the skeletal muscles is inhibited by ammonia metabolism accentuation. However, from this viewpoint, few previous studies have examined the effects of L-ornithine ingestion. Among them, Sugino et al. (2008) examined the effect of L-ornithine ingestion on the recovery from physical fatigue induced by extended exercise and accentuation of lipid metabolism, and reported that fatigue recovery and accentuation of lipid metabolism are identified by L-ornithine ingestion. However, the following two mixed viewpoints were found in Sugino et al.'s study: the above-stated fatigue reduction due to accentuating ammonia metabolism, and the accentuating lipid metabolism, which means the fatigue inhibition with the increase of energy substrate during exercise. It has not been clarified whether maintaining/enhancing performance and fatigue reduction by accentuating ammonia metabolism via the urea cycle with L-ornithine ingestion is found.

This study aimed to examine the effect of the L-ornithine hydrochloride ingestion on performance during maximal aerobic exercise and the subsequent recovery from fatigue in human beings.

Subjects and Methods

Subjects

Fourteen healthy young trained male adults who major in physical and health education participated in this study (age: 22.2 +/- 1.0 yr, height: 173.5 +/- 4.6 cm, body-mass: 72.5 +/- 12.5 kg). They had habitually performed sports such as track and field, swimming, soccer and basketball over 3 times per week (3.1 +/- 1.0 times/week), with moderate to high intensity over two hours per session (2.2 +/- 1.0 hour/time). Written informed consent was obtained from all subjects after a full explanation of the experimental purpose and protocol. Moreover, experimental protocol in this study was approved by the Kanazawa University Health & Sports Science Ethics Committee.

Experimental design

The experimental design was a double blinded cross-over method. Namely, subjects participated in both conditions: L-ornithine hydrochloride supplementation and placebo (indigestible dextrin aqueous solution) conditions. Due to the cross-over design, all subjects participated in both conditions at the same time with a week wash out period between conditions. Moreover, the test condition order was counter balanced to eliminate order effect. In addition, subjects were instructed to refrain from intensive exercise for two days prior to the experiment and to fast for at least two hours before starting exercise to avoid a nutritional imbalance created by eating and drinking. Subjects were also instructed not to consume beverages or food containing caffeine during the experiment period.

Ingestion conditions

Subjects ingested L-ornithine hydrochloride or an indigestible dextrin aqueous solution with the same flavor (placebo) at the ratio of 0.1g per kilogram body-mass. Isomers exist in almost all amino acids and are divided into levorotatory and dextrorotatory amino acids. L-ornithine corresponds to the former group of amino acids and is classified as naturally occurring. The hydrochloride of the L-ornithine was used in this study. In addition, the effect of L-ornithine hydrochloride ingestion was examined using the double blinded cross-over method stated above. Therefore, a placebo was required so that both subjects and testers were not biased as to the effects of L-ornithine hydrochloride. Nutrients that are included in indigestible dextrin aqueous solution with the same flavor as L-ornithine hydrochloride solution are difficult to digest. Hence, the influence of the nutrients is considered to be kept at a minimum.

Incremental exhaustive ergometer bicycle exercises

In both conditions, subjects conducted incremental exhaustive ergometer bicycle exercises following non-loaded pedaling (0 watt) for 3 min. They were instructed to maintain 60 pedal revolutions per min during incremental exercise. We judged exhaustion when they could not continue pedaling at a rate of 40 revolutions

per min (van Loon et al., 2000). In addition, a graded rate of exercise load was calculated using the following formulas considering each subjects' physical characteristics, and the exercise load was increased every minute during exercise (Wasserman, 1976).

・Incremental ratio of exercise load (Watts/min.) = (peak oxygen consumption (ml/min)[a] − oxygen consumption (ml/min)[b]) / 100

[a] Oxygen consumption during unloaded pedaling (ml/min) = 150 + (6 × body-mass (kg)), [b] Peak oxygen consumption (ml/min.) = (height (cm) – age (yr)) × 20

Experimental procedure

Subjects ingested L-ornithine hydrochloride and placebo aqueous solution after blood sampling. They then took a one hour rest in a sitting posture after the ingestion, followed by a second round of blood sampling. Then the incremental exhaustive ergometer bicycle exercise was conducted. After exhaustion, a third round of blood sampling was performed, and subjects took a 15 min rest in a sitting posture. The fourth round of blood sampling was conducted after the 15 min rest. All venous blood samples were obtained from an indwelling cannula in the antecubital vein.

Parameters

Each blood sample was analyzed for ornithine, ammonia, urea, lactic acid and glutamate. One milliliter of blood was transferred into 4 ml of chilled 0.6 M sodium tungstate for ammonia analysis, 1 ml of blood was transferred into 1 ml of chilled 0.8 M perchloric acid for lactate analysis, and 5 ml of blood were transferred into 65IU heparin sodium for ornithine, urea and glutamate analysis. These processes were carried out within 30 s of drawing the blood. The samples were immediately centrifuged and the supernatants were placed in chilled containers. These procedures were completed within 5 min for ammonia and lactic acid analyses and 30 min for ornithine, urea and glutamate analyses. The samples for ammonia and lactic acid determination were analyzed immediately after the experiment using a JCA-BM8000 (JEOL, Japan) and

Micro plate reader (Molecular Devices, Japan) according to the method of Okuda (1966) and enzymatic analysis by the lactic oxidase test. Sensitivity, inter-assay and intra-assay coefficients of variation (CV) of this assay were 3.33mmol/l, 2.47 and 2.0% for ammonia; and 2.12mmol/l, 0.46 and 0.49% for lactic acid. The samples for ornithine, urea and gluamic acid determination were analyzed by high performance liquid chromatography using HPLC system (Shimazu and Hitachi, Japan). Sensitivity, inter-assay and intra-assay coefficients of variation (CV) of this assay were 5.92nmol/l, 4.94 and 0.00%. The above stated serum parameters were measured before ingestion, one hour after ingestion, just after exhaustion and 15 min after exhaustion.

Expired gases were continuously sampled in a breath-by-breath method during exercise in both conditions. Oxygen consumption ($\dot{V}O_2$) was measured with an automatic expired gas analytic system (AE-280S; Minato Medical Science, Japan), and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) during incremental exhaustive ergometer bicycle exercises was calculated. Moreover, the heart rate during exercise was continuously measured (ML-1200; Fukuda, Japan) and peak heart rate was calculated as maximal heart rate. In addition, the exercise time (sec) of the incremental ergometer exercise and exercise intensity at exhaustion (watt) were selected as evaluation parameters.

Statistical analysis

A paired t-test was used to examine the difference in maximal aerobic performance between the L-ornithine hydrochloride and placebo ingestion conditions. Moreover, two-way repeated measures analysis of variance (ingestion condition × measurement time) was used to examine the effect of fatigue reduction in both conditions. When showing a significant main or interaction effect, Tukey's honestly significant difference was used as post-hoc analysis to examine specific mean differences. An alpha concentration of .05 was used for all tests.

Results

Table 1 shows the results of a paired t-test between both conditions for exercise time, exercise intensity, maximal oxygen consumption, and maximal heart rate during incremental ergometer exercise. All indices on maximal aerobic capacity showed no significant differences between both conditions.

Figure 1 shows the plasma ornithine, ammonia, urea, lactic acid and glutamate concentrations at times before ingestion, one hour after ingestion, just after exhaustion and 15 min after exhaustion in both conditions. In the plasma lactic acid concentration, significant main effect was found in measurement time ($F = 255.70$, $p < .000$), and from the results of post-hoc, it was the largest just after exhaustion and 15 min after exhaustion than before and one hour after ingestion in both conditions. Significant main effects were identified in ingestion condition and measurement time in the plasma ammonia concentration ($F = 7.78$, $p < .015$; $F = 45.25$, $p < .000$, respectively). It was the largest just after exhaustion and 15 min after exhaustion than before and one hour after ingestion in both conditions. Moreover, it was significantly higher just after exhaustion and at 15 min after exhaustion in the ornithine ingestion condition than in the placebo condition. Significant main effect in measurement time and interaction were found in the plasma urea concentration ($F = 13.37$, $p = .001$; $F = 3.58$, $p = .050$, respectively). It was significantly higher just after exhaustion and 15 min after exhaustion than those measured at one hour after ingestion in the ornithine ingestion condition. In the plasma glutamate concentration, significant main effect in measurement time and interaction were found ($F = 6.63$, $p = .008$; $F = 5.53$, $p = .015$, respectively). It was significantly higher in the ornithine ingestion condition than in the placebo condition at one hour after ingestion and 15 min after exhaustion. Moreover, it was higher at one hour after ingestion, just after exhaustion and 15 min after exhaustion than before ingestion in the ornithine ingestion condition. Significant main effects in ingestion condition and measurement time, and interaction were identified ($F = 268.24$, $p < .000$; $F = 105.95$, $p < .000$; $F = 99.98$, $p < .000$). They were significantly higher at one hour after ingestion, just after exhaustion, and 15 min after exhaustion than those measured before ingestion in the ornithine ingestion condition. And the concentration decreased significantly from just after exhaustion to 15 min after exhaustion in the ornithine ingestion condition. Moreover, it was greater

before ingestion, at one hour after ingestion and just after exhaustion than that at 15 min after exhaustion in the placebo ingestion condition. It was significantly greater at one hour after ingestion, just after exhaustion, and 15 min after exhaustion in the ornithine ingestion condition than those in the placebo condition.

Discussion

The present main result was that the plasma ammonia concentration immediately following and 15 min after the incremental ergometer exercise in the L-ornithine ingestion condition was significantly lower than that in placebo condition. In addition, this decrease in the plasma ammonia concentration may be attributed to L-ornithine hydrochloride ingestion before exercise due to the decrease in the plasma ornithine concentration in the same phase. Substantial ammonia and inosinic acid (IMP) are produced by the deamination of adenosine monophosphate (AMP) in skeletal muscle during high intensity exercise (Stathis et al., 1994). Moreover, the muscle's capacity to produce adenosine triphosphate (ATP) via oxidative phosphorylation decreases during this process, and muscle fatigue occurs (Sahlin et al., 1990; MacLean et al., 1991). Inhibited homeostasis produced by this ammonia production is recovered by ammonia metabolism in the urea cycle within the hepatocyte. The ammonia is metabolized by a number of amino acids, such as ornithine, arginine and citrulline, in the urea cycle. Therefore, ammonia metabolism is hypothesized to be accentuated by L-ornithine hydrochloride ingestion. The present results are suggested to inhibit the increase of ammonia concentration during and after incremental exhaustive ergometer bicycle exercises.

However, ammonia is eventually metabolized to urea by ammonia metabolism via the urea cycle. This is to say that urea has been hypothesized to increase by accentuating the metabolism of ammonia, which is produced by skeletal muscle during exercise with L-ornithine hydrochloride ingestion, but the present results differ from this hypothesis. The mechanism of in vivo ammonia detoxification is buffered in skeletal muscle in addition to its metabolism via the urea cycle. Skeletal muscle has a high capacity to buffer ammonia by the alanine produced from pyruvate and glutamine from glutamate and α-oxoglutarate in

transamination reactions (Graham and MacLean., 1992). Because the plasma glutamate concentration significantly increased an hour after the ingestion of L-ornithine hydrochloride and was significantly greater in the L-ornithine hydrochloride condition than that in the placebo condition immediately following and 15 min after exercise, the inhibition of ammonia concentration increase may be largely contributed to the ammonia buffering in the skeletal muscle. A difference between the results and the working hypothesis in this study was found also in the study by Meneguello et al. (2003). They reported that, although a decrease in the plasma ammonia concentration and a reduction in fatigue were found, the plasma urea concentration also decreased during maximal exhaustive exercise after the ingestion of arginine, citrulline and ornithine, which are intermediates of the urea cycle, in rats. Although the subjects and the rest period after exercise differed from those in the present study, their study is similar in the fact that an increase in the plasma urea concentration with accentuation of the rate of the urea cycle was not found. The time lag between creating and releasing urea into blood and the decrease in the serum ammonia concentration due to ammonia metabolism may exist, or the urea released into the blood may be immediately removed by the kidneys. However, this relationship between ammonia and the urea concentration was not clarified in this study because of the low frequency of blood drawing during and after exercise. It is believed that the relationship between the two will have to be studied more in-depth by increasing the frequency of blood drawing. Based on this data, it was found that the ammonia buffering ability during and after incremental exhaustive ergometer bicycle exercise may be enhanced by L-ornithine hydrochloride ingestion before exercise.

Meanwhile, exercise time, exercise intensity, maximal oxygen consumption, maximal heart rate and ratings of perceived exertion during incremental ergometer exercise showed insignificant differences between the ornithine and placebo conditions. As stated above, the substantial ammonia produced by the deamination of AMP in skeletal muscle during high intensity exercise (Stathis et al., 1994) decreases the muscle's capacity to produce ATP via oxidative phosphorylation decreases and results in muscle fatigue (Sahlin et al., 1990; MacLean et al., 1991). Performance was hypothesized to be maintained/enhanced because the decrease in ATP synthesis ability via oxidative phosphorylation in skeletal muscle is inhibited by accentuating ammonia

metabolism via the urea cycle after L-ornithine hydrochloride ingestion, but the present results did not suggest this. Enhancing ammonia buffering in skeletal muscle with L-ornithine hydrochloride ingestion was suggested in the present results. However, because of a decrease of α-ketoglutarate by ammonia buffering in skeletal muscle, oxoloacetic, which is the antecedent, also decreases. The production of NADH via the tricarboxylic acid (TCA) cycle and the ability of ATP production decrease with this change. These phenomena relate to ammonia buffering in skeletal muscle regardless of ornithine hydrochloride ingestion. Therefore, it is considered that the performance during the incremental exhaustive ergometer bicycle exercise is not to be improved by L-ornithine hydrochloride ingestion. The effect of exercise performance improvement by L-ornithine hydrochloride ingestion may not be expected in the case of the present relatively brief, high-intensity exercise. Many previous studies have reported the effects of amino acid ingestion on improving performance, but a variety of exercise types were used (Smith et al., 2008; Zoeller et al., 2007; de Araujo et al., 2006; Norton and Layman. 2006). Namely, there are studies which used short duration, high intensity exercise and studies which used long term, moderate intensity exercise. The selected exercise intensity and duration relate closely to the mechanism of action of amino acid selected in each study. Therefore, the selection of exercise intensity and duration, which induce ammonia accumulation, is important when examining the effect of amino acids found in the urea cycle, as in this study. Considering the above, although the production and accumulation of ammonia were observed by the present exercise intensity and time, improvement of performance induced by accentuating ammonia metabolism by L-ornithine hydrochloride ingestion may not be expected. Such an effect may be found if there is adequate exercise time to allow for blood from the skeletal muscle to be conveyed to the liver.

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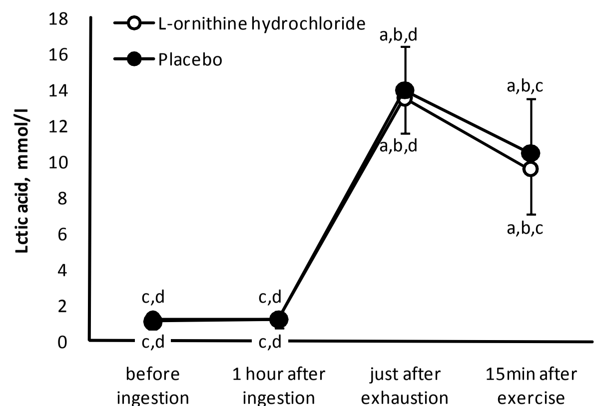
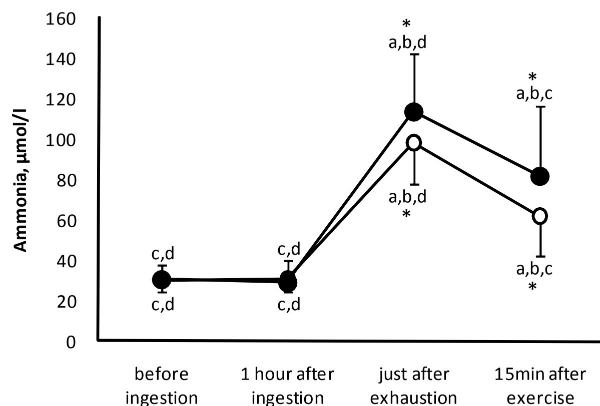
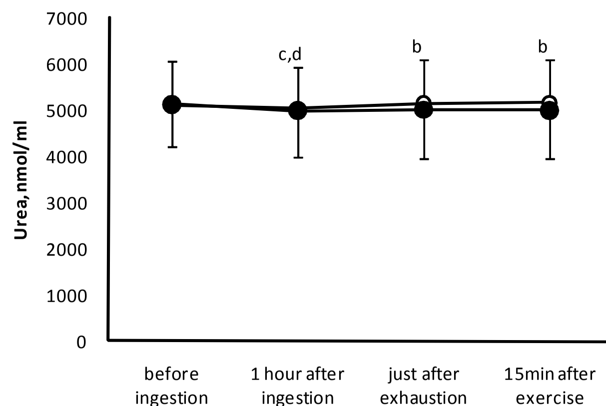
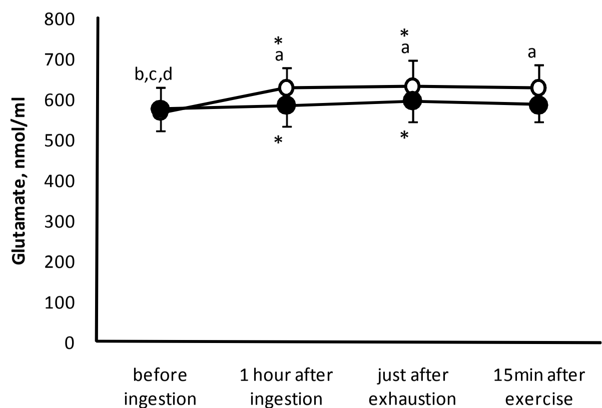
Table 1 Difference of maximal aerobic performance between both conditions

	L-ornithine hydrochloride		Placebo		t-value	*p*
	Mean	SD	Mean	SD		
Exercise time, sec	822.3	177.3	839.7	208.7	1.15	*0.269*
Exercise intensity, watt	319.1	27.9	322.4	25.3	0.66	*0.518*
Maximal oxygen consumption, ml/min/kg	55.7	8.3	55.2	8.0	0.60	*0.558*
Maximal heart rate, beat per min	186.1	11.8	185.9	10.4	0.09	*0.929*


L-ornithine hydrochloride
Placebo
Lctic acid, mmol/l
0
2
4
6
8
10
12
14
16
18
before ingestion
1 hour after ingestion
just after exhaustion
15min after exercise
c,d
c,d
a,b,d
a,b,c


Ammonia, μmol/l
0
20
40
60
80
100
120
140
160
before ingestion
1 hour after ingestion
just after exhaustion
15min after exercise
c,d
c,d
a,b,d
a,b,c
*


Urea, nmol/ml
0
1000
2000
3000
4000
5000
6000
7000
before ingestion
1 hour after ingestion
just after exhaustion
15min after exercise
c,d
b


Glutamate, nmol/ml
0
100
200
300
400
500
600
700
800
before ingestion
1 hour after ingestion
just after exhaustion
15min after exercise
b,c,d
a
*

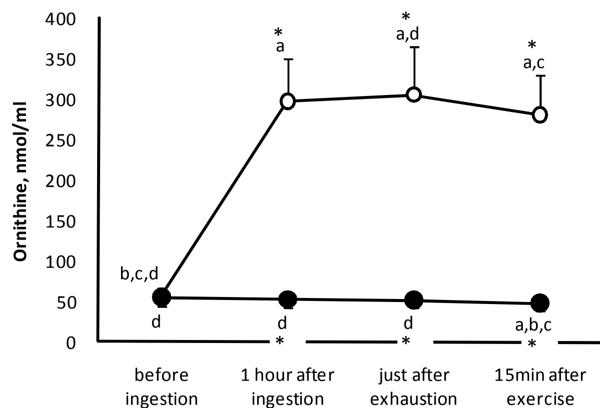

Ornithine, nmol/ml
0
50
100
150
200
250
300
350
400
before ingestion
1 hour after ingestion
just after exhaustion
15min after exercise
b,c,d
d
a
a,d
a,c
a,b,c
*

Figure 1 Plasma ornithine, ammonia, urea, lactic acid and glutamate concentrations at times before ingestion, one hour after ingestion, just after exhaustion and 15 min after exhaustion in both conditions. Values are means ± SD. *, significant difference between both conditions; a, significant difference with value at time before ingestion in each condition; b, significant difference with value at one hour after ingestion in each condition; c, significant difference with value just after exhaustion; d, significant difference with value 15 min after exhaustion.