Effect of L-ornithine hydrochloride ingestion on intermittent maximal anaerobic cycle ergometer performance and fatigue recovery after exercise

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Abstract

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 ammonia metabolism and performance after intermittent maximal anaerobic cycle ergometer exercise. Ten healthy young adults (age: 23.8 +/- 3.9 yr, height: 172.3 +/- 5.5 cm, body-mass: 67.7 +/- 6.1 kg) with regular training experience ingested L-ornithine hydrochloride (0.1g/kg, body-mass) or placebo after 30 seconds of maximal cycling exercise. Five sets of the same maximal cycling exercise were conducted 60 min after ingestion, and maximal cycling exercise was conducted after a 15 min rest. The intensity of cycling exercise was based on each subject's body-mass (0.74N・kg-1). Work volume (watt), peak rpm (rpm) before and after intermittent maximal ergometer exercise and the following serum parameters were measured before ingestion, immediately after exercise and 15 min after exercise: ornithine, ammonia, urea, lactic acid and glutamate. Peak rpm was significantly greater with L-ornithine hydrochloride ingestion than with placebo ingestion. Serum ornithine level was significantly greater with L-ornithine hydrochloride ingestion than with placebo ingestion immediately and 15 min after intermittent maximal cycle ergometer exercise. In conclusion, although maximal anaerobic performance may be improved by L-ornithine hydrochloride ingestion before intermittent maximal anaerobic cycle ergometer exercise, the above may not depend an increase of ammonia metabolism with L-ornithine hydrochloride.

Key words

Introduction

Ammonia, produced by deamination of adenylic acid (AMP) with skeletal muscle contraction and oxidative deamination of amino acids (Goodman and Lowenstein 1977; Lowenstein 1972; Lowenstein and Tornheim 1971) interferes with tissue oxidative metabolism (Lowenstein 1972; McKann and Tower 1961; Katanuma et al. 1966; Bryla and Niedzwiecka 1979; Worcel and Erecinska 1962), and may also induce pyruvate accumulation. Namely, it promotes anaerobiosis and subsequent lactic acid accumulation (Dutton and Berkman 1978; Hindfelt and Siesjo 1970; Schenker et al. 1967). Therefore, ammonia production and accumulation in skeletal muscle during intense exercise with a high contribution of anaerobic energy production triggers a performance decrease (Banister et al., 1983). However, such a decrease in performance may be prevented by the rapid metabolism of ammonia produced in skeletal muscle.

Ammonia produced in skeletal muscle is metabolized into harmless urea via the urea cycle in hepatocytes after transportation to the liver via blood (Hirai et al., 1995). The urea cycle is composed of free amino acids such as L-ornithine and relates closely to the production of urea and L-arginine by the action of the enzyme arginase. Thus, if the response speed of the urea cycle is accentuated, fatigue may be ameliorated by a reduction of the ammonia accumulation in skeletal muscle. Generally, the response speed of the urea cycle is accentuated by the promotion of carbamoyl phosphate syntase 1 activity with an increased concentration of glutamine and N-acetyle glutamate synthesized with ammonia accumulation. It has been reported that the ingestion or intravenous infusion of L-ornithine leads to an increase of the blood-ornithine level, resulting in passive uptakes of ornithine into mitochondoria and the accentuation of ammonia metabolism with an increase in the response speed of the urea cycle (Tsujino et al., 1991; Tsujino et al., 2002). L-ornithine is a free amino acid that plays a central role in the urea cycle and is also important for the disposal of excess nitrogen such as ammonia and ammonium ions (Rodwell, 2000).

Recently, the effect of L-ornithine ingestion on ammonia metabolism increase during intense exercise has been studied (Meneguello et al., 2003; Demura et al., 2010). Demura et al. (2010) examined the effect of L-ornithine ingestion on ammonia metabolism accentuation during an incremental exhaustive bicycle ergometer exercise by using young male adults. It was reported that the blood ammonia level immediately following, and 15 min after, exhaustion was lower in those participants who ingested L-ornithine than in those who ingested a placebo. This suggests that the metabolism of ammonia produced in skeletal muscle during

intense exercise is increased by the ingestion of L-ornithine. Moreover, although maintaining/improving performance is expected by the inhibition of lactic acid accumulation with such anaerobic metabolism, few have examined the effects of L-ornithine ingestion from this perspective.

Meanwhile, the contribution of energy via anaerobic metabolism increases gradually after reaching the anaerobic threshold during incremental exhaustive exercise, becoming greater at the point of exhaustion. However, because the exercise time is relatively long, there is greater reliance on aerobic energy. Fatigue caused by ammonia production and accumulation occurs more greatly in anaerobic metabolism as compared with aerobic metabolism (Majerczak et al., 2008). Therefore, although it has not been examined until now, the greater effect of ammonia metabolism accentuation is expected by L-ornithine ingestion before sprinting exercises which require greater anaerobic metabolism.

This study aimed to examine the effect of L-ornithine hydrochloride ingestion on ammonia metabolism and performance improvement after intermittent maximal anaerobic exercise.

Subjects and Methods

Subjects

Ten healthy young trained male adults who majored in physical and health education participated in this study (age: 23.8 +/- 3.9 yr, height: 172.3 +/- 5.5 cm, body-mass: 67.7 +/- 6.1 kg). They performed sports over three times per week habitually (3.1 +/- 1.0 times/week), with moderate and high intensity and over two hours duration (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, the experimental protocol in this study was approved by the "Kanazawa University Health & Sports Science Ethics Committee".

Experimental design

T The experimental design was a double blinded cross-over method. Subjects participated in the following conditions: L-ornithine hydrochloride supplementation and placebo (indigestible dextrin aqueous solution). Due to the cross-over design, all

subjects participated in both conditions at the same time with a week wash out period. 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 minimal.

Experimental procedure

Fig 1 shows experimental procedure of this study. In both conditions, subjects conducted intermittent maximal anaerobic cycle ergometer exercise after a blood draw. They were instructed to pedal a cycle ergometer, set at a load based on the each subject's body-mass (0.74N/kg) with maximal effort in 30 seconds by tester's signal. Following this, they had a 60 min rest after L-ornithine hydrochloride or placebo ingestion. Then, they conducted 5 sets of the above stated ergometer exercise with a minute rest between sets. They rested for 15 min following a blood draw immediately after intermittent exercise. Then, maximal ergometer exercise was conducted after the blood draw. All venous blood samples were obtained from an indwelling cannula in the antecubital vein. In addition, handlebar and saddle seat heights were based on each subject's physical characteristics (Arslan, 2005).

Parameters

1) Pedaling performances

Peak revolution (rpm) and mean power (watt) were measured during 30 second cycle ergometer exercise with maximal effort before and after intermittent ergometer exercise. Moreover, revolution every 5 seconds of each set of intermittent pedaling exercise was measured. Maximal anaerobic performance measurement was conducted in reference to the previous study (Bar-Or. 1987). In addition, the parameters that evaluate maximal anaerobic performance selected in this study (peak revolution and mean power) were suggested in previous studies (Narici et al., 1999; Gastin and Lawson. 1994).

2) Blood samples

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 after 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 the 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%, respectively. The above stated serum parameters were measured before ingestion of the test drug, one hour after ingestion, immediately after exhaustion and 15 min after exhaustion.

Statistical analysis

Moreover, two-way repeated measures analysis of variance (condition × measurement time) was used to examine the mean

difference of the peak rpm, work volume during 30 second cycle ergometer exercise with maximal effort before and after intermittent ergometer exercise, and blood parameters between 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 level of 0.05 was used for all tests.

Results

Fig 2 shows pedaling revolutions of each set during maximal intermittent anaerobic erogometer exercise. Pedaling revolutions decreased with an increase of set number, and by the fifth set, the number of revolutions was approximately 60% of the first set. Fig 3 shows changes in peak revolution and mean power of each set during maximal intermittent anaerobic erogometer exercise. The number of sets significantly affected both parameters based on the results of paired two-way analysis of variance (peak revolution: $F = 114.2$, $p < 0.05$; mean power: $F = 114.2$, $p < 0.05$), and both parameters in both conditions decreased with an increase of set number. In addition, no significant effect of ingestion condition was found in either parameter (peak revolution: $F = 1.726$, $p = 0.225$; mean power: $F = 1.726$, $p = 0.225$). Fig 4 shows serum lactic acid and ammonia concentrations before ingestion, immediately and 15 min after intermittent exercise in both conditions. Although time had a significant effect on both parameters by paired two-way analysis of variance (ammonia: $F = 92.99$, $p < .05$; lactic acid: $F = 157.22$, $p < .05$), no significant effect of ingestion condition was found (ammonia: $F = 0.11$, $p = 0.752$; lactic acid: $F = 0.23$, $p = 0.642$). Fig 5 shows plasma glutamic acid and ornithine concentrations before ingestion, immediately and 15 min after intermittent exercise in both conditions. The ingestion condition only had a significant effect on plasma ornithine concentration (ornithine: $F = 129.1$, $p < 0.05$; glutamic acid: $F = 0.75$, $p = 0.409$). Moreover, a significant effect of time was found in both parameters (ornithine: $F = 73.0$, $p < 0.05$; glutamic acid: $F = 15.5$, $p < 0.05$). Fig 6 shows mean power and peak revolution during maximal anaerobic performance before and after intermittent ergometer exercise in both conditions. The ingestion condition had a significant effect on peak revolution (peak revolution: $F = 9.0$, $p < 0.05$; mean power: $F = 4.8$, $p = 0.06$). Moreover, time had a significant effect on both parameters (peak revolution: $F = 12.3$, $p < 0.05$; mean power: $F = 19.7$, $p < 0.05$).

Discussion

The main result of this study was that peak pedaling revolution during maximal anaerobic ergometer exercise and subsequent maximal intermittent anaerobic ergometer exercise in the L-ornithine hydrochloride ingestion condition was significantly greater than that in the placebo ingestion condition. Namely, a decrease in performance after maximal intermittent exercise was suggested to be inhibited by L-ornithine hydrochloride ingestion. Generally, substantial ammonia and inosinic acid (IMP) are produced by the deamination of adenosine monophosphate (AMP) in skeletal muscles during high intensity exercise (Stathis et al., 1994; Goodman and Lowenstein 1977; Lowenstein 1972; Lowenstein and Tornheim 1971). Ammonia produced in skeletal muscles is buffered by synthesizing glutamic acid from α-ketoglutaric acid (Graham & MacLean., 1992). However, oxaloacetic acid, a precursor of α-ketoglutaric acid, also decreases with a marked decrease of α-ketoglutaric acid by the above stated ammonia buffering. Therefore, pyruvic acid accumulation is induced by inhibition of oxidative metabolism within tissue during exercise (Lowenstein 1972; McKann and Tower 1961; Katanuma et al. 1966; Bryla and Niedzwiecka 1979; Worcel and Erecinska 1962). Namely, there was an increase in anaerobic metabolism and subsequent lactic acid accumulation by inhibiting metabolism of acetyl CoA, which is generated by pyruvic acid within mitochondria (Dutton and Berkman 1978; Hindfelt and Siesjo 1970; Schenker et al. 1967). Therefore, ammonia production/accumulation in skeletal muscle during high intensity exercise with high contribution of anaerobic energy supply induces a decline in exercise performance (Banister et al., 1983). However, if ammonia produced in skeletal muscle is rapidly metabolized, the decline in exercise performance may be inhibited. Decline in exercise performance related to ammonia production is improved by metabolizing ammonia to urea via the urea cycle in hepatocyte mitochondria. L-ornithine plays an important role in the urea cycle, and ammonia metabolism in this study is assumed to be accentuated by its ingestion. Moreover, as the above results indicate, skeletal muscle fatigue reduces and maximal anaerobic performance improves. However, the present results showed no significant difference in plasma ammonia levels between ingestion of L-ornithine hydrochloride and placebo. Namely, the above stated improvement of maximal anaerobic performance was not related to accentuation of ammonia metabolism with L-ornithine hydrochloride ingestion. Although it is very difficult to explain the improvement of maximal anaerobic performance by the parameters selected in this study, glutamic acid produced by ammonia buffering action in skeletal muscles may be related to the above improvement. Glutamate and glutamic acid were produced in the aforementioned process of the ammonia buffering in skeletal muscles. Particularly, although

8

glutamic acid is reused for alanine production by transamination reactions, α-oxoglutarate is also produced from pyruvate. Alanine, glutamic acid and glutamate, which are released from skeletal muscles, are transferred to the liver in connection with carriers of nitrogen such as ammonia. Above all, the carbon structure of alanine is used for gluconeogenesis after its deamination. Ammonia buffering in skeletal muscles and its relationship with gluconeogenesis may improve anaerobic performance. Further studies will be required.

Conclusion

Although maximal anaerobic performance may be improved by L-ornithine hydrochloride ingestion before intermittent maximal anaerobic cycle ergometer exercise, this improvement may not depend on an increase of ammonia metabolism with L-ornithine hydrochloride ingestion.

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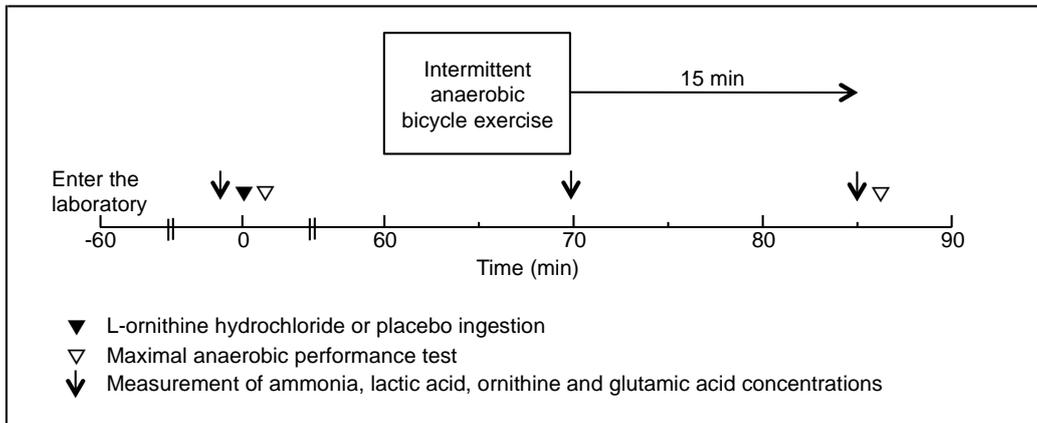
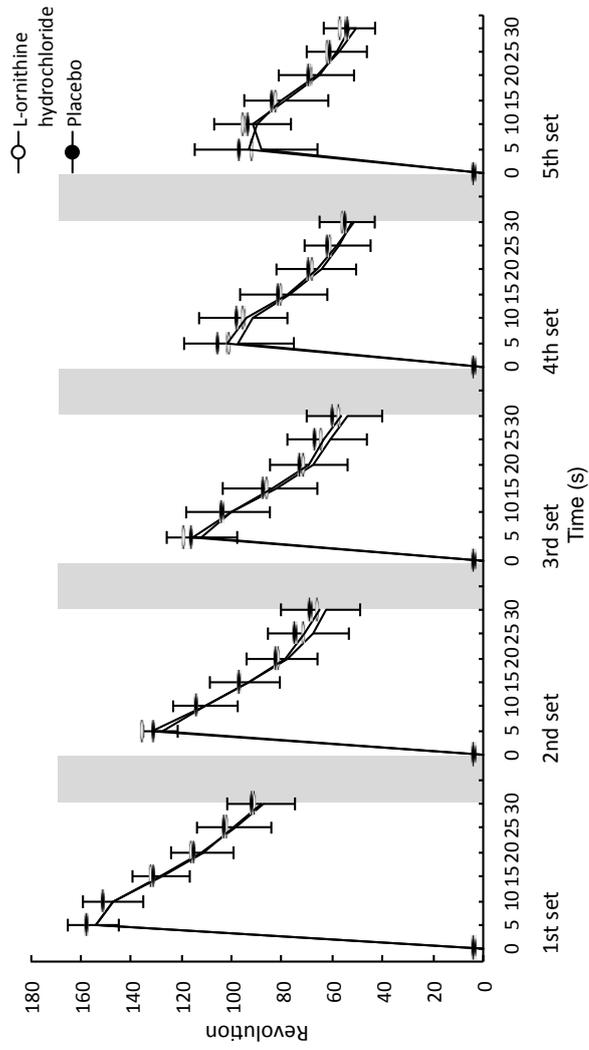Fig. 1   Protocol for exercise and blood sampling

Fig. 2 Revolutions during intermittent maximal anaerobic cycle ergometer exercise. 60s rest periods are denoted as shaded areas. Values are means +/- SD.
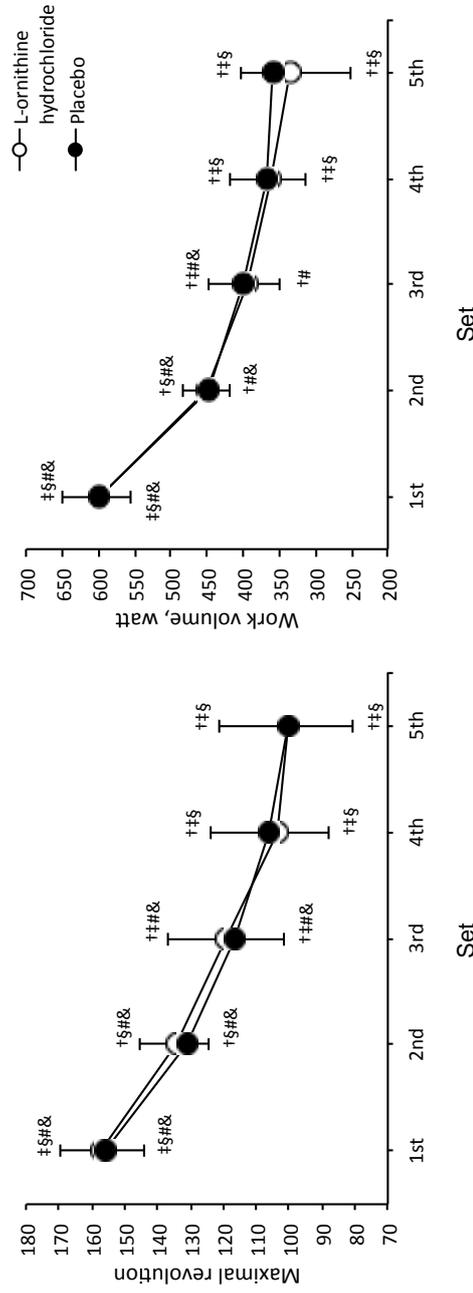
Fig. 3 Maximal revolution and work of volume of each set during intermittent maximal anaerobic cycle ergometer exercise. Values are means +/- SD. † significant difference was found with 1st set in each condition. ‡ significant difference was found with 2nd set in each condition. § significant difference was found with 3rd set in each condition. # significant difference was found with 4th set in each condition. & significant difference was found with 5th set in each condition.
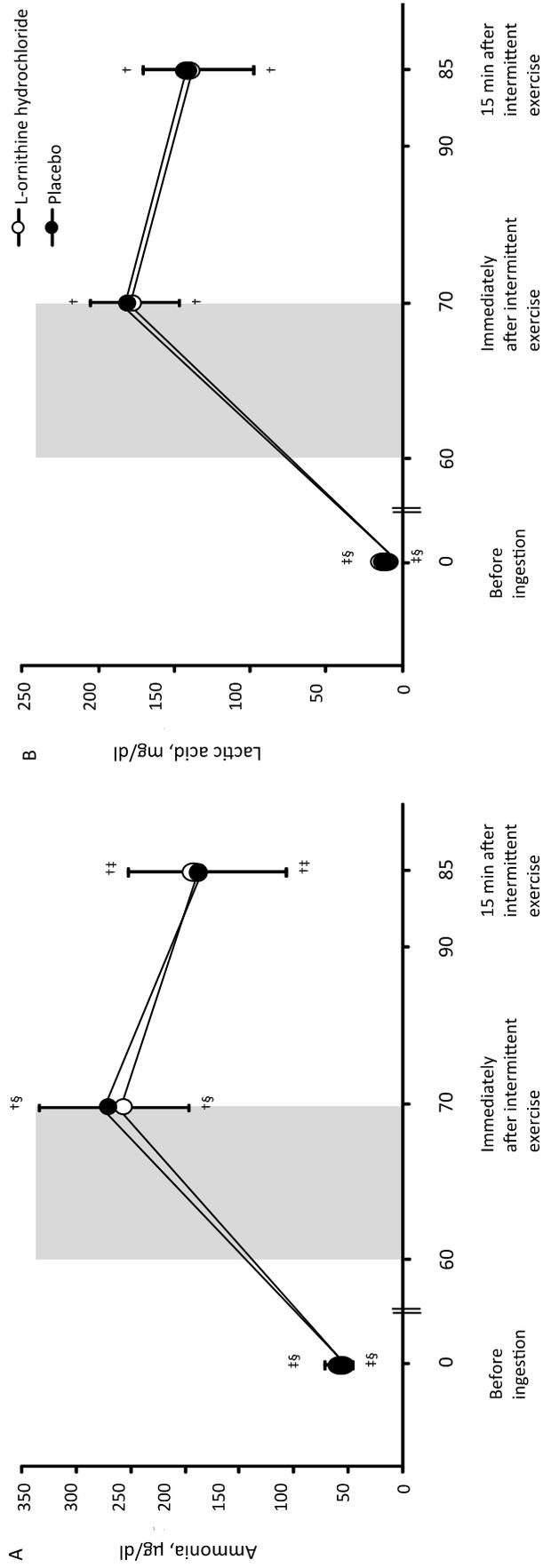
14

A

Ammonia, µg/dl

B

Lactic acid, mg/dl

Fig. 4 Serum ammonia (A) and Lactic acid (B) concentrations in both conditioins. Intermittent anaerobic bicycle exercise are denoted as shaded areas. Values are means +/- SD. † significant difference was found with before ingestion in each condition. ‡ significant difference was found with immediately after intermittent exercise in each condition. § significant difference was found with 15 min after intermittent exercise in each condition.
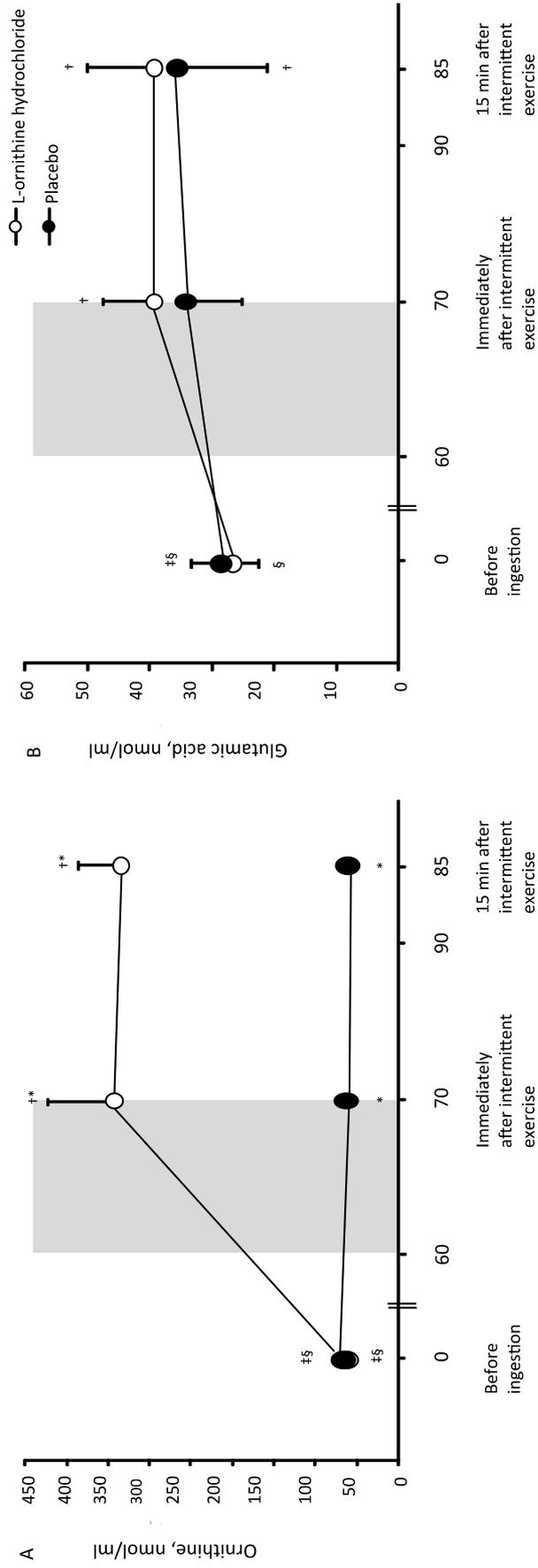
Fig. 5 Plasma ornithine (A) and glutamic acid (B) concentrations in both conditioins. Intermittent anaerobic bicycle exercise are denoted as shaded areas. Values are means +/- SD. † significant difference was found with before ingestion in each condition. ‡ significant difference was found with immediately after intermittent exercise in each condition. § significant difference was found with 15 min after intermittent exercise in each condition.
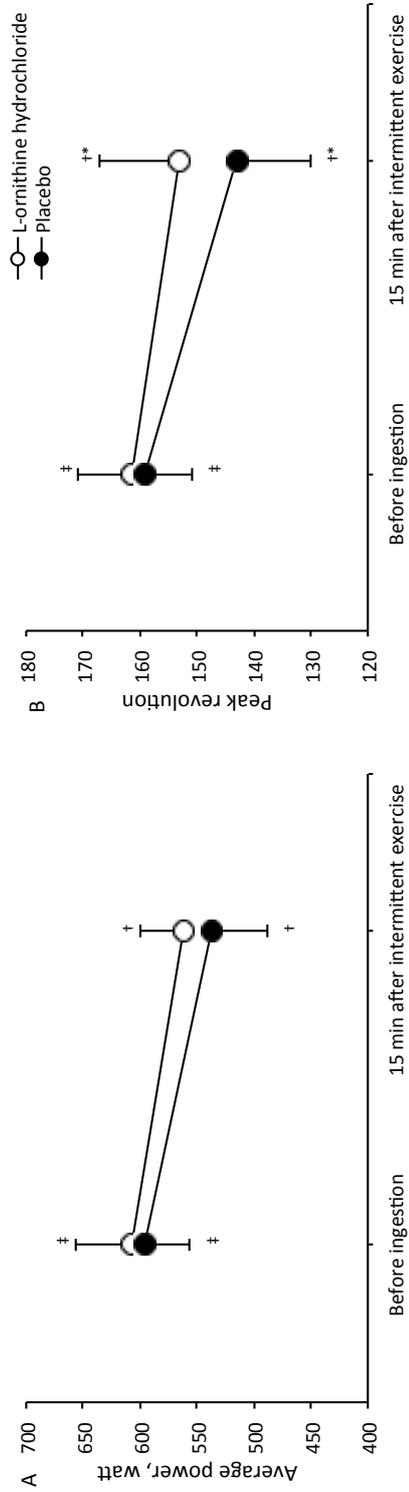
16

Fig. 6   Peak revolution (A) and work volume (B) during 30 seconds cycle ergometer exercise with maximal effort before and after intermittent ergometer exercise in both conditions. Values are means +/- SD. † significant difference was found with before ingestion in each condition. ‡ significant difference was found with 15 min after intermittent exercise in each condition. * significant difference was found between both conditions.