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Effects of Arginine Supplementation on Post-Exercise Metabolic Responses

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Abstract

This study investigated the effects of arginine supplementation on acute metabolic responses during recovery after a single bout of endurance exercise in trained athletes. Twelve healthy male judo athletes were randomly divided into two groups and performed a single bout of exercise at a speed estimated to correspond to 75% $\dot{V}O_2$max for 60 min, and then took either a placebo or arginine at 0.1 g/kg-wt. Blood samples of each athlete were collected before exercise, and 0, 15, 30, 45, 60, 90, 120 min after exercise, respectively. The experiment was repeated two weeks later, but treatments were exchanged for the two groups. The concentrations of glucose, insulin, free fatty acid (FFA), glycerol, lactate, ammonia, creatine kinase, and NO_x (NO_2^- + NO_3^-) in blood were examined. No differences in the levels of glycerol, lactate, ammonia, creatine kinase, or NO_x between the two groups were observed at any of the time points. However, the concentration of glucose was significantly higher in the arginine group as compared to that in the placebo group at the 15-min recovery point. The insulin concentration was also higher in the arginine group as compared to that in the placebo group at the 30-min recovery point. Furthermore, the free fatty acid levels at the 30, and 45-min recovery points were significantly lower in the arginine group compared to those in the placebo group. The results indicated that arginine supplementation during the exercise recovery period could increase glucose and insulin concentrations, and decrease FFA availability in the blood.

Key Words: arginine, exercise recovery, metabolic response

Introduction

Intravenous administration of L-arginine has been demonstrated to stimulate insulin secretion (8, 9), and to increase insulin-mediated whole-body glucose disposal (26). Arginine has been recognized as the most effective amino acid for the stimulation of insulin and glucagon secretions (9). From previous studies, this enhancement of insulin secretion is due to glucose, possibly by depolarizing the glucose-induced signal in the pancreatic β-cell membrane, thus transporting positively charged arginine into cells (17).

Arginine-induced insulin release was also demonstrated to be mediated by arginine-derived nitrogen oxides (29). Furthermore, L-arginine has

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been shown to increase insulin-mediated glucose uptake in healthy human subjects through these two effects (26). Because of its action, arginine has been used as a nonglucose secretagogue to elucidate pancreatic β-cell adaptations to training and to evaluate the insulin secretory capacity in man (7, 19). Thus, arginine supplementation might regulate the metabolism of protein, amino acids, glucose, and fatty acids (11).

The elevated serum insulin concentration might suppress fat metabolism, and increase muscle glucose uptake as well as carbohydrate oxidation during subsequent exercise (13). Plasma free fatty acids (FFA) are the main substrates used during exercise performed at half or less aerobic capacity. Moderate exercise induces endocrine and metabolic changes that seem to be associated with the improvement of FFA oxidation during the post-exercise recovery period.

The regulation mechanisms of FFA utilization are not well understood. Several hypotheses have been postulated. The hormonal milieu seems to play a role, particularly the concentrations of insulin and epinephrine (23). A classical concept has been FFA/TAG (triacylglycerol) recycling. It might play a role in the regulation of lipid metabolism during and after the exercise (32).

However, lipolysis and FFA mobilization in man have been less directly examined for prolonged periods after exercise. The fatty acids utilization changed during the recovery through the changes of the levels of insulin and glucose in plasma (23).

The purpose of this study is to examine whether arginine supplement plays a role in changing the hormonal and metabolic parameters, including involvement in the lipid mobilization, especially in healthy trained men in order to increase the utilization of FFA as an oxidative substrate.

Materials and Methods

Subjects

Fourteen male judo athletes during training participated in this study. Each athlete had been involved in judo training for 3 to 6 years. They were then informed about the procedures and the possible risks involved before giving their voluntary consent. The protocol was approved by the Human Safety Committee Review Board of National Taiwan Sport University. One subject was dropped from the study because of an injury and one for not completing the study protocol. Age, weight and height were recorded. Physical characteristics of the athletes are presented in Table 1.

Table 1. Characteristics of the judo athletes (n = 12)

Characteristics	Mean	SE
Age (y)	20.25	0.25
Weight (kg)	75.75	2.89
Height (cm)	175.33	1.32
Body mass index (BMI)	24.58	0.72
$\dot{V}O_2$max (ml/min/kg)	56.20	1.51

Diet History

A diet history and 3-d diet records were obtained from each subject before the commencement of the study. Dietary records were reviewed and analyzed by a registered dietitian. Subjects taking amino acid supplements were excluded from the study. Maintenance of established dietary patterns was encouraged throughout the study.

Study Design

The subjects were randomly divided into two groups and performed a single bout of exercise at an estimated speed corresponding to 75% $\dot{V}O_2$max for 60 min, and then, took either a placebo or arginine supplement. The arginine supplement group consumed 0.1 g/kg-wt of instant arginine powder (provided by Orient Europharma Co., Ltd. Taipei, Taiwan) with 150 ml of water. The arginine powder contanins 45.5% L-arginine, other components are orange flavor, Fibersol-2 *et al*. The purity of L-arginine is greater than 99%. The placebo group consumed 0.1 g/kg-wt of instant methylcellulose powder (provided by Orient Europharma Co., Ltd. Taipei, Taiwan) with 150 ml of water. The drinks were both orange flavoured. The appearance and flavor of the drinks were the same. The experiment was repeated two weeks later, but treatments were exchanged for the two groups.

The subjects were instructed to refrain from strenuous physical exercise on the day preceding the exercise test. On the day of the test, the subjects reported to the laboratory after a 10-h fast. After 15 min of seated rest, a catheter was placed in an antecubital vein, and blood samples of each athlete were collected before a single bout of endurance exercise, and 0, 15, 30, 45, 60, 90, 120 min after exercise. The concentrations of glucose, insulin, free fatty acid, glycerol, lactate, ammonia, creatine kinase, and NO_x in the blood samples were then examined.

Exercise Protocol

$\dot{V}O_2$max was determined in the pre-experimental period. Each subject came to the laboratory 7 d before the start of the actual study, and performed an

incremental running test on a motor-driven treadmill (Quinton Instruments, Model 18-60, Washington, USA) according to the Bruce protocol until exhaustion. In order to establish the baseline endurance performance time, maximum oxygen consumption ($\dot{V}O_2max$) was determined by the automated system (Model 29C, SensorMedics, Yorba Linda, CA,USA). The $\dot{V}O_2max$ was defined as the attainment of at least two of the three following criteria: [1] an increase of ≤ 140 ml $\dot{V}O_2max$ with an increasing workload; [2] heart rate within 10 beats of age-predicted maximum; and [3] rating of perceived exertion (RPE) greater than 18 using the Borg scale. Heart rate (HR) measured by the Sport Tester (PE 3000, Polar Electro, Kempele, Finland) was monitored during the treadmill exercise. The RPE was recorded using the modified Borg scale (5). Endurance performance time was also recorded at the end of the test for each subject. On the day of the experiment, subjects reported to the laboratory at 7-9 a.m. following a 10 h overnight fast.

Baseline physiological data was collected prior to the beginning of the single bout of exercise. Subjects then commenced a 5 min warm-up at a running speed equivalent to 55% $\dot{V}O_2max$. After that, the treadmill speed was increased to a pace equivalent to 75% $\dot{V}O_2max$, and the subjects ran for another 60 min.

Blood Collection and Analysis

For the analysis of free fatty acid, glycerol, and NO_x, 4 ml of blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). These tubes were then centrifuged, and the plasma was stored at –80°C and analyzed on a later date. For the analysis of glucose, insulin, lactate, ammonia, creatinine kinase, and hematocrit, 5 ml of blood was collected in tubes. These samples were obtained without stasis, allowed to clot, and the serum was assayed for glucose, lactate, ammonia, and creatinine kinase immediately with an Ektachem DT60 II chemistry analyzer (Johnson and Johnson, Rochester, NY, USA). A portion (500 μl) of serum was stored at –80°C for the analysis of insulin. Plasma free fatty acid and glycerol concentrations were determined with an ultraviolet and visible spectrophotometer (Randox laboratories Co., Ltd., Taipei, Taiwan). Insulin levels were determined using the DSL-10-1600 ACTIVE™ Insulin Enzyme-Linked Immunosorbent (ELISA) Kit (Diagnostic Systems Laboratories, Inc., Webster, TX), and all blood samples were run in duplicate and the mean of the two assays was used for statistical analyses. Plasma NO_x concentrations were determined by the Griess reaction (14) with Cayman chemical kit 780001 from Kuo Yang Sci. Corp. Hematocrit was determined by centrifuging a micro hematocrit tube of blood for five mins at 11,000 rpm. Hematocrit was read with a micro hematocrit reader.

This assay reduces all nitrate to nitrite and measures total nitrite by photoabsorbance at 540 nm of the deep purple azo conversion product.

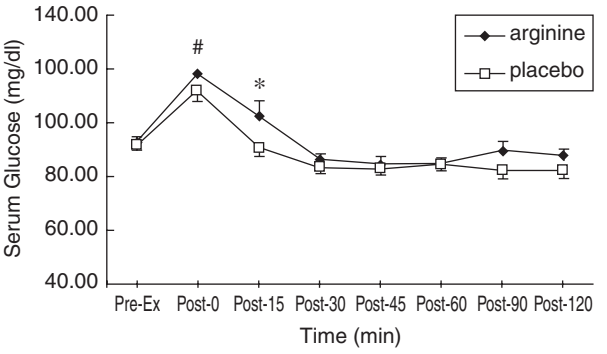

Fig. 1. Serum glucose concentrations (means ± SE) at pre exercise (Pre-Ex), and into recovery (Post-0, Post-15, Post-30, Post-45, Post-60, Post-90, Post-120: post exercise 0 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min) (n = 12). *Significantly ($P < 0.05$) higher mean value than that observed during the placebo trial. #Significantly ($P < 0.05$) higher mean value than that observed at rest in both trials.

Statistical Analyses

Data from the arginine group and placebo group are expressed as means ± standard error (SE). Statistical analyses were performed using 2 factor mixed analysis of variance (ANOVA) with repeated measures to study differences in blood parameters of timing (before exercise, and 0, 15, 30, 45, 60, 90, 120 min after exercise) and group (arginine, placebo). Where significant F ratios were found, a Tukey's *post hoc* test was used to determine the location of the variance. A paired *t*-test was then performed to compare the mean values between the arginine group and placebo group. Differences were considered significant when $P < 0.05$.

Results

Serum glucose concentration was significantly higher at 15 min after arginine was supplied. (Fig. 1). Serum glucose level increased in both trails after exercise.

Serum insulin concentration significantly increased at the 30-min recovery point after arginine supplementation (Fig. 2). Arginine supplementation resulted in significant suppression in plasma FFA concentrations at the 30, and 45-min recovery points (Fig. 3). FFA concentrations showed a marked elevation in placebo group, compared to those ob-

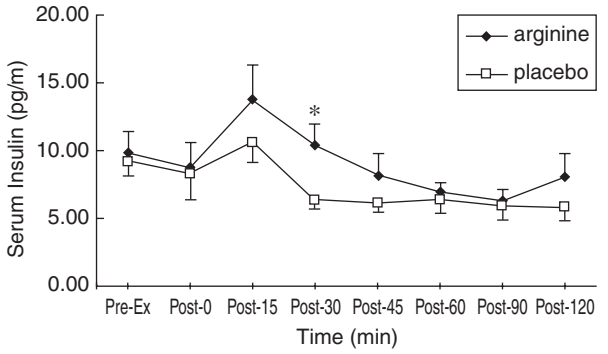

Fig. 2. Serum insulin concentrations (means ± SE) at pre exercise (Pre-Ex), and into recovery (Post-0, Post-15, Post-30, Post-45, Post-60, Post-90, Post-120: post exercise 0 min, 15 min, 30 min, 45 min, 60 min, 90min, 120 min) (n = 12). *Significantly ($P < 0.05$) higher mean value than that observed at rest in placebo trials.

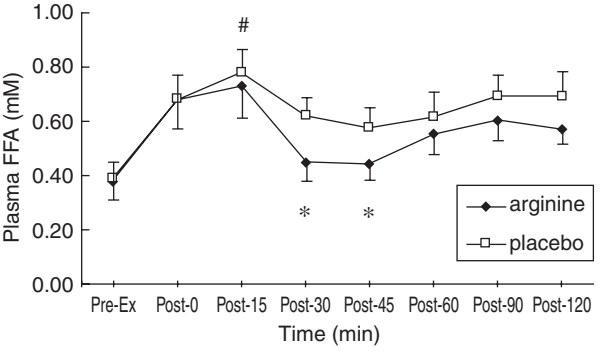

Fig. 3. Plasma FFA concentrations (means ± SE) at pre exercise (Pre-Ex), and into recovery (Post-0, Post-15, Post-30, Post-45, Post-60, Post-90, Post-120: post exercise 0 min, 15 min, 30 min, 45 min, 60 min, 90min, 120 min) (n = 12). *Significantly ($P < 0.05$) lower mean value than that observed during the placebo trial. #Significantly ($P < 0.05$) higher mean value than that observed at rest in placebo trials.

Table 2. Blood parameters (means ± SE) at pre exercise (Pre-Ex), and into recovery (Post-0, Post-15, Post-30, Post-45, Post-60, Post-90, Post-120: post exercise 0 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min) (n =12)

	Pre-Ex	Post-0	Post-15	Post-30	Post-45	Post-60	Post-90	Post-120
Hematocrit (%)								
arginine	41.75 ± 0.85	43.25 ± 0.80	42.42 ± 0.74	41.92 ± 0.72	41.58 ± 0.73	42.17 ± 0.81	41.83 ± 0.94	41.67 ± 0.94
placebo	42.33 ± 0.54	43.83 ± 0.51	43.00 ± 0.51	42.33 ± 0.66	42.04 ± 0.67	42.21 ± 0.56	41.50 ± 0.61	42.04 ± 0.75
Creatinine kinase activity (U/L)								
arginine	227.17 ± 53.46	277.75 ± 52.67	266.42 ± 52.55	255.67 ± 47.81	250.17 ± 47.84	255.08 ± 50.36	250.83 ± 49.46	245.00 ± 46.81
placebo	226.50 ± 76.53	272.75 ± 78.70	257.92 ± 78.17	259.50 ± 77.11	248.17 ± 76.83	248.33 ± 80.83	250.00 ± 73.28	237.75 ± 72.67
NO_x (μM)								
arginine	5.05 ± 0.65	5.65 ± 0.67	5.33 ± 0.68	5.58 ± 0.62	5.70 ± 0.62	5.65 ± 0.69	5.27 ± 0.60	5.16 ± 0.65
placebo	5.45 ± 0.60	6.19 ± 0.81	5.99 ± 0.72	5.99 ± 0.54	5.70 ± 0.60	5.52 ± 0.62	5.10 ± 0.59	5.27 ± 0.49
Lactate (mM)								
arginine	1.66 ± 0.10	5.33 ± 0.75*	2.55 ± 0.26	2.22 ± 0.14	2.03 ± 0.11	2.08 ± 0.20	1.85 ± 0.12	1.74 ± 0.13
placebo	1.56 ± 0.11	4.79 ± 0.59*	2.42 ± 0.15	1.98 ± 0.11	1.83 ± 0.12	1.73 ± 0.10	1.74 ± 0.11	1.76 ± 0.13
Ammonia (μM)								
arginine	13.08 ± 4.37	60.42 ± 10.01*	17.42 ± 4.05	14.67 ± 3.47	15.42 ± 4.29	14.83 ± 4.48	20.25 ± 4.75	7.33 ± 2.23
placebo	13.08 ± 2.86	64.17 ± 11.41*	17.50 ± 4.97	10.92 ± 2.78	13.33 ± 3.96	11.33 ± 2.10	18.92 ± 4.59	10.92 ± 3.96
Glycerol (μM)								
arginine	65.54 ± 7.02	238.33 ± 23.57*	115.59 ± 11.23	80.09 ± 7.87	75.40 ± 5.79	87.16 ± 8.08	92.64 ± 5.38	80.22 ± 5.15
placebo	63.49 ± 8.61	255.41 ± 26.53*	121.77 ± 14.92	93.15 ± 9.28	88.20 ± 7.03	94.68 ± 11.55	86.15 ± 8.86	89.55 ± 10.54

*Significantly ($P < 0.05$) higher mean value than that observed at per exercise.

served at rest and the 15-min recovery point. There were no differences in blood Hct, plasma NO_x, or serum creatinine kinase activity over time or between trials (Table 2). There were no concentrations differences in plasma lactate, ammonia, and glycerol between trials (Table 2). However, plasma lactate, ammonia, and glycerol concentrations increased from rest after endurance exercise in both trials, and the increase in plasma lactate, ammonia and glycerol compared to the pre-exercise time point was only seen at the zero time point post-exercise.

Discussion

Previous reports have demonstrated that an injection of arginine before exercise resulted in significant increases of both plasma insulin, and glucagon concentrations during exercising (7, 10, 30). The present study found that serum glucose concentration of the arginine group was significantly higher than that of the placebo trial 15 min after arginine oral ingestion. Furthermore, serum insulin concentration significantly increased 30 min after

arginine supplementation in the recovery period.

Robinson *et al*. (27) recently demonstrated that oral ingestion of L-arginine in men was insufficient to cause a significant increase in plasma glucose and insulin concentrations. In the present study, all subjects ingested 70 g carbohydrate (CHO) and either 10 g L-arginine or placebo. In our study, subjects did not ingest CHO. The stimulation of insulin secretion by arginine has been reported to be dependent on the ambient glucose concentration both *in vitro* (4, 22) and *in vivo* (21, 24, 31). Robinson *et al*. (27) reported that blood glucose concentrations increased significantly in all subjects under both treatments after CHO ingestion (ingestion of 10 g arginine or placebo). However, the responses of the arginine group were not different from those of the placebo group.

In studies by Gannon *et al*. (12), glucose concentration in blood increased after stimulation with L-arginine. When L-arginine was provided with glucose, blood glucose concentration decreased, compared with glucose ingestion alone. Based on their evidence, we proposed that glucose ingestion with arginine accelerated arginine removal rate. The simultaneous ingestion of glucose with arginine could have reduced arginine absorption rate or have accelerated its metabolism by enteral cells. Thus, when arginine was ingested with high doses of CHO, it could have caused the attenuation of the blood glucose concentration rise, and thus decreased the effect of arginine supplementation on glucose concentration.

Yaspelkis and Ivy (33) suggested that oral ingestion of high doses of arginine might cause intestinal cramping and diarrhea. They presented that L-arginine oral ingestion with CHO in man was insufficient to induce a significant increase in plasma, glucose and insulin concentrations. In this study instant arginine powder drink, having better absorption than arginine powder, was used. Oral ingestion of instant arginine powder drink could have avoided gastrointestinal problems, and enhanced glucose and insulin concentrations after exercise. Although results of this investigation are different from those of Robinson *et al*. (27), they suggest that oral ingestion, such as the powder drink, being easier to be absorbed by human bodies, could increase serum glucose and insulin concentrations.

Three different mechanisms have been postulated to explain the mechanism of the stimulation of insulin release by L-arginine (4, 29): 1) β-cell uptake of the positively charged L-arginine molecule, followed by depolarization of plasma membrane; 2) L-arginine metabolism through the action of argininase, resulting in the metabolism of arginine into ornithine and urea. Ornithine is then further metabolized, ending in the citric acid cycle; and 3) stimulation by nitric oxide (NO), derived from the metabolism of L-arginine through the action of a constitutive NO synthase (cNOS), this resulting in the production of NO and citrulline. Exactly how No influences hormone secretion is still uncertain (16). Both stimulatory (29) and inhibitory (25, 28) effects of insulin have been reported.

In this study, concentrations of NO were quantified by measuring the enzymatic oxidation to nitrite (NO_2^-) and nitrate (NO_3^-) (as shown in Table 2). In biological solutions, NO is rapidly oxidized to NO_2^-; however, in the presence of oxyhemoglobin, NO is completely oxidized to NO_3^- (18), which is subsequently excreted in the urine. Because NO is rapidly oxidized to stable end-products, $NO_2^- + NO_3^-$, direct measuring NO *in vivo* is difficult. The plasma level of NO_x ($NO_2^- + NO_3^-$) has been used as a biochemical marker of endogenous NO production *in vivo* (1, 20). In the present study, we found that the administered arginine did not stimulate an increase in NO_x concentration, compared with the placebo group. This indicates that insulin secretion is unlikely stimulated by NO, derived from the metabolism of arginine. The most plausible explanation for arginine-induced insulin secretion during recovery is that arginine is transported into the cell in a positively charged form, or that arginine-induced glucagon secretion stimulates glucose-induced insulin secretion. On the other hand, when arginine enters the system, it can be metabolized into ornithine and urea. When arginine enters the urea cycle, arginase converts it to ornithine and urea with no net increase of NO or insulin sensitivity (2). Ornithine metabolism ends up in the citric acid cycle, and is further metabolized to citrate in skeletal muscle. The glycolysis is regulated by the phosphofructokinase reaction, which is negatively modulated by ATP and citrate (15). The reduction in muscle glycolysis of arginine-treated subjects is due to the inhibitory effect of citrate on phosphofructokinase, thereby limiting the rate of glucose catabolism. It indicates that arginine supplementation has a glucose sparing effect (3).

A previous study suggested that arginine regulated the metabolism of glucose, lactate, and fatty acids during the recovery period (11). In this study, we found that the administered arginine did not stimulate an increase of glycerol, lactate, ammonia concentration, or creatinine kinase activity (as shown in Table 2) when compared with the placebo group during the recovery period, but a decrease in plasma FFA concentration was observed. Blood glycerol levels have been used in estimating lipolysis in many studies (23). Because glycerol cannot be reused in the adipose tissue after lipolysis, glycerol can easily diffuse into the blood. Similar glycerol levels of the two trials, but with lower FFA levels during the

recovery period in the arginine group, were observed in this study. These results suggest that arginine supplementation did not decrease the adipose tissue lipolysis during the recovery period, but instead increased the reesterification of the FFA to triacylglycerols or blocked the FFA passage from adipose tissue to blood. Two major lipolytic enzymes, hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL), act on intra-adipocyte and circulating lipoprotein triglyceride (TG), respectively. Other lipolytic processes occur in the muscle and the liver during exercise and recovery (6). HSL causes the release of glycerol and free fatty acids into systemic circulation, while some of the fatty acids might have also been reesterified within adipose tissue.

Some FFA may be taken up by the muscle and liver for either direct/partial oxidation to ketone bodies, or reesterification to triacylglycerols. Coppack *et al*. (6) suggested that insulin is the most potent antilipolytic hormone. In this study, we observed that insulin concentrations increased in the arginine group. It indicates that arginine supplementation could inhibit HSL activity and enhance FFA esterification in adipose tissue. However, this study showed that there were no significant differences in glycerol between the two trials, and FFA levels were significantly lower in the arginine group, compared with the placebo group 30, 45 min after exercise. Thus, we suggest that the administered arginine may increase the reesterification of the FFA to triacylglycerols.

In conclusion, this study indicates that consuming 0.1 g/kg-weight of arginine during the exercise recovery period will provide the muscle with an anabolic environment by increasing glucose concentration and stimulating insulin secretion. Moreover, the decrease in the FFA availability in the blood decreases the fraction fat oxidation during recovery from endurance exercise may benefit exercise recovery.

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