

GLUTAMINE SUPPLEMENTATION: EFFECTS ON GROWTH HORMONE AND TIME-TRIAL PERFORMANCE AFTER PROLONGED EXERCISE

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ABSTRACT

Dietary glutamine supplementation has been proposed as a potential aid to athletic performance. The purpose of the investigation was to determine if glutamine supplementation might improve cycling performance, perhaps by stimulating growth hormone release, in athletes consuming a high-carbohydrate diet. Six trained young adult men participated in two four-day supplementation trials. In both trials, the subjects underwent an identical carbohydrate-loading regimen of controlled diet (75% carbohydrate) and exercise. The only difference in the trials was whether the subjects ingested dietary supplements of glutamine or glucose placebo. During the first three days of each trial, the diets were supplemented with either glutamine or glucose placebo at a dosage of 150 mg/kg body weight (~10.4 g/d). On the fourth day, the supplements were dissolved in a sports drink (4 g/L). The sports drink initially contained a 6% solution of carbohydrate. On the fourth day the subjects ingested 7 ml/kg of the supplemented sports drink 2 h before exercise, 5 ml/kg 1 h before exercise, and 3 ml/kg after 15, 30, 45, 60, and 90 min of exercise. The total amount of glutamine supplement or placebo consumed on the fourth day was 108 mg/kg or about 7.4 g, of which 3.3 g were consumed before and 4.1 g during exercise. On the fourth day, subjects completed 90 min of cycle ergometry, divided into six 15-min periods, each of which included in sequence 12 min @75% VO_{2max} , 1 min @ 120% VO_{2max} , and 2 min @ 50% VO_{2max} . The 90 min exercise session was immediately followed by 15 min of rest and then a final 20-km time trial at ~85% VO_{2max} . Plasma concentrations of glycerol and free fatty acids and serum growth hormone concentrations were measured before and during exercise, and time-trial performance was recorded. Times (\pm SEM) to complete the 20-km performance rides were 31.03 ± 0.58 min for the placebo trial and 30.93 ± 0.60 min for the glutamine trial and were not significantly different ($P>0.05$). There were also no

significant effects on serum growth hormone or on plasma glycerol or free fatty acids attributable to glutamine supplementation at any sampling time. In summary, we found no evidence that glutamine supplementation in moderate dosages has any effect on circulating growth hormone, glycerol, or free fatty acids or on time-trial performance in athletes who consume a high-carbohydrate diet.

Key words: Exercise, effect, final, Time.



Introduction

The concentration of glutamine in the blood is greater than that of any other amino acid and, as reviewed by Castell (2003) and Walsh et al. (1998), it usually declines during the late stages of prolonged exercise and/or during many hours of recovery. It has been suggested that this reduction in circulating glutamine following prolonged exercise may play a role in the impairment of immune function observed after prolonged exercise, especially in “overtrained” athletes (Castell, 2003; Rohde et al., 1998; Walsh et al., 1998).

Glutamine also has properties that indicate it might be useful as a dietary supplement to enhance exercise performance (Antonio & Street, 1999). For example, glutamine contributes to acid-base balance during exercise and recovery by removing hydrogen ions as urea and producing bicarbonate ions in the kidneys (King et al., 1983; Welbourne, 1995). Glutamine supplementation also increases the concentration of intermediate compounds of the tricarboxylic acid cycle in exercising muscles, and theoretically this could improve aerobic energy production during prolonged exercise (Bruce et al., 2001). Moreover, glutamine supplementation increases the plasma concentration of glutamate (Ziegler et al., 1990), a major metabolite of glutamine.

Glutamate can cross the blood-brain barrier and act as an excitatory neurotransmitter in the brain (Newsholme & Leech, 1983, p. 775), perhaps serving to attenuate fatigue processes originating in the central nervous system. Finally, in non-exercising subjects, supplements of glutamine alone (Welbourne, 1995) or a mixture of glutamine, glycine, and niacin (Arwert et al., 2003) can stimulate the secretion of growth hormone. Growth hormone secretion is increased during many types of strenuous exercise (Wideman et al., 2002) and can mobilize fatty acids for energy and spare muscle glycogen (Newsholme & Leech, 1983, p. 689; Welbourne, 1995), which theoretically could lead to improved exercise endurance. Whether or not glutamine supplementation can enhance the growth hormone response to exercise is unknown.

The purpose of the present investigation was to determine the effect of glutamine supplementation before and during 90 min of cycling exercise on concentrations of free fatty acids and glycerol in plasma and growth hormone in serum and on time-trial performance following the 90-min exercise bout.

Methods

Subjects

Six adult endurance athletes recruited from local cycling and triathlon clubs participated as subjects. Means (\pm SEM) for body mass, percent body fat (hydrostatic weighing) and maximal oxygen uptake ($VO_2\max$) during progressive cycle ergometry on Velodyne™ ergometers (Velodyne Sports, Laguna Hills, California, USA) were 68.7 ± 3.0 kg, 11.2 ± 1.2 %, and 64.5 ± 1.5 ml·kg⁻¹·min⁻¹.

Diet and Exercise Protocol:

The cross-over experimental design required that all subjects participate in two trials, each lasting four days. One week during which subjects exercised normally and consumed their normal diets separated the two trials. In both trials, the subjects underwent an identical carbohydrate-loading regimen of controlled diet and exercise. The only difference in the trials was whether the subjects ingested dietary supplements of glutamine or glucose placebo. Three subjects ingested glutamine in the first trial, and the remaining three ingested placebo.

Diet and Glutamine Supplementation:

All meals were provided for the subjects, and consumption of breakfasts and dinners was supervised in a laboratory setting. Mean daily values for the diet composition were as

follows: total energy = 4,130 kcal; carbohydrate (75% of energy) = 12.7 g/kg lean body mass (LBM); fat (15% of energy) = 1.13 g/kg LBM; protein (10% of energy) = 1.69 g/kg LBM. During the first three days of each trial, the diets were supplemented with either glutamine or glucose placebo at a dosage of 150 mg/kg body weight (~10.4 g/d). A similar dosage was previously shown to produce marked increases in plasma glutamine concentrations (Bowtell et al., 1999). Supplements were dissolved in carbohydrate beverages and administered in a double-blind fashion. On the fourth day, the supplements were dissolved in a sports drink (4 g/L). The sports drink initially contained a 6% solution of carbohydrate. On the fourth day the subjects ingested 7 ml/kg of the supplemented sports drink 2 h before exercise, 5 ml/kg 1 h before exercise, and 3 ml/kg after 15, 30, 45, 60, and 90 min of exercise. The total amount of glutamine supplement or placebo consumed on the fourth day was 108 mg/kg or about 7.4 g, of which 3.3 g were consumed before and 4.1 g during exercise.

Exercise :

On the first day of each four-day trial, subjects completed 90 continuous minutes of Velodyne™ ergometry, divided into six 15-min periods, each of which included in sequence 12 min @75% VO_{2max} , 1 min @ 120% VO_{2max} , and 2 min @ 50% VO_{2max} . On the second and third days, subjects completed only two of these 15-min periods for a

total of 30 min of exercise. The protocol on the fourth day was identical to that on the first day, but the 90 min exercise session was immediately followed by 15 min of rest and then a final 20-km time trial at ~85% VO_2 max.

Blood Collection:

Before beginning exercise on the fourth day, subjects reported to the laboratory and sat quietly for 15 min. A 20-gauge 3-cm Teflon cannula was inserted into an antecubital forearm vein from which blood samples were obtained by stopcock and syringe. The cannula was kept patent with periodic injections of isotonic saline. Samples were collected during rest 120 min before exercise (-120 min), immediately before exercise (0 min), after 30, 60, and 90 min of cycling, just before the time trial, and immediately following the time trial. To save costs, assays for growth hormone were conducted neither at -120 min nor after the time trial. The blood was processed and centrifuged, and plasma or serum was stored at -84°C until analyzed.

Biochemical Assays

Plasma glycerol was analyzed by a spectrophotometric assay (Sigma Chemical, St. Louis, Missouri, USA) and plasma free fatty acids by an enzymatic colorimetric method

(Wako Chemicals, Richmond, Virginia, USA). Serum growth hormone was measured with an Immunoradiometric assay (Diagnostic Products Corp., Los Angeles, California, USA), and the minimal detectable dose for the assay was approximately 0.9 ng/ mL. Samples for individual subjects were analyzed in the same assay run. Intra-assay and inter-assay coefficients of variation for all assays were $\leq 5\%$ and 7% , respectively).

Statistical Analysis:

Data were analyzed with a one-way analysis of variance (ANOVA) with repeated measures (time). Neuman- Keuls posthoc pairwise comparisons of means were used to locate any significant effects detected by the analysis of variance. Statistical significance was accepted when $P \leq 0.05$. All values were reported as means and standard errors.

Results:

The data for plasma glycerol and free fatty acids are shown in Figures 1 and 2, respectively, and show a gradual rise in both glycerol and fatty acids with a more pronounced increase after the time trial. No significant effects of the glutamine supplementation were detected.

The serum growth hormone response to the exercise challenge (Figure 3) was similar to previously reported responses to strenuous aerobic exercise (Wideman et al., 2002). However, the mean values for placebo and glutamine treatments were not statistically different.

The intensity of the time trials is evidenced by the fact that the average heart rate of the subjects was 169 ± 2 beats/min. Time trials were completed in 31.03 ± 0.58 and 30.93 ± 0.60 min for the control and glutamine treatments, respectively, and were not significantly different. Three subjects performed slightly better and three slightly worse after glutamine supplementation.

Discussion :

We hypothesized that glutamine supplementation would enhance exercise performance by one or more mechanisms, perhaps including a greater secretion of growth hormone and a secondary elevation of plasma free fatty acids that could be used for energy by the exercising muscles. Our results did not support any of these hypotheses.

Diet

One of the unique features of the present study is that under both supplementation conditions, our subjects followed a three-day carbohydrate-loading regimen prior to each exercise performance trial and consumed a 6% carbohydrate-electrolyte beverage before and during the performance trial. This protocol is presumably similar to that followed by many athletes who compete in prolonged sports events. We did not include a control group without carbohydrate loading or consumption of sports drinks because such a group would not have practical validity for endurance athletes. Moreover, previous comparisons of the effects on plasma glutamine of moderate- versus high-carbohydrate diets for three days (Blanchard et al., 2001; Gleeson et al., 1998) showed no important differences. Likewise, ingestion of carbohydrate drinks during exercise did not affect plasma glutamine when compared to ingestion of water (vanHall et al., 1998).

Glutamine Dosage

For the glutamine phase of the experiment, our subjects consumed 10.4 g of glutamine supplement during each of the three days before the time trial, 3.3 g during 2 h of rest immediately before exercise on the day of the time trial, and 4.1 g during 90-min of exercise just before the time trial. Although we did not measure plasma glutamine, previous research strongly suggests that the glutamine dosage was sufficient to cause a marked increase in plasma glutamine concentration. Welbourne (1995) showed that a

single dose of 2 g of glutamine resulted in a 19% increase in plasma glutamine concentration; Ziegler et al. (1990) found a 50% increase in plasma glutamine after a single 7.4 g dose, Bowtell et al. (1999) reported a 46% increase following a single dose of 8 g of glutamine; Arwert et al. (2003) showed that 5 g of glutamine ingested twice daily for three weeks increased plasma glutamine by 48%; and Castell and Newsholme (1997) doubled plasma glutamine after administering glutamine supplements of 5 g or Krzywkowski 7 g.

Glycerol and Free Fatty Acids

Compared to a fasted condition, the slow rise in glycerol and free fatty acids throughout the 90-min exercise sessions (Figures 1,2) was quite modest, which is consistent with our subjects having consumed a high-carbohydrate diet (Wagenmakers et al., 1991). To be effective in stimulating the mobilization of fatty acids and glycerol the hypothesized action of growth hormone would have had to overcome the inhibitory effect of high levels of insulin and lactic acid (not shown) during the exercise sessions (Campbell et al., 1992).

The large responses of both glycerol and free fatty acids following the time trial were presumably caused by adrenergic stimulation associated with the maximal exhaustive exercise.

Growth Hormone

Although exercise stimulated the expected large rise in serum growth hormone (Figure 3) there was no systematic difference between placebo and glutamine supplementation. Two previous studies in rested subjects have shown that glutamine supplementation can produce marked increases in circulating growth hormone (Arwert et al., 2003; Welbourne, 1995), and a third reported a trend toward greater serum growth hormone with increasing doses of glutamine supplementation (Ziegler et al., 1990). However, experiments in exercising subjects have generally failed to demonstrate any effect of glutamine supplementation on growth hormone. For example, in one experiment, Krzywkowski et al. (2001) required fasted male athletes to cycle for 2 h at 75% VO_2 max. In three trials, the athletes ingested a protein solution or 3.5 g glutamine or 3.5 g carbohydrate placebo after the first hour of exercise and then every 45 min through 4 h of recovery. The total amount of glutamine or carbohydrate ingested was 17.5 g, and no effect of the supplementation on plasma growth hormone was detected immediately after or 2 h after exercise.

Exercise Performance

Only three previous reports were found that investigated the effects of glutamine supplementation on exercise performance. In a cycle-ergometry experiment, athletes

ingested either a placebo or a small dose of glutamine (0.03 g glutamine/kg body weight; ~2 g) 90 min before exercise (Haub et al., 1998). They completed four 60-s bouts of cycling at 100% VO_2 max, each bout separated by 60 s of rest, and then cycled at the same load until fatigue (~263 s). No effects of glutamine supplementation were detected on performance or blood acid-base balance.

In two studies of weightlifting, glutamine supplementation also failed to improve performance. Subjects of Antonio et al. (2002) ingested 23 g of glutamine or glycine or a fruit juice placebo 60 min before completing two sets of maximal bench presses at 100% body weight and leg presses at 200% body weight. No differences in number of repetitions completed were found among the treatments. In a training study, Candow et al. (2001) administered 61 g of glutamine or carbohydrate placebo daily for 6 weeks to young men who were undergoing a heavy resistance training regimen. Before and after training, the subjects were tested on three measures of maximal strength, on body composition, and on muscle protein degradation. There were no significant effects of glutamine supplementation on any of the variables tested.

Conclusion

It could be argued that the power of the statistical tests used in the present study may have been insufficient to detect any important effects of glutamine that might have existed. However, the absolute differences that did exist were small, the putative effects typically occurred in only three or four of the six subjects at most, and the effects in a given subject were not consistent across the sampling times. Moreover, the absence of statistically significant effects in the present investigation is consistent with the results of previous studies of glutamine supplementation in resistance-trained athletes and in subjects who performed brief, high-intensity cycle ergometry.

In summary, we found no evidence that for athletes consuming a high-carbohydrate diet, supplementation with moderate amounts of glutamine before and during exercise has any effect on serum growth hormone, plasma glycerol and free fatty acids, and time-trial performance after prolonged cycling exercise.

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Figure 1. Means (\pm SEM) for plasma glycerol concentrations. Samples were collected 120 min before beginning 90 min of cycle ergometry, at 30-min intervals during the exercise, after 15 min of recovery (immediately before the time trial), and immediately after a 20-km time trial at 85% VO_{2max} . There were no significant differences ($P < 0.05$) due to glutamine supplementation.

Figure 2. Means (\pm SEM) for plasma free fatty acid concentrations. Samples were collected 120 min before beginning 90 min of cycle ergometry, at 30-min intervals during the exercise, after 15 min of recovery (immediately before the time trial), and immediately after a 20-km time trial at 85% VO_{2max} . There were no significant differences ($P < 0.05$) due to glutamine supplementation.

Figure 3. Means (\pm SEM) for serum growth hormone concentrations. Samples were collected immediately before beginning 90 min of cycle ergometry, at 30-min intervals during the exercise, and after 15 min of recovery (immediately before a 20-km time trial at 85% VO_2 max). There were no significant differences ($P < 0.05$) due to glutamine supplementation.

