Glutamine supplementation further enhances exercise-induced plasma IL-6

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Recent studies have demonstrated that acute exercise elevates plasma levels of interleukin-6 (IL-6) up to 100-fold (12). This increase has been attributed to an increase in the release of IL-6 from contracting skeletal muscle (17). It has been suggested that muscle-derived IL-6 works in a hormone-like fashion, exerting its effect on liver and fat tissue (13); however, the exact mechanisms for the production, release, and biological roles of IL-6 are not known.

The amino acid glutamine is produced predominantly in skeletal muscle and, within muscle, plays a role in the synthesis and breakdown of proteins (14). Glutamine transport in skeletal muscle occurs predominantly via the insulin-sensitive Nm transport system, and it has been shown that muscle glutamine uptake is largely regulated by glutamine availability (4), such that a decrease in arterial glutamine concentration decreases the rate of glutamine uptake into skeletal muscle. Considering its role within skeletal muscle in protein synthesis, it is possible that a decrease in glutamine uptake may inhibit the production of IL-6, although this relation has not been shown previously.

Oral glutamine supplementation has been shown to elevate glutamine uptake by skeletal muscle (9), the small intestine, and the splanchic area (8). In response to acute, exhaustive exercise, oral glutamine supplementation has been shown to attenuate the exercise-induced decrease in plasma glutamine concentration when administered during exercise and in recovery (15). In this situation, glutamine supplementation after exercise may prevent the decrease in glutamine availability to skeletal muscle and further elevate the production and release of IL-6 from skeletal muscle.

This study aimed to measure the effect of oral glutamine and glutamine-rich protein supplementation on plasma IL-6 concentration in response to acute exercise. It was hypothesized that supplementation with glutamine and glutamine-rich protein would attenuate the exercise-induced decrease in plasma glutamine concentration and result in a further elevation in plasma IL-6 concentration via enhanced release from working skeletal muscle.

METHODS

Subjects. Eight healthy, highly trained men [25–48 yr of age, 47.4–68.4 ml · kg⁻¹ · min⁻¹ peak O₂ uptake (VO₂ peak)] volunteered to participate in the investigation. After a full written and verbal explanation of all procedures and possible risks associated with the study, each subject signed a written consent form. The Ethical Committee of the Copenhagen Community approved the investigation.

Experimental protocol. Subjects performed a graded, continuous exercise test to volitional exhaustion for determination of VO₂ peak. This test was performed on a cycle ergometer (Krogh, Copenhagen, Denmark). Subjects cycled at progressively higher work rates (increasing 25 W every 2 min) until volitional exhaustion. Expired O₂ and CO₂ concentrations...
were recorded each minute by using gas analyzers calibrated against known gas mixtures before each test, and inspired volume was also recorded each minute (CPF-S and CPX metabolic system, MedGraphics, St. Paul, MN). Heart rate was also recorded each minute (NV System, Polar Advantage, Kempele, Finland).

Each subject reported to the exercise laboratory at 0800 on three occasions, separated by ≥2 wk. On each occasion, subjects had fasted overnight and had not performed strenuous exercise for 24 h. Subjects performed cycle ergometry at a power output of 75% \( \dot{V}O_2 \) peak for 2 h. \( \dot{O}_2 \) uptake and \( \dot{CO}_2 \) output were measured intermittently throughout the exercise period, and the ergometer load was adjusted when necessary to maintain the desired power output. After exercise, subjects were asked to remain in the laboratory for 2 h of passive recovery. Subjects were allowed to consume water ad libitum throughout the exercise and recovery period.

**Supplementation.** Supplementation was undertaken in a double-blind, crossover, placebo-controlled manner. In each experimental trial, subjects were asked to consume one of three types of beverages on five occasions throughout the trial period. These supplements were as follows: glutamine (Gln, 3.5 g glutamine in 500 ml water), protein (Pro, 13.7 g protein from sodium caseinate, containing 1.23 g protein-bound glutamine in 375 ml water), and a placebo (Plac, 3.5 g maltodextrin in 500 ml water). The Gln and Plac beverages were first consumed after 60 min of exercise and then at 45-min intervals after consumption of the first beverage. These two beverages (Gln and Plac) were identical in appearance and taste. The Pro beverage was first consumed at the onset of exercise and then at 1-h intervals after the consumption of the first beverage. This beverage was different in appearance and taste and contained 2% sucrose, 0.2% citric acid, and 0.15% lemon flavor to enhance the taste. At all times, subjects were asked to consume the beverage provided within 5 min. The frequency of consumption of all the trial beverages was designed to maintain plasma glutamine concentration throughout the exercise and recovery period and was based on pilot studies performed in our laboratory. All products were provided by the Nestlé Research Center and were prepared for consumption on the morning of each experimental trial. After consumption of the final beverage, subjects were provided a standardized meal consisting of ~200 g white bread, 65 g cheese, 150 g tomato, 50 g lettuce, 150 g cucumber, and one average-sized banana.

**Blood collection.** Blood samples (10 ml) were collected on three occasions by venipuncture of an antecubital vein before the onset of exercise, immediately after exercise, and 2 h after the cessation of exercise. EDTA-treated whole blood samples were centrifuged for 15 min at 2,500 g at 4°C, and the supernatant was transferred to 1.6-ml cryovials and stored at −80°C for later analysis.

**Analytic techniques.** Plasma glutamine concentration was measured by high-performance liquid chromatography as outlined previously (3). Plasma IL-6 concentration was corrected for changes in plasma volume according to the equation of Dill and Costill (1) using measurements of hematocrit and hemoglobin. Plasma IL-6 was measured by high-sensitivity enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions.

**Statistics.** Statistical analysis was performed by using SigmaStat for Windows (version 2.03). Values are means ± SE. A two-way analysis of variance for repeated measures was used to determine an effect of treatment and time on plasma IL-6 concentration. Tukey’s test for pairwise multiple comparison procedure was performed to identify the source of any significant differences. At all times, \( P < 0.05 \) was used to indicate statistical significance.

**RESULTS**

**Plasma glutamine concentration.** There was no effect of time on plasma glutamine concentration in the Gln and Pro experimental groups (Fig. 1; \( P > 0.05 \)). That is, glutamine and protein supplementation were able to attenuate the exercise-induced decrease in plasma glutamine concentration. However, in the Plac group, there was a significant decrease in plasma glutamine concentration at 2 h after exercise compared with pre-exercise and immediately after exercise.

**Plasma interleukin-6 (IL-6) concentration before exercise, immediately after exercise, and 2 h after exercise in groups supplemented with Glu, Pro, and Plac. Values are means ± SE. *Significantly different from Pre and Post 2 h (\( P < 0.005 \)); **Significantly different from Pre and Post 2 h (\( P < 0.001 \)); § significantly different from Pro and Plac (\( P < 0.005 \)).**
Plasma IL-6 concentration. Plasma IL-6 concentration is presented in Fig. 2. Plasma IL-6 concentration increased from preexercise to immediately after exercise in all groups (P < 0.001). At 2 h after exercise, plasma IL-6 concentration had almost returned to preexercise levels in all groups (P < 0.005). There was no difference in plasma IL-6 concentration between groups before exercise (P > 0.05). However, immediately after exercise, plasma IL-6 concentration was significantly greater in the Gln group (18-fold increase from preexercise) than in the Pro (14-fold) and Plac (11-fold) groups (P < 0.005). There was no difference in plasma IL-6 between the Pro and Plac groups immediately after exercise. At 2 h after exercise, plasma IL-6 concentration was not significantly different between groups. Plasma glucose levels did not differ between the groups (data not shown).

DISCUSSION

The main finding of this study was that the exercise-induced increase in circulating IL-6 was further enhanced by glutamine and glutamine-rich protein supplementation, administered at doses that attenuated the exercise-induced decrease in plasma glutamine concentration.

In all groups, plasma IL-6 concentration increased in response to acute exercise. This has been shown in previous studies, and this increase is likely due to an increase in the production and release of IL-6 from the working skeletal muscle (17). The augmented increase in plasma IL-6 in the Gln and Pro groups, however, may be explained by an increase in skeletal muscle glutamine uptake, which then stimulates a further increase in IL-6 production. A decrease in skeletal muscle glutamine uptake may result in a decrease in its metabolism within the muscle, lowering the production and release of IL-6, and also attenuate the release of glutamine into the circulation. However, when glutamine concentration is maintained during exercise by supplementation, skeletal muscle uptake is maintained, and the production and release of IL-6 are further enhanced.

However, the actual difference in plasma glutamine concentrations between the supplemented groups and the placebo group was <100 μM, and in none of the groups was plasma glutamine <500 μM. It could be questioned whether the enhanced IL-6 level in the supplemented groups is due to an enhanced production of IL-6. An alternative explanation of the data could be that glutamine supplementation influences the clearance of IL-6 during exercise. Lyngsø et al. (7) recently showed that human recombinant IL-6 infusion increases the clearance of IL-6 by the splanchnic area. A recent study from our group (unpublished observations) demonstrates that the splanchnic area clears IL-6 during acute exercise, when circulating levels of IL-6 are elevated. It is known that IL-6 is involved in hepatic amino acid transport, including the transport of glutamine (2, 18). Thus it may be hypothesized that a decrease in plasma glutamine concentration, via a decrease in skeletal muscle release (or an increase in uptake by other tissues, such as the kidneys or immune cells), results in a decrease in IL-6-mediated uptake by the liver. As a result, the demand for IL-6 release from skeletal muscle may decrease, inasmuch as less IL-6 is required to mediate hepatic glutamine uptake. This may be reflected by lower plasma levels of IL-6.

Several studies have demonstrated that carbohydrate loading attenuates the exercise-induced increase in plasma IL-6 (10) and that low muscle glycogen further enhances the transcription and release of muscle-derived IL-6 (5). In the present study, the dietary intake was highly controlled, and the subjects performed the three trials in a randomized fashion. Thus muscle glycogen content is unlikely to be a contributing factor. The placebo drink contained a very small amount of sucrose, equivalent to only ~10% of the doses used in carbohydrate-loading studies (11), and the blood glucose levels were identical in all three trials (6). Therefore, the observed changes in plasma IL-6 concentration cannot be attributed to changes in carbohydrate metabolism.

In conclusion, the present study has demonstrated that an exercise-induced increase in plasma IL-6 is further enhanced by glutamine supplementation.

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REFERENCES


