

Research article

GLUTAMINE SUPPLEMENTATION DID NOT BENEFIT ATHLETES DURING SHORT-TERM WEIGHT REDUCTION

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ABSTRACT

The purpose was to determine if glutamine supplementation would prevent a loss of lean mass in athletes during a 12-day weight reduction program. It was hypothesized that supplementation would spare lean body mass. Subjects (n=18) exercised and dieted to create a 4186kJ· day⁻¹ energy deficit and a 8372 kJ· day⁻¹ energy deficit on days 1-5, days 6-12, respectively. The glutamine (GLN) group (n=9) ingested 0.35 g· kg⁻¹ body mass of glutamine while a placebo was administered to the remaining subjects. Body mass (BM), lean body mass (LBM) and fat mass (FM), were measured at days 0, 6, and 12. GLN and placebo groups both lost significant amounts of BM, LBM and FM. There were no significant differences between groups. The findings indicate little benefit for retention of lean mass with supplementation of glutamine during a short-term weight reduction program.

KEY WORDS: Amino acid, protein metabolism, anticatabolic

INTRODUCTION

Glutamine is the most abundant amino acid in human plasma and the intracellular pool of free amino acids in skeletal muscle. It is synthesized and stored primarily in skeletal muscle and released into the blood when the metabolic need for this amino acid is present. Its role in the physiology of humans includes catabolism in the gut, liver, kidneys, and cells of immune function. It is classified as a non-essential amino acid due to the body's ability to synthesize it from metabolic intermediates using transamination from branched-chained amino acids. However researchers have suggested that its role in the body during catabolic states would indicate that it as a "conditionally essential" amino acid (Lacey and Wilmore, 1990). Studies on post-surgical patients demonstrated the potential for glutamine as an anticatabolic agent (Blomqvist et al., 1995; Boelens et al., 2001; Coster et al., 2003). Additionally, glutamine supplementation has been used to prevent endogenous protein breakdown in

very low birth weight infants (Neu et al., 2002). The addition of glutamine through total parenteral nutrition (TPN) at a dose of 0.28 g kg⁻¹ body mass improved nitrogen balance and prevented the decline of muscle free glutamine, a sign of whole body protein catabolism (Hammarqvist et al., 1989). Hickson (1995) suggested that glutamine supplementation is an effective antagonist of glucocorticoid-mediated muscle atrophy. The atrophy prevention is associated with maintenance of intramuscular glutamine levels and a partial reversal of the declines in myosin heavy chain and total protein synthesis. Thus, glutamine supplementation has clinical relevance as a therapy against muscle atrophy.

The preservation of skeletal muscle during a weight reduction program is extremely important for athletic performance, since the level of skeletal muscle has been linked to measures of strength, muscular endurance, and anaerobic power. In the sport of wrestling, where deliberate and voluntary weight reduction is so prominent, a large amount of

the weight loss can be in the form of fat-free mass. Freischlag (1984) examined a group of wrestlers during two consecutive wrestling seasons and found that wrestlers had a total weight loss of 2.7 kg, of which approximately 2.1 kg was fat-free mass. Utter et al (1998) recently reported with an average body mass reduction of 4.9 kg from pre-season to mid-season in college wrestlers, 2.1 kg (43%) was from fat-free mass. With very low daily energy intakes ($\leq 5000 \text{ kJ} \cdot \text{day}^{-1}$) and the high daily energy expenditure ($>9000 \text{ kJ} \cdot \text{day}^{-1}$), values that have been reported for a wrestler preparing for competition, it would seem that the potential for loss of fat-free mass is evident (Widerman and Hagan, 1982).

Due to the clinical evidence of glutamine supplementation, it has been suggested that glutamine has potential utility as a dietary supplement for athletes engaged in heavy exercise training (Antonio and Street, 1999). Two studies have investigated the effect of oral glutamine supplementation during resistance training (Antonio et al., 2002; Candow et al., 2002). No significant differences between the glutamine groups and placebo groups were reported for any of the variables of strength or body mass. Perhaps, the application of oral glutamine supplementation for improvements in strength and lean body mass is not appropriate. The clinical applications of glutamine suggest retention of muscle mass during periods of severe stress would be the expected outcome. Therefore, the purpose of this study was to determine if glutamine supplementation in addition to a high-protein diet compared to high-protein alone would attenuate a loss of lean body mass in athletes during a weight reduction program. It is hypothesized that the presence of glutamine via endogenous pathways will spare the loss in fat-free mass associated with a weight reduction program using restricted energy intake and exercise.

METHODS

This study involved a randomized trial to examine the effects of glutamine supplementation on body composition during a 12-day weight reduction program conducted by athletes. The protocol was reviewed and approved by the University's Human Subjects Committee and written informed consent was obtained.

Subject Selection

Male wrestlers from the college wrestling team at a mid-sized University in the United States were recruited. As part of the pre-season screening, all wrestlers were assessed for body mass and body composition (skinfold measurements). Subjects were excluded if they failed medical clearance, had body

fat $\leq 7\%$, and body mass $> 100 \text{ kg}$. Sixteen of the wrestlers that met inclusion criterion volunteered to participate. Two male Reserve Officer Training Corp (ROTC) members were recruited that were engaged in a regular conditioning program in order to obtain a 80% statistical power and an alpha level of 0.05. Baseline age, weight, height, and body composition for the subjects are listed in Table 1. Subjects were separated by baseline body mass into two equally numbered categories (heavyweight or lightweight). Within these categories, subjects were randomly assigned to either a glutamine or placebo group.

Table 1. Mean (SEM) baseline subject demographic and body composition data.

Parameters	Glutamine (n=9)	Placebo (n=9)
Age (yrs.)	21.3 (1.2)	20.7 (1.2)
Height (m)	1.74 (.02)	1.75 (.02)
Body mass (kg)	73.5 (2.9)	77.3 (2.9)
Lean mass (kg)	63.3 (2.2)	66.4 (2.2)
Fat mass (kg)	10.1 (.8)	10.9 (.8)
% Body fat	13.7 (.7)	13.9 (.7)

Dietary Intervention

All meals were planned using a composition high in carbohydrates (55%) throughout the study period with both groups receiving an energy intake value relative to their pre-study body weight. The food was measured, weighed and served to each individual subject. All subjects were fed three times per day at the campus-dining center and monitored to ensure they consumed the meal. During two days prior to the weight reduction period, the subjects were fed a control diet consisting of $171.6 \text{ kJ} \cdot \text{kg}^{-1}$ body mass. This control diet was used to ensure all subjects began the weight reduction program with similar nutritional status and was based upon previous weight reduction research involving wrestlers (Horswell et al., 1990; Rankin et al., 1996). The maintenance of protein content for this study was used to control the effect of protein content in diet on urinary urea nitrogen levels. The value $1.0 - 1.5 \text{ g} \cdot \text{kg}^{-1}$ body mass has been suggested when training weight-restricted wrestlers attempting to lose body fat (ACSM, 1996).

On day 1 of the weight reduction period, the energy intake was decreased by 25% while on day 6, another 25% of energy intake was omitted. Carbohydrate percentage and protein volume ($1.5 \text{ g} \cdot \text{kg}^{-1}$ body mass $\cdot \text{day}^{-1}$) was maintained for all subjects throughout the study. Fat intake was reduced (percentage and absolute volume) throughout the study to lower energy intake. There were no differences in total energy intake or macronutrient composition between groups (Table

2). Subjects were instructed to refrain from other food consumption during the period of the study. All subjects were encouraged to hydrate using water.

Exercise Program

Throughout the study, all subjects underwent two physical training sessions per day to increase energy expenditure. One session grouped 4-8 subjects together to exercise under supervision in the Human Performance Laboratory. The other session involved a total group workout conducted by the wrestling staff as part of the pre-season conditioning program. The ROTC subjects performed their group physical training program with their military peers. All sessions were evaluated to estimate energy expenditure. An exercise professional prescribed daily exercise volume and intensity for the session in the Human Performance Laboratory using estimates of daily energy expenditure (Benedict-Harris Equations), practice logs from the previous day's group activity, and known energy intakes for that day. The goal was to provide a caloric balance (energy intake = energy expenditure) for the two days prior to weight reduction, produce an energy deficit (energy intake < energy expenditure) of 4,186 kJ· day⁻¹ for the next six days and 8,372 kJ· day⁻¹ for the final six days.

A daily record was kept to determine compliance to the supervised exercise sessions. Any subject missing a group or individual session would be subject to dismissal from the study. Subjects were instructed not to perform other vigorous exertion outside of the prescribed training as part of the study. All subjects were engaged in typical daily activities of college students.

Treatment

Glutamine was given in a dose of 0.35 g· kg⁻¹ body mass· day⁻¹ to the treatment group (n=9) split between two daily doses for 12 days. The volume of glutamine was selected since it had been tested and found safe for oral ingestion (Ziegler et al., 1990). The splitting of the doses was utilized in order to provide two periods for plasma glutamine rise

through endogenous means and to minimize the detection of the glutamine in solution. The glutamine was mixed in a colored container with an artificially sweetened beverage and served at the time of the meal. The periods of oral administration occurred during the afternoon and evening meals after the scheduled exercise session. The placebo group received the same artificially sweetened beverage in a similar container without glutamine.

Measurements

Body composition was assessed by dual energy x-ray absorptiometry using a Hologic QDR 4500. The Hologic QDR 4500 is a multiple detector, fan beam DXA using low dose radiation (≤ 1 mrem) to detect bone mineral content and soft tissue composition. The coefficient of variance for total lean mass and total percent fat is 1.0%. Total body scans were performed after the clinical laboratory tests in the late-morning of days 0, 6, and 12. The Whole Body V8.23 software provided total body mass, bone mineral content (BMC), lean (non-bone) mass, lean + BMC mass, and fat mass. The measures of body mass, lean + BMC (lean body mass), and fat mass were used in the analysis.

Statistical Analysis

Data were entered on a spreadsheet and analyzed using SPSS for Windows Version 9.0. All measures of body composition (body mass, lean body mass, fat mass) were tested for between and within group differences using Analysis of Variance (ANOVA) with repeated measures. A probability level of 0.05 was chosen for statistical analysis. First, groups x time interactions were evaluated. If no significant interactions were evident, simple main effects were examined. Paired sample comparisons were used to test differences within the treatment group and independent T-test were used to compare each simple main effect between the two groups. A Bonferroni adjustment was made to the probability level for within group comparisons ($0.05/3 = 0.0167$).

Table 2. Mean (SEM) energy intakes over the study period.

Days	Group	Energy Intake (kJ)	Protein* (g)	Fat (g)	Carbohydrate (g)
-1 – 0	Glutamine	12571 (486)	110 (4)	108 (4)	436 (19)
	Placebo	13148 (486)	115 (4)	107 (4)	462 (19)
1 – 5	Glutamine	9427 (364)	110 (4)	69 (4)	313 (11)
	Placebo	9862 (364)	115 (4)	74 (4)	323 (11)
6 – 12	Glutamine	6283 (243)	110 (4)	33 (2)	194 (8)
	Placebo	6576 (243)	115 (4)	33 (2)	207 (8)

*dietary protein only

Table 3. Means (SEM) from body composition data.

Variables	Groups	n	Day 0	Day 6	Day 12
Body Mass* (kg)	Combined	18	75.39 (2.04)	74.05 (1.98) ^a	73.40 (1.96) ^{a,b}
	Glutamine	9	73.46 (2.43)	72.44 (2.33)	71.59 (2.29)
	Placebo	9	77.33 (3.23)	75.85 (3.22)	75.21 (3.19)
Lean Mass* (kg)	Combined	18	64.85 (1.54)	64.12 (1.50) ^a	64.16 (1.46) ^a
	Glutamine	9	63.30 (1.85)	62.70 (1.79)	62.68 (1.73)
	Placebo	9	66.39 (2.46)	65.53 (2.43)	65.64 (2.34)
Fat Mass* (kg)	Combined	18	10.55 (.58)	9.90 (.56) ^a	9.25 (.58) ^{a,b}
	Glutamine	9	10.15 (.74)	9.54 (.72)	8.92 (.71)
	Placebo	9	10.94 (.92)	10.26 (.89)	9.58 (.95)

* $p < .05$ for time effect, a = significant difference vs day 3, b = significant difference vs day 8 ($p < .0167$, Bonferroni adjusted 0.05/3)

RESULTS

Eighteen subjects that volunteered completed all the study requirements. The means (\pm SEM) for body mass, lean body mass, and fat mass over the study period for the two groups are listed on Table 3. Repeated measures analysis indicated no significant interaction between group and time for any of the body composition parameters. Simple main effects of time were significant on all body composition measures however there was no significant group effect. Both treatment and placebo groups lost significant amounts of body mass ($p < .001$), lean ($p < .001$), and fat mass ($p < .001$) over the 12-day period. Pair-wise comparisons determined that both groups experienced a significant reduction in body mass and fat mass between day 0 and day 6, day 0 and day 12, and between day 6 and day 12. However, significant reductions in lean body mass were observed only between day 0 and either day 6 or day 12 ($p < .05$). No significant differences in lean body mass were observed for either group between day 6 and day 12 ($p = .581$).

DISCUSSION

In this study, all subjects lost a significant amount of body mass during the 12-day weight loss program, as well as a significant amount of fat-free mass and fat mass as measured by DXA. However the ingestion of glutamine had no significant effect on the amount of lean mass retained or fat loss as compared to the placebo group. Since this is one of the first studies to examine these variables during oral glutamine supplementation, offering comparison to other studies is not possible and thus evaluating the benefits of oral glutamine supplementation is difficult. Further examination of the dosage procedure and protein intake elicited observations that might offer potential explanation to the findings.

For supplementation to be effective, the endogenous intake should raise the plasma glutamine to a level that is significantly greater than the placebo group. That is, during times of stress, the consumption of glutamine exceeds skeletal muscle glutamine synthesis, which results in depletion of skeletal muscle glutamine stores (Boelens et al., 2001). Considering that there is a strong correlation between glutamine muscle concentration and protein synthesis rates in skeletal muscle (Jepson et al., 1988), the maintenance of muscle glutamine stores maybe critical in maintaining lean body mass during weight loss. Further analysis of the literature indicates that following oral ingestion of glutamine, plasma glutamine levels peak at 30 minutes and return to baseline at ~ 2 hours (Castell and Newsholme, 1997). However, the study by Castell and Newsholme (1997) observed a 50% increase in plasma glutamine levels after 30 minutes of ingestion, with a dose of $0.1 \text{ g} \cdot \text{kg}^{-1}$. The doses administered in this study were $0.175 \text{ g} \cdot \text{kg}^{-1}$ (ingested twice per day) and therefore should have been sufficient to cause a transient increase in plasma glutamine levels.

Assuming that the dosages were high enough to raise the plasma glutamine levels, issues arise in regards to the fate of the glutamine. Due to the high levels of glutaminase in the splanchnic tissues, it is most likely that those tissues consumed the majority of the ingested glutamine. More specifically, it has been observed that as much as 76% of infused glutamine is taken up by the splanchnic tissue during the first pass in healthy men and women (Mitterdorfer et al., 2001). Because of this reaction, it would be impossible to maintain elevated plasma glutamine levels via two doses. According to one study, $0.57 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of glutamine, administered via TPN is required to maintain elevated plasma levels (Weingartmann et al., 1996). Furthermore, it has been observed that glutamine supplementation must occur each 30 minutes in order to maintain

elevated plasma glutamine levels (Hiscock and Pedersen, 2002). Clearly, two doses of glutamine ($0.175 \text{ g} \cdot \text{kg}^{-1}$) daily may not have been sufficient to maintain elevated plasma glutamine levels in the glutamine group.

In regards to protein intake, all subjects consumed a relatively high protein content throughout the study period ($1.5 \text{ g} \cdot \text{kg}^{-1}$ body weight). This diet may have provided the body with adequate levels of protein as to allow for weight loss without protein catabolism. Additionally, several studies have described the efficacy of high protein diets in attenuating the loss of lean body mass during rapid weight loss (Piatti et al., 1994). It was the intent of this investigation to test the effect of glutamine with a high protein on the retention of lean body mass during weight loss. To ensure that any effect of glutamine consumption on body composition was not due to an increase in protein intake alone, all subjects received similar high protein content in their diets.

Another possible explanation for the finding may be related to the total amount of body mass (and thus lean body mass) that was lost during this study period. The weight loss program may not have had sufficient time or produced the necessary energy deficit to induce the catabolic state which glutamine supplementation could benefit. The placebo group and glutamine group experienced a mean weight loss of 2.12 and 1.87 kg over the 12-day period respectively. The weight reduction program was designed to reduce energy intake and increase energy expenditure to create a large energy deficit ($>8000 \text{ kJ} \cdot \text{day}^{-1}$) after the 6th day of the 12-day period. It was decided not to create this energy deficit immediately during the first week of the study so as not to promote protein catabolism. Therefore only six days of extreme energy deficit was experienced. By increasing the duration of this deficit, greater weight loss and fat-free weight loss may have been observed, however it maybe difficult for athletes to adhere to such a program for an extended period of time. It is uncommon for wrestling to severely restrict calories for more than one week (Steen and Brownell, 1990). In a practical sense, increasing the duration of the deficit is not recommended. In regards to increasing the energy deficit via further restricting energy intake, wrestlers are recommended to consume $7116 - 10465 \text{ kJ} \cdot \text{day}^{-1}$ (ACSM, 1996). The subjects in this study were fed only $6283 - 6576 \text{ kJ} \cdot \text{day}^{-1}$, therefore further reducing the energy intake is not recommended.

CONCLUSIONS

In conclusion, the findings of this study indicate little benefit for retention of muscle mass with oral

supplementation of glutamine, during a short-term weight reduction program commonly used in wrestling. It is likely that this study did not provide the same catabolic state or decreased plasma glutamine levels that are experienced in patients during sepsis or trauma. In addition, the high levels of protein provided in the diet of these exercising subjects may have provided adequate nitrogen to the body as to offset the protein catabolism. Since this is one of the first studies to investigate the effects of glutamine supplementation on retention of lean body mass in athletes, further studies should be conducted to determine whether these conclusions are supported.

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