

Original Article

The effects of glutamine supplementation on performance and hormonal responses in non-athlete male students during eight week resistance training

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ABSTRACT

Hakimi M, Mohamadi MA, Ghaderi Z. The effects of glutamine supplementation on performance and hormonal responses in non-athlete male students during eight week resistance training. *J. Hum. Sport Exerc.* Vol. 7, No. 4, pp.770-782, 2012. The aim of this study was to determine the effects of glutamine supplementation on performance, and hormonal changes during an 8-week resistance training program in non athlete male students. Thirty healthy non athlete male (age 21.25 ± 1.6 years, height 173.2 ± 3.2 cm, body mass 72.8 ± 2.8 kg, VO_{2max} 43.48 ± 2.38 ml·kg⁻¹·min⁻¹) were randomly divided into a glutamine supplementation (GL) group (n=15), and a placebo (PL) group (n=15). Each group was given either glutamine or a placebo in a double blind manner to be taken orally for eight weeks (0.35 g/kg/day). GL and PL groups performed the same weight training program 3 days, each week for 8 weeks. The training consisted of 3 sets of 8 repetitions, and the initial weight was 80% of the pre-1RM. Subjects were tested for performance and blood hormone concentrations before and after the 8-week period. Both groups increased their performance however the GL group showed significantly greater increases in upper and lower body strength, explosive muscular power, blood testosterone, GH and IGF-1 when compared to the PL group; however, cortisol concentrations were significantly more reduced in GL group when compared to the PL group. It can, therefore, be concluded that within 8 weeks glutamine supplementation during resistance training was found to increase performance (explosive muscular power, muscle strength) and improved body composition (increased body mass, fat-free mass and reduced body fat). **Key words:** GLUTAMINE SUPPLEMENTATION, RESISTANCE TRAINING, HORMONAL CHANGES.

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INTRODUCTION

Nowadays the high level of muscular fitness can aid to achieve best performance. On this basis, coaches are searching ways to improve performance. Resistance training has become a frequently chosen method for increasing strength, flexibility, muscle mass, power and speed, local muscular endurance, balance and for improving athletic performance (American College of Sports Medicine, 2001). Also amino acids are theorized to enhance performance in a variety of ways, such as increasing the secretion of anabolic hormones, modifying fuel use during exercise, preventing adverse effects of overtraining, and preventing mental fatigue (Melvin, 2005). Glutamine is the most abundant non-essential amino acid in human muscle and plasma (Phillips, 2007). Glutamine is one of the most popular dietary supplements marketed to athletes and physically active individuals (Varnier et al., 1995; Bowtell et al., 1999). Also glutamine is involved in so many physiological processes, it has been suggested that glutamine supplementation may assist athletes by: providing nutritional support for the immune system and preventing infection, improving cellular fluid retention, increasing water absorption from the gut, encouraging muscle glycogen synthesis, stimulating muscle protein synthesis, therefore enhance muscle growth, reducing muscle soreness and enhancing tissue repair and enhancing buffering capacity (Varnier et al., 1995; bowtell et al., 1999).

Glutamine is an important component of protein and is involved in many physiological roles such as nucleotide synthesis, gluconeogenesis, maintaining acid-base balance, and regulation of protein production and destruction (Wernerman et al., 2008; Gleeson, 2008; Karogotich et al., 2007; Lagranha et al., 2007). In a study by Lacey et al. (1990) short-term glutamine ingestion had no effect on muscular strength; however, long-term supplementation showed to be a more effective application of glutamine in regards to strength gains.

Candow et al. (2001) assessed the effect of oral glutamine supplementation combined with resistance training in young adults. Strength and muscular skeletal markers were examined before and after the six week study in both the placebo and experimental group. It appears as though there was a slight increase in one repetition squat, force production in the knee extensor, and lean muscle mass. Although these numbers were slightly higher than the placebo group they were not enough to be a "significant" difference. It must be noted that many world class athletes may work years for small increases in performance that may or may not seem "significant" to those in a laboratory setting but may be of utmost importance to the elite athlete. However to the average weightlifter results from this specific study may not warrant supplementation with glutamine.

One study has investigated the effect of oral glutamine supplementation during resistance training (Antonio 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. Several studies indicate that glutamine supplementation increases cell volume and stimulates protein and glycogen synthesis (Antonio, 1999; Low et al., 1996; Varnier et al., 1995). Theoretically, glutamine supplementation prior to and/or following exercise (e.g., 6-10 g) would help to optimize cell hydration and protein synthesis during training leading to greater gains in muscle mass and strength. However, although there is strong scientific rationale, additional research is needed to determine the impact of glutamine supplementation during training on body composition and strength before definitive conclusions can be made. The scientific evidence suggests that acute consumption of 20-30g per day in healthy adults has been tolerated without adverse effects (Walsh et al., 2000). For a full review on dosages utilised in scientific studies, please refer to Gleeson (2008).

Hormonal concentrations in blood have been widely used to study the association of training programmes with performance in a multitude of exercise training including resistance training. Anabolism is the metabolic pathway by which complex tissues such as fat and muscle are synthesized from simple compounds. Anabolic hormone is primarily responsible for protein synthesis resulting in the promotion of muscle growth and the growth of other complex living tissue in the body. There are 4 major anabolic hormones that indirectly or directly affect protein synthesis. They are growth hormone (GH), insulin-like growth factor-1 (IGF-1) and testosterone. Also catabolic hormones such as cortisol are secreted by the human body and act to erode muscle tissue. Both anabolic and catabolic hormones are needed by the human body to maintain homeostasis, or regulation of a stable internal environment (Hadley & Hinds, 2002; Demling & Desanti, 2001). However, there have only been a few studies that have examined the effect of prolonged glutamine supplementation (e.g. length of a typical off-season resistance training program) on changes in hormonal concentrations in non athlete men during resistance training. The main hormones which have been studied in this protocol training are anabolic hormones such of growth hormone (GH), testosterone, insulin like growth factor (IGF-1) and catabolic hormones such of cortisol hormone.

Thus, the purpose of this study was to examine the effect of glutamine supplementation on upper and lower body strength, explosive muscular power, body composition (body mass, body fat, fat-free mass) and hormonal adaptations (Testosterone, GH, IGF-1 and cortisol) during an 8-week resistance training program in non athlete healthy young male students.

MATERIAL AND METHODS

Participants

Thirty non athlete healthy young male students volunteered to participate in this study. Subjects were randomly assigned to either a glutamine supplement group (GL; n =15) or a placebo group (PL; n =15). After signing the informed consent, demographic information regarding each subject was collected. This information included the subject's age, height, VO_{2max} and body composition (see Table 1). Before undergoing the tests, the subjects were given explanations about the assessment procedures, study objectives, and the possible benefits and risks. The Institutional Review Board of the University approved of the research protocol. According to the medical information questionnaire, all subjects were healthy and none complained of hypertension, a cardiovascular disease, diabetes, lipid disorders, a kidney disease a liver disease, respiratory and bone injuries and did not report any supplement use in past 6 months. None of them had continuous exercise history. They were non athlete and did not follow a specific diet. The study protocol was explained to the volunteers, and all of them signed a written consent for the study. Before the study, the subjects were informed about the type, severity and number of days in a week and the time of activities, and they were asked to keep the diet and the intensity of activities constant during the study period, and not to use any other dietary supplements. Their body mass was measured to the nearest 0.1 kg using an electronic body mass scale (Seca 707; Seca GmbH & Co. KG., Hamburg, Germany) also height was measured by rod (Iran). Standing height and weight was used to calculate the BMI (kg/m^2).

Table 1. Study participants' demographics.

Parameters	GL group (N=15)	PL group (N=15)
Age (yrs)	20.8 ± 1.3	21.7 ± 3.1
Height (m)	1.74 ± 2.18	1.72 ± 3.32
Body mass (kg)	74.2± 3.45	71.4± 2.36
Body fat (kg)	11.3± 4.38	10.6± 5.45
Body fat (%)	15.22± 5.12	14.84± 3.68
Fat-free mass (kg)	62.9± 2.31	60.8± 4.18
BMI (Kg/m ²)	24.50± 1.58	24.13± 1.18
VO _{2max} (ML·Kg ⁻¹ ·Min ⁻¹)	42.54 ± 5.84	44.42 ± 6.46

Note: Data are presented as means ± standard deviations.

GL: glutamine supplementation group, PL: placebo supplementation group.

Data collection procedures

Approval to conduct this research was granted by the Institutional Review Boards of the two participating institutions. Coaches from various sport teams were contacted to inform them of the study and to gain their support. An online survey was posted on SurveyMonkey, a web-based Internet survey engine. An email was sent directly to potential participants via SurveyMonkey with an invitation to participate in the study. In the email a link was provided to directly access the survey. Once the link was clicked, participant rights were presented along with a description of the study. If participants consented to participate they continued to fill out the survey. Once the survey was completed the window automatically closed and the data was saved on SurveyMonkey for export and analysis.

Experimental design

A double-blind, randomized study was employed using two experimental groups (glutamine and placebo supplementation) who underwent 8 weeks' supplementation. Before starting the training, pre-1 repetition maximum (1RM) values were obtained on the following exercises: leg extension, leg flexion, squat, bench press, lateral pull down, and triceps pushdown. Six different lifts were performed and they were identical to those used in the 1RM measurements. GL and PL groups performed the same weight training program 3 days (Tuesday, Thursday and Saturday) each week for 8 weeks. The training consisted of 3 sets of 8 repetitions, and the initial weight was 80% of the pre-1RM. The warm-up prior to each session consisted of 2 sets of 12 repetitions of the first exercise at 40% of the 1RM load, and then 80% of the 1RM load was selected as the load used in testing (Burke et al., 2001; Mayhew et al., 1992). When participants were able to perform more than 8 repetitions on the third set, they were instructed to increase their resistance for the next workout. Rest times between sets were 2-3 minutes, and 3-5 minutes elapsed between the 6 different lifts. After selection for either the supplement group or the placebo group, the subjects were required to participate in an 8-week training program, details of which are outlined below. After the 8-week training program, post-testing for 1RM were repeated in the same manner in which they were performed during pretesting.

Strength measures

Lower and upper body maximal strength was assessed by using 1RM actions. During each testing session subjects performed a 1-repetition maximum (1-RM) strength test for the squat and bench press exercises. The 1 RM tests were conducted as described by Hoffman (2006). Each subject performed a warm-up set using resistance that was approximately 40-60% of his perceived maximum, and then performed three to four subsequent attempts to determine the 1-RM. A 3-5 minute rest period was provided between each lift. No bouncing was permitted, as this would have artificially boosted strength results. Bench press testing was performed in the standard supine position: the subject lowered an Olympic weightlifting bar to midchest and then pressed the weight until his arms were fully extended. The squat exercise required the subject to rest an Olympic weightlifting bar across the trapezius at a self-chosen location. The squat was performed to the parallel position, which was achieved when the greater trochanter of the femur was lowered to the same level as the knee. The subject then lifted the weight until his knees were extended.

Vertical jump

The vertical jump has become a standard measure of athletic ability and power performance. Vertical jump height was measured via a Vertec vertical jump tester (Sports Imports, Hilliard, OH, USA) to give an indication of explosive muscular power (Canavan & Vescovi, 2004). Each subject performed three trials with one minute of rest in between each jump and the highest jump height was used in the data analysis. The following procedure was used for each subject during data collection. The Vertec was adjusted to match the height of the individual subject by having them stand with their dominant side to the base of the testing device. Their dominant hand was raised and the Vertec was adjusted so that their hand was the appropriate distance away from the marker based on markings on the device itself. At that point, subjects performed a countermovement jump. Arm swings were allowed but no preparatory step was performed.

Body composition

Body composition was determined from seven skinfold sites (triceps, subscapular, midaxillary, chest, suprailiac, abdomen, and thigh) according to the method of Lohman, et al. (1988) using a Lange skinfold caliper. Skinfold measurements were based on the average of two trials and obtained on the right side in serial fashion by the same investigator. Body density was estimated using the age-adjusted equation of Pollock and Jackson (1984). The three-compartment Siri equation was used for % body fat (Siri, 1961).

Blood collection and analyses

The subjects were asked to fast for 10 hours before the study. Immediately after the first resistance training session, blood samples were drawn from an antecubital forearm vein using a 20-gauge needle and Vacutainers to determine serum testosterone, GH, IGF-1 and cortisol concentration. For each subject blood samples were obtained, before and after 8 weeks of supplementation (immediately after the first and the last resistance training sessions), in the early morning hours and after a 10-h overnight fast in order to minimize the effects of diurnal hormonal variations. The blood was processed and centrifuged, and the resultant serum was stored at -80°C until analyzed. Serum total testosterone, GH, IGF-1 and cortisol were determined in duplicate by using standard RIA procedures and were assayed via ELISA kits obtained from Diagnostic Systems Laboratories (Webster, TX, OH).

Supplement schedule

The glutamine supplement (ultimate nutrition, Farmington Inc, CT, USA) and placebo (the placebo content of the supplement consisted of starch) was in powder form and provided in individual packets. The only permitted supplement was glutamine powder containing no additional ingredients. Daily glutamine supplementation was 0.35g/kg/day parceled into three equal dosages to be consumed with each major

meal (Finn et al., 2003). The contents of each packet were mixed with 500 ml of water. Subjects consumed one drink every morning, the second daily drink following their exercise session and the third daily drink in the evening. On non-training days, the GL and PL groups ingested 1 dose of the GL or PL supplement in the morning and once again in the evening. The subjects consumed the supplements for 8-week.

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows software (version 17.0; SPSS Inc.). Descriptive statistics were calculated as the mean and standard deviations (Mean \pm SD). Changes from baseline were assessed using the paired sample t-test. In addition, PRE – POST comparisons between groups in performance measures were analyzed with independent student's t-tests. The level of significance for this investigation was set at $p < 0.05$.

RESULTS

Performance

Significant increases in strength and explosive muscular performance from PRE occurred for both GL and PL in the 1-RM squat; 1-RM bench press and vertical jump (see Table 2). However, strength and explosive muscular comparisons showed that subjects in GL had significantly greater improvement in 1-RM squat, 1-RM bench press strength and vertical jump compared to the PL group.

Table 2. Measures of body strength (Upper and lower body strength) in the GL (N=15) and PL (N=15) groups during the pre and post-supplementation period. Data presented as mean \pm SEM.

	GL		PL	
	Pre	post	Pre	post
Upper body Strength (kg)	81.25 \pm 6.07	87.5 \pm 5.48* **	82.12 \pm 5.53	85.65 \pm 6.54*
Lower body Strength (kg)	89.76 \pm 7.27	95.78 \pm 6.45* **	90.37 \pm 7.87	93.91 \pm 6.52*
Vertical Jump (cm)	62.31 \pm 5.22	67.11 \pm 4.44* **	65.31 \pm 7.34	67.47 \pm 5.43*

GL: glutamine supplementation group, PL: placebo supplementation group

* Significantly different from corresponding to pre training value; ** Significantly different between GL and PL.

Body composition

The GL group gained significantly more body mass (2.3 \pm 0.08 kg) and fat-free mass (2.9 \pm 1.05 kg) than the PL group. Also GL group showed significantly greater decrease in body fat (0.6 \pm 0.11) compared to GL group (Table 3).

Table 3. Measures of body composition (body mass, Body fat and Fat-free mass) in the GL (N=15) and PL (N=15) groups during the pre and post-supplementation period. Data presented as mean ± SEM.

	GL		PL	
	Pre	post	Pre	post
Body mass (kg)	74.2± 3.45	76.5± 4.53***	71.4± 2.36	72.1± 2.36*
Body fat (kg)	11.3± 4.38	10.7 ± 6.11***	10.6± 5.45	10.4± 5.28
Body fat (%)	15.22± 5.12	13.98± 3.71***	14.84± 3.68	14.42± 5.31
Fat-free mass (kg)	62.9± 2.31	65.8 ± 7.14***	60.8± 4.18	61.7± 4.51

GL: glutamine supplementation group, PL: placebo supplementation group

* Significantly different from corresponding to pre training value; ** Significantly different between GL and PL.

Hormonal responses

A significant increase in blood testosterone, GH and IGF-1 from PRE occurred for both GL and PL groups (see Table 4). However, blood testosterone, GH and IGF-1 comparisons showed that subjects in GL had significantly greater increase in blood testosterone, GH and IGF-1 compared to the PL group. A significant decrease in blood cortisol from PRE occurred for both GL and PL groups (see Table 4). However, blood cortisol comparisons showed that subjects in GL had significantly greater decrease in blood cortisol compared to GL group.

Table 4. Serum growth hormone, testosterone, insulin like growth factor and cortisol concentrations in the GL (N=15) and PL (N=15) groups during the pre and post-supplementation period. Data presented as mean ± SEM.

	GL		PL	
	Pre	post	Pre	post
Serum GH (ng/ml)	10.25± 1.42	11.94± 1.73***	11.12± 2.23	11.54± 2.35*
Serum Testosterone (ng/ml)	5.41 ± 0.42	6.32 ± 0.14***	5.92 ± 0.42	6.32 ± 0.31*
Serum IGF-1(ng/ml)	511.52± 16.52	602.32± 22.42 ***	503.14± 41.42	545.41± 65.42*
Serum Cortisol (mg %)	18.91 ± 1.68	17.37 ± 2.11***	19.21 ± 2.28	18.92 ± 1.76*

GL: glutamine supplementation group, PL: placebo supplementation group

* Significantly different from corresponding to pre training value; ** Significantly different between GL and PL.

DISCUSSION

In the present study we investigated the effects of glutamine supplementation on performance (explosive muscular power, body upper and lower muscle strength (body composition (body mass, fat-free mass and body fat) and hormonal changes) Testosterone, GH, IGF-1 and cortisol (during an 8-week resistance training program in non athlete male students.

The results of this study demonstrated that even though both groups demonstrated significant performance (explosive muscular power, body upper and lower muscle strength (increases over time, the glutamine supplemented (GL) group showed greater improvements in upper and lower body strength and explosive muscular power when compared to the placebo (PL) group.

These findings were similar to the study by Candow et al. (2001). Although two studies have investigated the effect of oral glutamine supplementation during resistance training (Antonio et al., 2002; Lacey et al., 1990). No significant differences between the glutamine groups and placebo groups were reported for any of the variables of strength or body mass. Most of these studies have shown short-term effects of glutamine supplementation however, long-term supplementation showed to be a more effective application of glutamine in regards to strength gains. Although glutamine may simulate muscle glycogen synthesis, Varnier et al. (1995) and Bowtell et al. (1999) demonstrated that infusion and oral glutamine supplementation promotes storage of muscle glycogen. On this basis one current widely used supplement is L-glutamine, which increases protein synthesis within skeletal muscle leading to enhanced muscle growth. By increasing muscle mass, the contractile force of a muscle can be increased furthermore, protein sparing and synthetic action of glutamine can result in improved markers of sport performance as a direct result of increases in muscle strength (Phillips, 2007).

The vertical jump test is a simple and reliable test that can provide useful information about explosive muscular power and performance characteristics of athletes (Canavan & Vescovi, 2004). Significant changes were seen during the 8-week resistance training program in any of the power performance measures for either group. Although GL has been shown to significantly enhance power performance. Comparison of our results in the vertical jump test with other studies is difficult because the magnitude of exercise induced loss in muscle power has not been reported systematically.

Also, the results of this study demonstrated a significant increase in blood testosterone, GH and IGF-1 from PRE in both GL and PL groups. However, blood testosterone, GH and IGF-1 comparisons showed that subjects in GL had a significantly greater increase in blood testosterone, GH and IGF-1 compared to the PL group.

Blood concentrations of testosterone stimulate muscle protein accretion (Kraemer et al., 2007). Testosterone also increases protein synthesis by binding to the androgen receptor for the complex to become a transcription factor and thirdly by possibly activating muscle satellite cells, which is important because gene transcription is an initial target for the modulation of protein synthesis (Herbst & Bhasin, 2004, Olsen et al., 2006). Resistance training is associated with significant elevations in anabolic hormones such as testosterone (Kraemer & Ratamess, 2005). The findings about testosterone hormone changes in this study were similar to the study by Volek et al. (1997).

Growth hormone has major effects on metabolism and affects the utilization of substrates and changes the tissue specific metabolism (Hammarqvist et al., 2010). Findings in this study were similar to the study by Zou et al. (2006) and Welbourne (1995). Parts of the action of growth hormone are mediated through the insulin-like growth factor-1 (IGF-1). Administration of growth hormone induces a rise in circulating IGF-1 (Kimbrough et al., 1991) that has important metabolic effects in stimulating glucose and amino acid uptake in muscle and improving muscle protein synthesis (Fryburg, 1994; Russell-Jones et al., 1994). In catabolic situations the levels of IGF-1 decrease while its binding proteins increases leading to a lower local IGF-1 activity, contributing to the decreased insulin sensitivity seen in catabolism (Van den Berghe et al., 2000; Bang et al., 1998). These results similar to the study by Marcelo et al. (2011).

Cortisol is an adreno-cortical steroid hormone released into the body from the adrenal cortex in response to stressful physical or psychological stimuli (Fleck & Kraemer, 2004). Resistance training may also have led to an overall reduction (Kraemer et al., 2007; Staron et al., 1994) or similar (Ahtiainen et al., 2005; Ahtiainen, 2003) cortisol responses to exercise loading in men. Although the results of this study demonstrated a significant decrease in blood cortisol after supplementation. This improved hormonal response may help to reduce the significant decrease in muscle glycogen that occurs during exercise, and result in an enhanced anabolic environment where muscular adaptations and recovery can occur. Furthermore, glutamine supplementation increases the availability of amino acids which may result in an increased uptake of amino acids by the muscle. Increased uptake of amino acids by the muscle enhances net muscle protein balance and improves the anabolic environment (Finn et al., 2003). On the other hand D-Roshan and Barzegarzadeh (2009) reported no significant changes in cortisol concentration between glutamine and placebo groups, although this study was implemented in the short term.

8-week of glutamine supplementation resulted showed significant increase in both body mass (2.30 ± 0.08 kg) and fat-free mass (2.90 ± 2.25 kg) these findings similar to the study by Yan and Zhao-Han (2002) and Shabert (1999). On the other hand one study has investigated the effect of glutamine supplementation during resistance training (Antonio et al., 2002). No significant differences between the glutamine groups and placebo groups were reported for body mass. A limitation of the current study was that muscle mass were not measured. Nevertheless, the increase in body mass is most likely due to an increase in muscle protein or muscle mass (Wernerman et al., 2008; Gleeson, 2008; Karogotich et al., 2007).

CONCLUSIONS

In conclusion, the results of this investigation suggested that 8-week resistance training combined with the timed ingestion of glutamine supplementation increase explosive muscular power and muscle strength (upper and lower body strength) in non athlete male students. Additionally, the strategic consumption of a daily glutamine supplementation (0.35g/kg/day) parceled into three equal dosages represents a simple but effective strategy that enhances performance during resistance training. Also, this strategic (glutamine supplementation combined with resistance training) improved body composition.

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