SciMedCentral

Journal of Human Nutrition & Food Science

Research Article

Low Dose Glutamine Supplementation Preserves the Biological Stress Response among US University Students

Taylor Szczygiel[1], Mary Moore[1], Ryan Pettit-Mee[1], Amanda Slezsak[1], and Micah Zuhl[1,2]*

[1]*School of Health Sciences, College of Health Professions, Central Michigan University, USA*

[2]*Department of Health, Exercise, and Sport Sciences, University of New Mexico, USA*

***Corresponding author**

Micah Zuhl, Department of Health, Exercise, and Sport Sciences, College of Education, Johnson Center MSC 04 2610, University of New Mexico, Albuquerque, NM87131, USA, Tel: (269) 767-0003; Email: zuhl09@unm.edu

Submitted: 24 August 2017

Accepted: 13 September 2017

Published: 15 September 2017

ISSN: 2333-6706

Keywords

- Glutamine
- Students
- Stress

Abstract

The purpose of this study was to examine the affects of 8-weeks low dose (0.09 g/kg/day) oral glutamine supplementation in healthy university-age adults on markers of oxidative and cellular stress. Twenty healthy adults, enrolled as full-time undergraduate students (5 male, 15 female), were separated into glutamine (Gln) or placebo (Pla) groups. Low dose glutamine resulted in higher plasma glutamine and higher serum antioxidant capacity at 4-weeks compared to pre in the glutamine group. In addition, peripheral blood mononuclear cell expression of heat shock stress protein (HSF-1) and inhibitor of the pro-inflammatory nuclear kappa-B alpha (IκB) decreased at 4-weeks compared to the pre time point in the placebo group. Levels did not decrease in the Glutamine group. In conclusion, low dose glutamine may provide short-term protective benefits by increasing antioxidant levels and preserving the heat shock stress system among university students. This may indicate a use for the supplement for students during high stress times (i.e. mid-terms and finals).

INTRODUCTION

Chronic exposure to psychological, physical, bacterial, viral, or environmental stressors increase risk for illnesses such as upper respiratory tract infections and the common cold, along with aggravation of auto immune disorders such as irritable bowel disease [1,2]. The mechanisms appear to be based upon hormonal modulation of immune function resulting in possible systemic immune suppression or excessive activation [3,4]. Suppression of leukocyte proliferation and cytokine release (both pro- and anti-inflammatory) in response to a pathogen may increase susceptibility to illness [5]. Further, exposure to various forms (e.g. mental and physical) of prolonged stress leads to low-grade inflammation due to over production of pro-inflammatory agents from immune cells, which promotes development of disease [3]. Immune dysfunction can lead to overactive neutrophil phagocytosis and the buildup of reactive oxygen species leading to increased oxidative stress [6]. Biological markers of oxidative stress are higher after exposure to stressful events [7]. Those who experience chronic stress have shown lengthened healing time once illness occurs [8-10].

Glutamine is the most abundant non-essential amino acid in the human body, and is considered a major nutrient for healthy immune function [11]. It is biosynthesized, highly regulated, and serves important biological roles in ammonia regulation and acid base balance [12]. However, under conditions of biological stress, glutamine levels may decrease as the amino acid provides nutrients to support leukocyte activation, and modulates immune cell levels of heat shock protein (HSP) stress system [13,14]. The HSP system is ubiquitous, and protects cells against environmental, pathological, and physiological damaging stimuli; but in immune cells it has been shown to regulate intracellular inflammatory pathways [15]. Glutamine works through up regulation of the transcription factor, heat shock factor-1 (HSF-1), resulting in increased expression of HSP 70. The immune regulatory mechanism of HSP70 is through suppression of the inflammatory mediator, nuclear factor-kappa B (NF-κB) transcription factor, which upon activation promotes pro-inflammatory cytokine production [16]. In *in vitro* models over expression of HSP70 inhibits NF-κB up regulation by preventing the degradation of its inhibitor, kappa B (Iκ-Bα) [17]. In previous studies, we have demonstrated that a high dose of orally ingested glutamine increases peripheral blood mononuclear cell expression of HSF-1, HSP70, and Iκ-Bα levels in human PBMCs [18,19].

Glutamine also provides substrate to support the antioxidant defense system [20]. The conversion to glutamate contributes to the glutathione system, suggesting that glutamine supplementation may improve total antioxidant capacity [21,22]. Glutamine supplementation has been shown to preserve plasma antioxidant levels among surgical patients susceptible to infectious complications [23].

A potential target for glutamine therapy is university students, who are exposed to psychological stress associated with their studies, social interactions, and financial strain [24]. Students are also commonly exposed to close quarters containing large numbers of individuals such as classrooms, laboratories, and apartments. These environments may expose them to bacterial, viral, or chemical pathogens [25]. These various exposures may compromise their immune function and decrease their ability to fight infection, making them vulnerable to illness [8,25,26]. Students have also demonstrated elevations in free radical levels during periods of psychological strain, which indicates oxidative stress [7,27]. It may be difficult to reduce or eliminate stress among this unique population; therefore, another option is to attempt to improve their ability to cope with the multitude of stressors though nutritional supplementation.

Therefore, purposes of this study are to examine the effects of 8-weeks of low-dose glutamine supplementation on markers of the biological stress response among university subjects, who are enrolled in more than twelve academic credit hours. The identified markers include serum antioxidant levels and peripheral blood mononuclear cell expression of HSF-1, HSP70, and Iκ-Bα levels. In previous studies, we have observed beneficial HSP regulatory results at high glutamine dosages (0.90 g/kg); however, it is unknown if a low dose will have similar effects [18,19].This is important as glutamine supplement manufacturers recommend ingestion at a low dosage.

MATERIALS AND METHODS

Subjects

The present study was approved by the Human Research Review Board of Central Michigan University, Mt. Pleasant, MI, USA. Twenty healthy college-age adult men (n=5) and women (n=15) ages 19-22 years volunteered for this study. Each participant was an undergraduate student enrolled in 12 or more academic credit hours. Subjects were excluded if they had known cardiovascular, kidney, liver, or GI disease (such as Crohn's disease, colitis, or celiac disease). In addition, those prescribed antidepressants, been diagnosed with a food allergy, and pregnant women were excluded. Upon meeting study requirements, subjects read and signed the informed consent. All testing was performed in the Exercise Science Laboratory at Central Michigan University. Data collection occurred during the winter months (January-March), and all participants were residents of Mt. Pleasant, MI.

Experimental Design

Using a double-blinded research design, each subject was randomly placed into a glutamine (Gln) (n=10) or placebo (Pla) (n=10) group for 8-weeks of supplementation. Subjects were recruited into the study at the beginning of winter semester (January) with the study terminating mid-way through the academic semester. The subjects reported to the laboratory after an overnight fast on three occasions, which included baseline, after 4-weeks, and after 8-weeks of supplementation. At each time point, height, weight, and body composition were measured. After 10-minutes of seated rest, a 20mL blood sample was taken to measure baseline plasma levels of glutamine and serum levels of total antioxidant capacity, along with peripheral blood mononuclear cell expressions of HSP70, HSF-1, and IκBα. At baseline and 4-week time points, subjects were provided a supplement bag containing 4-weeks of a daily dose of glutamine or placebo.

Glutamine and Placebo Supplementation: Subjects ingested 0.09 g/kg of body weight each day of glutamine (Jarrow Formulas, Los Angeles, CA) or placebo in pill form (Capsuline Inc., Pompano Beach, FL), but no subject consumed more than nine pills (0.750 g/d per pill)per day, or exceeded 6.75 grams per day. Glutamine and placebo were both in capsule form; however, the placebo capsule was empty and vegetarian based. The supplements were divided into three separate does per day, taken in the morning, early afternoon, and evening.

Body Composition

A three-site skin fold measurement was administered to estimate body density (Lange; Beta Technology Inc, Cambridge, MD, USA) and then used to determine percent body fat using the appropriate Jackson equation for men and women [28,29]. Each site was measured three times and the average of the two closest values was used for the calculation.

Blood sampling and analysis

After 10-minutes of seated rest, venous blood was collected during the baseline, 4-week, and 8-week visits from an antecubital vein. Blood samples were drawn into sterile syringes and immediately transferred into serum vacutainers containing EDTA (BD Biosciences, Franklin Lakes, NJ). The serum vacutainers clotted for approximately twenty minutes, and were then centrifuged (968g, 20min, 4°C). Serum was pipette into 1.5 ml micro tubes and frozen at -80°C for further analysis of total antioxidant capacity. Blood from the vacutainers containing EDTA were added to Histopaque (1077, Sigma-Aldrich, St. Louis, MO) in a 1:1 ratio (15 ml/15 ml) and centrifuged (968 g, 30 minutes, 20°C). Plasma was pipetted into 1.5 ml micro tubes and frozen at -80°C for further analysis of glutamine levels. The buffy coat containing the mononuclear cells was collected, and after a wash, the cell pellet was stored at -80°C for cellular level analysis of HSP70, HSF-1, and Iκ-Bα.

Plasma glutamine

Plasma glutamine was assessed with a quantitative colorimetric enzyme assay kit (EGLN-100, Bio Assay Systems, and Hayward, CA). The materials and chemicals used were provided by the manufacturer, and the manufacturer directions were followed. Glutamate was measured in each sample and subtracted from the glutamine absorbance of the respective sample. Results were calculated based on data from the standard curve. Plasma glutamine was detected within a linear range of 0.023-2 mM glutamine.

Serum total antioxidant capacity

A quantitative colorimetric antioxidant assay kit was used to determine total antioxidant capacity (TAC) (DTAC-100, Bioassay Systems, and Hayward, CA). Samples were diluted 1:2 with distilled water. The materials and chemicals used were provided by the manufacturer, and the manufacturer directions

were followed. Through this procedure, Cu2+ is reduced by antioxidant to Cu+. The resulting Cu+ specifically forms a colored complex with a dye reagent. The color intensity at 570nm is proportional to TAC in the sample. Serum total antioxidant capacity is expressed in mM Trolox equivalents and is detected within the linear range of 1.5-1000 mM Trolox equivalents.

Western Blot Gel Electrophoresis: Mononuclear cells were homogenized for 25 minutes with 150µl of RIPA lysis buffer (89901, Thermo Scientific, Rockford, IL) containing 25mM Tris-Hcl pH 7.6, 150 mMNaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS, as well as Halt protease/phosphatase inhibitors (78442, Thermo Scientific, Rockford, IL). The samples then were centrifuged (20,000g, 10 min, 4°C), the supernatant was collected, and protein measurement was performed using Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). Laemmli gel loading buffer (161-0737, Bio-Rad Laboratories, Hercules, CA) was added to the lysate containing 20µg of protein. The microtube containing this mixture was then boiled at 100°C for 10 minutes. Proteins were then separated bytris-glycine sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (162-0094, Bio-Rad Laboratories, Hercules, CA). Following the transfer, the membrane was placed in Ponceau S staining solution (P7170-1L, Sigma-Aldrich, St. Louis, MO) for 5 minutes, and the stain was removed with 0.1 M sodium hydroxide solution (319481, Sigma-Aldrich, St. Louis, MO). The membrane was then incubated for 1 hour in blocking solution (5% dry milk in Tris-buffered saline Tween 20 buffer) followed by incubation with HSP 70, HSF-1, and Iκ-Bα (4872, 4356, and 4814, Cell Signaling Technology, Danvers, MA) antibody in a blocking solution. Each membrane was cut and the upper half of the first membrane was treated with HSF-1 and the lower half was treated with β-actin (3700, Cell Signaling Technology, Danvers, MA). The second cut membrane was treated with HSP70 on the upper half, and β-actin and IκBα on the lower half. After incubation, the membranes were washed with TBS-Tween and placed in a horseradish peroxidase-conjugated secondary antibody (7074, 7076, Cell Signaling Technology, Danvers, MA). The membrane was developed using Luminata Forte Western HRP substrate (WBLUF0500, Millipore, and Darmstadt, Germany) on the FluorChem E protein imager (FE-0683, Protein Simple, and San Jose, CA). Adobe Photoshop (San Jose, CA) was used to quantify protein expression and standardized to β-actin and Ponceau S stain to control for protein loading. Protein levels were also expressed relative to the baseline visit.

Statistical Analysis: Sample size was based on apriori calculation using a power of 0.80 and alpha level of 0.05. All results are expressed as means ± SD and checked for homogeneity of variance and normality. A mixed model repeated measures ANOVA was used to compare within group differences (time: baseline, 4-weeks, 8-weeks). Non-paired student t-test was used to compare between group differences (Gln vs. Pla).

RESULTS

Twenty adults completed the study, with ten subjects in the glutamine (2 male, 8 female) and placebo (3 male, 7 female) groups. Subjects were similar in age (20.20 ± 0.0 vs. 19.6 ± 1.0, Gln and Pla, respectively), and were enrolled in same number of undergraduate credit hours (14.90 ± 2.0 vs. 15.3 ± 0.9, Gln and Pla, respectively). There were no differences in BMI, body weight, and body fat percentage between the groups (Table 1).

Plasma glutamine levels (Figure 1 and 2). The subjects in the glutamine group consumed 0.09g/kg of glutamine each day, for an average intake of 5.94g/day. Baseline (pre) glutamine levels were not different between Gln and Pla groups (0.390 ± 0.08 vs. 0.436 ± 0.034 mM, respectively). Plasma glutamine was higher at the 4-week time point compared to pre in the Gln group (0.4666 ± 0.06 mM vs. 0.3901 ± 0.08mM, $p<0.05$), indicating that the supplement increased glutamine levels in the subjects. Glutamine was trending higher at 4-weeks in the Gln group compared to 4-weeks in the Pla group, but this was not statistically significant ($p=0.061$). No other comparisons were different. Plasma glutamine data was also compared based on percent change from baseline (pre) at each time point (Figure 2) with baseline (pre) set to 100 in both Gln and Pla. Percent change was significantly higher from baseline in the Gln group at 4-weeks (100 ± 0.0% vs. 123 ± 6.9%). The percent change at 4-weeks in the Gln group was higher when compared to 4-weeks in Pla (123 ± 6.9% vs. 0.913 ± 6.9%, respectively).

Serum total antioxidant capacity (Figure 3). Serum levels of trolox were not different at baseline (pre) between groups. Trolox levels were higher at the 4-week time point compared

Table 1: Subject characteristics.

	Glutamine (n=10)	Placebo (n=10)
Sex	2 male, 8 female	3 male, 7 female
Age	20.20 ± 0.90	19.60 ± 1.0
Height (cm)	166.60 ± 6.90	165.90 ± 8.20
Weight (kg)	66.20 ± 16.10	66.70 ± 12.60
Body fat (%)	27.50 ± 8.80	27.20 ± 9.40
Body mass index (kg/m^2)	23.60 ± 4.20	24.20 ± 4.10
Undergraduate credit hours enrolled	14.90 ± 2.0	15.30 ± 0.90

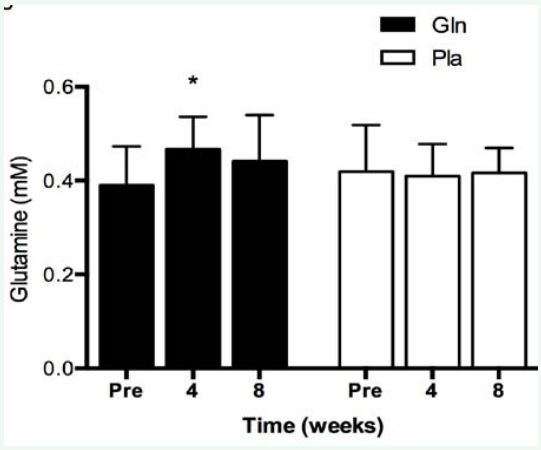

Figure 1 Plasma glutamine levels. Low dose oral glutamine supplementation increased plasma glutamine levels at 4-weeks compared to pre in the Gln group (shaded bars) (0.4666 ± 0.06 mM vs. 0.3901 ± 0.08 mM). * $p<0.05$ compared to pre time point within the same group. Data are mean ± SD, n=20.

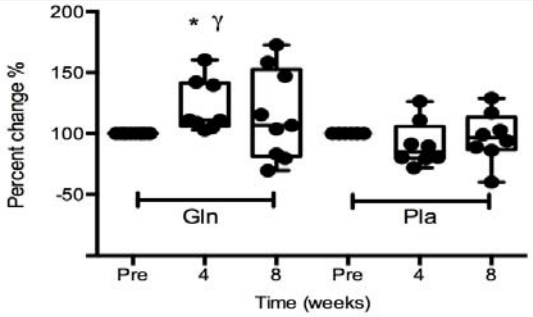

Figure 2 Plasma glutamine percent change from baseline (pre). Glutamine percent increase in the Gln trial was higher when compared to pre (123 ± 6.9% vs. 100 ± 0.0%) and when compared to 4-weeks in the Pla trial (123 ± 6.9% vs. 0.913 ± 6.9%). * p<0.05 compared to pre time point within the same group. γ p<0.05 compared to same time point in Pla trial. Data are mean ± SD, n=20.

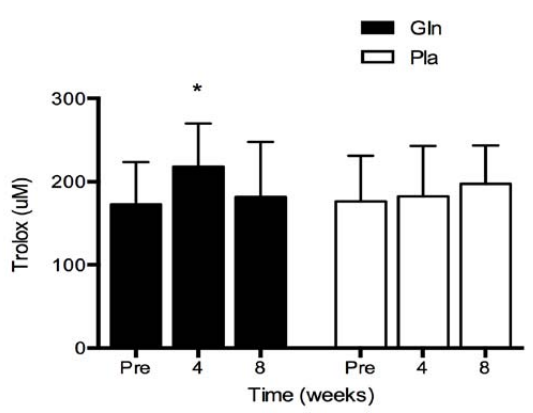

Figure 3 Serum total antioxidant capacity. Trolox was higher at the 4-week time point compared to pre in the Gln group (shaded bars) (218.20 ± 51.62 vs. 172.50 ± 51.12 uM). * p<0.05 compared to pre time point within the same group. Data are mean ± SD, n=20.

to pre in the Gln group (218.20 ± 51.62 vs. 172.50 ± 51.12 uM, p<0.05). Levels did not change at any time point in the Pla group, and there were no differences between groups.

Peripheral blood mononuclear cell proteins (Figure 4). HSF-1 levels did not change in the Gln group, but decreased in the Pla group at the 4-week time point when compared to baseline (pre) (0.64 ± 0.39 vs. 1.00 ± 0.00, p<0.05). Iκ-Bα followed a similar trend, levels decreased in the Pla group and increased marginally in the Gln group. Levels were significantly lower at the 4-week time point compared to baseline (pre) in the Pla group (0.68 ± 0.21 vs. 1.00 ± 0.00, p<0.05). In addition, a significance difference was detected at the 4-week time point between the groups (Gln vs. Pla) (1.18 ± 0.47 vs. 0.68 ± 0.21, p<0.05). No within or between group differences were detected for HSP70.

DISCUSSION

The major finding of this study is that 8-weeks of low dose oral glutamine supplementation support the stress response among university undergraduate students. This was evident by the increase in total antioxidant capacity and the preservation of the heat shock protein pathway. College students are exposed to variety of environmental and social stressors, including poor sleep habits, dietary adjustments, academic challenges, and social interactions [24]. The response to this challenging environment may elicit an increase in mental stress, anxiety, and depression, which have all been linked to illness among college students [30]. Further, biological free radicals have been shown to be elevated among university students when exposed to mental stress [7]. The current study is one of only a handful of studies to analyze

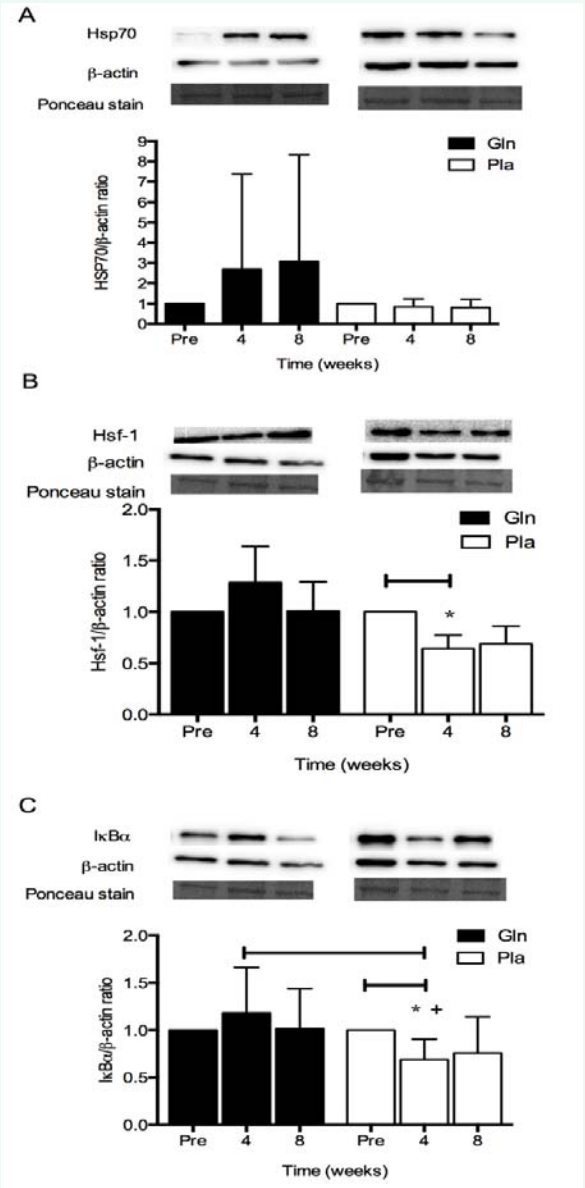

Figure 4 Peripheral blood mononuclear protein expression. HSF-1 (B) levels decreased in the Pla (open bars) at the 4-week time point compared to pre (0.64 ± 0.39 vs. 1.00 ± 0.00). Levels did not decrease in the Gln group (shaded bars). IκBα protein expression decreased in the Pla group at the 4-week time point compared to pre (0.68 ± 0.21 vs. 1.00 ± 0.00). Levels were higher at the 4-week time in the Gln group compared to the Pla group (1.18 ± 0.47 vs. 0.68 ± 0.21). * p<0.05 compared to pre time point within the same group. + p<0.05 compared to same time point between groups (Gln vs. Pla). Data are mean ± SD, n=20.

the effects of a dietary supplement on markers of stress among college students. Smith et al. (2013) examined the effects of 12-weeks probiotic supplementation on the severity of upper respiratory infection among college students, and reported shorter infection time and less missed school days [31]. Kiecolt-Glaser et al. (2011) demonstrated lower levels of IL-6 release from LPS stimulated leukocytes and a reduction in anxiety symptoms after 12-weeks of omega-3 supplementation among medical students [32]. To our knowledge, the current study is the first to report on the biological stress markers antioxidant capacity and cellular stress protein (HSF-1, HSP70, Iκ-Bα) levels after amino acid supplementation.

Oral glutamine ingestion has been shown to substantially increase plasma glutamine levels in humans [33]. However, in many of these studies, the subjects were critically ill [34] and/or the dosage levels were markedly high, ranging from 0.30- 0.90 g/kg/day; this equates to 21-63 g/day of glutamine ingestion for a 70kg body weight human [35]. In the current study, subjects ingested 0.09 g/kg/d for an average intake of 5.94 g/d among subjects. The dosage chosen resembles a common supplemental dose as recommended by glutamine manufacturers (e.g. Ajinomoto). Plasma glutamine levels did increase in the glutamine group compared to the baseline measurement, but levels did not rise substantially compared to the Pla group. This indicates that larger dosing may have an added affect, or recommendations from manufacturers should be further evaluated. However, the percent increase in plasma glutamine from baseline (pre) levels increased significantly at 4-weeks compared to placebo. This may be because baseline levels in the Pla group were slightly higher, although not significant. The increase in glutamine levels was only seen at the 4-week time point then returned to near baseline levels at the 8-week time point. This may imply that the body adjusted to dosage and reduced the amount of glutamine produced [36], further explanations maybe dietary changes; however, food intake was not measured or tracked. Another explanation may be noncompliance among subjects. Interestingly, baseline plasma glutamine levels appear low among these subjects, which could represent greater glutamine utilization, or inadequate nutrition. Low levels of plasma glutamine have been associated with illness, and considered a marker of overtraining among athletes [37,38]. In summary, low dose glutamine may preserve and slightly increase plasma glutamine levels, but the increase may only be short term, which provides support for shorter dosing periods and higher concentrations. Students may only need to ingest glutamine during periods of elevated stress.

When antioxidant levels decrease in the human body, cells and tissues become more vulnerable to developing dysfunction and potentially disease. Total antioxidant capacity is a reliable test used to monitor these levels [39]. Pathological states such as lung disease and diabetes show a decrease in the total antioxidant capacity [39,40]. The glutathione system is one of the main sources of antioxidant defense at the cellular level. Total antioxidant capacity, measured by trolox equilvalents, increased in the Gln group at the 4-week mark, but returned to near baseline levels at the 8-week time point. The trolox equivalent provides evidence of the overall ability of a fluid to counteract reactive oxygen species. The main biological antioxidants that have a similar antioxidant capacity as trolox are bilirubin, ascorbate, urate, glutathione, and α-tocopherol [41]. The most likely candidate among these molecules is glutathione as dimidiation of glutamine via glutaminase generates glutamate, which is a substrate in the synthesis of glutathione [42]. This is speculative, as glutathione levels were not measured in the current study. However, oral glutamine supplementation has been shown to increase glutathione levels in rats [43]. In addition, an anlanyl-glutamine supplementation for 7 days has been shown to increase plasma glutathione among intensive care patients [44]. Glutathione works as an antioxidant by reducing reactive oxygen species hydrogen peroxide, and lipid peroxide. Reduced glutathione has been shown to affect immune function by reducing lymphocyte proliferation, and the generation of cytotoxic T-cells and natural killer cells [45]. Those suffering from panic disorders and anxiety have been shown to have elevated markers of free radicals, indicating enhance oxidative stress [46]. Low dose glutamine increased antioxidant levels in the current study, and may provide protection against oxidative damage during periods of high stress.

Glutamine has been shown to activate the heat shock protein stress pathway. In several animal studies, Wischmeyer's group has demonstrated that oral glutamine enhances the expression of HSF-1, the transcription factor for HSPs, and protects against thermal stress, endotoxic insult, and ischemia-reperfusion damage [47-49]. Our group was the first to show that oral glutamine supplementation increases protein expression of HSF-1 and HSP70 in human peripheral blood mononuclear cells [18,50]. The heat shock protein system provides cellular maintenance by refolding damaged proteins to their native state upon exposure to stressors such as heat, free radicals, and bacteria [51]. In a normal physiological model, an acute exposure to stress activates the HSP system resulting in up regulation of HSP70, which provides protection against subsequent exposures. This is commonly referred to stress pre-conditioning. The same level of protection against cellular stress is demonstrated by over expressing HSPs using various supplements or adenovirus [17]. For example, mice over expressing HSP70 are better protected against oxidative stress and neurodegerative diseases [52,53]. The HSP protection against oxidative stress may be a result of two mechanisms: (1) the repair of misfolded protein as a result of the oxidative stress; and (2) the ability of HSPs to preserve glutathione levels, and improve the antioxidant defense system [54,55]. HSPs also play an important anti-inflammatory role, demonstrated through the inhibition of the pro-inflammatory NF-κB pathway. Up regulation of HSP70 in human PBMCs has been shown to preserve cytosolic levels of IκBα, which inhibits nuclear translocation of NF-κB, and prevents transcription of inflammatory cytokines (e.g. TNFα, IL-6) [17].

In the current study, low dose glutamine preserved HSF-1 and Iκ-Bα protein levels in PBMCs. This provides evidence that glutamine may support the stress response, and serve an anti-inflammatory role through stabilization of Iκ-Bα. In the placebo group, the expression of HSF-1 and Iκ-Bα both decreased significantly from the baseline measurement at the 4-week time point, which resulted in Iκ-Bα levels being different between groups at the same 4-week mark. Lower levels of HSF-1 may indicate an inadequate response to cellular stress, and put one at greater risk for illness or disease [56]. In addition, degradation of

Iκ-Bα may also imply a pro-inflammatory state among the placebo group; however, no other measures of inflammation (e.g. plasma cytokines) were measured. In previous studies, we have shown that acute and 7-days of glutamine supplementation increases HSF-1, HSP70, and Iκ-Bα levels in human PBMCs after exercise in the heat [18,19,50]. Plasma levels of TNFα was also reduced 4-hours into recovery from the exercise bout, supporting an anti-inflammatory response. In addition, glutamine supplementation has also been shown to reduce the suppression of HSP70 among children with severe malarial anemia (SMA) and further reduced NF-κB activation. The authors also observed an association between low plasma glutamine levels and risk of developing SMA among children, and may serve as a targeted therapy [57]. These results demonstrate that preserving HSF-1 and HSP70 through glutamine supplementation may provide benefits against biological stress.

In conclusion, low dose glutamine may protect undergraduate students, who are exposed to a variety of stressors through several mechanisms: (1) increased antioxidant levels, enhancing the defense against free radicals; (2) preserving the stress response and a reduced inflammatory state by stabilizing HSF-1 levels, and Iκ-Bα levels, respectively. However, larger dosing and shorter periods of supplementation may have an added affect.

AUTHORS CONTRIBUTIONS

TS and MZ participated in the research design of the study. TS, MM, RPM, AS, and MZ all participated in data collection and analyses, and interpretations. TS drafted the manuscript. TS, MM, RPM, AS, and MZ all contributed to revisions. MZ supervised the experiment. All authors approved the final draft.

DECLARATION OF CONFLICTING INTERESTS

The authors report no conflicts of interest. All methods, results, and analyses presented are representative of the raw data collected during the study. All authors contributed to, and are responsible for the interpretation and writing of the manuscript

REFERENCES

1. Cohen S, Doyle WJ and Skoner DP. Psychological stress, cytokine production, and severity of upper respiratory illness. Psychosom Med. 1999; 61: 175-180.
2. Mawdsley JE, Rampton DS. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. Gut. 2005; 54: 1481-1491.
3. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull. 2004; 130: 601-630.
4. Tian R, Hou G, Li D, Yuan T-F. A possible change process of inflammatory cytokines in the prolonged chronic stress and its ultimate implications for health. Sci World J. 2014.
5. Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol. 2005; 5: 243-251.
6. Irie M, Asami S, Ikeda M, Kasai H. Depressive state relates to female oxidative DNA damage via neutrophil activation. Biochem Biophys Res Commun. 2003; 311: 1014-1018.
7. Sivonová M, Zitnanová I, Hlincíková L, Skodácek I, Trebatická J, Duracková Z. Oxidative stress in university students during examinations. Stress. 2004; 7: 183-188.
8. Assaf AM. Stress-induced immune-related diseases and health outcomes of pharmacy students: A pilot study. Saudi Pharm J. 2013; 21: 35-44.
9. Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. Brain Behav Immun. 2007; 21: 993-999.
10. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. Lancet. 1995; 346: 1194-1196.
11. Souba WW, Smith RJ, Wilmore DW. Glutamine metabolism by the intestinal tract. JPEN J Parenter Enteral Nutr. 1985; 9: 608-617.
12. Taylor L, Curthoys NP. Glutamine metabolism: Role in acid-base balance. Biochem Mol Biol Educ. 2004; 32: 291-304.
13. Walsh NP, Blannin AK, Robson PJ, Gleeson M. Glutamine, exercise and immune function. Links and possible mechanisms. Sports Med. 1998; 26: 177-191.
14. Castell L. Glutamine supplementation in vitro and in vivo, in exercise and in immunodepression. Sports Med. 2003; 33: 323-345.
15. Muralidharan S, Mandrekar P. Cellular stress response and innate immune signaling: integrating pathways in host defense and inflammation. J Leukoc Biol. 2013; 94: 1167-1184.
16. Tieri P, Termanini A, Bellavista E, Salvioli S, Capri M, Franceschi C. Charting the NF-κB pathway interactome map. PLoS One. 2012; 7: 32678.
17. Tieri P, Termanini A, Bellavista E, Salvioli S, Capri M, Franceschi C. Charting the NF-kappaB pathway interactome map. PLoS One. 2012; 7: 32678.
18. Dokladny K, Lobb R, Wharton W, Ma TY, Moseley PL. LPS-induced cytokine levels are repressed by elevated expression of HSP70 in rats: possible role of NF-kappaB. Cell Stress Chaperones. 2010; 15: 153-163.
19. Zuhl M, Dokladny K, Mermier C, Schneider S, Salgado R, Moseley P. The effects of acute oral glutamine supplementation on exercise-induced gastrointestinal permeability and heat shock protein expression in peripheral blood mononuclear cells. Cell Stress Chaperones. 2015; 20: 85-93.
20. Zuhl MN, Lanphere KR, Kravitz L, Mermier CM, Schneider S, Dokladny K, et al. Effects of oral glutamine supplementation on exercise-induced gastrointestinal permeability and tight junction protein expression. J Appl Physiol (1985). 2014; 116: 183-191.
21. Newsholme P, Lima MM, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, et al. Glutamine and glutamate as vital metabolites. Braz J Med Biol Res. 2003; 36: 153-163.
22. Gouvêa Junior VT, Caporossi C, Salomão AB, Côrtes E, Munhoz MF, Nascimento JE. Effect of glutamine on the total antioxidant system of rats subjected to renal ischemia and reperfusion. Acta Cir Bras. 2011; 26: 445-450.
23. Bollhalder L, Pfeil AM, Tomonaga Y, Schwenkglenks M. A systematic literature review and meta-analysis of randomized clinical trials of parenteral glutamine supplementation. Clin Nutr. 2013; 32: 213-223.
24. Fan YP, Yu JC, Kang WM, Zhang Q. Effects on glutathione of patients received glutamine dipeptide enriched parenteral nutrition post abdominal surgery. Zhonghua Wai Ke Za Zhi. 2005; 43: 1383-1386.
25. Ross SE, Niebling BC and Heckert TM. Sources of stress among college students. Soc Psychol. 1999; 61: 841-846.
26. Kemeny ME, Schedlowski M. Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise

progression. Brain Behav Immun. 2007; 21: 1009-1018.

27. Gadzella BM. Student-Life Stress Inventory: identification of and reactions to stressors. Psychol Rep. 1994; 74: 395-402.

28. Wang L, Muxin G, Nishida H, Shirakawa C, Sato S, Konishi T. Psychological stress-induced oxidative stress as a model of sub-healthy condition and the effect of TCM. Evid Based Complement Alternat Med. 2007; 4: 195-202.

29. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr. 1978; 40: 497-504.

30. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Med Sci Sports Exerc. 1980; 12: 175-181.

31. Rawson HE, Bloomer K, Kendall A. Stress, anxiety, depression, and physical illness in college students. J Genet Psychol. 1994; 155: 321-330.

32. Smith TJ, Rigassio-Radler D, Denmark R, Haley T, Touger-Decker R. Effect of Lactobacillus rhamnosus LGG(R) and Bifidobacterium animalis ssp. lactis BB-12(R) on health-related quality of life in college students affected by upper respiratory infections. Br J Nutr. 2013; 109: 1999-2007.

33. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun. 2011; 25: 1725-1734.

34. Hankard RG, Darmaun D, Sager BK, D'Amore D, Parsons WR, Haymond M. Response of glutamine metabolism to exogenous glutamine in humans. Am J Physiol. 1995; 269: 663-670.

35. Bollhalder L, Pfeil AM, Tomonaga Y, Schwenkglenks M. A systematic literature review and meta-analysis of randomized clinical trials of parenteral glutamine supplementation. Clinical nutrition. 2013; 32: 213-223.

36. Liaw SH, Pan C, Eisenberg D. Feedback inhibition of fully unadenylylated glutamine synthetase from Salmonella typhimurium by glycine, alanine, and serine. Proc Natl Acad Sci USA. 1993; 90: 4996-5000.

37. Bongers T, Griffiths RD, McArdle A. Exogenous glutamine: the clinical evidence. Crit Care Med. 2007; 35: 545-552.

38. Rowbottom DG, Keast D, Morton AR. The emerging role of glutamine as an indicator of exercise stress and overtraining. Sports Med. 1996; 21: 80-97.

39. Kusano C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol. 2008; 7: 1-15.

40. Tsai PH, Liu JJ, Yeh CL, Chiu WC, Yeh SL. Effects of glutamine supplementation on oxidative stress-related gene expression and antioxidant properties in rats with streptozotocin-induced type 2 diabetes. Br J Nutr. 2012; 107: 1112-1118.

41. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond). 1993; 407-412.

42. Tapiero H, Mathé G, Couvreur P, Tew KD. II. Glutamine and glutamate. Biomed Pharmacother. 2002; 56: 446-57.

43. Denno R, Rounds JD, Faris R, Holejko LB, Wilmore DW. Glutamine-enriched total parenteral nutrition enhances plasma glutathione in the resting state. J Surg Res. 1996; 61: 35-38.

44. Eroglu A. The effect of intravenous alanyl-glutamine supplementation on plasma glutathione levels in intensive care unit trauma patients receiving enteralnutrition: the results of a randomized controlled trial. Anesth Analg. 2009; 109: 502-505.

45. Dröge W, Breitkreutz R. Glutathione and immune function. Proc Nutr Soc. 2000; 59: 595-600.

46. Kuloglu M, Atmaca M, Tezcan E, Ustundag B, Bulut S. Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. Neuropsychobiology. 2002; 46: 186-189.

47. Singleton KD, Wischmeyer PE. Oral glutamine enhances heat shock protein expression and improves survival following hyperthermia. Shock. 2006; 25: 295-299.

48. Singleton KD, Beckey VE, Wischmeyer PE. Glutamine prevents activation of nf-kappab and stress kinase pathways, attenuates inflammatory cytokine release, and prevents acute respiratory distress syndrome (ards) following sepsis. Shock. 2005; 24: 583-589.

49. Wischmeyer PE, Vanden Hoek TL, Li C, Shao Z, Ren H, Riehm J, et al. Glutamine preserves cardiomyocyte viability and enhances recovery of contractile function after ischemia-reperfusion injury. JPEN J Parenter Enteral Nutr. 2003; 27: 116-122.

50. Dokladny K, Zuhl MN, Mandell M, Bhattacharya D, Schneider S, Deretic V, et al. Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. J Biol Chem. 2013; 288: 14959-14972.

51. Morimoto RI, Kline MP, Bimston DN, Cotto JJ. The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones. Essays Biochem. 1997; 32: 17-29.

52. Kalmar B, Greensmith L. Activation of the heat shock response in a primary cellular model of motoneuron neurodegeneration-evidence for neuroprotective and neurotoxic effects. Cell Mol Biol Lett. 2009; 14: 319-335.

53. Dedeoglu A, Ferrante RJ, Andreassen OA, Dillmann WH, Beal MF. Mice overexpressing 70-kDa heat shock protein show increased resistance to malonate and 3-nitropropionic acid. Exp Neurol. 2002; 176: 262-265.

54. Gu XH, Hao Y, Wang XL. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poult Sci. 2012; 91: 790-799.

55. Arrigo AP, Firdaus WJ, Mellier G, et al. Cytotoxic effects induced by oxidative stress in cultured mammalian cells and protection provided by Hsp27 expression. Methods. 2005; 35: 126-138.

56. Hooper PL, Hooper JJ. Loss of defense against stress: diabetes and heat shock proteins. Diabetes Technol Ther. 2005; 7: 204-208.

57. Kempaiah P, Dokladny K, Karim Z, Raballah E, Ong'echa JM, et al. Reduced Hsp70 and Glutamine in Pediatric Severe Malaria Anemia: Role of Hemozoin in Suppressing Hsp70 and NF-κB activation. Mol Med. 2016.

Cite this article

Szczygiel T, Moore M, Pettit-Mee R, Slezsak A, Zuhl M (2017) Low Dose Glutamine Supplementation Preserves the Biological Stress Response among US University Students. J Hum Nutr Food Sci 5(3): 1114.