Oral Glutamine Supplement Reduces Subjective Fatigue Ratings during Repeated Bouts of Firefighting Simulations

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Oral Glutamine Supplement Reduces Subjective Fatigue Ratings during Repeated Bouts of Firefighting Simulations

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Abstract: Wildland firefighting requires repetitive (e.g., consecutive work shifts) physical work in dangerous conditions (e.g., heat and pollution). Workers commonly enter these environments in a nonacclimated state, leading to fatigue and heightened injury risk. Strategies to improve tolerance to these stressors are lacking. Purpose: To determine if glutamine ingestion prior to and after consecutive days of firefighting simulations in the heat attenuates subjective ratings of fatigue, and evaluate if results were supported by glutamine-induced upregulation of biological stress responses. Methods: Participants (5 male, 3 female) ingested glutamine (0.15 g/kg/day) or a placebo before and after two consecutive days (separated by 24 h) of firefighter simulations in a heated chamber (35 °C, 35% humidity). Perceived fatigue and biological stress were measured pre-, post-, and 4 h postexercise in each trial. Results: Subjective fatigue was reduced pre-exercise on Day 2 in the glutamine group (p < 0.05). Peripheral mononuclear cell expression of heat shock protein 70 (HSP70) and serum antioxidants were elevated at 4 h postexercise on Day 1 in the glutamine trial (p < 0.05). Conclusions: Ingestion of glutamine before and after repeated firefighter simulations in the heat resulted in reduced subjective fatigue on Day 2, which may be a result of the upregulation of biological stress systems (antioxidants, HSPs). This response may support recovery and improve work performance.

Keywords: fatigue; glutamine; heat; firefighter; recovery; heat shock proteins

1. Introduction

Many labor-intensive occupations require workers to perform extreme physical tasks. Fatigue, which is a multifactorial condition, has been defined as an overwhelming and excessive whole-body tiredness that leads to reduced physical and mental performance [1]. Both mental and physical fatigue, along with injury risk, are exacerbated when heavy work tasks are performed in inclement conditions [2]. Factors such as hot temperatures, air pollution, and dangerous terrains are common challenges for agricultural and forest workers, military personnel, and wildland firefighters [3]. In these groups, a valid method of evaluating fatigue is through subjective evaluations [4].

In addition to the heavy physical demands and environmental stressors of outdoor occupations, another factor that heightens the injury risk of workers is the initial and repetitive exposure to
harsh environments [5]. Among wildland firefighters, heat exhaustion occurs in response to high physical exertion despite adequate fluid intake [6]. Further, nearly 40% of injuries among wildland firefighters have been reported to occur during the first five days of fire deployment [7]. A highlighted mechanism that contributed to these injuries included overexertion, which is possibly linked to excess fatigue. Methods such as heat-acclimation protocols have been implemented in preparatory training programs for some skilled workers, (e.g., army soldiers), and have been recommended for athletes [8]. These programs improve tolerance to hot environments through thermoregulatory and thermotolerance mechanisms [9]. Additionally, exercise training and acclimation appear to improve tolerance against oxidative stress, which is commonly elevated among workers in various occupations, and has also been linked to injury [10–12]. Conversely, heat acclimation and exercise programs have not been established for occupations such as wildland firefighting; therefore, workers may be unprepared for the first several days of heavy-work schedules. In addition, larger fires and longer fire seasons have increased the need for wildland firefighters, along with heightened injury risk [13]. Interventions during initial deployments or when seasonal temperatures rise may provide protection or improve tolerance against harsh environments.

Glutamine is the most abundant amino acid in the human body and plays a biological role in the prevention and treatment of physiological stress [14,15] and critical illness [16]. Supplementation with glutamine has been associated with improvements in biological markers of heat stress in both human and animal models, and with reductions in perceived fatigue among athletes [17–20]. Glutamine has also been linked to improved survivability of broiler chickens exposed to hot and humid temperatures [19]. We have previously shown that both acute and seven days of oral glutamine supplementation prior to exercise in the heat reduces biological markers of heat damage and cellular injury [20,21]. The mechanism of glutamine protection is thought to be through the upregulation of the heat-shock-protein (HSP) pathway, namely, the heat inducible family, heat shock protein 70 (HSP70, HSPA1A) [20–25]. HSP70 provides protection to cells by refolding damaged proteins to their native state, and its activation results in protective benefits to an organism against mild toxic stresses, such as heat shock or the stress caused by intense physical activity [26].

Glutamine may also support antioxidant defenses during situations of high reactive-oxygen-species production, such as physical exercise in the heat [27,28]. For example, short-term oral glutamine supplementation increased circulating glutathione levels both before and after long-duration exercise among healthy humans, which aligned with lower levels of oxidative damage [27–29]. In addition, high levels of reactive oxygen species have been linked to physical fatigue [30].

Therefore, the major aim of this study was to assess whether acute oral glutamine dosage prior to and after laboratory-based firefighting simulation exercises in the heat on consecutive days attenuates fatigue among healthy volunteers. A secondary aim was to evaluate whether glutamine supplementation enhanced systemic antioxidant defenses and the heat-shock-protein pathway response. Laboratory-based firefighting simulations completed by healthy volunteers have previously been used to evaluate the potential benefits of oral-supplement interventions for firefighters [31,32]. This study’s approach provides a foundational insight into whether or not these therapies are effective without putting actual wildland firefighters under undue physical stress.

2. Subjects and Methods

Subjects: Eight moderately trained men (n = 5) and women (n = 3) volunteered to participate (Table 1). This study was conducted between the months of November and March, and participants were not acclimated to heat throughout the study duration. All subjects completed a health questionnaire, and procedures, discomforts, and risks were discussed before written informed consent was obtained; all study procedures were conducted in accordance with the Declaration of Helsinki. The participants reported no cardiovascular, pulmonary, or metabolic disorders. Subjects had no gastrointestinal, liver, seizure, or kidney disorders. Exclusions were applied to anyone with previously reported heat illness, or if they were taking certain medications (nonsteroidal anti-inflammatory drugs, antidepressants,
or diuretics) or nutritional supplements. Participants were requested to abstain from ingesting any nutritional supplements containing glutamine for at least one month prior to beginning the study. Participants were asked to abstain from physical exercise for 24 h prior to and during the study days. All testing was performed in the Sports Medicine Laboratory at Central Michigan University (235 m above sea level), and protocol was approved by the Central Michigan University Institutional Review Board for Human Subject Research (protocol 897539).

Study protocol/experiment design: The study design is represented in Figure 1. Using a double-blinded cross-over design, each participant completed baseline testing, and both a randomized glutamine (Gln) and placebo (Pla) trial. Both trials consisted of two consecutive days of testing and were separated by a 4-week washout period. Baseline measures included: a maximal oxygen-consumption treadmill test using a mask and metabolic cart (Parvo Medics, Sandy, UT, USA) that was determined based on previously established criteria [33]; a 15 mL blood sample; and an exercise-familiarization period. The participants reported at 7:00 on each day of the experimental trials (both Gln and Pla). Urine was collected to measure urine-specific gravity (USG) using a refractometer (A300 model; ATAGO Co., Tokyo, Japan). If participants reported a USG ≥ 1.025, they were deemed dehydrated and required to drink fluids [34,35]. Another urine sample was collected after 60 min to verify USG. A standardized meal was then provided (peanut butter and jelly sandwich plus 250 mL of orange juice, 550 kcal) and combined with the glutamine or placebo supplement. After 60 min of seated rest, a 15 mL blood sample was taken and a rectal thermistor (YSI 4600 precision thermometer with a 401 probe; Advanced Industrial Systems Inc, Prospect, KY) was inserted for the measurement of core body temperature. Subjects wore standard exercise clothing (shorts and t-shirt; females also wore a sports bra) and a heart-rate monitor (Polar, Oulu, Finland, USA) underneath protective coverall clothing (13341, Lion Apparel Inc., Dayton, Ohio), typically used by wildland firefighters. Participants then entered a heated environmental chamber (35 °C, 35% relative humidity) for the exercise portion of the trial (total exercise time = 78 min). The standardized meal with the supplement (Gln or Pla) was ingested after completion of exercise. Blood samples were taken immediately post- and 4 h postexercise. Exact procedures were repeated the next calendar day, beginning again at 7:00. All blood measurements were corrected for plasma volume shifts [36]. Approximately 1 month later, subjects returned and performed the second arm of the study trial by undergoing the identical protocol with the opposite supplement (glutamine or placebo). During the 1 month washout period, subjects were asked to maintain normal physical-activity habits.

### Table 1. Subject characteristics.

<table>
<thead>
<tr>
<th>Sex (male, female)</th>
<th>Age (years)</th>
<th>Body Mass (kg)</th>
<th>Height (cm)</th>
<th>Body Fat (%)</th>
<th>Maximal Aerobic Capacity (mL/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male n = 5</td>
<td>24 ± 1.0</td>
<td>75.46 ± 8.6</td>
<td>174.13 ± 6.9</td>
<td>18.22 ± 10.3</td>
<td>52.61 ± 6.2</td>
</tr>
<tr>
<td>female n = 3</td>
<td></td>
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</table>

Figure 1. Exercise protocol. Subjects completed consecutive days of physical exercise in a heated environmental chamber. The supplement (either glutamine or placebo) was ingested before and after each exercise bout on both days.
Exercise protocol: The exercise protocol was adapted from Zhang et al., and was completed on 2 consecutive days [31]. Total exercise time was 78 min, and began with treadmill exercise (model 1850; Proform Performance, Logan, UT, USA) performed at 60% VO$_2$max (based on a maximal treadmill test) and consisted of 4 bouts of 4 min walking followed by 1 min of volitional barbell arm curls (bar weighing 20 lbs/9.1 kg). The 5 min routine (4 min walking, 1 min barbell curls) was followed by a 4 min rest interval. After completing 4 intervals, a 6 min rest period was given. The participants then performed 4 x 4 min of stepping exercise (40 cm high platform) at a step cadence rate of 96 steps·min$^{-1}$, followed by 1 min of volitional shoulder press exercise (5 lb/2.3 kg dumbbells). The 5 min routine (4 min stepping, 1 min shoulder press) was also followed by a 4 min rest interval. The arm curls and shoulder presses was counted and recorded by a technician. The stepping exercise was intended to simulate hill climbing, a common activity for those involved in outdoor activities, while the weighted exercises (arm curls and shoulder press) simulated the carrying or heavy lifting that these occupational activities also often entail [31].

Glutamine or placebo supplementation: Subjects ingested 0.075 g/kg of body weight of glutamine (Gln; L-glutamine, Ajinomoto) mixed with 2 g of sugarfree lemon powder delivered in 250 mL of water, or the lemon-powder drink alone mixed in 250 mL of water (Pla). Gln or Pla was administered in a nontranslucent bottle to blind participants from Gln or Pla. Gln and Pla were taken 1 h prior to and immediately post exercise trials. The total amount of ingested glutamine was 0.15 g/kg/day, split between the two doses. We previously supplemented glutamine at dosages ranging from 0.90 to 0.075 g/kg/day. The decision to dose at 0.15 g/kg/day was to balance between preventing adverse responses (e.g., gastrointestinal complaints, headaches) to high supplementation while providing an effective amount [20,21,29,37].

Standard meal: The standard meal was ingested 60 min prior to exercise (after an overnight fast) and after the postexercise blood draw on both days. Participants were not allowed to eat again until after the 4 h blood draw. No additional dietary restrictions were given. The meal consisted of a peanut-butter (32 g) and jelly (40 g) sandwich (2 slices of whole wheat bread), and orange juice (250 mL).

Physiological measurements: Core body temperature and heart rate were recorded during the last minute of each bout of aerobic exercise (treadmill walking and stair stepping) to evaluate thermoregulation and hemodynamics. Oxygen consumption was also sampled during the last minute of each treadmill or stair-stepping bout using a metabolic cart to control for exercise intensity and ensure the participant was working at 60% of VO$_2$ max. Oxygen consumption and carbon dioxide production were based on a 30 s average. Workload (speed or grade) was adjusted accordingly to maintain 60% intensity, and replicated during the second arm of the study (either Gln or Pla).

Fatigue and perceived exertion measurements: The Samn–Perelli Fatigue Scale was used to monitor subjective ratings of fatigue, and has been validated in occupational settings, such as aviation operations [4]. The paper-printed scale was completed pre-, post-, and 4 h postexercise during both exercise days. The fatigue scale was administered at the same time and prior to blood collection in an effort to control for any added stress. For the analyses, the Samn–Perelli Fatigue Scale Daily Profile was determined for each participant from their score on the scale at the 3 daily time points. A lower value calculated from the Samn–Perelli scale indicates less fatigue. A subject’s rating of perceived exertion (RPE) during exercise was assessed using the Borg 6–20 scale, and measured throughout each exercise trial (end of each 5 min interval) [38]. Perception of thermal stress was also assessed every 5 minutes during exercise using a 10 point Likert scale (1 = unbearably cold, 2 = very cold, 3 = cold, 4 = a little cold, 5 = warm, 6 = too warm, 7 = hot, 8 = very hot, 9 = extremely hot, 10 = unbearably hot) [39].

Blood sampling: After 10 min of rest in a seated position (15 mL), venous blood (from an antecubital vein) was collected at baseline, pre-, post- and 4 h postexercise (for each exercise bout). Blood samples were drawn into EDTA (10 mL blood), heparin (2 mL), and serum (3 mL) vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA). The serum remained at room temperature for approximately 20 min to promote clotting, and then centrifuged (2200 × g, 20 min, 4 °C). The serum was then pipetted
into 1.5 mL microtubes and frozen at −80 °C. EDTA blood was centrifuged (3000× g, 25 min, 4 °C), and plasma was removed into 1.5 mL microtubes and frozen at −80 °C for further analysis. Blood samples in the heparin vacutainers were stored in a refrigerator at 4 °C for subsequent analysis of hemoglobin concentrations. Hemoglobin concentration was measured using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA) sensitive to 0.005 g/dL. Concentration was calculated from standard curve.

3. Biological Measurements

Plasma glutamine: Plasma glutamine was assessed using a quantitative colorimetric enzyme assay kit (EGLN-100, BioAssay Systems, Hayward, CA, USA). Manufacturer materials and chemicals were used, and directions followed. Glutamate was measured in each sample and subtracted from the glutamine absorbance (565 nm) of each sample using a microplate reader (Tecan GENios, Tecan Trading, Switzerland). A standard curve and results were calculated. Plasma glutamine was detected within a linear range of 0.023–2 mM glutamine, and the assay had a sensitivity range of 0.023–2 mM. Levels were corrected for plasma volume shifts using haematocrit and haemoglobin assessments, according to Dill and Costill [36].

Serum total antioxidant capacity: A quantitative colorimetric assay kit was used to measure total antioxidant capacity (TAC) (DTAC-100, BioAssay Systems, Hayward, CA, USA). Serum samples were diluted with distilled water (1:2). Manufacturer materials and chemicals were used and directions followed. Through this procedure, Cu^{2+} (cupric form of copper ion) was reduced by antioxidants to Cu^{+} (cuprous form of copper ion). The resulting Cu^{+} formed a colored complex when combined with the dye reagent. Color intensity at 570 nm was proportional to TAC in the sample. Values were expressed in mM Trolox-equivalent and detected within the linear range of 1.5–1000 mM Trolox-equivalent.

Peripheral blood mononuclear-cell (PBMC) protein measurements: A Luminex performance multiplex bead assay (48-615MAG, Millipore Sigma, Burlington, VA, USA) was used to measure the PBMC expression of HSP70. Microparticles were read using a Bio-Plex analyzer (Bio-Rad Laboratories, Hercules, CA, USA) with an intra-assay CV across all analytes of 5.3%. A 5 parameter logistic curve was created for each analyte, and results were calculated in pg/mL. Values were adjusted for percentage changes in plasma volume based on hemoglobin and hematocrit measurements as previously described [40].

Statistical analysis: The sample-size estimation of the 8 subjects was based on a priori calculation using a power of 0.80 and an alpha level of 0.05 for the selected variables of interest. These included changes in plasma glutamine, human PBMC expression of HSP70, and serum total antioxidant in response to acute exercise. Effort was made to include acute glutamine supplementation trials [20,41]. All statistical analyses were performed using IBM SPSS (version 19, Chicago, IL, USA). A mixed model repeated measures analysis of variance (ANOVA) was used to assess differences between supplement (Gln or Pla) and time (baseline, pre-, post-, and 4 h postexercise). Where there was a main significant effect, a paired t-test with Bonferroni correction analysis was used to assess differences between groups. For the rating of perceived exertion (RPE) data, the average at each time point during the exercise trials was averaged. The influence of glutamine was tested using a measure of effect size to compare the results. For this, the pooled Cohen’s d method was used and calculated using software (Meta-Essentials, Eramus University Rotterdam, Rotterdam, Netherlands). All data are reported as mean ± standard deviation with a significance level set to a probability value <0.05.

4. Results

Subject physiological characteristics: All subjects completed the full protocol (no dropouts) in all trials without the core temperature reaching termination criteria (40 °C). There was no difference in exercise intensity (reported as VO_{2}\text{max}) between Pla and Gln consecutive trials (62.83 ± 0.01% vs. 63.13 ± 0.01%, respectively). Final core temperature was not different between trials (Days 1 and 2) in either condition (Pla or Gln). Peak core temperature across both exercise trials in each condition was
not different (Gln Trial 1 = 38.26 ± 0.39 °C, Gln Trial 2 = 38.28 ± 0.12 °C, Pla Trial 1 = 38.28 ± 0.31 °C, Pla Trial 2 = 38.22 ± 0.20 °C). Both the shoulder-press and arm-curl repetitions were added together within each trial for a total number of repetitions. Subjects completed significantly higher number of total repetitions on Day 2 compared to Day 1 in the glutamine trial (40 ± 2 vs. 36 ± 3, ES = 1.59, p < 0.001), which translated into an 11% increase. In the placebo trial, the total number of repetitions was significantly lower on Day 2 compared to Day 1 (39 ± 3 vs. 41 ± 2, ES = −0.79, p < 0.05), which equaled a 5% decline.

Oral glutamine supplementation increased plasma glutamine levels: Plasma glutamine levels were significantly higher in Gln trials at the pre-exercise time points of Days 1 and 2 when compared to pre-exercise Days 1 and 2 in Pla (Day 1: 0.543 ± 0.165 mmol/L vs. 0.367 ± 0.082 mmol/L, ES = 1.35, p < 0.05; Day 2: 0.578 ± 0.144 mmol/L vs. 0.367 ± 0.274 mmol/L, ES = 0.96, p < 0.05, respectively; see Figure 2).

Subjective measures: The effects of glutamine on fatigue are shown in Figure 3. The Samn–Perelli fatigue index was reverse-scored, where a lower value indicates less fatigue. Fatigue was significantly lower (lower values indicate less fatigue) at the pre-exercise time point of Day 2 between the Gln and Pla groups (4 ± 2 vs. 6 ± 3, ES = −1.04, p < 0.05). The average RPE during each of the exercise trials was not different between groups. There were no significant differences in thermal comfort observed between the Gln and Pla trials.
Figure 3. Samn–Perelli Fatigue Index. Subjects felt more fatigued in the Pla trial vs. the Gln during Ex 2 pre (Ex 2 pre = prior to the second day of exercise when participants had ingested glutamine). * $p < 0.05$ significance compared to the same time point in the Pla trial. Data are mean ± SD, n = 8.

Total body antioxidants: The effects of glutamine on total body antioxidants are shown in Figure 4. Total body antioxidants were significantly increased at 4 h post on Day 1, as well as post and 4 h post on Day 2 in the Gln trial compared to the baseline ($p < 0.05$). Values did not increase in the Pla trial ($p > 0.05$).

Figure 4. Plasma Trolox. Measurement of antioxidant capacity of the serum was higher at 4 h post on Day 1 (Ex 1), and immediately post as well as 4 h post on Day 2 (Ex 2) in the Gln trial compared to the baseline. Values were not higher in the Pla trial. * $p < 0.05$ significance compared pre-time point. Data are mean ± SD, n = 8.
Heat-shock-protein response: HSP70 expression was higher at the 4 h postexercise time point of Day 1 in the Gln group compared to pre-exercise on that same day (1.33 ± 0.26 vs. 1.00 ± 0.00, \( p < 0.05 \)). HSP70 was also higher between groups (Gln vs. Pla) at the 4 h postexercise time point of Day 1 (1.33 ± 0.26 vs. 1.07 ± 0.34, ES = 0.85, \( p < 0.05 \); see Figure 5).

![HSP70 expression](image)

**Figure 5.** HSP70 expression. Effect of glutamine supplementation on HSP70 levels in peripheral blood mononuclear cells (PBMCs). Densitometric values of protein content were obtained using a Luminex performance multiplex bead assay, normalized to pre-time points of Day 1 in each trial and set to 1. *\( p < 0.05 \), significantly different from the same time point in the placebo trial; \( \tau \) \( p < 0.05 \), significantly different from the pre-exercise level in the same trial. Data are mean ± SD, \( n = 8 \).

5. Discussion

Glutamine ingestion has been shown to support antioxidant defenses and the HSP response during heavy physical exercise in the heat [27,42], and has also been linked to reduced perceived fatigue during prolonged athletic events among humans [17,18]. Here, we report lower ratings of subjective fatigue on the second day of repeated bouts (back-to-back days) of wildland firefighting simulation exercises in the glutamine trial compared to the placebo. In accordance with previous research on glutamine, we further demonstrated the upregulation of systemic total antioxidant capacity and heat shock response after glutamine ingestion, which may provide some insight into how glutamine mitigates fatigue. The time course of the biological responses may support the improvement in fatigue perception, which was shown to be reduced at the beginning of the second day of physical exercise in the glutamine trial. More specifically, in the glutamine group, HSP70 upregulation occurred 4 h postexercise on the first day, and fatigue was improved at the start of exercise on the second day compared to the placebo trial. Lower fatigue on the second day also corresponded with an increase in the total number of exercise repetitions on Day 2 (11% increase) in the glutamine trial, while repetitions decreased on Day 2 in the placebo trial. Enhanced cellular stress and antioxidant response may improve recovery after initial exposure to physical exercise and heat stress (first bout), and further improve tolerance against repeated (second bout) exposure to a similar environment. In turn, this upregulated heat-stress response may benefit workers by decreasing fatigue in the day following initial heat exposure.

Low levels of HSP expression have been linked with conditions such as mild or severe heatstroke, and certain genetic polymorphisms that may progress heat stress to heat stroke [43,44]. The heat-shock-protein system primarily works by refolding damaged proteins to their conventional...
state during and after exposure to stressors such as heat and physical activity. Prestress conditioning has been established as a mechanism to activate the HSP70 system. Through the initial HSP70 induction, an organism is protected from subsequent exposure in harmful or stressful environments [29]. Workers subjected to extended periods of stress like heat, noise, dust, or a combination of these in an occupational setting had the highest levels of HSP70 antibodies in comparison to office workers, who had the lowest levels [45]. Glutamine is a known inducer of the heat-shock-protein system. The results in the current study, along with those of other research teams, have demonstrated similar glutamine supplementation results in both human and animal models [22,25,46,47]. Glutamine ingestion may enhance the prestress-conditioning activation of HSP70. More specifically, glutamine protects against cellular stress through the transcriptional regulation of heat shock factor-1 (HSF-1), and control of HSP70 induction [48,49]. The regulation of HSF-1 occurs through the activation of O-linked N-acetylglucosamine, for which glutamine serves as the key substrate [48]. Oral glutamine supplementation has been shown to improve recovery and inhibit fatigue. For example, amateur soccer players who ingested 5 g of glutamine in a multi-ingredient supplement experienced attenuated perceived fatigue after a 90 min intermittent sprint test [18]. In addition, acute rehydration with an L-alanyl-L-glutamine drink improved reaction time after exhaustive treadmill running [50]. Similar to the current study, we recently reported that oral glutamine prior to, and after firefighting simulations increased HSP70, which aligned with lower subjective fatigue on the second day of physical work [51]. In this previous study, we also identified that glutamine may have immunoregulatory properties, and this, combined with evidence that wildland firefighters are under a low-grade inflammatory state, suggests another possible mechanism for glutamine ingestion among wildland firefighters [52]. It is important to mention that similar research exploring the role of glutamine on fatigue has used multi-ingredient supplements, so the reported benefit could not be solely due to glutamine [17,18]. In addition, research regarding the physical-performance-enhancing benefits of glutamine is mixed, but the aim of this study was not to investigate work performance [53,54].

Reduced fatigue may also, in part, be due to glutamine-induced increases in observed plasma antioxidant levels. In the current study, total plasma antioxidant levels were quantified via a Trolox equivalent, which provides evidence of the overall ability of a fluid to counteract reactive oxygen species [55]. Oxidative stress is often a scenario resulting from an unchecked increase in free oxygen radicals or an inadequacy in antioxidant systems under certain disordered states [56]. These free radicals have deleterious toxic effects by directly inducing tissue damage through the generation of proinflammatory molecules. Excessive oxidative stress is linked to reduced muscle performance along with mental fatigue [57–59]. In addition, higher plasma and skeletal muscle antioxidant levels have been shown to attenuate both physical and mental fatigue [60,61]. Glutamine is thought to increase the antioxidant defense systems via the antioxidant properties of the amino acid or due to its conversion to glutathione [62–64]. The findings posted in the current study suggest that oral glutamine may protect against oxidative stressors by increasing antioxidant capacity, which may then delay fatigue during heavy physical work.

High physical demands in harsh environmental conditions is common for wildland firefighters [65]. The negative physiological and psychological impact may be exacerbated during the early days of exposure (in a nonacclimatized state) and from consecutive work shifts [66,67], thus increasing the risk for injury [6]. The most reported nonfatal injuries among wildland firefighters are from common hazards such as slips, trips, and falls, which may be due to fatigue, along with smoke inhalation as a result of pollution exposure [68]. In a large occupational study (N = 58,495) in Thailand, it was found that 20% of workers developed occupational heat stress that was strongly associated with injury. The authors further concluded that safety programs and strategies should be developed in response to high occurrences of heat-related injury in tropical regions [69]. In addition, efforts have been made to identify the characteristics of those susceptible to heat-related injury to prevent public health risks [70]. This may include older workers and those working for successive days in the heat [71]. Injury prevention among wildland firefighters is of high priority, but the bulk of the research has explored various
technological (e.g., clothing, devices) and fire-strategy advancements [72,73]. The implementation of effective nutritional strategies may also support wildland firefighters and other occupations that are exposed to similar environments.

A limitation of the current study is the short duration of the physical-work protocol, which is considerably shorter than a real-world wildland-firefighter work shift. Divergence between clinical and physical exertion/exercise nutrition data has resulted in the idea that, perhaps, L-glutamine stores within the body cannot sufficiently be depleted by physical exercise [74]. The short duration of the utilized physical-work protocol may explain why we did not observe decreases in plasma levels in the placebo trial. An extended exercise protocol, or field work similar to real-world work exposures [6], may have modulated a reduction in glutamine plasma levels in the placebo group, as well as a more pronounced HSP and antioxidant response in the glutamine trial. An additional limitation is that only subjective fatigue was measured in the study, where a more accurate measure of fatigue would be a physical-performance assessment. Further, the implemented physical exercises were designed to represent occupational activities, such as marching and lifting objects; however, these do not characterize all physical movements of firefighters. Lastly, the participation of healthy volunteers as a substitute for wildland firefighters and the small sample size are additional limitations to the current study.

In conclusion, we report that glutamine supplementation during repetitive exercise and work bouts in the heat has the ability to reduce ratings of subjective fatigue in healthy college-aged adults. These positive effects may have occurred through upregulation of the heat-shock response and antioxidant protection, therefore possibly mitigating subjective fatigue rating, which is associated with heat-related occupational injuries. Future studies should explore the efficacy of glutamine supplementation to modulate the heat-shock response and its impact on perceptions of fatigue among wildland firefighters in the field.


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References


12. Ho, E.; Galougahi, K.K.; Liu, C.-C.; Bhindi, R.; Figtree, G.A. Biological markers of oxidative stress: Applications to cardiovascular research and exercise. *Redox Biol.* 2013, 1, 483–491. [CrossRef]


43. Milner, C.; Campbell, R. Polymorphic analysis of the three MHC-linked HSP70 genes. *Immunogenetics* 1992, 36, 357–362. [CrossRef]


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