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# Effects of glutamine supplementation on maximal performance and recovery from high-intensity exercise.

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# EFFECTS OF GLUTAMINE SUPPLEMENTATION ON MAXIMAL PERFORMANCE AND RECOVERY FROM HIGH-INTENSITY EXERCISE

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ABSTRACT

EFFECTS OF GLUTAMINE SUPPLEMENTATION ON MAXIMAL PERFORMANCE AND RECOVERY FROM HIGH-INTENSITY EXERCISE

Mandy Lynn Jacobs

August 7, 2008

The purpose of this study was to determine the effects of acute glutamine supplementation on maximal performance and recovery from high-intensity exercise. In a placebo-controlled, crossover study, seven (six males and one female) healthy subjects performed maximal treadmill exercise one-hour after the ingestion of 0.03 g·kg body mass$^{-1}$ glutamine mixed with caffeine-free fruit juice or placebo. Expired gases, respiratory exchange ratios (RER), heart rate (HR), ratings of perceived exertion (RPE) and blood samples were collected pre-, during, and post-exercise. In addition, urine and saliva samples were obtained pre- and post-exercise. No significant differences in oxygen consumption, $CO_2$ production, HR, RPE, urine and saliva pH were found between treatments. The GLN trial (1.20 ± 0.07) produced a higher RER value than the PLC trial (1.11 ± 0.09) at 5-min post-exercise, resulting in a significant difference ($p = 0.042$). However, the significant difference was due to the inclusion of the female in the group analysis. This study demonstrates that acute glutamine supplementation does not enhance maximal performance or recovery from high-intensity treadmill running.

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# CHAPTER 1

# INTRODUCTION

In today's culture, athletes are continuously searching for means beyond traditional training techniques that will give them an additional advantage over their competitors. Thus, athletes often rely on dietary supplementation in order to improve their performance. Although limited information is available on the extent of dietary supplement use among athletes, in 1999 it was estimated that the annual sales of supplement products in the United States totaled $12 billion[1]. Athletes account for a significant fraction of the total market and a wide range of products are aimed at both the active population and at those engaged in competitive sport. The problem with this reliance on dietary supplements is that the industry is completely unregulated by the government in the United States due to the Dietary Supplement Health and Education Act of 1994[57]. As a consequence, an abundance of supplement products of dubious value, content and quality are now available to athletes all over the world[105]. More recently gaining popularity among athletes are individual amino acids- specifically, glutamine- due to its use in the area of human nutrition[133]. Results of clinical studies have suggested that glutamine supplementation may improve nitrogen balance[54] and support immune function[102].

In a brief review of glutamine as a potential supplement for athletes, Antonio and Street suggest that glutamine might provide ergogenic effects for athletes that engage in intense exercise training[4]. They hypothesize that when athletes engage in high-intensity

exercise, a significant increase in plasma lactate and hydrogen ion ($H^+$) concentration occurs within the body due to glycolysis. This increased production of $H^+$ can cause a fall in plasma pH from approximately 7.4 to 6.9-7.0, resulting in a state known as metabolic acidosis (pH < 7.35). The associated fall in pH with acidosis can hinder muscular contraction and ultimately performance by limiting the rate of resynthesis of ATP by inhibiting key glycolytic enzymes, such as phosphfructokinase[29, 125]; inhibiting the release of calcium ions from the sarcoplasmic reticulum and the binding of these ions to the protein troponin[33, 37]; and impairing the neural impulse propagation[32, 124]. Under normal physiological conditions, glutamine is converted to α-ketoglutarate, which produces ammonium ions ($NH_4^+$) that in turn buffer the acidotic state. However, when the decrease in pH is too drastic for this buffering system too work efficiently, such as during high-intensity exercise, the athlete's performance and recovery may be compromised[56].

Past studies have demonstrated that increasing plasma pH via sodium bicarbonate or sodium citrate ingestion can improve anaerobic exercise performance[92, 93, 103, 144,15, 18, 27, 90, 125]. Hence some researchers have stated that it is logical to believe glutamine supplementation would also be able to increase pH as well due to its base generating potential[3, 56]. However, very few studies have been conducted to investigate the effects of glutamine supplementation on athletic performance and/or recovery from high-intensity exercise[3, 56, 104]. Making matters even more complicated, the studies have solely examined the effects of glutamine supplementation on short intermittent bouts of exercise, such as those observed during weightlifting[3] and cycling (sprints)[56, 104]. No

reported literature was found examining the effects of glutamine supplementation on maximal performance and recovery during high-intensity running.

## Purpose of the Study

The purpose of this study was to determine if glutamine supplementation has an ergogenic effect on high-intensity treadmill running.

### Primary Aim:

1) To determine the effects of acute glutamine supplementation on plasma glutamine concentration and lactate during and after a high-intensity running protocol.

### Secondary Aims:

1) To determine the effects of acute glutamine supplementation on maximal performance ($VO_2max$) and associated parameters (ratings of perceived exertion, heart rate, $CO_2$ production) during a high-intensity running protocol.

2) To determine the effects of acute glutamine supplementation on recovery parameters ($O_2$ consumption, $CO_2$ production, heart rate, respiratory exchange ratio) after a high-intensity running protocol.

3) To further investigate the changes in plasma glutamine concentration during and after high-intensity running.

## Hypotheses

The following hypotheses will be tested:

### Primary Hypothesis:

1) The acute supplementation of glutamine will increase plasma glutamine concentration and decrease lactate levels during and after high-intensity running via a buffering effect.

**Secondary Hypotheses:**

1) The acute supplementation of glutamine will increase $VO_2$max and decrease ratings of perceived exertion during exercise.

2) The acute supplementation of glutamine will increase $CO_2$ production and respiratory exchange ratios post-exercise.

3) Plasma glutamine concentrations will increase during and post-exercise from resting (baseline) values.

**Significance of Study**

This study is significant as it will determine if acute glutamine supplementation one-hour prior to exercise has an ergogenic effect on high-intensity running. Several studies have indicated the use of oral glutamine supplementation to improve exercise performance during high-intensity and prolonged exercise. To date no literature has addressed the potential effects of glutamine supplementation on maximal performance and recovery from high-intensity treadmill running. This research is important in validating the use of glutamine supplementation prior to high-intensity running.

# CHAPTER II

## LITERATURE REVIEW

Glutamine is a five-carbon amino acid[76] that is found in the human body at relatively high levels[117]. In fact, glutamine is the most abundant non-essential amino acid in human plasma[76], with normal plasma concentrations ranging between 500 and 750 μmol/L[40]. There are two principal enzymes of glutamine metabolism: glutaminase and glutamine synthetase. Glutaminase catalyses the hydrolysis of glutamine to glutamate and ammonia, and glutamine sythetase catalyses the synthesis of glutamine from ammonia and glutamate. The direction and rate of flux through the substrate cycle is tissue-dependent, as different tissues vary considerably in the forward and reverse rates of reaction. Therefore, the net direction of the reaction will determine whether a particular tissue is a net consumer or producer[75, 97]. A constant level is maintained under normal conditions due to a balance between release and utilization of glutamine by various organs within the body. By simultaneously sampling arterial and venous blood various organs, one can classify an organ as either predominantly a consumer or a producer of glutamine[117]. Those organs considered to be involved in the synthesis of glutamine include primarily skeletal muscle, the lungs, liver, brain and possibly adipose tissue[117]. The main consumers appear to be the kidneys, intestines and cells of the immune system[40, 75, 97, 117]. Under certain conditions, the liver may also become a net consumer of glutamine[136].

Although numerous organs within the body use glutamine as a fuel, it is prominently used by the immune system and gastrointestinal tract. In addition, glutamine accounts for more than 60% of the total intramuscular free amino acid[122] and because skeletal muscle represents a large mass of tissue, it is quantitatively the most important site of glutamine synthesis despite the fact that glutamine synthetase activity is relatively low per unit mass in skeletal muscle[140].

Glutamine fulfills many vital roles in the tissues and organs of the body. Such roles include maintenance of the acid-base balance during acidosis[28, 46, 106], transfer of nitrogen between organs and detoxification of ammonia[97], acting as a nitrogen precursor for the synthesis of nucleotides[75], a fuel for gut mucosal cells[55, 123, 145, 146] and cells of the immune system[7] and a possible direct regulator of protein synthesis and degradation[85].

As previously stated, plasma glutamine concentrations remain at constant level under normal physiological conditions. Most naturally occurring food proteins contain four to eight percent of their amino acid residues as glutamine, so the daily consumption of glutamine is usually less than 10 grams[122]. However, during various catabolic states, such as sepsis[9], surgery[54, 65, 108], trauma, burns[102] and acidosis[142], glutamine homeostasis is placed under stress, and glutamine reserves are reduced. In addition, some studies have shown similar states of depletion due to both prolonged and high-intensity exercise stress[23, 24]. It has been suggested that it may be necessary to consume between 20 and 40 grams of glutamine per day to maintain homeostasis after a severe catabolic insult[76].

**The Effects of Exercise on Plasma Glutamine Concentrations**

Skeletal muscle is quantitatively the most important source of glutamine since not only can it synthesize glutamine but it also provides storage[23]. The store of free glutamine

within the skeletal muscle is estimated at 20 mmol/L of intracellular water[13], which accounts for the majority of the body's total glutamine stores[122]. The rate of glutamine synthesis in human skeletal muscle is higher than that for any other amino acid and in the fed state is approximately 50 mmol/h[44, 137]. Parry-Billings et al. speculates that this high synthetic rate is essential to maintain glutamine homeostasis due to the fact that skeletal muscle provides the majority of glutamine required by other tissues[100]. Thus, it is suggested that the failure of skeletal muscle to provide sufficient glutamine due to exercise stress could result in a hindered performance and state of recovery.

**Plasma Glutamine Measurement**

Unlike the many other amino acids, glutamine has been shown to be relatively unstable in aqueous solution[95] and is known to readily break down to glutamate and ammonia in the presence of heat, as well as either strong acidic or basic environment[5]. Specific preparations of samples and storage methods have been developed to minimize degredation[117]. To date, three different measurement techniques are primarily used for the quantification of glutamine levels: enzymatic methodology[21, 35, 83, 101, 118, 119, 146, 149], high performance liquid chromatography (HPLC)[10, 12, 30, 36, 47, 68, 110, 115], and bioassay[70, 116]. Quantification of glutamine by enzyme assay involves conversion of glutamine to glutamate[83], the subsequent degradation of the product to α-ketoglutarate at pH 9.0[14], and the concomitant conversion of nicotinamide adenine dinucleotide ($NAD^+$) to its reduced form NADH, which is detected spectrophotometrically. The plasma level of glutamate is then subtracted to obtain the original level of plasma glutamine, on the principal that the conversion of glutamine to glutamate is on a molar to molar basis[117]. Automated amino acid analyzers have also been developed, based on this enzyme assay[71]. HPLC has also

been used to determine plasma glutamine[10, 110] but generally require acidified samples in analytical procedures[135]. The recently developed bioassay method, uses a strain of *Escherichia coli* (*E. coli*) known to be dependent on glutamine for replication[91]. Growth of *E. coli* has been shown to be directly proportional to the amount of glutamine available[70]. The normal range of values obtained for human plasma samples differ to some degree depending on the methodology used, thus it is important to consider which methodology is being used when comparing particular studies with one another[117].

**Exercise & Plasma Glutamine Concentrations**

There have been various studies that investigated the effects of exercise on plasma glutamine concentrations (Table 1), although a clear consensus does not exist within the literature. Data has shown that during exercise, plasma glutamine concentrations may increase, decrease or remain unchanged depending on the type, duration, and intensity of exercise. Walsh et al. feels the equivocal data is also due to the nutritional status of the individuals and differences in measurement technique, blood sampling times and sample storage[137].

**Prolonged Exercise**

Studies that have analyzed the effects of prolonged exercise on plasma glutamine concentration have led to ambiguous conclusions. In a recent experiment, continuous cycling at 55% of maximal oxygen consumption ($VO_2max$) for three hours in 18 healthy men led to a 23% fall in plasma glutamine one hour after exercise (580 μmol/L pre-exercise compared with 447 μmol/L after one hour of recovery)[112]. Producing similar results, Rennie et al. monitored plasma glutamine levels for 270-minutes following 195-minutes of cycling at 50% $VO_2max$[110]. A fall from 557 μmol/L at rest to 470 μmol/L

immediately after exercise was reported. After two hours of recovery, plasma glutamine levels continued to fall its' lowest level of 391 μmol/L, but after 270-minutes of recovery, levels increased to 482 μmol/L[110]. In addition, Parry-Billings et al. reported a 16% decrease in mean circulating glutamine levels in 22 trained distance runners following a marathon race (592 to 495 μmol/L)[101].

In contrast, both a 30km treadmill run and cycle ride to exhaustion at 73% $VO_2$max had no effect on plasma glutamine levels when samples were collected immediately before and after exercise[101]. Similarly, Lehmann et al. reported no changes in venous plasma glutamine concentrations following an ultra triathlon (7.5km swimming, 360km cycling and 85km running)[80]. However, in that study blood samples were taken within 30 minutes of completing the race and it is possible that the glutamine concentration may have decreased after this time[114]. In addition, continuous cycling to exhaustion at 80% $VO_2$max (which occurred within one hour) in 18 healthy men did not alter the plasma glutamine level at exercise test cessation from the pre-exercise value[112].

**Acute/High-Intensity Exercise**

Numerous studies have demonstrated an increase in plasma glutamine concentration following brief (less than one hour) high-intensity exercise in humans. Eriksson et al. found plasma glutamine levels increased from 538 μmol/L to 666 μmol/L during 45-minutes of incremental exercise at 80% $VO_2$max[36] and Babij et al. observed increases from 575 μmol/L at rest to 734 μmol/L during exercise at 100% $VO_2$max[10]. These findings were further supported by a study conducted by Parry-Billings et al. in which a series of ten six-seconds sprints resulted in an increase in plasma glutamine levels from 556 μmol/L pre-exercise to 616 μmol/L immediately post-exercise[101]. Hood

and Terjung claim that an increase in plasma glutamine concentrations during acute exercise suggest that glutamate acts as a sink for $NH_3$ in the formation of glutamine during enhanced ammonia ($NH_3$) production, which consequently occurs during high-intensity exercise due to an increased reliance on anaerobic metabolism and an increased production of lactic acid[62].

In contrast, Keast et al. found a significant decrease in plasma glutamine levels due to acute high-intensity exercise. The study consisted of seven trained males that participated in fifteen one-minute bouts at 90% $VO_2$max on a treadmill, which resulted in a 44% decrease in plasma glutamine levels immediately after exercise. Additionally, the same seven subjects showed a decrease of 55% immediately after participating in fifteen one-minute bouts at 120% $VO_2$max on a treadmill[70]. There has been one study to date that has aimed to establish the pattern and time course of plasma glutamine recovery after exercise and any diurnal variations. Kargotich et al. conducted a study in which eight elite male swimmers completed 15 x 100m intervals at 70% and 95% of their maximal exercise intensity. Venous blood samples were obtained at rest, immediately post-exercise and 30-minutes, 60-minutes, 120-minutes and 150-minutes post-exercise. No changes were observed in plasma glutamine following the 70% interval, however, following the 95% interval, the means plasma glutamine concentration changed significantly from 1140 μmol/L at rest to 1002 μmol/L post-exercise. This data suggests that at least six hours between intensive training sessions must be allocated to ensure adequate plasma glutamine recovery. This study further emphasizes the necessity to establish appropriate times within training programs to screen for specific training related changes, as glutamine has been suggested as a marker of overtraining[67].

**Recovery from Exercise**

Similar to the changes that occur during exercise, the return of plasma glutamine levels to pre-exercise values during recovery (post-exercise) are dependent upon the intensity and duration of the exercise bout. Décombaz et al. reported that the changes in plasma glutamine levels observed in trained athletes following a 100km run had not returned to pre-exercise values even after 24 hours of recovery[31]. In addition, during a 225-minute exercise session at 50% $VO_2$max, a mean resting glutamine level of 557 μmol/L, fell to 470 μmol/L immediately after exercise and was further decreased to 391 μmol/L after 120-minutes of recovery. It had increase to only 482 μmol/L after 270 minutes of recovery[110]. Similarly, in a study involving eight trained male swimmers whom participated in fifteen one-minute bouts at 95% $VO_2$max, the lowest levels were observed between four and six hours after exercise, 84 and 86% of the resting plasma glutamine level respectively[66]. Consequently, it has been suggested that considerable periods of recovery may be required between exercise training sessions, particularly those of high-intensity exercise, to allow complete recovery of plasma glutamine levels[117]. Moreover, following a triathlon in which eight volunteers swam 2.5km, cycled 81km and ran 19km, mean serum glutamine levels declined from 468 μmol/L (pre-race) to 318 μmol/L at two-hours post-exercise[114].

The fall in plasma glutamine observed post-exercise may be due to an increased uptake of glutamine by the kidneys in attempt to buffer metabolic acidosis[96, 141, 143]. Acidosis can arise from increased lactic acid production associated with high-intensity exercise and an accumulation of other organic acids including free fatty acids, acetoacetate and 3-hydroxybutyrate. Ammonia production in the kidneys and its

secretion into the distal tubules together with excretion of excess protons in the urine protects against acidosis. By the action of glutaminase, glutamine is hydrolyzed to glutamate in the distal tubules, producing $NH_3$, which can be combined with a hydrogen ion to form ammonium ions[137]. Alternatively, the immune system may be stimulated to increase glutamine utilization as a result of exercise-induced early inflammation and cytokine activation[109, 121], concomitant with lymphocyte activation[19] and mobilization of natural killer cells with an increased glutamine oxidation rate[42]. It has also been postulated that glutamine uptake by the liver and gastrointestinal tract for gluconeogenesis occurs simultaneously with constant or decreased glutamine release by muscle, resulting in a decreased post-exercise plasma glutamine concentration[137].

**Overtraining Syndrome**

Given the changes in plasma glutamine levels that are commonly observed after prolonged and/or high-intensity exercise and the function of glutamine in immune cells, the role of glutamine in overtraining has recently gained attention[52]. The Overtraining Syndrome (OTS) has been defined as prolonged fatigue and under-performance following periods of heavy training[98]. The plasma concentration of glutamine has been reported to be lower in overtrained athletes than in well trained athletes and sedentary individuals[73, 84, 101, 116]. Rowbottom et al. observed a lower mean plasma glutamine level in a group of ten athletes classified as overtrained (703 μmol/L) compared with sedentary (1030 μmol/L) and athletic age-matched controls (1179 μmol/L)[116]. While Parry-Billings et al. reported mean plasma glutamine levels of 503 μmol/L in a group of 40 athletes diagnosed as having the OTS, compared with a mean level of 550 μmol/L in a group of controls[101]. Plasma glutamate (the precursor to glutamine) levels were,

however, reported to be significantly higher (161 *vs.* 125 μmol/L ) in the group suffering from the OTS. In another study, glutamine responses in trained cyclists who completed two weeks of intensified training were examined and while no significant changes in glutamine were noted across the two week period, a decline in the glutamine/glutamate ratio was found[53]. This relationship has also been noted by Smith and Norris who hypothesize that glutamine concentration decreases when the volume of work exceeds the athlete's capacity to tolerate work[120]. Various reasons have been suggested as the cause of the decline in glutamine concentration over time, such as increased levels of glucocortiocods[6, 81]; decreased nutritional intake of protein[82]; mitochondrial lesions within skeletal muscle[116]; and increased rate of utilization of glutamine by other tissues rather than decreased production[120].

**Exercise and the Acid-Base Balance**

During high-intensity exercise, glycolytic energy production is elevated with a concomitant increase in lactate ($La^-$) and hydrogen ion concentration ($H^+$) within the working tissues[58, 59, 74]. Consequently, the accumulation of $H^+$ results in a decrease in pH which is thought to (a) reduce force production through hindered excitation-contraction coupling[34, 37], (b) inhibit glycolytic energy production[25, 125, 129], and (c) decrease cross-bridge cycling[87, 138]. Therefore, it is believed that an artificially induced alkalosis (increase in pH) may enhance performance by improving the buffering capacity of the blood and tissues to withstand the decrease in pH associated with high-intensity exercise[86].

Past studies have demonstrated that an induced alkalosis (using sodium bicarbonate or sodium citrate) can delay the onset of fatigue by buffering the

accumulation of $H^+$ and aiding the efflux of lactate from contracting tissues during high-intensity exercise lasting between 30-seconds and 4-minutes[15, 18, 27, 90, 92, 93, 103, 125, 144]. In one study, six trained male athletes were studied under control, sodium bicarbonate ($NaHCO_3$) and placebo conditions to study the effect of induced alkalosis on 400-m racing time. In the alkalotic condition the subjects ran significantly faster (1.52-sec) than either the control or placebo conditions. In addition, the post-exercise pH, bicarbonate and base excess levels were all lower in the alkalotic condition than in the others[45]. Thus suggesting that $NaHCO_3$ can be used an ergogenic aid by facilitating the efflux of $H^+$ from the skeletal muscles. Moreover, several investigators have reported a decrease in perceived exertion[111, 126]. Sodium citrate ingestion has also been found to improve anaerobic performance of 120-sec and 240-sec on a cycle ergometer by increasing bicarbonate concentrations and decreasing pH levels[94]. However, some would argue that the evidence for any ergogenic effect resulting from ingestion of alkanizers is equivocal. Several authors found no improvement in the performance of high-intensity exercise following the ingestion of either sodium citrate or sodium bicarbonate) [69, 72, 78]. The variation in findings may partially be explained by differences in the dosages of alkalinizing agents and in the exercise intensity and duration[11].

**Dietary Influences on Plasma Glutamine Concentration**

There is substantial evidence that dietary intake can influence plasma glutamine levels[43, 49, 89, 134, 148]. Gleeson et al. investigated the relationship between carbohydrate (CHO) intake and plasma glutamine; subjects exercised twice for 60-minutes at 70% $VO_2$max: once after consumption of a normal diet and once after three days on either a high (75%) or low (4%) CHO diet. Exercise after the low CHO diet resulted in a lower

post-exercise plasma glutamine concentrations when compared with the high CHO diet[43]. This is most likely due to resting metabolic acidosis, which has been shown to occur with very low CHO diets[89]. Circulating acidosis leads to an increased uptake of glutamine by the kidneys for the purpose of buffering $H^+$ and this reduces plasma levels of glutamine[16]. In addition, glutamine is used as a gluconeogenic precursor[117] and gluconeogenesis increases when CHO availability is low[16]. Similar results were discovered by Blanchard et al. who investigated the relationship between muscle glutamine, muscle glycogen, and plasma glutamine concentrations over three days of high-intensity exercise during which CHO intake varied. Mean plasma glutamine concentration was significantly higher during the 70% CHO exercise trial when compared to the 45% CHO trial. However, glycogen decreased by the same magnitude during both trials and there was no relationship between changes in plasma glutamine and changes in muscle glycogen concentration. Because neither muscle glycogen nor muscle glutamine concentrations differed between diets and exercise trials, the authors suggested that the lowered CHO and increased protein intake (40% higher than that on the high-CHO diet) may have increased removal of glutamine from the circulation[16]. These results are in agreement with data of Zanker et al., which showed that consumption of an 80% CHO meal before intense endurance exercise resulted in higher glutamine levels compared with fasting for 14-hours before exercise[148]. Studies in rats also indicate that pre-exercise CHO availability is an important factor, since 24-hour fasted rats exhibited a much greater fall in plasma glutamine after 30-minutes swimming compared with the same exercise performed in the fed state[26]. In contrast, van Hall et al. reported that CHO supplementation prior to and during exercise had no effect on plasma glutamine

concentration during exercise and did not prevent the post-exercise decrease in plasma glutamine[132].

Research has also shown that protein intake can influence plasma glutamine concentration, as increases of plasma glutamine levels of up to 29% were observed following meals[21] especially those with substantial protein content. Alternatively, it has been demonstrated that a diet rich in protein (24% protein, 72% fat, 3% CHO) consumed for four days results in a 25% lowering of muscle and plasma glutamine levels[50]. Furthermore, in a study aimed at determining the relationship between resting plasma glutamine concentration and dietary protein intake for athletes of different sports (runners, swimmers, cyclists, nonathletes) dietary protein intake expressed relative to body mass ($g \cdot kg^{-1} \cdot d^{-1}$) was significantly inversely related to plasma glutamine concentration. This negative relationship may be explained by an increase in glutamine uptake in the kidney, in an attempt to maintain acid-base balance in athletes consuming a high protein diet[61]. Ammonia produced via the hydrolysis of glutamine to glutamate binds to a hydrogen ion to form ammonium ion[49, 63, 117]. In combination with an anion, commonly chloride, ammonium is excreted in urine. In this way, the kidney is able to effectively excrete excess protons, and thus prevent cell acidosis associated with a high protein diet[48, 49].

**Diurnal Variations of Plasma Glutamine Concentration**

It is important to note that intra-individual plasma glutamine concentrations can vary widely during the day[21]. During starvation or an overnight fast, the supply of glutamine from the intestinal lumen is reduced because the glutamine present in the protein of the diet is mainly metabolized by the absorptive cells of the small intestine. It

is believed that 50-60% of glutamine is utilized in this way[40]. Early studies shave identified a diurnal variation in plasma total amino acid level[38], as well as a similar variation for individual amino acids, including glutamine[39]. Although dietary influences may have been a major contributor in these studies, diurnal changes have been reported in healthy young adults following an overnight fast[99]. More recently, it seems that studies examining possible diurnal variations in glutamine concentration have published conflicting results. Plasma glutamine levels were monitored for a period of 21-hours in four healthy volunteers (n = 4) with no dietary restrictions, which resulted in a plasma glutamine increase two-hours after ingesting a meal containing protein. Castell et al. reported that during a 21-hour fast there was almost no change in plasma glutamine levels throughout the day, compared with the normal day (free of dietary restrictions), thus suggesting the absence of diurnal variations. However, it should be noted that only one subject participated in the 21-hour fast within the study[21].

**Glutamine Supplementation**

Antonio and Street state that it would seem plausible that glutamine supplementation may exert a beneficial effect for individuals engaged in chronic and intense exercise training. They believe that because glutamine concentrations decline in a dose-dependent manner[77], one could hypothesize that exercise training would increase the requirements for glutamine such as that exogenous self-administration may be necessary for ultimate performance and complete recovery[4]. Antonio and Street state their hypothetical reasoning for glutamine supplementation by athletes on established effects of exercise stress and glutamine. Since glutamine plays a crucial role in the functioning of the immune system and its' cells, it may be suggested that glutamine

supplementation may prevent or lessen severity of illness or infection after an intense bout of exercise, thus enabling the athlete to resume intense training more quickly. In addition, glutamine supplementation may offset catabolic effects of elevated glucocorticoid levels that are produced during exercise. Moreover, the provision of glutamine to fuel other organs (gastrointestinal tract, liver, kidney, and immune cells) could spare the potential loss of glutamine due to inadequate dietary intake, thus sparing muscle protein[4].

Of particular importance is the theoretical effect glutamine may have on the acid-base balance within the body by producing a buffering effect. It is suggested that glutamine alters the acid-base balance by increasing plasma bicarbonate ($HCO_3$) retention within the kidneys, via deamination, when glutamine enters the epithelial cells of the kidneys[139, 142, 147]. The ammonium that is formed from the deamination of glutamine, then binds with a $H^+$ to form an ammonium ion. This loss from the epithelial cell drives the carbonic anhydrase reaction to yield $HCO_3$ and $H^+$. The excess intracellular $HCO_3$ diffuses into the extracellular regions with a sodium ion and subsequently enters systemic circulation[51]. The ammonium ion combines with an anion (usually chloride) and is then excreted in the urine[97], thus allowing the kidneys to effectively excrete excess protons and protect the body from acidosis[46]. More simply, once glutamine is oxidized in the Krebs cycle, it is transported into the kidneys to dispose of nitrogen and recycle the carbon skeleton.

Although Ziegler et al. confirmed that acute oral glutamine ingestion did not show any signs of toxicity in patients receiving glutamine for several weeks[149], very few studies have investigated the effects of glutamine supplementation on athletic

performance and recovery from high-intensity exercise. One study performed by Antonio and colleagues[3], aimed to determine if high-dose glutamine ingestion affected weightlifting performance. In a double blind, placebo-controlled, crossover study, six resistance trained men performed weightlifting exercises after ingestion of glutamine or glycine (0.3 g·kg body mass$^{-1}$) mixed with calorie-free fruit juice or placebo. One hour after ingestion, subjects performed four total sets of exercise to momentary muscular failure. The findings indicated that short-term ingestion of glutamine does not enhance weightlifting performance in resistance-trained men. Antonio et al. further suggested that future work should examine long-term supplementation of glutamine in athletes during a competitive season[3].

In a similar study, Haub et al.[56] investigated the potential of glutamine supplementation to alter the acid-base balance of the blood and subsequently increase the time to exhaustion during high-intensity exercise. The researchers based their investigations on the theory that glutamine alters acid-base status by increasing $HCO_3$ retention in the kidneys. Ten trained males performed five exercise bouts on a cycle ergometer at 100% $VO_2$peak. The first four bouts were 60-sec in duration, while the fifth bout was continued to fatigue. Each bout was separated by 60-seconds recovery. The exercise bouts were initiated 90-minutes after ingesting 0.03 g·kg body mass$^{-1}$ of either glutamine or placebo. Venous blood samples showed no significant difference in pH, [$HCO_3$], and lactate concentration ([$La^-$]) between pre-ingestion, pre-exercise, bout four and bout five. In addition, time to fatigue was not significantly different between conditions. Overall, the data indicated that acute glutamine ingestion did not enhance either buffering potential or exercise performance in trained males. However, the

researchers suggest that the environment may need to be acidic in order to activate glutaminase (the enzyme responsible for the breakdown of glutamine in the kidney) and ultimately use glutamine to increase $HCO_3$[56].

In a recent study Piattoly investigated the effects of glutamine supplementation on recovery from intense exercise among elite cyclists. Specifically, the influence of glutamine supplementation (0.3 g·kg body mass$^{-1}$ for six days) on time to exhaustion and power after a prolonged bout of exercise was analyzed. The results showed no group differences in $VO_2$peak, peak power and time to exhaustion before supplementation, and participants in both the glutamine group and placebo group experienced similar declines in performance immediately and 24-hours after an exhaustive bout of exercise. However, it was discovered that participants in the glutamine group increased time to exhaustion following six days of supplementation and appeared to recover from exhaustive exercise earlier than those participants that did not ingest glutamine (placebo group)[104].

In addition, data showing low plasma glutamine and an increased occurrence of upper respiratory tract infections in some groups of highly trained athletes has raised interest in glutamine supplementation for those investigating immune function during periods of intense training and post-exercise recovery[137]. One study specifically examined a possible prophylactic effect of oral glutamine supplementation on the occurrence of infection[24]. Castell et al. gave ultra-marathon and marathon runners participating in races either a placebo drink (malo-dextrin) or glutamine solution (5-grams glutamine in 330mL of water) immediately upon completion and two-hours after the race. Athletes were given questionnaires to self-report the occurrence of symptoms of infection for seven days after the race. In those receiving the glutamine supplements (n =

72), 81% experienced no infection in that period. In those receiving the placebo drink (n = 79), only 49% experienced no infection in the same period. The authors concluded that the provision of two glutamine drinks in the first two hours post-race decreased the incidence of infection in the week after the event[24]. Similarly, another study found that a glutamine solution (0.1 g·kg body mass$^{-1}$) given at 0, 30, 60, and 90-min following a marathon race prevents the fall in plasma glutamine concentration[113].

**Glutamine Absorption**

In a glutamine supplementation study, Castell et al. found that after administering glutamine, plasma glutamine concentrations increased by at least 50%, peaking at 30-minutes after ingestion and remaining elevated for one to two-hours before returning to the normal range of 500-700 μmol/L. Subjects were either given 0.1 or 5 grams L-glutamine per kilogram of body weight[22]. In another study Ziegler found similar results in whole blood. Rested subjects were given either 150-ml distilled water (zero glutamine dose), 150-ml water containing 0.1-gram glutamine per kilogram of body weight (low glutamine dose) or 150-ml distilled water containing 0.3 g·kg body weight (high glutamine dose). Whole blood concentrations of glutamine rose in proportion to administered oral glutamine load. Blood glutamine levels peaked 30-45 minutes after ingestion and then decline steadily to the normal range within 90-120 minutes (low dose) or 180-240 minutes (high dose)[149].

**Summary**

In summary, glutamine is an amino acid that is crucial for many important homeostatic functions and for the functioning of a number of tissues in the body. During various catabolic states including surgery, infection and acidosis, glutamine homeostasis is placed under stress and glutamine reserves can become depleted. Similar to a catabolic

stress, high-intensity exercise may result in an increase, decrease or no change in plasma glutamine levels during and after exercise. The resulting changes have been found to be dependent upon the type, duration and intensity of exercise. Based upon previous human research studies of glutamine supplementation, some researchers believe glutamine has a potential utility as a dietary supplement for athletes participating in high-intensity exercise. The mechanism behind this potential has been suggested to be due to glutamine's influence on the acid-base balance within the body. To date, only one study has reported an ergogenic effect (specifically, an increased time to exhaustion) due to glutamine supplementation, thus warranting additional research in this area in attempts to elucidate the equivocal data.

The present study will add to the current literature by examining a comprehensive set of variables, which include plasma glutamine, glutamate, alanine and lactate concentrations before, during and after exercise. Of the three supplementation studies previously mentioned, the study conducted by Haub et al. was the only one to analyze any blood work throughout the testing protocol. Blood was analyzed for pH, $La^-$ and $HCO_3$ concentrations at baseline, pre-exercise (90-minuntes post-ingestion), and after the fourth and fifth bouts of exercise[56]. The studies conducted by Antonio et al. and Piattolly et al. did not collect blood samples at any time throughout data collection[3, 104]. By collecting blood samples, we hope to gain a better understanding for how high-intensity exercise influences plasma glutamine, glutamate, alanine and lactate concentrations before, during and after exercise. In addition, we will be analyzing expired gases for $VO_2max$, $CO_2$ production, and respiratory exchange ratios, which have not been looked at in previous supplementation studies. We will also be utilizing a treadmill running

protocol, which is a novel exercise testing modality, as previous studies have only examined the effects of supplementation on weight lifting performance and cycling[3, 56, 104]. More specifically, our study will be utilizing an exercise protocol that should rely on a combination of aerobic and anaerobic pathways to produce energy as the protocol is designed to induce fatigue within 8-14 minutes. This is in contrast to the exercise protocols used in previous studies, which could be classified as purely anaerobic. Lastly, our study will be taking into account the average dietary intake of each subject, which despite recent literature demonstrating the influence high protein/low carbohydrate consumption on plasma glutamine concentration, has not been considered in any of the previous supplementation studies.

# CHAPTER III

# METHODS

## Subjects

Seven healthy subjects (six males and one female) between the ages of 18 and 30 participated in this study. The subjects participated in a minimum of thirty minutes of moderate to high-intensity aerobic exercise at least four days a week and were free of metabolic, cardiovascular, and orthopedic limitations as determined through a self-reported health survey (Appendix A) and participation questionnaire (Appendix B). Individuals taking supplements (e.g. creatine, amino acid) were excluded from the study. Prior to participation, subjects were informed of potential risks and benefits, familiarized with all testing procedures and asked to sign an informed consent (Appendix C) approved by the Institutional Review Board of the University of Louisville. The descriptive characteristics (mean ± SD) of the subjects were as follows: age (23.0 ± 1.0 yrs), height (173.1 ± 8.2 cm), weight (74.5 ± 13.3 kg), BMI (24.7 ± 3.0 $kg/m^2$), body fat percentage (12.7 ± 7.1%), resting systolic blood pressure (112.5 ± 8.2 mm/Hg), resting diastolic blood pressure (80.2 ± 6.4 mm/Hg), and resting heart rate (72.2 ± 6.6 $b \cdot min^{-1}$).

*Session 1*

Subjects participated in three sessions. During the orientation session (session 1), the research project and procedures were explained to the subject and consent was signed. In addition, the general health and overall body composition of the subject was assessed by obtaining each subject's height, weight, heart rate, blood pressure and skin fold

measurements. Subjects were instructed to maintain current levels of exercise and to not alter dietary habits for the duration of the study. Each subject was asked to complete and turn in a three-day food diary before the first day of exercise testing. The food diary included everything the subject consumed for three days (one weekend day and two week days). Instructions to complete a three-day food diary and food logs were provided for the subjects (Appendix D). Food diary data was then analyzed by Diet Analysis™ software (Version 7.0).

*Session 1 Measurements*

1. *Height & Weight*

Height was measured without shoes in centimeters. Weight was measured without shoes to the nearest 0.5 pound.

2. *Heart Rate*

Heart rate was measured via radial artery pulse palpation. The pulse was counted for 15 seconds and then multiplied by four, to determine the per-minute heart rate.

3. *Resting Blood Pressure*

Resting blood pressure was measured with the subjects seated in a chair with their feet on the floor.

4. *Body composition*

Body composition was determined using a three-site skin fold measurement. Skin fold measurements were obtained at the chest, abdomen, and thigh for male subjects. Measurements were obtained at the tricep, suprailiac, and thigh for female subjects. Gender specific regression equations were then used to convert the sum of skin folds to percent body fat[64].

*Sessions 2 & 3(Exercise Testing Sessions)*

Subjects then participated in two separate exercise testing sessions. Before the first exercise testing session (session two), all subjects were assigned to one of two treatment groups (Group A or Group B). Treatment order was randomly assigned and subjects were not informed as to which treatment group they had been assigned. Group A participated in the experimental (glutamine supplementation) condition during the second session and the control (placebo) condition during the third session. Group B participated in the control condition during the second session and the experimental condition during the third session. All subjects participated in both the experimental and control treatment, with a minimum of seven days between testing sessions.

For each exercise testing session, subjects were asked to abstain from exercise and to fast (water only) for two-hours prior to reporting to the laboratory. Upon reporting to the laboratory for the exercise testing sessions, height, weight, resting blood pressure and heart rate were recorded. Next, a research registered nurse trained and credentialed in intravenous catheter (IV) placement, prepared the subject for the blood draws. The baseline blood sample was collected via an IV catheter that was inserted by the research nurse. The subject was then instructed to consume 0.03 g·kg body mass$^{-1}$ of either glutamine (Progressive Laboratories, Inc., Irving, TX) mixed with 250 ml of caffeine-free fruit juice or 250 ml of placebo matched for caloric input (caffeine-free fruit juice). All drinks were pre-mixed by a research assistant before the subject's arrival and to ensure compliance, subjects were asked to consume the drink under the supervision of laboratory personnel within ten-minutes. Once the assigned solution had been consumed subjects rested for one hour in a sitting position.

At the end of one hour, the research nurse obtained the second blood draw (60-min post ingestion). Subjects then provided urine and saliva samples which were analyzed using a pH test strip (Sigma, St. Louis, MO). Next, the subjects performed a maximal exercise test on the treadmill and $VO_2$max was determined via indirect open circuit spirometry (ParvoMedics, Sandy, UT). The Bruce treadmill protocol consisted of incremental increases in workload and was designed to induce fatigue within 8-14 minutes (Appendix E)[2]. Ratings of perceived exertion (RPE) were obtained every three-minutes using the Borg's Category Scale[17](Appendix F) and heart rate (Polar 610i HR Monitor, Polar Electro, Finland) was recorded every minute throughout the exercise test. Determination of $VO_2$max was ascertained if the subject met two of the following criteria: 1) A plateau in oxygen uptake (or failure to increase oxygen uptake by 150 mL/min) with increasing workload; 2) a respiratory exchange ratio (RER) $\geq$1.15; or 3) a RPE of >17 on the Borg 6-20 scale[60]. RER values are a ratio between the amount of carbon dioxide produced and the amount of oxygen consumed by the body ($VCO_2/VO_2$)[20]. In addition, the research nurse obtained blood samples every four-minutes, resulting in a possible maximum of four separate blood draws (4-min exercise, 8-min exercise, 12-min exercise, 16-min exercise) during exercise. The actual number of blood draws was unique to the subject and dependent upon the duration of the exercise test.

Upon the completion of the exercise test, subjects immediately moved to a chair while remaining connected to the metabolic cart for 10-minutes. After, the initial 10-minute recovery period subjects were disconnected from the cart and then reconnected for 5-minute intervals at 25-min and 55-min post exercise in order to obtain recovery data

at 30-and 60-min post exercise. During the one-hour recovery period, heart rate (Polar 610i HR Monitor, Polar Electro, Finland) was recorded 1-min post exercise, 3-min post exercise, 5-min post exercise, 10-min post exercise, 30-min post exercise and 60-min post exercise. An RPE value was also recorded upon completion of exercise. In addition, blood samples were obtained 5-min post-exercise, 10-min post-exercise, 30-min post-exercise and 60-min post exercise. At the commencement of the one-hour recovery period, subjects provided urine and saliva samples which were analyzed for pH values.

*Blood Draw Procedure:*

Blood samples were collected pre-exercise, during exercise and post-exercise testing. To collect multiple blood draws with minimal discomfort to the subject, an IV catheter was inserted by the research nurse. The following procedure was used: Subjects were asked to sit with either left or right arm resting on the table and a tourniquet was applied by the research nurse. The subject's forearm was reviewed for the optimal site to insert the IV. Once determined, the area was cleaned with an alcohol prep pad saturated with 70% isopropyl alcohol. Depending on the subject's veins, either an 18-gauge or 20-gauge IV catheter (Becton Dickinson, Sandy, UT) was inserted. An IV catheter extension set (Baxter Health Care Corporation, Deerfield, IL) was connected to the end of the IV and blood work was drawn using a 5-mL syringe. Once drawn, the IV was flushed with 5-mL of 0.9% sodium chloride (saline solution) in order to maintain patency and ensure there was no discomfort or adverse effects to the IV placement. Surgical tape was used to secure the catheter to the subject's arm. The blood was then transferred into a vacutainer blood collection tube with sodium fluoride potassium oxalate (5mg/4mg) (BD Vacutainer®, Franklin Lakes, NJ). Blood was collected intermittently throughout

each exercise testing session, resulting in a possible maximum of ten separate blood draws (baseline, 60-min post ingestion, 4-min into exercise test, 8-min into exercise test, 12-min into exercise test, 16-min into exercise test, 5-min post exercise, 10-min post exercise, 30-min post exercise, and 60-min post exercise) and no more than 80-ml of blood was obtained from a subject during each exercise testing session. Following the initial blood draw, each subsequent blood draw began with drawing 3-ml of 'waste' fluid (saline flush and blood) from the IV using a 5-mL syringe. This was followed by a second syringe being used to withdraw approximately 5-ml of blood from the catheter which was then transferred into a vacutainer blood collection tube with sodium fluoride potassium oxalate (5mg/4mg). The IV was flushed with 5-mL saline solution to ensure patency for future blood draws. The blood samples were given time to clot and then centrifuged (Smith Kline Beechman Clinical Laboratories, Model 6500) at 3400 rpm for approximately 20 minutes in order to separate the serum from the cellular components. Serum was then pipette transferred into a microcentrifuge tube (Fischer Scientific, Pittsburgh, PA) and stored at -80°C until ready for analysis. If during exercise, the research nurse was unable to obtain a sufficient (3-mL) amount of 'waste fluid' and/or blood, the sample was not obtained. If post-exercise, the research nurse was unable to obtain a sufficient (3-mL) amount of 'waste fluid' and/or blood, the sample was collected via venipuncture. A different venous site was used to draw blood using a 21-gauge "butterfly needle" (Becton Dickinson, Franklin Lakes, NJ) and transferred into the vacutainer blood collection tube with sodium fluoride potassium oxalate (5mg/4mg). The "butterfly needle" was inserted after the site was cleaned with an alcohol prep pas

saturated with 70% isopropyl alcohol and was immediately removed after collecting the blood sample.

**Statistical Analyses**

SPSS (Version 16.0) was used for all statistical analyses with significance set at $p<0.05$. Basic descriptive statistics were calculated for all data and values are reported as mean ± standard deviation. Dependent *t* tests were performed to assess differences between means for the experimental (glutamine) and control (placebo) trials and are reported as mean ± standard deviation (SD).

# CHAPTER IV

# RESULTS

## Oxygen Consumption

No significant difference was observed between treatments for $VO_2$max or post-exercise oxygen consumption at any measurement time during recovery (Table 2).

## $CO_2$ Production

No significant difference was found between treatments for $CO_2$ production (Table 3). Carbon dioxide values were recorded every 15-seconds during exercise, however the values used for analyses were derived from an average of the second and third minute of each completed stage of the protocol to ensure values represented a physiological steady state. Since only one subject was able to complete Stage 5, only stages 1 through 4 were included in the analyses. Post-exercise $CO_2$ production values were not significantly different between treatments at any measurement time during recovery.

## Respiratory Exchange Ratio

Maximum respiratory exchange ratios (RER) during exercise and post-exercise values are displayed in Table 4 for the seven subjects (six males and one female). No significant treatment effect was observed for the maximum RER value during exercise, nor for values at 10-min post, 30-min post and 60-min post-exercise. Significance was observed between treatments for the RER value at 5-min post exercise, with the GLN trial producing a higher RER value than the PLC trial ($p = 0.042$).

**Heart Rate**

Heart rate responses during exercise and post-exercise are shown in Table 5. No significant treatment effect was observed for heart rate during exercise or at any measurement time post-exercise. Similarly, there was no significant difference observed between treatments for heart rate immediately upon the completion of exercise.

**Ratings of Perceived Exertion**

Ratings of perceived exertion (RPE) responses can be found in Table 6. No significant treatment effect was found for RPE at any time measurement during exercise or immediately upon completion of exercise.

**Urine & Saliva pH**

The pH values for both urine and saliva pre- and post-exercise can be found in Table 7. There was no significant difference between the two treatments.

**3-Day Dietary Intake**

Three-day dietary averages can be seen in Table 8 for the seven subjects (six males and one female). Each subject's food diary was analyzed for 1) average intake of carbohydrates, proteins, and fats (in kcal and percentage of total intake); 2) average daily dietary intake in kcal; and 3) average intake of carbohydrates, proteins, and fats in grams.

**Blood Analysis**

All results pertaining to serum glutamine, glutamate, alanine and lactate concentrations are currently pending analysis.

**Gender Differences**

When the one female subject was removed from all statistical analysis, significance was no longer observed at 5-min post-exercise for RER. Similarly, no

significant differences in $O_2$ consumption, $CO_2$ production, RER, HR, RPE, and urine pH were found between treatments at any measurement time (Tables 10-15). The pH values for saliva pre- and post-exercise can be found in Table 15. No significant treatment effect was observed for saliva pH pre-exercise, however, significance was observed between treatments for post-exercise saliva pH, with the GLN trial producing a higher pH than the PLC trial ($p=0.025$). Lastly, the three-day dietary averages for male subjects can be found in Table 16.

**Table 1. Plasma glutamine concentration following exercise in humans**

| Reference | Study Participants | EX Intensity/Duration | Methodology | Changes in plasma glutamine level (%) | |
|---|---|---|---|---|---|
| | | | | After EX | Recovery |
| *Continuous or intermittent high-intensity EX* | | | | | |
| Parry-Billings et al.[101] | 10 RA | 10 x 6 sec treadmill sprint | Enzymatic (A) | ↑11 | |
| Sewell et al.[119] | 9 RM (2F) | 60 sec treadmill running at 20 km/h<br><br>20 km/h treadmill run to exhaustion | Enzymatic (G) | ↑5<br><br>↑14 | Return to baseline at 5min after |
| Robson et al.[112] | 18 TM | Cycling to exhaustion at 80% $VO_2max$ | Enzymatic (G) | ↑3 | ↑2 at 5h |
| Katz et al.[68] | 8 RA M | Cycling to exhaustion at 100% $VO_2max$ | HPLC | ↑26 | ↑13 at 10min |
| van Hall et al.[132] | 8 TM | 3 min cycling at 50% $W_{max}$ and 6 min at 80% $W_{max}$ alternated to exhaustion | Enzymatic (-) | ↓9 | ↓16 at 2h |
| van der Schoor et al.[131] | 8 TM | 2 min cycling at 90% $W_{max}$ alternated with 2 min at 50% $W_{max}$ until exhaustion | Enzymatic (-) | ↓20 | ↓24 at2h |
| Keast et al.[70] | 7 TM | 15 x 1 min treadmill exercise at 90% $VO_2max$<br><br>15 x 1 min treadmill exercise at 120% $VO_2max$ | Bioassay | ↓44<br><br>↓55 | |
| Walsh et al.[136] | 8 TM | 20 x 1 min cycling at 100% VO2max | Enzymatic (G) | ↓2 | ↓16 at 5h |

**Table 1 (cont.)**

| | | | | | |
|---|---|---|---|---|---|
| *Prolonged light-moderate intensity EX* | | | | | |
| Parry-Billings et al.[101] | 22 T (20 M)<br>12 TM<br>4 TM | Marathon ≈ 150 min | Enzymatic (A) | ↓16 | |
| | | 30 km self-paced treadmill run | | ↑8 | |
| | | Cycling to exhaustion ay 73% $VO_2max$ | | ↑8 | |
| Rennie et al.[110] | 4 RA M | 3.75h cycling at 50% $VO_2max$ | HPLC | ↓16 | ↓30 at 2h |
| Babij et al.[10] | 8 RA M | 10 min incremental cycling at 25, 50, and 75% $VO_2max$ and to exhaustion | Column chromatography | ↑28 | Return to baseline at 10min |
| Eriksson et al.[36] | 11 RA M | 45 min incremental cycling to 75% $VO_2max$ | HPLC | ↓24 | ↑12 at 1h |
| Robson et al.[112] | 18 TM | Cycling at 55% $VO_2max$ for 180 min | Enzymatic (G) | ↓11 | ↓23 at 1h |
| Maughan & Gleeson et al.[88] | 5 RA M | 90min cycling at 70% $VO_2max$ | Enzymatic (G) | ↑3 | |
| Decombaz et al[31] | 8 TM | 100 km run | Column chromatography | ↓16 | ↓ at 24h |
| Rohde et al.[114] | 8 TM | 2500m swim, 81km cycle, 19km run | HPLC | ↓20 | ↓32 at 2h |

a = percentage difference from pre-exercise plasma glutamine level (not corrected for plasma volume changes); A = asparaginase used to determine plasma glutamine; F = female; G = glutamine; HPLC = high performance liquid chromatography; M = male; RA = recreationally active but not specifically endurance trained; T = trained; $W_{max}$ = maximal work rate attained during an incremental exercise test; ↑ = increased; ↓ = decreased; EX = exercise

**Table 2. $VO_2$ (ml/kg/min$^{-1}$) during and post-exercise (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| $VO_2$max | 61.5 ± 11.8 | 58.6 ± 10.1 | 0.065 |
| 5-min$PVO_2$ | 8.82 ± 1.73 | 9.12 ± 2.31 | 0.412 |
| 10-min$PVO_2$ | 7.61 ± 2.08 | 7.24 ± 1.00 | 0.414 |
| 30-min$PVO_2$ | 6.56 ± 2.37 | 6.05 ± 1.46 | 0.399 |
| 60-min$PVO_2$ | 4.94 ± 0.79 | 4.62 ± 1.09 | 0.557 |

$VO_2$ = volume of oxygen consumption; P = post-exercise. n = 7 (6 males and 1 female), except for 30-min$PVO_2$ (n = 6; 5 males and 1 female).

**Table 3. $CO_2$ Production (L/min) during and post-exercise (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| Stage 1 | 1.18 ± 0.27 | 1.14 ± 0.18 | 0.581 |
| Stage 2 | 1.77 ± 0.34 | 1.66 ± 0.32 | 0.362 |
| Stage 3 | 2.93 ± 0.53 | 2.55 ± 0.57 | 0.167 |
| Stage 4 | 4.37 ± 0.78 | 4.15 ± 0.65 | 0.483 |
| 5-minP | 0.79 ± 0.18 | 0.76 ± 0.24 | 0.366 |
| 10-minP | 0.53 ± 0.23 | 0.47 ± 0.12 | 0.237 |
| 30-minP | 0.33 ± 0.16 | 0.31 ± 0.09 | 0.447 |
| 60-minP | 0.27 ± 0.05 | 0.26 ± 0.03 | 0.613 |

Stage 1 = average of minutes 2-3; Stage 2 = average of minutes 5-6; Stage 3 = average of minutes 8-9; Stage 4 = average of minutes 11-12; P = post-exercise. n = 7 (6 males and 1 female), except for 30-minP (n = 6; 5 males and 1 female).

**Table 4. Respiratory Exchange Ratio during and post-exercise (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| Max | 1.13 ± 0.04 | 1.13 ± 0.07 | 0.944 |
| 5-minP* | 1.20 ± 0.07 | 1.11 ± 0.09 | 0.042* |
| 10-minP | 0.91 ± 0.08 | 0.87 ± 0.08 | 0.122 |
| 30-minP | 0.70 ± 0.08 | 0.72 ± 0.09 | 0.564 |
| 60-minP | 0.73 ± 0.10 | 0.76 ± 0.10 | 0.688 |

Max = maximal RER during exercise; P = post-exercise, n = 7 (6 males and 1 female), except for 30-minP and 60-minP (n = 6; 5 males and 1 female), *$p<0.05$, significant difference between treatments.

**Table 5. Heart Rate ($b \cdot min^{-1}$) during and post-exercise (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| 3-minE | 110.1 ± 11.8 | 103.0 ± 16.4 | 0.379 |
| 6-minE | 132.7 ± 13.4 | 127.4 ± 16.5 | 0.109 |
| 9-minE | 158.8 ± 14.8 | 154.5 ± 16.8 | 0.462 |
| 12-minE | 182.0 ± 8.54 | 172.6 ± 23.1 | 0.403 |
| End | 180.5 ± 8.26 | 181.8 ± 5.81 | 0.579 |
| 1-minP | 132.6 ± 12.7 | 132.3 ± 20.0 | 0.962 |
| 3-minP | 113.1 ± 11.3 | 112.6 ± 10.3 | 0.875 |
| 5-minP | 107.0 ± 8.30 | 107.0 ± 8.40 | 1.000 |
| 10-minP | 104.1 ± 9.83 | 105.0 ± 10.8 | 0.773 |
| 30-minP | 96.8 ± 11.9 | 92.2 ± 15.4 | 0.255 |
| 60-minP | 82.8 ± 17.4 | 81.8 ± 14.5 | 0.705 |

E = during exercise; End = upon completion of exercise; P = post-exercise, n = 7 (6 males and 1 female), except for 12-minE (n = 3 males), End (n = 6; 5 males and 1 female), 1-minP (n = 6; 5 males and 1 female), and 3-minP (n = 6; 5 males and 1 female).

**Table 6. Ratings of Perceived Exertion (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| 3-minE | 6.71 ± 0.75 | 6.57 ± 0.53 | 0.604 |
| 6-minE | 8.86 ± 1.34 | 8.57 ± 1.71 | 0.654 |
| 9-minE | 12.0 ± 1.73 | 11.5 ± 2.14 | 0.407 |
| 12-minE | 15.2 ± 0.95 | 15.0 ± 3.26 | 0.854 |
| End | 18.0 ± 1.52 | 17.1 ± 1.86 | 0.356 |

E = during exercise; End = upon completion of exercise, n = 7 (6 males and 1 female), except for 12-minE (n = 4 males).

**Table 7. Urine and Saliva pH Values (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| PreUrine | 6.67 ± 0.56 | 6.50 ± 0.38 | 0.511 |
| PostUrine | 6.11 ± 0.19 | 6.14 ± 0.37 | 0.805 |
| PreSaliva | 6.74 ± 0.52 | 6.77 ± 0.44 | 0.886 |
| PostSaliva | 6.91 ± 0.41 | 6.78 ± 0.33 | 0.200 |

Pre = 1-hour post-ingestion, immediately pre-exercise; Post = 1-hour post exercise; n = 7 (6 males and 1 female).

**Table 8. Average Dietary Intake from the Three-Day Food Diary (mean ± SD)**

| | Mean ± SD |
|---|---|
| Total (kcal) | 3296.0 ± 1113.4 |
| Carbohydrate (kcal) | 1657.1 ± 593.3 |
| Protein (kcal) | 581.7 ± 162.7 |
| Fat (kcal) | 931.2 ± 376.0 |
| Carbohydrate % | 50.5 ± 6.7 |
| Protein % | 17.7 ± 3.9 |
| Fat % | 28.1 ± 5.6 |
| Carbohydrate (grams) | 431.2 ± 166.7 |
| Protein (grams) | 145.4 ± 40.6 |
| Fat (grams) | 113.9 ± 60.5 |

n = 7 (6 males and 1 female)

**Table 9. Descriptive characteristics of male subjects (mean ± SD)**

| | Mean ± SD |
|---|---|
| **Age (yrs)** | 23.1 ± 0.98 |
| **Height (cm)** | 175.6 ± 5.2 |
| **Weight (kg)** | 76.5 ± 13.4 |
| **BMI ($kg/m^2$)** | 24.6 ± 3.3 |
| **BF (%)** | 10.6 ± 5.0 |
| **RSBP (mm/Hg)** | 113.0 ± 8.9 |
| **RDBP (mm/Hg)** | 80.6 ± 7.0 |
| **RHR ($b \cdot min^{-1}$)** | 73.3 ± 6.6 |

BMI = body mass index; BF = percentage of body fat; RSBP = resting systolic blood pressure; RDBP = resting diastolic blood pressure; RHR = resting heart rate; n = 6.

**Table 10. $VO_2$ (ml/kg/min$^{-1}$) during and post-exercise for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| $VO_2$max | 62.3 ± 12.7 | 59.4 ± 10.9 | 0.109 |
| 5-min$PVO_2$ | 9.21 ± 1.53 | 9.60 ± 2.13 | 0.371 |
| 10-min$PVO_2$ | 8.08 ± 1.83 | 7.50 ± 0.80 | 0.237 |
| 30-min$PVO_2$ | 6.90 ± 2.49 | 6.36 ± 1.40 | 0.475 |
| 60-min$PVO_2$ | 4.86 ± 0.83 | 4.66 ± 1.19 | 0.745 |

$VO_2$ = volume of oxygen consumption; P = post-exercise, n = 6, except for 30-minP (n=5)

**Table 11.$CO_2$ Production (L/min) during and post-exercise for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| Stage 1 | 1.24 ± 0.25 | 1.18 ± 0.15 | 0.553 |
| Stage 2 | 1.84 ± 0.32 | 1.71 ± 0.32 | 0.401 |
| Stage 3 | 3.02 ± 0.53 | 2.59 ± 0.62 | 0.195 |
| Stage 4 | 4.48 ± 0.79 | 4.25 ± 0.65 | 0.547 |
| 5-minP | 0.83 ± 0.16 | 0.80 ± 0.23 | 0.554 |
| 10-minP | 0.57 ± 0.23 | 0.49 ± 0.12 | 0.144 |
| 30-minP | 0.35 ± 0.18 | 0.32 ± 0.09 | 0.489 |
| 60-minP | 0.27 ± 0.05 | 0.27 ± 0.03 | 0.937 |

Stage 1 = average of minutes 2-3; Stage 2 = average of minutes 5-6; Stage 3 = average of minutes 8-9; Stage 4 = average of minutes 11-12; P = post-exercise, n =6, except for 30minP (n=5)

**Table 12. Respiratory Exchange Ratio during and post-exercise for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| Max | 1.13 ± 0.04 | 1.13 ± 0.08 | 0.945 |
| 5-min | 1.18 ± 0.04 | 1.10 ± 0.09 | 0.102 |
| 10-minP | 0.91 ± 0.08 | 0.85 ± 0.08 | 0.106 |
| 30-minP | 0.69 ± 0.09 | 0.71 ± 0.10 | 0.619 |
| 60-minP | 0.73 ± 0.10 | 0.79 ± 0.12 | 0.363 |

Max = maximal RER during exercise; P = post-exercise, n = 6, except for 30-minP (n = 5).

**Table 13. Heart Rate ($b \cdot min^{-1}$) during and post-exercise for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| 3-minE | 108.1 ± 11.6 | 108.1 ± 10.0 | 1.00 |
| 6-minE | 130.1 ± 12.7 | 124.0 ± 15.1 | 1.08 |
| 9-minE | 156.5 ± 14.7 | 152.5 ± 17.4 | 0.562 |
| 12-minE | 182.0 ± 8.54 | 172.6 ± 23.1 | 0.403 |
| End | 179.6 ± 8.90 | 181.8 ± 6.49 | 0.435 |
| 1-minP | 135.8 ± 11.3 | 132.6 ± 22.4 | 0.690 |
| 3-minP | 114.0 ± 12.4 | 112.6 ± 11.6 | 0.713 |
| 5-minP | 107.1 ± 9.08 | 107.1 ± 9.19 | 1.00 |
| 10-minP | 103.5 ± 10.6 | 104.3 ± 11.7 | 0.814 |
| 30-minP | 95.6 ± 12.6 | 91.5 ± 16.7 | 0.374 |
| 60-minP | 84.1 ± 18.7 | 83.3 ± 15.3 | 0.790 |

E = during exercise; End = upon completion of exercise; P = post-exercise, n = 6, except for 12-minE (n = 3), End (n = 5), 1-minP (n = 5), and 3-minP (n = 5).

**Table 14. Ratings of Perceived Exertion for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| 3-minE | 6.67 ± 0.81 | 6.50 ± 0.54 | 0.611 |
| 6-minE | 8.67 ± 1.36 | 8.33 ± 1.75 | 0.661 |
| 9-minE | 11.8 ± 1.83 | 11.3 ± 2.25 | 0.415 |
| 12-minE | 15.2 ± 0.95 | 15.0 ± 3.26 | 0.854 |
| End | 18.7 ± 1.6 | 17.0 ± 2.00 | 0.272 |

E = during exercise; End = upon completion of exercise, n = 6, except for 12-minE (n = 4)

**Table 15. Urine and Saliva pH Values for male subjects (mean ± SD)**

| | GLN | PLC | p-value |
|---|---|---|---|
| PreUrine | 6.61 ± 0.59 | 6.51 ± 0.41 | 0.734 |
| PostUrine | 6.13 ± 0.20 | 6.16 ± 0.40 | 0.809 |
| PreSaliva | 6.86 ± 0.45 | 6.90 ± 0.30 | 0.889 |
| PostSaliva* | 7.00 ± 0.37 | 6.80 ± 0.36 | 0.025* |

Pre = 1-hour post-ingestion, immediately pre-exercise; Post = 1-hour post exercise; n = 6.

*$p < 0.05$, significant difference between treatments

**Table 16. Average Dietary Intake from the Three-Day Food Diary for male subjects (mean ± SD)**

| | Male |
|---|---|
| Total (kcal) | 3545.8 ± 981.5 |
| Carbohydrate (kcal) | 1764.5 ± 570.7 |
| Protein (kcal) | 632.0 ± 102.5 |
| Fat (kcal) | 1024.1 ± 311.7 |
| Carbohydrate % | 49.8 ± 7.02 |
| Protein % | 18.0 ± 4.19 |
| Fat % | 29.3 ± 5.08 |
| Carbohydrate (grams) | 460.1 ± 162.4 |
| Protein (grams) | 158.0 ± 25.6 |
| Fat (grams) | 125.9 ± 56.6 |

n = 6

# CHAPTER V

# DISCUSSION

The aim of this study was to examine the effects of glutamine supplementation on high-intensity exercise and recovery from exercise. More specifically, the primary purpose was to determine if acute glutamine supplementation improved performance during a high-intensity running bout on a treadmill by altering the acid-base balance (buffering capacity) of the blood. No significant difference in $VO_2$max, HR, or RPE was detected between the GLN and PLC trials. A secondary purpose was to determine if glutamine supplementation improved recovery parameters one-hour post exercise. No significant difference in HR, $CO_2$ production or RER was observed between trials. Thus, results of this study demonstrated no significant difference in maximal treadmill running performance or recovery between GLN and PLC treatments following acute glutamine supplementation. Unfortunately, due to the pending analysis of all blood work, it is not possible at this time to address the effects of high-intensity running on plasma glutamine, alanine, glutamate and lactate concentrations during and post-exercise and consequently, how acute glutamine supplementation may or may not influence these parameters.

In this study, the only significant difference observed between the GLN and PLC treatments when both male and female subjects were included in analysis, occurred within the RER values at 5-min post exercise ($p = 0.042$). The GLN trial ($1.20 \pm 0.07$) produced a higher RER value than the PLC trial ($1.11 \pm 0.09$), which indicates at 5-min post-exercise subjects were producing greater amounts of $CO_2$ relative to the amount of

$O_2$ consumed during the GLN treatment. During recovery from high-intensity exercise, it is common that RER values increase due to the rise in lactic acid which consequently gives rise to $CO_2$. $CO_2$ is then eliminated from the body via the lungs, as the lactic acid in the blood is buffered by forming $H_2CO_3$. Although it would seem logical to contribute the higher value in the GLN trial to an increased buffering capacity due to the supplementation of glutamine, because this was the only RER value to show significance, future research is warranted in this area.

No significant differences in oxygen consumption were observed between treatments at maximal efforts during exercise or at any time throughout the one-hour recovery period. However it should be noted that the difference between $VO_2max$ (61.5 ± 11.8 and 58.6 ± 10.1 ml/kg/min for the experimental and control treatments, respectively) was approaching significance at $p = 0.065$. Although this fails to meet the established criterion for statistical significance ($p < 0.05$), it suggests that the subjects tended to use less oxygen at maximal intensity during the GLN treatment. In addition, no significant differences in heart rate, ratings of perceived exertion, $CO_2$ production, urine and saliva pH were found between treatments.

The nutrient analysis from the three-day food diary for the six males and one female showed that the subjects' average intake consisted of a diet that was 50.5 ± 6.7% carbohydrate, 17.7 ± 3.9% protein and 28.1 ± 5.6% fat. Their average carbohydrate, protein and fat intake were 1657.1 ± 593.3kcal, 581.7 ± 162.7 kcal, and 931.2 ±376.0 kcal, respectively, resulting in a total caloric intake of 3296.0 ± 1113.4 kcal. These values indicate that on average the subjects consume what is considered a 'balanced' diet for people who live an active lifestyle. According to the 2005 U.S. Department of

Agriculture's "Dietary Guidelines for Americans", acceptable macronutrient distribution ranges (AMDR) are 45-65% carbohydrate, 20-35% fat and 10-35% protein[130]. The subjects in this study displayed values that were within these recommendations, hence the dietary habits of the subjects in the present study should not have influenced plasma glutamine concentration before, during or post-exercise, as past studies[43, 49, 89, 134, 148] have only demonstrated that high protein/low carbohydrate and high carbohydrate/low protein diets alter plasma glutamine concentration,. However, at this point in time, we can only speculate due to the pending blood analysis.

To our knowledge, no other studies have examined the effects of acute glutamine supplementation on performance and recovery from a high-intensity running bout. This study indicates that acute glutamine supplementation does not improve high-intensity exercise performance or recovery of this type. The comprehensive lack of significant differences between treatments could be contributed to numerous factors and without the blood analyses it is difficult to determine the mechanism(s) supporting the performance and recovery results.

High-intensity exercise brings about dramatic alterations in acid-base status of intracellular and extracellular regions of working tissues[56]. Previous work has demonstrated that increasing the buffering capacity and pH of extracellular fluid and blood improves performance during high-intensity exercise[27, 45, 74, 79, 107]. In addition, research indicates that L-glutamine has potential alkalinizing properties which may elicit improvements in exercise performance[139, 142]. Welbourne demonstrated that the oral ingestion of 2-grams of glutamine significantly increased plasma bicarbonate ($HCO_3$)

concentration in normal, healthy subjects, thus indicating the use for glutamine as an ergogenic aid[139]. However, the results of this study do not support this theory.

It may be plausible that the administered dosage of glutamine (0.03 g·kg body mass$^{-1}$) one-hour prior to exercise may not have been a great enough dosage to increase plasma glutamine concentrations. Castell et al. found that after orally administering 0.1 g·kg body mass$^{-1}$ or 5-grams glutamine, plasma glutamine concentrations increased by at least 50%, peaking at 30-minutes after ingestion and remaining elevated for one to two hours before returning to the normal range of 500-700 μmol/L[22]. This is three times the amount of glutamine administered in the current study. If there was not an increase in plasma glutamine concentration post-ingestion, then the subsequent increase in plasma $HCO_3$ concentration[139] may not have occurred. However, in a study by Antonio et al.[3], similar performance results were discovered despite to use of dosage ten times greater than that used in the current study. Subjects were given 0.3 g·kg body mass$^{-1}$ one-hour prior to performing four total sets of exercise to momentary muscular fatigue, yet no ergogenic benefit was shown[3]. More similar to our study, Haub et al.[56] gave 0.03 g·kg body mass$^{-1}$, and 90-minutes after ingestion, subjects performed four bouts of 60 seconds cycling at 100% $VO_2$ peak followed by a fifth bout to exhaustion. There was no difference in performance in the fifth bout between glutamine and placebo groups. It should be noted that plasma glutamine and bicarbonate concentrations were not measured in either of these supplementation studies[56, 139]. Since we did not investigate the changes in plasma $HCO_3$ concentrations and do not currently know how plasma glutamine concentrations responded to the study, it is impossible to determine if this is an influential factor at this point in time. There is also the possibility that an increase in $HCO_3$ may not

have occurred because glutaminase, the enzyme responsible for the breakdown of glutamine in the kidney to initiate $HCO_3$ production, was not in a highly active state[143]. Furthermore, for glutamine to elevate $HCO_3$ the physiological environment may have to be acidic. It has been demonstrated that endogenous L-glutamine shifts from a component for purine and pyramidine formation to one of base formation in the kidney during a state of metabolic acidosis[101, 143]. This supports the rationale that the environment may need to be acidic in order to activate glutaminase and subsequently use glutamine to increase plasma $HCO_3$[56]. In this study, subjects were not in a state of metabolic acidosis prior to glutamine supplementation.

**Gender Differences**

Due to the fact that there was only one female in this study in comparison to six males, we thought it was important to examine the results controlling for gender. As previously stated, when the female subject was removed (n=6), no significant difference between the two treatments was observed for $O_2$ consumption, $CO_2$ production, RER, RPE, HR, or urine pH. A significant treatment effect (p=0.025) was shown in the post-exercise saliva pH, with the GLN trial producing a higher (more alkaline) value of 7.00 ± 0.37 compared to a more acidic value of 6.80 ± 0.36 in the PLC trial. It is logical to assume the more alkaline value in the GLN treatment may be due to the acute supplementation of a potential alkanizing agent (glutamine) prior to exercise, yet more research is needed to make a conclusive observation.

As expected, the dietary intake averages changed drastically when the female was removed. The males' average intake consisted of a diet that was 49.8 ± 7.02% carbohydrate, 18.0 ± 4.19% protein and 29.3 ± 5.08% fat. Their average carbohydrate,

protein and fat intake were 1764.5 ± 570.7 kcal, 632.0 ± 102.5 kcal and 10241.1 ± 311.7 kcal, respectively, resulting in a total caloric intake of 3545.8 ± 981.5 kcal. Despite the increase in total kcal, the male subjects still displayed values that met the AMDR's, thus plasma glutamine concentrations should not have been influenced[130]. To our knowledge the literature has not suggested a gender difference in glutamine metabolism. However, it seems plausible that dietary intake may influence glutamine metabolism and ultimately plasma glutamine concentration as differences in fuel substrate utilization between males and females have been observed during exercise. For a given relative work intensity, free fatty acids compromise a greater proportion of the source of energy in woman than in men, thus resulting in greater lipid utilization[41, 127, 128]. It appears that there is a connection between estrogen and increased lipid mobilization, such as estrogen's ability to inhibit lipoprotein lipase (responsible for the breakdown of triglycerides in the blood stream for storage or fuel) or enhance epinephrine production, since a higher concentration of epinephrine increases the activity of hormone sensitive lipase (responsible for adipose tissue lipolysis)[8]. It has also been shown that muscle glycogen (storage form of carbohydrate that is located within the muscle cell) depletion is 25% greater in males compared with females when subjects were matched for training status and performance[127]. This is in agreement with females having a greater reliance on fats as fuel during exercise.

**Limitations**

The most obvious limitation to this study was the lack of blood analysis and hence, plasma glutamine, glutamate, alanine and lactate measurements. Without these measurements it was very difficult to assess the effectiveness of glutamine as an

ergogenic aid based upon a theoretical buffering capacity. Another limitation to this study was the small sample size (n = 7; n = 6, when female excluded) to detect differences between the GLN and PLC treatment. Power and sample size analyses for each variable for n = 7 and n = 6 (males only) can be found in Appendix G. The exercise protocol is another potential limitation because previous studies used different modalities (strength training and cycling), durations, and intensities of exercise to examine the effects of glutamine supplementation[3, 56, 104] which makes it difficult to compare results. Furthermore, the blood draw procedure used during testing proved to be problematic as it was often difficult to obtain the desired amount to blood from the subject, especially during exercise. This is most likely due to the fact that during exercise, blood is redirected away from areas were it is not needed (i.e. forearms) and to the working muscles. Lastly, the lack of diet logs prior to reporting to the lab on testing days could have been another possible limitation, as subjects were not asked to report what they consumed on exercise testing days. Although, all subjects reported to the lab after a two-hour fast, it might have been beneficial to use a control meal to exclude the possibility of a high-protein/low-carbohydrate or high-carbohydrate/low-protein meal influencing plasma glutamine concentrations.

**Future Research**

The findings from our research suggest that other research could be conducted to better understand the effects of acute glutamine supplementation on high intensity exercise and recovery from exercise. Future studies should examine the changes that occur during and post-exercise to plasma glutamine, glutamate, alanine, $HCO_3$, pH and lactate values. Studies should also examine the effects of acute supplementation at

various dosages on performance and recovery parameters, such as $VO_2$max, RER, $CO_2$ production, heart rate, and ratings of perceived exertion. Research is also warranted in the area of long-term glutamine supplementation for athletes participating in numerous high-intensity performances separated by only a few hours, as supplementation may allow for a more favorable acid-base status prior to subsequent performances.

**Summary**

In summary, this study demonstrated no significant difference in maximal treadmill running performance between GLN and PLC treatments following acute glutamine supplementation. Although, there was a significant difference in the 5-min post-exercise RER value this was due to the inclusion of the female in the group analysis. More research is needed to make a conclusive observation about the effects of acute glutamine supplementation on recovery from high-intensity exercise. Therefore, the potential of acute glutamine supplementation as an ergogenic aid due to an increased buffering capacity has yet to be observed.

## REFERENCES


1. Herbal treatments: the promise and pitfalls. *Consumer Reports*. 64:44-48, 1999.

2. American College of Sports Medicine. *ACSM's guidelines for exercise testing and prescription*. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000.

3. Antonio, J., M. Sanders, D. Kalman, D. Woodgate, and C. Street. The effects of high-dose glutamine ingestion on weightlifting performance. *J Strength Cond Res*. 16:157-160, 2002.

4. Antonio, J. and C. Street. Glutamine: A potentially useful supplement for athletes. *Can J Appl Physiol*. 24:1-14, 1999.

5. Archibald, R. Chemical characteristics and physiological roles of glutamine. *Chem Rev*. 37:161-208, 1945.

6. Ardawi, M. and E. Newsholme. Glutamine metabolism in lymphocytes of the rat. *Biochem J*. 212:835-842, 1983.

7. Ardawi, M. and E. Newsholme. Metabolism in lymphocytes and its importance to the immune response. *Essays Biochem*:1-44, 1985.

8. Ashley, C., M. Kramer, and P. Bishop. Estrogen and substrate metabolism. *Sports Med*. 29:221-227, 2000.

9. Askanazi, J., Y. Carpentier, C. Michelsen, D. Elwyn, P. Furst, L. Kantrowitz, F. Gump, and J. Kinney. Muscle and plasma amino acids following injury. Influence of intercurrent infection. *Ann Surg*. 192:78-85, 1980.

10. Babij, P., S. M. Matthews, and M. J. Rennie. Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. *Eur J Appl Physiol & Occ Physiol*. 50:405-411, 1983.

11. Ball, D. and R. Maughan. The effect of sodium citrate ingestion on the metabolic response to intense exercise following diet manipulation in man. *Experimental Physiol*. 82:1041-1056, 1997.

12. Bergström, J., P. Fürst, and E. Hultman. Free amino acids in muscle tissue and plasma during exercise in man. *Clin Physiol*. 5:155-160, 1985.

13. Bergström, J., P. Fürst, L. Norée, and E. Vinnars. Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol.* 36:693-697, 1974.

14. Bernt, E. and H. Bergmeyer. UV-assay with glutamate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis.* H. Bergmeyer (Ed.) New York: Academic Press, 1974, pp. 1704-1708.

15. Bird, S., J. Wiles, and J. Robbins. The effect of sodium bicarbonate ingestion on 1500-m racing time. *J Sports Sci.* 13:399-403, 1995.

16. Blanchard, M., G. Jordan, B. Desbrow, L. MacKinnon, and D. Jenkins. The influence of diet and exercise on muscle and plasma glutamine concentrations. *Med Sci Sports Exerc.* 33:69-74, 2001.

17. Borg, G. A. *Borg's Perceived Exertion and Pain Scales.* Champaign, IL: Human Kinetics, 1998.

18. Bouissou, P., G. Defer, C. Guezenne, P. Estrade, and B. Serrurier. Metabolic and catecholamine responses to exercise during alkalosis. *Med Sci Sports Exerc.* 20:228-232, 1988.

19. Brand, K., W. Leibold, P. Luppa, C. Schoener, and A. Schultz. Metabolic alterations associated with proliferation of mitogen activated lymphocytes and of lymphoblastoid cell lines: evaluation of glucose and glutamine metabolism. *Immunology.* 173:23-24, 1986.

20. Brooks, G., T. Fahey, and T. White. *Exercise Physiology: Human Bioenergetics and Its Application.* 2nd ed. Mountain View, CA: Mayfield Publishing Company, 1996

21. Castell, L., C. Liu, and E. Newsholme. Diurnal variation of plasma glutamine and arginine in normal and fasting humans. *Proc Nutr Soc.* 54:118A, 1995.

22. Castell, L. and E. Newsholme. The effects of oral glutamine supplementation on athletes after prolonged, exhaustive exercise. *Nutrition.* 13:738-742, 1997.

23. Castell, L. and E. Newsholme. Glutamine and the effects of exhaustive exercise upon the immune response. *Can J Physiol Pharmacol.* 76:524-532, 1998.

24. Castell, L., J. Poortmans, and E. Newsholme. Does glutamine have a role in reducing infections in athletes? *Eur J Appl Physiol* 73:488-490, 1996.

25. Chasiotis, D., E. Hultman, and K. Sahlin. Acidotic depression of cyclic AMP accumulation and phosphorylase b to a transformation in skeletal muscle of man. *J Physiol.* 335:197-204, 1983.

26. Christophe, J., J. Winand, R. Kutzner, and M. Hebbelinck. Amino acid levels in plasma, liver, and muscle and kidney during and after exercise in fasted and fed rats. *Am J Physiol.* 221:453-457, 1971.

27. Costill, D., F. Verstappen, H. Kuipers, E. Janssen, and W. Fink. Acid-base balance during repeated bouts of exercise; influence of HCO3. *Int J Sports Med.* 5:228-231, 1984.

28. Damian, A. and R. Pitts. Rates of glutaminase I and glutamine synthetase reactions in rat kidney *in vivo*. *Am J Physiol.* 218:1249-1255, 1970.

29. Danforth, W. Activation of glycolytic pathway in muscle. In: *Control of energy metabolism*. B. Chance, R. Estabrook, and J. Williamson (Eds.) New York: Academic Press, 1965.

30. Déchelotte, P., D. Darmaun, M. Rongier, B. Hecketsweiler, O. Rigal, and J. Desjeux. Absorption and metabolic effects of eternally administered glutamine in humans. *Am J Physiol.* 260:G677-682, 1991.

31. Décombaz, J., P. Reinhardt, K. Anantharaman, G. von Glutz, and J. Poortmans. Biochemical changes in a 100km run: Free amino acids, urea, and creatinine. *Eur J Appl Physiol* 41:61-72, 1979.

32. Del Castillo, J., T. Nelson Jr., and V. Sanchez. Mechanisms of the increased acetylcholine sensitivity of skeletal muscle in low pH solutions. *J Cell Comp Physiol.* 59:35-49, 1962.

33. Donaldson, S. and L. Hermansen. Differential direct effects of $H^+$ and $Ca^{2+}$-activated force of skinnes fibres from the soleus, cardiac, adductor magnus muscles of rabbits. *Pflugers Arch.* 376:55-65, 1978.

34. Donaldson, S., L. Hermansen, and L. Bolles. Differential direct effects of W on $Ca^{2+}$ activated force of skinnes fibers from the soleus, cardiac and adductor magnus muscles of rabbits. *Pflugers Arch.* 376:55-65, 1983.

35. Elia, M., P. Folmer, A. Schlatmann, A. Goren, and S. Austin. Amino acid metabolism in muscle and in the whole body of man before and after ingestion of a single mixed meal. *Am J Clin Nutr.* 49:1203-1210, 1989.

36. Eriksson, L., S. Broberg, O. Bjorkman, and J. Wahren. Ammonia metabolism during exercise in man. *Clinical Physiol.* 5:325-336, 1985.

37. Fabiato, A. and F. Fabiato. Effects of pH on the myofilaments and sarcoplasmic reticulum of skinnes cells from cardiac and skeletal muscles. *J Physiol.* 276:233-235, 1978.

38. Feigin, R. Circadian periodicity of blood amino-acids in adult men. *Nature.* 215:512-514, 1967.

39. Feigin, R., A. Klainer, and W. Beisel. Factors affecting circadian periodicity of blood amino acids in man. *Metabolism.* 17:764-775., 1968.

40. Felig, P. Amino acid metabolism in man. *Ann Rev Biochem.* 44:933-955, 1975.

41. Friedlander, A., G. Casazza, M. Horning, M. Huie, M. Piacentini, J. Trimmer, and G. Brooks. Training-induced alterations of carbohydrate metabolism in women: women respond differently than men. *J Appl Physiol.* 85:1175-1186, 1998.

42. Frisina, J., S. Gaudieri, T. Cable, D. Keast, and T. Palmer. Effects of acute exercise on lympocyte subsets and metabolic activity. *Int J Sports Med.* 15:36-41, 1994.

43. Gleeson, M., A. Blannin, N. Walsh, N. Bishop, and A. Clark. Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int J Sports Med.* 8:49-59, 1998.

44. Golden, M., P. Jahoor, and A. Jackson. Glutamine production rate and its contribution to urinary ammonia in normal man. *Clin Sci.* 62:299-305, 1982.

45. Goldfinch, J., L. McNaughton, and P. Davies. Induced metabolic alkalosis and its effects on 400-m racing time. *Eur J Appl Physiol.* 57:45-48, 1988.

46. Goldstein, L., H. Schrock, and C. Cha. Relationship of muscle glutamine production to renal ammonia metabolism. *Biochem Soc Trans.* 8:509-510, 1980.

47. Graser, T., H. Godel, S. Albers, P. Földi, and P. Fürst. An ultra rapid and sensitive high-performance liquid chromatographic: method for determination of tissue and plasma free amino acids. *Anal Biochem.* 151:142-152, 1985.

48. Greenhaff, P. L., M. Gleeson, and R. Maughan. Diet-induced metabolic acidosis and the performance of high intensity exercise in man. *Eur J Appl Physiol.* 57:583-590, 1988.

49. Greenhaff, P. L., M. Gleeson, and R. Maughan. The effects of diet on muscle pH and metabolism during high intensity exercise. *Eur J Appl Physiol.* 57:531-539, 1988.

50. Greenhaff, P. L., M. Gleeson, and R. Maughan. The influence of an alteration in diet composition on plasma and muscle glutamine levels in man [abstract]. *Clin Sci.* 74:20P, 1988.

51. Guyton, A. *Textbook of medical physiology.* 8th ed. Philadelphia: WB Saunders Co, 1991, 330-335.

52. Halson, S. and A. Jeukendrup. Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Med.* 34:967-981, 2004.

53. Halson, S., G. Lancaster, A. Jeukendrup, and M. Gleeson. Immunological responses to overraching in cyclists. *Med Sci Sports Exerc.* 35:854-861, 2003.

54. Hammarqvist, F., J. Wernerman, R. Ali, A. von der Decken, and E. Vinnars. Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. *Annals of surgery.* 209:455-461, 1989.

55. Hanson, P. and D. Parsons. Metabolism and transport of glutamine and glucose in vascularly perfused small intestine rat. *Biochem J.* 166:509-519, 1977.

56. Haub, M., J. Potteiger, K. Nau, M. Webster, and C. Zebas. Acute L-glutamine ingestion does not improve maximal effort exercise. *J Sports Med Phys Fitness.* 38:240-244, 1998.

57. Hawes, K. The culture of supplements: Pills, powders and potions have become ingrained in athletes' way of life. In: *The NCAA News*, 2003.

58. Heigenhauser, G. and N. Jones. Bicarbonate loading. In: *Perspectives in exercise science and sports medicine.* D. Lamb and M. Williams (Eds.) Carmel, MI: Cooper Publishing Group, 1991, pp. 183-207.

59. Heigenhauser, G., N. Jones, J. Kowalchuk, and M. Lindinger. The role of physicochemical systems in plasma in acid-base control in exercise. In: *Biochemistry of exercise VII.* A. Taylor, P. Gollnick, H. Green, C. Lanuzzo, E. Noble, G. Metivier, and J. Sutton (Eds.) Champaign, IL: Human Kinetics, 1991, pp. 359-374.

60. Heyward, V. *Advanced Fitness Assessment and Exercise Prescription.* 5th ed, 1998

61. Hiscock, N. and L. MacKinnon. A comparison of plasma glutamine concentration in athletes from different sports. *Med Sci Sports Exerc.* 30:1693-1696, 1998.

62. Hood, D. and R. Terjung. Amino acid metabolism during exercise and following endurance training. *Sports Med.* 9:23-35, 1990.

63. Houston, M. *Biochemistry Primer for Exercise Science.* Champaign, IL: Human Kinetics, 1995, 117-122.

64. Jackson, A. and M. Pollock. Practical assessment of body composition. *Phys Sport Med.* 13:76-90, 1985.

65. Kapadia, C., M. Colpoys, Z. Jiang, D. Johnson, R. Smith, and D. Wilmore. Maintenance of skeletal muscle intracellular glutamine during surgical trauma. *J Parenter Enteral Nutr.* 9:583-589, 1985.

66. Kargotich, S., C. Goodman, B. Dawson, A. Morton, D. Keast, and D. Joske. Plasma glutamine responses to high-intensity exercise before and after endurance training. *Res Sports Med.* 13:287-300, 2005.

67. Kargotich, S., D. Rowbottom, D. Keast, C. Goodman, and A. Morton. Plasma glutamine changes after high intensity exercise in elite male swimmers. *Res Sports Med.* 13:7-21, 2005.

68. Katz, A., S. Broberg, K. Sahlin, and J. Wahren. Muscle ammonia and amino acid metabolism during dynamic exercise in man. *Clinical Physiol.* 6:365-379, 1986.

69. Katz, A., D. Costill, D. King, M. Hargreaves, and W. Fink. Maximal exercise tolerance after induced alkalosis. *Int J Sports Med.* 5:107-110, 1984.

70. Keast, D., D. Arstein, W. Harper, R. Fry, and A. Morton. Depression of plasma glutamine concentration after exercise stress and its possible influence on the immune system. *Med J Aust.* 162:15-18, 1995.

71. Khan, K., E. Blaak, and M. Elia. Quantifying intermediate metabolites in whole blood after a simpe deproteinization step with sulfosalicylic acid. *Clin Chem.* 37:718-733, 1991.

72. Kindermann, W., J. Keul, and G. Huber. Physical exercise after induced alkalosis (bicarbonate or trisbuffer). *Eur J Appl Physiol.* 37:197-204, 1977.

73. Kingsbury, K., L. Kay, and M. Hjelm. Contrasting plasma free amin acid patterns in elite athletes: association with fatigue and infection. *Br J Sports Med.* 32:25-33, 1998.

74. Kowalchuk, J., G. Heigenhauser, M. Lindinger, J. Sutton, and N. Jones. Factors influencing hydrogen ion concentration in muscle after intense exercise. *J Appl Physiol.* 65:2080-2089, 1988.

75. Krebs, H. Glutamine metabolism in the animal body. In: *Glutamine: metabolism, enzymology, and regulation.* J. Mora and R. Palacios (Eds.) New York: Academic Press, 1980, pp. 319-329.

76. Krebs, H. The synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. *Biochem J.* 29:1951-1969, 1935.

77. Lacey, J. and D. Wilmore. Is glutamine a conditionally essential amino acid? *Nutr Rev.* 48:297-309, 1990.

78. Lambert, C., P. L. Greenhaff, D. Ball, and R. Maughan. Influence of sodium bicarbonate ingestion on plasma ammonia during incremental exercise in man. *Eur J Appl Physiol.* 63:338-344, 1992.

79. Lavender, G. and S. Bird. Effect of sodium bicarbonate ingestion upon repeated sprints. *Br J Sports Med.* 23:41-45, 1989.

80. Lehmann, M., M. Huonker, F. Dimeo, N. Heinz, U. Gastmann, N. Treis, J. Steinacker, J. Keul, R. Kajewski, and D. Haussinger. Serum amino acid concentrations in nine athletes before and after the 1993 Colmar Ultra Triathlon. *Int J Sports Med.* 16:155-159, 1995.

81. Lehmann, M., E. Makob, U. Gastmann, J. Steinacker, and J. Keul. Unaccustomed high mileage vs high training-related performance and neuromuscular response in distance runners. *Eur J Appl Physiol.* 70, 1995.

82. Lemon, P. Effect of exercise in protein requirements. *J Sports Sci.* 9:53-70, 1991.

83. Lund, P. Determination with glutaminase and glutamate dehydrogenase. In: *Methods of enzymatic analysis*. H. Bergmeyer (Ed.) New York: Academic Press, 1974, pp. 1719-1722.

84. MacKinnon, L. and S. Hooper. Plasma glutamine and upper respiratory tract infection during intensified training in swimmers. *Med Sci Sports Exerc.* 28:285-290, 1996.

85. MacLennan, P., R. Brown, and M. J. Rennie. A positive relationship between protein synthesis rate and intracellular glutamine concentration in perfused rat skeletal muscle. *FEBS Lett.* 215:187-191, 1987.

86. Mainwood, G. and P. Worsley-Brown. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J Physiol.* 250:1-22, 1975.

87. Margaria, R. Effect of alkalosis on performance in supramaximal exercise. *Int Z Angew Physiol.* 29:215-218, 1971.

88. Maughan, R. and M. Gleeson. Influence of a 36 h fast followed by refeeding with glucose, glycerol or placebo on metabolism and performance during prolonged exercise in man. *Eur J Appl Physiol.* 57:570-576, 1988.

89. Maughan, R., P. L. Greenhaff, J. Leiper, D. Ball, C. Lambert, and M. Gleeson. Diet composition and the performance of high-intensity exercise. *J Sports Sci.* 15:265-275, 1997.

90. Maughan, R., J. Leiper, and P. Litchfield. The effects of induced acidosis and alkalosis on isometric endurance capacity in man. In: *Exercise Physiology: Current Selected Research.* C. Dotson and J. Humphreys (Eds.) New York: AMS Press, 1986, pp. 73-82.

91. Mayers, E., O. Smith, W. Fredricks, and M. McKinney. The isolation and characterisation of glutamine-requiring strains of *Escherichia coli* K12. *Mol Gen Genet.* 137:131-142, 1975.

92. McKenzie, D., K. Coutts, D. Stirling, H. Hoeben, and G. Kuzara. Maximal work production following two levels of artificially induced metabolic alkalosis. *J Sports Sci.* 4:35-38, 1986.

93. McNaughton, L. Sodium citrate and anaerobic performance: implications of dosage. *Eur J Appl Physiol.* 61:392-397, 1990.

94. McNaughton, L. and R. Cedaro. Sodium citrate ingestion and its effects on maximal anaerobic exercise on different durations. *Eur J Appl Physiol.* 64:36-41, 1992.

95. Meister, A. *Biochemistry of amino acids.* 2nd ed. New York: Academic Press, 1965, I-57.

96. Newsholme, E., J. Lang, and A. Relman. Control of rate of glutamine metabolism in the kidney. *Contrib Nephrol.* 31:1-4, 1982.

97. Newsholme, E. and A. Leech. *Biochemistry for the medical sciences.* Chichester: John Wiley, 1983

98. Noakes, T. *The Lore of Running.* 2nd ed. Capetown: Oxford University Press, 1986

99. Palmer, T., M. Rossiter, B. Levin, and V. Oberholzer. The effect of protein loads on plasma amino acid levels. *Clin Sci Molec Med.* 45:827-832, 1973.

100. Parry-Billings, M., E. Blomstrand, N. McAndrew, and E. Newsholme. A communicational link between skeletal muscle, brain, and cells of the immune system. *Int J Sports Med.* 11:S122-S128, 1990.

101. Parry-Billings, M., R. Budgett, Y. Koutedakis, E. Blomstrand, S. Brooks, C. Williams, P. Calder, S. Pilling, R. Baigrie, and E. Newsholme. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. *Med Sci Sports Exerc.* 24:1353-1358, 1992.

102. Parry-Billings, M., J. Evans, P. Calder, and E. Newsholme. Does glutamine contribute to immunosuppression after major burns? *Lancet.* 336:523-525, 1990.

103. Parry-Billings, M. and D. MacLean. The effect of sodium bicarbonate and sodium citrate ingestion on anaerobic power during intermittent exercise. *Eur J Appl Physiol.* 55:524-529, 1986.

104. Piattoly, T. and M. Welsch. L-glutamine supplementation: Effects on recovery from exercise. *Med Sci Sports Exerc.* 36:S127, 2004.

105. Pipe, A. and C. Ayotte. Nutritional supplements and doping. *Clin J Sport Med.* 12:245-249, 2002.

106. Pitts, R., L. Pilkington, M. MacLeod, and E. Leal-Pinto. Metabolism of glutamine by the intact functioning kidney of the dog. Studies in metabolic acidosis and alkalosis. *J Clin Invest.* 51:557-565, 1972.

107. Potteiger, J., G. Nickel, M. Webster, M. Haub, and R. Palmer. Sodium citrate ingestion enhances 30 km cycling performance. *Int J Sports Med.* 17:7-11, 1996.

108. Powell, H., L. Castell, M. Parry-Billings, J. Desborough, G. Hall, and E. Newsholme. Growth hormone suppression and glutamine flux associated with cardiac surgery. *Clin Physiol.* 14:569-580, 1994.

109. Pyne, D. Exercise-induced muscle damage and inflammation: A review. *Aust J Sci Med Sport.* 26:49-58, 1994.

110. Rennie, M. J., R. Edwards, S. Krywawych, C. Davies, D. Halliday, J. Waterlow, and D. Millward. Effect of exercise on protein turnover in man. *Clin Sci* 61:627-639, 1981.

111. Robertson, R., J. Falker, A. Drash, A. Swank, K. Metz, S. Spungen, and J. LeBoeuf. Effect of blood pH on peripheral and central signals of perceived exertion. *Med Sci Sports Exerc.* 18:114-122, 1986.

112. Robson, P., A. Blannin, and N. Walsh. Effect of exercise intensity and duration on plasma glutamine responses following exercise and the time course of recovery in physically active men. *J Physiol.* 506:118P-119P, 1998.

113. Rohde, T., S. Asp, D. MacLean, and B. Pedersen. Competitive sustained exercise in humans, and lymphokine activated killer cell activity- an intervention study. *Eur J Appl Physiol.* 78:448-453, 1998.

114. Rohde, T., D. MacLean, A. Hartkopp, and B. Pedersen. The immune system and serum glutamine during a triathlon. *Eur J Appl Physiol.* 74:428-434, 1996.

115. Roth, E., J. Funovics, F. Mühlbacher, M. Schemper, W. Mauritz, P. Sporn, and A. Fritsch. Metabolism disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin Nutr.* 1:25-41, 1982.

116. Rowbottom, D., D. Keast, C. Goodman, and A. Morton. The haematological, biochemical and immunological profile of athletes suffering from the overtraining syndrome. *Eur J Appl Physiol.* 70:502-509, 1995.

117. Rowbottom, D., D. Keast, and A. Morton. The emerging role of glutamine as an indicator of exercise stress and overtraining. *Sports Med.* 21:80-97, 1996.

118. Sahlin, K., A. Katz, and S. Broberg. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am J Physiol.* 259:C834-841, 1990.

119. Sewell, D., M. Gleeson, and A. Blannin. Hyperammonaemia in relation to high-intensity exercise duration in man. *Eur J Appl Physiol.* 69:350-354, 1994.

120. Smith, D. and S. Norris. Changes in glutamine and glutamate concentrations for tracking training tolerance. *Med Sci Sports Exerc.* 32:684-689, 2000.

121. Smith, L. Cytokine hypothesis of overtraining: A physiological adaptation to excessive stress. *Med Sci Sports Exerc.* 32:317-331, 2000.

122. Souba, W. *Glutamine: physiology, biochemistry and nutrition in critical illness.* Austin: RG Landes, 1992.

123. Souba, W., R. Smith, and R. Wilmore. Glutamine metabolism by the intestinal tract. *J Parenter Enteral Nutr.* 9:608-617, 1985.

124. Stephens, J. and A. Taylor. Fatigue of maintained voluntary muscle contraction in man. *J Physiol.* 22:1-18, 1972.

125. Sutton, J., N. Jones, and C. Toews. Effect of pH on muscle glycolysis during exercise. *Clin Sci.* 61:331-338, 1981.

126. Swank, A. and R. Robertson. Effect of induced alkalosis on perception of exertion during intermittent exercise. *J Appl Physiol.* 67:1862-1867, 1989.

127. Tarnopolsky, L., J. MacDougall, S. Atkinson, M. Tarnopolsky, and J. Sutton. Gender differences in substrate for endurance exercise. *J Appl Physiol.* 68:302-308, 1990.

128. Tarnopolsky, M., S. Atkinson, S. Phillips, and J. MacDougall. Carbohydrate loading and metabolism during exercise in men and women. *J Appl Physiol.* 78:1360-1368, 1995.

129. Trivedi, B. and W. Danforth. Effect of pH on the kinetics of frog muscle phosphofructokinase. *J Biol Chem.* 241:4110-4112, 1966.

130. U.S. Department of Health and Human Services and U.S. Department of Agriculture. *Dietary Guidelines for Americans.* 6th ed. Washington, D.C.: U.S. Government Printing Office, 2005

131. van der Schoor, P., G. van Hall, and W. Saris. Ingestion of drinks containing protein hydrolysate prevents the postexercise reduction in plasma glutamine. *Int J Sports Med.* 18:S115, 1997.

132. van Hall, G., W. Saris, and A. Wagenmakers. Effect of carbohydrate supplementation on plasma glutamine during prolonged exercise and recovery *Int J Sports Med.* 19:82-86, 1998.

133. Wagenmakers, A. Amino acid supplements to improve athletic performance. *Curr Opin Clin Nutr Metab Care.* 2:539-544, 1999.

134. Wagenmakers, A., E. Beckers, F. Brouns, H. Kuipers, P. Soeters, G. van der Vusse, and W. Saris. Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. *Am J Physiol.* 260:E883-890, 1991.

135. Walker, V. and G. Mills. Quantitative methods for amino acid analysis in biological fluids. *Ann Clin Biochem.* 32:28-57, 1995.

136. Walsh, N., A. Blannin, A. Clark, L. Cook, P. Robson, and M. Gleeson. The effects of high intensity intermittent exercise on the plasma concentration of glutamine and organic acids of severe exercise stress. *Eur J Appl Physiol.* 77:434-438, 1998.

137. Walsh, N., A. Blannin, P. Robson, and M. Gleeson. Glutamine, exercise and immune function: links and possible mechanisms. *Sports Med.* 26:177-191, 1998.

138. Wasserman, K., A. Kessel, and G. Burton. Interaction of physiological mechanisms during exercise. *J Appl Physiol.* 22:71-85, 1967.

139. Welbourne, T. Increased plasam bicarbonate and growth hormone after an oral glutamine load. *Am J Clin Nutr.* 61:1058-1061, 1995.

140. Welbourne, T. Interorgan glutamine flow in metabolic acidosis. *Am J Physiol.* 253:F1069-1076, 1987.

141. Welbourne, T., D. Childress, and G. Givens. Renal regulation of interorgan glutamine flow in metabolic acidosis. *Am J Physiol.* 251:R858-R866, 1986.

142. Welbourne, T. and S. Joshi. Enteral glutamine spares endogenous glutamine in chronic acidosis. *J Parenter Enteral Nutr.* 18:243-247, 1994.

143. Welbourne, T. and S. Joshi. Interorgan glutamine metabolism during acidosis. *J Parenter Enteral Nutr.* 14:77S-85S, 1990.

144. Wilkes, D., N. Gledhill, and R. Smyth. Effect of acute induced metabolic alkalosis on 800m racing time. *Med Sci Sports Exerc.* 15:277-280, 1983.

145. Windmueller, H. and A. Spaeth. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. *Arch Biochem Biophys.* 171:662-672, 1975.

146. Windmueller, H. and A. Spaeth. Uptake and metabolism of plasma glutamine by the small intestine. *J Biol Chem.* 249:5070-5079, 1974.

147. Wright, P. and M. Knepper. Glutamate dehydrogenase activities in microdissected rat nephron segments: effects of acid-base loading. *Am J Physiol.* 259:F53, 1990.

148. Zanker, C., I. Swaine, L. Castell, and E. Newsholme. Responses of plasma glutamine, free tryptophan and branch-chained amino acids to prolonged exercise after a regime designed to reduce muscle glycogen. *Eur J Appl Physiol.* 75:543-548, 1997.

149. Ziegler, T., K. Benfell, R. Smith, L. Young, E. Brown, E. Ferrari-Baliviera, D. Lowe, and D. Wilmore. Safety and metabolic effects of L-glutamine administration in humans. *J Parenter Enteral Nutr.* 14:137S-146S, 1990.

# APPENDIX A

## THE UNIVERSITY OF LOUISVILLE
## EXERCISE PHYSIOLOGY LABORATORIES

### Health Survey

Name_____ Local Phone_____

Local Address_____

Today's Date_____ Birth date_____

Age_____ Sex_____ Weight_____ Height_____

1. *Have you ever been diagnosed as having any of the following (if there is a family history of any of the following, please check the Family History column)?*

| | Never | In Past | Presently | Family History |
|---|---|---|---|---|
| a. Heart Diseases | | | | |
| b. Rheumatic Fever | | | | |
| c. High Blood Pressure | | | | |
| d. Hemophilia | | | | |
| e. Other Vascular Disorders | | | | |
| f. Diabetes | | | | |
| g. Kidney Disease | | | | |
| h. Liver Disease | | | | |
| i. Asthma | | | | |
| j. Allergies (in general) | | | | |
| k. Chronic Bronchitis | | | | |
| l. Other Respiratory Illness | | | | |
| m. High Serum Lipids | | | | |
| n. Anemia | | | | |
| o. Low Blood Sugar | | | | |
| p. Neuro-Musculo-Skeletal Problems | | | | |

2. *Please indicate any surgery that you have undergone and the approximate date(s):*

_____

_____

3. *Please indicate recent illnesses or major injuries that you have had & the approximate date(s):*

_____

_____

4. *Please list any and all medications that you are presently taking:*

| Medication | Dosage | Dosage per day |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |

5. *Do you smoke?* _____ *Packs per day* _____

6. *Describe your present training program (the activity, amount per day, days per week, and the length of time you have been training at this level):*

| Activity | Minutes/Day | Days/Week | Weeks of Training |
|---|---|---|---|
| _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ |

*The information provided above is correct to the best of my knowledge:*

_____ _____

Signature Date

## APPENDIX B

### Subject Participation Questionnaire

1. Name _____

2. Please describe in *detail* your current exercise program. Please specify the activity, amount per day, days per week, and the length of time you have been training at this level

_____

_____

_____

_____

3. Have you recently experienced an illness or injury that has required you to take time off from training? If yes, please explain.

_____

_____

_____

4. Have you previously taken dietary supplements (protein powder, creatine, amino acids, etc.)? If yes, please specify.

_____

_____

5. Are you currently taking dietary supplements? If yes, please specify.

_____

## APPENDIX C

### Subject Informed Consent

*Title of the research study:* The effects of glutamine supplementation on maximal performance and recovery from high-intensity exercise.

*IRB assigned number:* 07.0174

*Investigator(s) name & address:* Jacks, Dean, Department of Health & Sport Sciences University of Louisville Crawford Gym, Room 114Louisville, Kentucky 40292

*Site(s) where study is to be conducted:* Belknap Campus Laboratory, Laboratory

*Phone number for subjects to call for questions:* Dean Jacks, Ph.D. at (502) 852-8352.

**Introduction and Background Information**

You are invited to take part this research study because you are 18-30 years of age and participate in thirty minutes of moderate to high-intensity aerobic exercise at least four days a week. In addition, you are free of metabolic, cardiovascular, and orthopedic limitations. The study is being conducted under the direction of Dean Jacks, Ph.D., Assistant Professor for the Department of Health & Sport Sciences and Mandy Jacobs, B.S., graduate student. Approximately twelve local subjects will be invited to participate. Your participation in this study will consist of three separate sessions on three different days.

**Purpose**

The purpose of this study is to investigate the effects of glutamine supplementation on maximal performance during high-intensity exercise and on recovery from high-intensity exercise.

**Procedures**

Your participation in this study will consist of three separate sessions. You will be asked to abstain from any dietary supplementation and maintain current levels of exercise for the duration of the study. The first session will last approximately one-hour and will consist of completing a health survey and questionnaire which ask about your physical activity habits, supplementation history and recent illness and/or injuries. You may decline to answer any questions that make you feel uncomfortable. To assess general and overall body composition, height, weight, and skin folds will be taken. In addition, your resting heart rate and blood pressure will be measured. You will then be asked to complete and turn in a 3-day food diary before the first day of exercise testing. The food diary should include dietary intake for two weekdays and one weekend day. We will explain how to complete the food diary at the end of the first session and give you examples to take home.

The second and third sessions will last approximately 4 hours and a minimum of 7 days should exist between the second and third testing session. You will be asked to fast 2-

hours prior to reporting the laboratory for each testing session. In addition, you will be asked not to perform any exercise prior to reporting to the laboratory. Before the second testing session, you will be randomly assigned to one of two treatments, however you will not be informed as to which treatment you have been assigned. Treatment assignments will be made using randomization which is a process similar to "flipping a coin". One group will participate in the experimental condition during the second testing session and the control condition the third testing session. The second group will participate in the control condition during the second testing session and the experimental condition during the third testing session.

Upon reporting to the laboratory for the second and third testing sessions, we will measure your resting blood pressure and heart rate. A research registered nurse trained and credentialed in intravenous catheter placement will then insert a temporary catheter into a vein on the back of your hand. The catheter is similar to an intravenous catheter used to collect blood samples and administer intravenous medications. This catheter will be used to draw blood before exercise, during exercise and after exercise testing. A maximum of ten blood samples will be obtained throughout each testing session. The blood samples will be allowed to clot and then centrifuged for 20-30 minutes in order to separate the serum from the cellular components. The serum will then be pipette transferred to storage tubes and stored at -70F for future analysis. Specifically, we will be analyzing the blood samples for glutamine, glutamate, alanine and lactate concentrations. The samples will be stored for approximately 7 months before analysis is conducted. After analysis is conducted, blood samples will be destroyed.

You will then be asked to drink 0.03 g/kg of body weight of glutamine mixed with 250 ml of caffeine-free fruit juice or 250 ml of caffeine-free fruit juice (placebo). You will be given 10-minutes to consume the drink, which will be premixed by a research assistant. You will then rest for 1-hour in the sitting position. After 1-hour you will perform a maximal exercise test on the treadmill. The exercise test will consist of incremental increases in workload and is designed to induce fatigue within 8-14 minutes. Upon completion of the exercise test you will be asked to sit in a chair for 1-hour. In order to measure expired gases during and after the exercise test, you will be asked to wear a headpiece and mouthpiece that is comparable to a snorkel. At the commencement of 1-hour, a research nurse will remove the catheter from your hand and a research assistant will remove the headpiece and mouthpiece. Additionally, you will be asked to provide a urine sample pre-and post-exercise. All blood and urine samples will be de-identified (your name will not be linked to the samples), thus if you withdraw from the study, specimens cannot be destroyed.

**Potential Risks**

There are known physical risks linked with high—intensity exercise. Physical risks include elevated heart rate, muscle fatigue, and shortness of breath. In rare instances, high—intensity exercise may lead to cardiac events and/or death. It is likely (greater than 40%) that you will experience elevated heart rate, muscle fatigue and shortness of breath. In rare instances (less than 1%) high—intensity exercise may lead to cardiac events and/or death. There are known physical risk linked with having blood drawn. Risk may

include excessive bleeding, fainting or feeling light headed, hematoma (blood accumulating under the skin) and infection. To date, oral supplementation of glutamine have not demonstrated any side effects when taken in small dosages. Glutamine has shown to be well tolerated in healthy subjects when administered orally in small dosages and is readily metabolized and cleared from the body. Non physical risk (psychological, social, economic, legal) are not anticipated with this study. However, you may suffer harms that we have not seen before.

**Benefits**
It is not clear that you will benefit directly from this study, but it is hoped that your participation will help others in the future.

**Alternatives**
Instead of taking part in this study, you could choose to not participate as a subject in this study.

**Research Related Injury**
If you are injured by being in this research study, the study doctor will arrange for you to get medical treatment. The sponsor, the study site, or your study doctor has not set aside money to pay for treatment of any injury. You and your insurance will be billed for the treatment of these injuries. Before you agree to take part in this research study you should find out whether your insurance will cover an injury in this kind of research. You should talk to the study doctor or staff about this. If you are injured, there is no money set aside for lost wages, discomfort, disability, etc. You do not give up your legal rights by signing this form. If you think you have a research related injury, please call your study doctor.

**Compensation**
You will not be compensated for your time, inconvenience, or expenses while you are in this study.

**Costs**
There will be no additional costs to you for participating. However, you or your insurance company will be billed for all office visits and procedures that are part of routine medical care. It is your responsibility to find out what costs, if any, your insurance company will cover before taking part in the study.

**HIPAA Research Authorization**
The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for protected health information (PHI). Examples of PHI are your name, address, and birth date. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI cannot be used or shared without your agreement, unless it meets one of the HIPAA exceptions. You will be asked to sign a "Research Authorization" form. This allows the use and sharing of your PHI by those listed in the "Research Authorization."

**Confidentiality**

Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public. The following may look at your research and medical records:
*The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University.
*People who are responsible for research and HIPAA oversight at the institutions where the research is conducted.
•Government agencies, such as: Office for Human Research Protections (OHRP), Office of Civil Rights, and other appropriate regulatory government agencies.

**Security**
All data will be protected from disclosure by being kept in a locked file and all identifying information will be excluded from data forms. In addition, blood and urine samples will be de-identified (your name will not be linked to the samples), thus if you withdraw from the study, specimens cannot be destroyed.

**Voluntary Participation**
Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be penalized or lose any benefits for which you qualify. You will be told about any new information learned during the study that could affect your decision to continue in the study.

**Termination**
Your study doctor has the right to stop this study at any point. Your study doctor may take you out of this study with or without your okay. Reasons why this may occur include: failure to follow the experimental procedures and instructions provided by the investigators and failure to attend the experimental sessions. If you wish to withdraw from the study, there will be no consequences to you. We only ask that you tell us of your desire to stop participation in the study and you will be immediately withdrawn from the study.

**Participation in Other Research Studies**
You may not take part in this study if you are currently in another research study. It is important to let your doctor know if you are in another research study.

**Contact Persons**
Dean Jacks, Ph.D. at (502) 852-8352.

**Research Subject's Rights**
If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You can discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB).

You may also call this number if you have other questions about the research, and you cannot reach the study doctor, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has reviewed this research study.

**Concerns and Complaints**
If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville.

**Acknowledgment and Signatures**
This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights by signing this informed consent document. You will be given a signed copy of this consent form to keep for your records.

Do you want your primary care physician notified that you are a subject in this study?
☐Yes ☐No

| Printed Name of Subject/Legal Representative | Signature of Subject/Legal Representative | Date Signed |
|---|---|---|
| Printed Name of Person Explaining Consent Form (if other than investigator) | Signature of Person Explaining Consent Form | Date Signed |
| Printed Name of Investigator | Signature of Investigator | Date Signed |

**Note: If the investigator is not the person explaining the consent form, then the investigator must sign the Informed Consent Document within two weeks of the subject or subject's legal representative.*

**Specimen Informed Consent**

*Title of the research study:* The effects of glutamine supplementation on maximal performance and recovery from high-intensity exercise.

*IRB assigned number:* 07.0174

*Investigator(s) name & address:* Jacks, Dean, Department of Health & Sport Sciences University of Louisville Crawford Gym, Room 114Louisville, Kentucky 40292

*Site(s) where study is to be conducted:* Belknap Campus Laboratory, Laboratory

*Phone number for subjects to call for questions:* If you have any questions, concerns, or complaints about the research study, please contact Dean Jacks at (502) 852-8352.

**Introduction and Background Information**
You are invited to participate in a research study. The study is being conducted by Dr. Dean Jacks, Ph.D., Assistant Professor for the Department of Health & Sport Sciences and Mandy Jacobs, B.S, graduate student. The study is sponsored by the University of Louisville, Department of Health & Sport Sciences. The study will take place at Crawford Gym on Belknap Campus. Approximately 12 local subjects will be invited to participate. Your participation in this study will consist of three separate sessions on three different days.

**Purpose**
The purpose of this research study is to investigate the effects of glutamine supplementation on maximal performance during high—intensity exercise and on recovery from high—intensity exercise. Glutamine is an amino acid that is found in most naturally occurring food proteins.

**Procedures**
A research registered nurse trained and credentialed in intravenous catheter placement will insert a temporary catheter into a vein on the back of your hand. The catheter is similar to an intravenous catheter used to collect blood samples and administer intravenous medications. Approximately 5.5 tablespoons of blood will be removed from your vein for the experimental purpose of determining the concentrations of various amino acids (glutamine, alanine, glutamate) and lactate, which is a byproduct of exercise. A tourniquet will be placed on your arm, a needle placed in your vein, and approximately 5.5 tablespoons of blood will be removed. The blood will be removed intermittently, meaning not all of the blood will be removed at once. About 1.5 teaspoons of blood will be removed at a time, resulting in a maximum of 10 separate blood draws. The blood samples will be allowed to clot and then centrifuged for 20-30 minutes in order to separate the serum from the cellular components. The serum will then be pipette transferred to storage tubes and stored at -70F for future analysis. In addition, you will be asked to provide a urine sample pre- and post-exercise testing. The samples will be stored for approximately 7 months before analysis is conducted. All samples will be de-identified (your name will not be linked to the samples), thus if you withdraw from the study, specimens cannot be destroyed. Once all analyses have been conducted, the samples will be destroyed.

**Risks**
There may be some pain or discomfort during the procedure. It is possible there may be some bleeding, bruising or infection at the puncture site.

**Benefits**

The possible benefits of this study include more information about the efficacy of glutamine as an ergogenic aid. The information collected may not benefit you directly. The information learned in this study may be helpful to others.

**Compensation**
You will not be compensated for your time, inconvenience, or expenses while you are in this study.

**Costs**
There will be no additional costs to you for participating. However, you or your insurance company will be billed for all office visits and procedures that are part of routine medical care. It is your responsibility to find out what costs, if any, your insurance company will cover before taking part in the study.

**HIPAA Research Authorization**
The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for protected health information (PHI). Examples of PHI are your name, address, and birth date. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI cannot be used or shared without your agreement, unless it meets one of the HIPAA exceptions. You will be asked to sign a "Research Authorization" form. This allows the use and sharing of your PHI by those listed in the "Research Authorization."

**Security**
All data will be protected from disclosure by being kept in a locked file and all identifying
information will be excluded from data forms. In addition, blood and urine samples will be de-identified (your name will not be linked to the samples), thus if you withdraw from the study, specimens cannot be destroyed.

**Voluntary Participation**
Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be penalized or lose any benefits for which you qualify.

**Termination**
Your study doctor has the right to stop this study at any point. Your study doctor may take you out of this study with or without your okay. Reasons why this may occur include: failure to follow the experimental procedures and instructions provided by the investigators and failure to attend the experimental sessions. If you wish to withdraw from the study, there will be no consequences to you. We only ask that you tell us of your desire to stop participation in the study and you will be immediately withdrawn from the study.

**Participation in Other Research Studies**
You may not take part in this study if you are currently in another research study. It is important to let your doctor know if you are in another research study.

**Confidentiality**
Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public. The following may look at your research and medical records:
*The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University.
*People who are responsible for research and HIPAA oversight at the institutions where the research is conducted.
*Government agencies, such as: Office for Human Research Protections (OHRP), Office of Civil Rights, and other appropriate regulatory government agencies.

**Contact Persons**
If you have any questions, concerns, or complaints about the research study, please contact Dean Jacks at (502) 852-8352.

**Research Subject's Rights**
If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You can discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB). You may also call this number if you have other questions about the research, and you cannot reach the study doctor, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has reviewed this research study.

**Concerns and Complaints**
If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville..

**Acknowledgment and Signatures**
This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights by signing this informed consent document. You will be given a signed copy of this consent form to keep for your records.

Printed Name of Subject/Legal Representative  Signature of Subject/Legal Representative  Date Signed

_____  _____  _____
Printed Name of Person Explaining Consent Form  Signature of Person Explaining Consent Form  Date Signed

(if other than investigator)

| Printed Name of Investigator | Signature of Investigator | Date Signed |
|---|---|---|

**Note: If the investigator is not the person explaining the consent form, then the investigator must sign the Informed Consent Document within two weeks of the subject or subject's legal representative.*

***Consent version date: 11/26/2007***

## APPENDIX D

*Instructions for 3-Day Nutritional Diary*

- Record everything you consume for 3 days (1 weekend day, 2 week days) in the logs provided. Be sure to include the date on each dietary log.
- **'Amount'** – In this space indicate the amount of the particular food/drink item you ate/drank. Estimate the size (2"x 1"x1"), the volume (1/2 cup), the weight (2 ounces) and/or the number of items (12) of that type of food/drink.
- **'What Kind'** – In this column, write down the type of food/drink you ate/drank. Don't forget to write down extras, such as candy, butter, alcohol and sugar.
- **'Brand'** – In this space indicate the brand of the particular food/drink you ate/drank.
- **'Time'** – Write down the time of day you ate/drank the food/drink.
- Be sure to note if you were taking any medication, vitamins, supplements, etc. in the space provided.

*Tips for Keeping a Nutritional Diary*

**1. Write down EVERYTHING**

Keep your form with you all day and write down everything you eat and drink. A piece of candy, a handful of pretzels, a can of soda or small cookie may not seem like much at the time, but over 3 days these calories add up.

**2. Do it NOW**

Don't depend on your memory at the end of the day. Record your eating/drinking as you go.

**3. Be SPECIFIC**

Make sure to include extras, such as ketchup, salad dressing, gravy, pickles on your sandwich, etc. Also include how the item was prepared – fried, baked, breaded, scrambled, steamed, etc.

**4. Estimate amounts**

Remember, a fist = 1 cup; palm or deck of cards = 3 oz.; handful = 1-2 oz.

**5. Tell the Truth**

There's nothing to be gained by trying to look good on these forms. Do not change your eating habits while you're keeping your food diary.

## Three-Day Nutritional Diary

| Food or Drink | | | |
|---|---|---|---|
| **Amount** | **What Kind** | **Brand** | **Time** |
| | | | |
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**Supplements/Vitamins/Medications (BE SPECIFIC):**

## APPENDIX E

*Treadmill Protocol*

| TIME (minutes) | SPEED (mph) | GRADE (%) |
|---|---|---|
| 3 | 1.7 | 4.0 |
| 6 | 2.5 | 6.5 |
| 9 | 3.4 | 10.0 |
| 12 | 4.2 | 12.5 |
| 15 | 5.0 | 15.0 |
| 18 | 5.5 | 17.5 |

## APPENDIX F

Category Scale for Ratings of Perceived Exertion[17]

| | |
|---|---|
| 6 | |
| 7 | Very, very light |
| 8 | |
| 9 | Very light |
| 10 | |
| 11 | Fairly light |
| 12 | |
| 13 | Somewhat hard |
| 14 | |
| 15 | Hard |
| 16 | |
| 17 | Very hard |
| 18 | |
| 19 | Very, very hard |
| 20 | |

## APPENDIX G

**Power & Sample Size Analysis**

| **n = 7 (6 males & 1 female)** | Power Analysis* | Sample Size Calculation# |
|---|---|---|
| $VO_2$max | 0.078 | 19 |
| 5-minP $VO_2$ | 0.059 | 60 |
| 10-minP $VO_2$ | 0.071 | 25 |
| 30-minP $VO_2$ | 0.077 | 19 |
| 60-minP $VO_2$ | 0.096 | 11 |
| Stage 1 $CO_2$ | 0.057 | 42 |
| Stage 2 $CO_2$ | 0.096 | 12 |
| Stage 3 $CO_2$ | .253 | 3 |
| Stage 4 $CO_2$ | 0.088 | 14 |
| 5-minP $CO_2$ | 0.058 | 65 |
| 10-minP $CO_2$ | 0.094 | 12 |
| 30-minP $CO_2$ | 0.060 | 54 |
| 60-minP $CO_2$ | 0.074 | 22 |
| Max RER | 0.051 | 466 |
| 5-minP RER | 0.551 | 1 |
| 10-minP RER | 0.155 | 5 |
| 30-minP RER | 0.072 | 23 |
| 60-minP RER | 0.087 | 14 |
| 3-minE HR | 0.153 | 5 |
| 6-minE HR | 0.101 | 10 |
| 9-minE HR | 0.080 | 17 |
| 12-minE HR | 0.673 | 4 |
| End HR | 0.063 | 39 |
| 1-minP HR | 0.050 | 4024 |
| 3-minP HR | 0.051 | 603 |
| 5-minP HR | X | X |
| 10-minP HR | 0.053 | 170 |
| 30-minP HR | 0.096 | 12 |
| 60-minP HR | 0.052 | 331 |
| 3-minE RPE | 0.069 | 28 |
| 6-minE RPE | 0.064 | 36 |
| 9-minE RPE | 0.077 | 20 |
| 12-minE RPE | 0.053 | 186 |
| End RPE | 0.168 | 5 |
| Pre Urine | 0.102 | 10 |
| Post Urine | 0.054 | 124 |
| Pre Saliva | 0.052 | 333 |
| Post Saliva | 0.100 | 11 |

| **n = 6 (Males)** | Power Analysis | Sample Size Calculation |
|---|---|---|
| $VO_2$max | 0.071 | 21 |
| 5-minP $VO_2$ | 0.065 | 29 |
| 10-minP $VO_2$ | 0.11 | 8 |
| 30-minP $VO_2$ | 0.075 | 18 |
| 60-minP $VO_2$ | 0.063 | 34 |
| Stage 1 $CO_2$ | 0.080 | 15 |
| Stage 2 $CO_2$ | 0.108 | 8 |
| Stage 3 $CO_2$ | 0.252 | 2 |
| Stage 4 $CO_2$ | 0.105 | 13 |
| 5-minP $CO_2$ | 0.058 | 56 |
| 10-minP $CO_2$ | 0.117 | 7 |
| 30-minP $CO_2$ | 0.065 | 29 |
| 60-minP $CO_2$ | 0.051 | 548 |
| Max RER | 0.051 | 573 |
| 5-minP RER | 0.552 | 1 |
| 10-minP RER | 0.255 | 2 |
| 30-minP RER | 0.065 | 2 |
| 60-minP RER | 0.156 | 4 |
| 3-minE HR | X | X |
| 6-minE HR | 0.118 | 7 |
| 9-minE HR | 0.071 | 18 |
| 12-minE HR | 0.155 | 4 |
| End HR | 0.078 | 16 |
| 1-minP HR | 0.061 | 40 |
| 3-minP HR | 0.055 | 95 |
| 5-minP HR | X | X |
| 10-minP HR | 0.052 | 251 |
| 30-minP HR | 0.077 | 17 |
| 60-minP HR | 0.051 | 589 |
| 3-minE RPE | 0.071 | 21 |
| 6-minE RPE | 0.066 | 27 |
| 9-minE RPE | 0.071 | 22 |
| 12-minE RPE | 0.052 | 186 |
| End RPE | 0.369 | 1 |
| Pre Urine | 0.063 | 33 |
| Post Urine | 0.053 | 143 |
| Pre Saliva | 0.059 | 118 |
| Post Saliva | 0.158 | 4 |

$VO_2$= volume of $O_2$ consumption; P = post-exercise; Stage 1 = average of minutes 2-3; Stage 2 = average of minutes 5-6; Stage 3= average of minutes 8-9; Stage 4= average of minutes 11-12; E = during exercise; End = upon completion of exercise; Pre = 1-hour post-ingestion; Post = 1-hour post exercise; X = could not calculate; * $\alpha$ = 5%; # $\beta$ = 80%

# CIRRICULUM VITAE

**Amanda Lynn Jacobs**
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## Education

| | | |
|---|---|---|
| August 2006 – present | University of Louisville<br>M.S. Exercise Physiology<br>Expected to graduate in August 2008<br>GPA: 4.0 | Louisville, KY |
| August 2003 – May 2006 | University of Louisville<br>B.S. Health & Human Performance<br>Concentration: Exercise Science<br>Cumulative GPA: 3.76, Cum Laude | Louisville, KY |

## Professional Experience

### Graduate Teaching Assistant

August 2006- May 2008 University of Louisville Louisville, KY
Mentor: Dean Jacks, PhD

- Responsible for teaching two sections of HSS 180: First Aid & Safety Education each semester
- Assisted with data collection in various research projects
- Recruited subjects for research studies
- Served as a laboratory teaching assistant (TA) for HSS 396: Health Fitness Instructor Lab

### Academic Tutor

July 2007- May 2008 Olga S. Peers Academic Center Louisville, KY

- Provided academic support to student-athletes participating in the University of Louisville's intercollegiate athletic programs by promoting self-responsibility, personal growth and academic development.

**Internship**

January 2006- May 2006 The Physique Institute Louisville, KY

- Designed and conducted individualized exercise programs for clients
- Conducted health assessments for clients
- Instructed clients and members in aerobic and resistance training

**Certifications & Memberships**

American Red Cross

- Instructor – Adult/Child/Infant CPR and First Aid, AED

National Strength and Conditioning Association

- Certified Personal Trainer: 05-11-09-024

American College of Sports Medicine

- Member: 617949

**Manuscripts in Review**

Bibeau, W.S., Mitchell, N., **Lynn, A.**, Jacks, D.E., Topp, R., Fee, R., Moore, J.B. (Submitted July 2008). Physical activity moderates the effect of neighborhood stress on adiposity among ninth grade females. Submitted to *Journal of Physical Activity and Health.*

**Published Abstracts**

Bibeau, W.S., Mitchell, N., **Lynn, A.**, Jacks, D.E., Topp, R., Fee, R., Moore, J.B. Physical activity moderates the effect of chronic stress on adiposity among ninth grade females.

Bibeau, W.S., Moore, J.B., Mitchell, N., **Lynn, A.**, Jacks, D.E. Self efficacy moderates the effect of peer social support on physical activity in rural elementary school children. *Med & Sci. Sports Exerc.* 39(5):S87, 2007.

Jacks, D., Moore, J., Bibeau, W., **Lynn, M.** Over weight rural elementary school children have significantly higher blood pressure than normal weight children. *Med & Sci Sports Exerc.* 39 (5):S166, 2007

**Presentations**

Bibeau, W.S., Mitchell, N., **Lynn, A.**, Jacks, D.E., Topp, R., Fee, R., Moore, J.B. Physical activity moderates the effect of chronic stress on adiposity among ninth grade females. Annual meeting, The Obesity Society, New Orleans, LA. 2007.

Bibeau, W.S., Mitchell, N., **Lynn, A.**, Cerrito, P., Moore, J.B. The relationship between sedentary behaviors and adiposity among rural children. Annual meeting, Southeast American College of Sports Medicine, Charlotte, NC. 2007.

Bibeau, W.S., Moore, J.B., Mitchell, N., **Lynn, A.**, Jacks, D.E. Understanding the influence of psychosocial factors on weight status through physical activity. Annual meeting, American College of Sports Medicine, New Orleans, LA. 2007.

**Lynn, A.**, Bibeau, W.S., Mitchell, N., Jacks, D.E., Moore, J.B. Self-efficacy moderates the effect of peer social support on physical activity in rural elementary school children. Annual meeting, Southeast American College of Sports Medicine, Charlotte, NC. 2007.

Bibeau, W.S., Mitchell, N., **Lynn, A.**, Cerrito, P., Moore, J.B. The predictive value of sedentary activity on adiposity among rural children. Research Louisville, University of Louisville, Louisville, KY. 2006.

Mitchell, N., Bibeau, W.S., **Lynn, A.**, Moritz-Rudasill, K., Moore, J. The self-concept of rural elementary school children: contributions of adiposity and fitness. Research Louisville, University of Louisville, Louisville, KY. 2006.