

L-GLUTAMINE SUPPLEMENTATION: EFFECTS ON RECOVERY FROM
EXERCISE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

In

The Department of Kinesiology

by
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B.S., Louisiana State University, 1999
August, 2005

ACKNOWLEDGEMENTS

There have been numerous roadblocks during my path to complete this project and the thought of not continuing to pursue this has crossed my mind on several occasions. Without the help of the following people, completing this project would have not been possible.

I would first like to thank Dr. Arnold Nelson and Dr. Robert Wood for serving as members of the committee and offering their professional guidance.

To my parents Thomas Piattoly and Deborah Piattoly Reeves who gave me the opportunity to where and who I am today. Without their guidance and assistance, this wouldn't be possible.

To my wife Lindsey Ware Piattoly, whose patience, persistence, and constant motivation helped me see the light ahead of the tunnel. I love you.

Finally, I would like to thank Dr. Michael Welsch, who gave me a chance to prove myself, although I didn't have on paper, the requirements to be accepted as a graduate student. Thank you for believing in me, guiding me, and having the patience to keep me moving forward. None of this would have been possible without you. I can't thank you enough.

This thesis is dedicated to my late father, Thomas Joseph Piattoly who always emphasized the importance of education. I love you and know you would be proud.

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ABSTRACT

Clinical evidence supports the use for exogenous glutamine in the maintenance of muscle mass and immune system function in critically ill patients. Relatively little research has examined the benefits of glutamine for athletes engaged in heavy exercise training, despite a possible link between overtraining and glutamine. **PURPOSE:** To examine the influence of Glutamine on time to exhaustion and power after a prolonged bout of exercise. **METHODS:** Twelve men (Age: 19 to 30y) involved in cycle training were asked to participate in the study. All participants performed a Symptom-Limited Graded Exercise Test (SL-GXT) using the Astrand Cycle protocol. On a subsequent visit participants performed two Wingate tests on a cycle ergometer to assess Peak Power, Mean Power, and Fatigue Index. The tests were separated by an exhaustive bout of exercise at 70% of VO₂R. Twenty-four hours later another Wingate test was performed. Immediately after performing the last Wingate test, subjects were randomized to: 1. Glutamine plus carbohydrate drink (0.3 grams/kg of body weight/ for 6 days) or 2) Placebo (Carbohydrate drink). After 6 days the Wingate and exhaustive bout of exercise were repeated in each individual. **RESULTS:** There were no group differences in VO₂peak (Glu: 44.53±8.75; Pla: 43.83±5.26 ml/kg/min), PP (Glu: 717.71±118.90; Pla: 593.66±117.08), TR (Glu: 38.50±2.26; Pla: 35.50±5.65) and time to exhaustion (Glu: 46.33±10.80; Pla: 41.90±3.82 ml/kg/min) before supplementation. Both groups showed a significant drop in PP (-27%, p=0.001), and TR (-22%, p=0.001) after the exhaustive exercise bout. Incomplete recovery was noted at 24h PP (-17%, p=0.03 vs. baseline), and TR (-13%, p=0.09 vs baseline). Following supplementation TE improved by 3.16±0.75min in the Glu group compared to no change in the Pla (p=0.001). Lastly,

the Glu group had similar PP prior to each exhaustive bout of exercise, the PP in the Pla group was still significantly lower after 6 days. CONCLUSION: Participants in the Glu group increased time to exhaustion following 6 days of supplementation, and appeared to recover from exhaustive exercise earlier than the Pla group.

CHAPTER 1

INTRODUCTION

After an intense bout of exercise, whether aerobic (i.e. marathon) or anaerobic (weight lifting), sufficient rest and recovery is required for the particular muscle(s) utilized. It is that period of recovery that may have the greatest influence on subsequent bouts of exercise. For the exercised muscle to sufficiently recover, a 48-72 hour rest period is recommended. On the other hand, athletes such as football players and cyclists, who perform subsequent bouts of intense physical exercise, train a particular muscle two to three times a week in order to achieve and enhance training goals (i.e. increase muscular strength and endurance, muscle mass). With these athletes, it is not unusual for them to turn to dietary supplements.

1.1 Dietary Supplementation and Ergogenic Aids

Ergogenic refers to the application of a nutritional, physical, mechanical, psychologic, or pharmacologic procedure or aid to improve physical work capacity or athletic performance (McArdle, W., Katch, F., and Katch, V., 1996). Amphetamines, carbohydrates, hormones, proteins, amino acids, steroids, caffeine, additional red blood cells, phosphates, and music are just a few of the aids that have been examined in the literature to determine if a possible ergogenic benefit occurs. Athletes use a few of these aids routinely, and only a few cause real controversies.

Many male and female athletes use a variety of dietary supplements in the belief that a specific drug will have a positive influence on skill, strength, power, or endurance. At some point during an exercise program, whether training to increase muscular strength, cardiovascular performance, or improve recovery from an intense bout of

exercise, an individual may reach a performance plateau. With the increasing demands placed on elite and often on young athletes to achieve high levels of exercise performance, manipulation of diet through nutritional supplementation has increased. For several years, nutritional strategies such as overfeeding and ingesting carbohydrate/protein before and after exercise have been the foundation for enhancing exercise performance and muscular strength. On the other hand, in the past decade, dietary supplements have emerged and has become the cornerstone for improving performance. Some dietitians and exercise scientists believe an adequate diet, which meets the recommended dietary allowance (RDA) for macronutrients, vitamins and minerals, is sufficient to achieve top performance. However, other experts believe that supplementation is necessary to restore specific nutrient, vitamin, or mineral levels that may be suppressed during prolonged or intense exercise. For example, Vitamin E supplementation before exercise has shown to maintain normal values that usually decrease during prolonged endurance exercise, prevents free radical damage during intensive exercise, and decreases serum creatine kinase levels, a measure of muscle damage (Rokitzi, L., Logemann, E., Sagvedos, A., Murphy, M., Roth, W., and Kuel, J, 1994). In addition, there's an abundance of evidence that plasma values of the amino acid glutamine fall substantially during and/or after very prolonged exercise (Parry Billings, M., Budget, R., and Koustedakis, Y., 1992, Rennie, M., Tadros, L., Khogli, S., Ahmed, A., and Taylor, P., 1994, and Walsh, N., Blannin, K., Robson, P., and Gleeson, M., 1998).

During the last few years, the emergence of creatine and nutritional formulations containing creatine, have been the most popular nutritional strategies employed by

resistance-trained athletes to promote gains in strength and fat-free mass. The rationale is that creatine supplementation (20-25g/day for 4 to 7 days then 2 to 25 g/day) has been reported to increase total body mass, (Becque, B., Lochmann, J., and Melrose, D., 1997, Earnest, C., Snell, P., and Rodriguez, R., 1995, Greenhaff, P., Bodin, K., and Soderlund, K., 1994, and Kreider, R., Ferreira, M., and Wilson, M., 1998, Kreider, M., Klesges, R., and Harmon, K., 1996), fat-free mass, (Greenhaff, P., Casey, A., and Short, A., 1993, Kirksey, K., Warren, B., and Stone, M., 1997, and Kreider et al. 1996) single-effort and/or repetitive sprint capacity, (Balsom, P., Soderlund, K., Sjoding, B., 1995, Becque et al. 1997, Earnest et al. 1995, and Kreider et al. 1996.), strength and/or power, (Volek, J., Kraemer, W., and Bush, J., 1997), and work performed during sets of maximal effort muscle contraction. Recently, (Cottrell, G., Coast, J., and Herb R., 2002), it has been discovered that 6 days of creatine supplementation (0.3 g/kg/day) was sufficient to increase mean power when the between bout recovery interval was 3 minutes or less.

In addition to creatine supplementation, glutamine is a common ingredient currently found in many of the weight-gain supplements marketed to athletes and has been considered to increase muscular strength and improve recovery. Even though popular to many college and professional athletic organizations, there has been a lack of research examining the effects of glutamine supplementation on muscular strength, exercise performance, and recovery from exercise.

1.2 Glutamine Metabolism

Glutamine, originally classified as a non-essential amino acid, is the most abundant amino acid in plasma as well as the skeletal muscle (Antonio, J., and Street, C., 1999, Kreider, R., 1999, Rowbottom, D., Keast, D., and Goodman, C., 1995, and

Watford, M., Darcey-Vrillon, B., and Duee, P., 2000). However, more recently it has been considered that glutamine is “conditionally essential” (Lacey, J. and Wilmore, M., 1990) particularly after clinical trauma such as major surgery or burns where a marked decrease in the concentration of plasma glutamine occurs and is often maintained for several days (Parry Billings et al. 1992). Glutamine accounts for more than 60% of the total intramuscular free amino acid pool, and because skeletal muscle represents such a large mass of tissue, it is the most important site for glutamine synthesis.

1.3 Relationship between Glutamine and Exercise

It is considered that periods of severe stress such as burns, infection, trauma, major surgery, and sepsis can cause a significant reduction in skeletal muscle and plasma glutamine concentration. One might consider an intense bout of exercise (heavy workload resistance training, marathon, prolonged cycling) a “stressful event”. During exercise, increases and decreases in plasma glutamine levels have been demonstrated and these variations are reflected upon the type, duration, and intensity of exercise. A number of studies have shown an increase in plasma glutamine level following brief (< 1 hour) high intensity exercise in humans (Babij, P., Matthews, S., and Rennie, M., 1983, and Eriksson, L., Broberg, S., and Bjorkman, O., 1985). On the other hand, after prolonged exhaustive exercise, such as a marathon, a significant decrease in plasma glutamine has been observed during and post exercise (Parry-Billings, M., Evans, J., Calder, P., and Newsholme, E., 1990, and Castell, L., Poortmans, J., and Newsholme, E., 1996). This decrease is relatively transient, lasting approximately 6-9 hours after a marathon. Other studies have demonstrated similar effects to plasma glutamine with levels returning to baseline within 2-3 hours post-exercise and others reporting plasma glutamine levels

below normal at 24 hours post-exercise (Decombaz, J., Reinhardt, P., Anantharaman, K., Von Glutz, G., and Poortmans, J., 1979). It has yet to be determined whether plasma glutamine levels decrease during repeated bouts of anaerobic (Sprinting) or aerobic (Tour de France) exercise, possibly decreasing recovery time to exercise leading an athlete or individual into an over-trained state. In over-trained individuals, also known as the Over-Training Syndrome (OTS), recurrent infections, fatigue, impaired immune function, and reduced exercise performance have been observed (Walsh, N., Blannon, K., Robson, P., and Gleeson, M., 1998). Consequently, glutamine levels can be significantly reduced, remaining low for several weeks (Parry Billings et al. 1990). Such athletes' increased susceptibility to infection may result from impaired immune function caused by prolonged low levels of plasma glutamine due to intense or prolonged exercise training. Furthermore, the reduction in plasma glutamine levels following very prolonged exercise may result from an increased demand and uptake of glutamine by the tissues of the body that require it (skeletal muscle, adipose tissue, liver, kidney, and immune cells). The fall in plasma glutamine could be due to a combination of increased uptake and decreased production/altered transport kinetics (Walsh et al., 1998).

The links between a decrease in plasma glutamine during exercise and an increased risk of infection (Budgett, R., 1990, Newsholme, E., and Parry-Billings, M., 1990, Rowbottom et al., 1995, Rowbottom, D., Keast, D., and Morton, A., 1996, and Walsh et al. 1998) could pose a need for exogenous glutamine supplementation to stimulate faster recovery time, prevent OTS, and allow an individual to participate in multiple bouts of exercise more frequently and prolonging time to fatigue.

1.4 Glutamine and the Immune System

As previously mentioned, skeletal muscle is believed to be the primary source of glutamine released to the bloodstream and this release may play an important role in delivering glutamine to the immune system. During catabolic states such as trauma, major surgery, sepsis, and infection, all which are associated with muscle wasting, plasma glutamine levels are insufficient to meet increased demands by the skeletal muscle and other tissues that utilize glutamine. The stressed catabolic patient has a compromised immune system (Newsholme, E., Newsholme, P., and Curi, R., 1987). Many investigations have shown that during severe stress the consumption of glutamine exceeds glutamine synthesis, resulting in depletion of glutamine stores (Lacey, J. and Wilmore, M., 1990, and Rennie, M., 1985). It also may be that during prolonged, high intense exercise, severe stress is imposed upon the skeletal muscle, possibly compromising the immune system, and requiring exogenous glutamine supplementation. It is known that glutamine is a key substrate for cells of the immune system particularly lymphocytes (Ardawi, M. and Newsholme, E., 1983) and macrophages, and monocytes (Ardawi M. and Newsholme, E., 1985) which utilize glutamine for fuel at very high rates. Glutamine metabolism in immune cells fulfills two major components, as an energy source via oxidation, and also as a precursor for purines and pyrimidines in nucleotide synthesis- essential to cell replication (Ardawi, M. and Newsholme, E., 1985). These nucleotides are needed for the synthesis of new DNA and RNA during proliferation of lymphocytes, and for mRNA synthesis and DNA repair in macrophages. A decrease in glutamine concentration below normal in human plasma not only decreased the maximum rate of proliferation in response to mitogenic stimulation in peripheral blood

lymphocytes but also slowed the response time (Parry Billings et al. 1990). Castell and Newsholme (1998) concluded that circulating lymphocytes and neutrophils were restored to baseline levels the next morning in marathon runners who supplemented with glutamine compared to the placebo group, in whom they were still slightly elevated.

Given that the concentration of plasma glutamine decreases during and after prolonged, high intense exercise, and in overtrained individuals, the question arises whether muscle, together with other tissues, can respond sufficiently to release enough glutamine to maintain the normal blood concentration. This may be of importance especially during repeated bouts of intense exercise, which may prevent sufficient recovery time. Recovery time is essential for maximizing muscular strength and improving performance. In addition, this would be particularly important in the event of muscle damage during excessive exercise. Damage to muscle fibers results in an inflammatory response that causes a transfer of fluid and cells to the damage tissue (Clarkson and Sayers, 1999). An increase in fluid produces swelling after injury. Furthermore, muscle damage may present an area of tissue to which immune cells might migrate. Neutrophils constitute 60% of the circulation leukocytes and are speculated to be the first cells to infiltrate damaged muscle fibers (Abrams, 1997, and Smith, 1991). They act as first line of defense cells in the blood and at sites of infection. As the number of immune cells increase, activity increases and/or proliferation of some cells which, in turn, increases local demand for glutamine. It is reasonable to suggest that the provision of glutamine may elicit a positive affect if ingested before or after an intense bout or repeated bouts of high-intensity exercise to enhance recovery. As a result of supplementation, glutamine will be available to important fuel sites (skeletal muscle,

immune cells), plasma and intramuscular levels that require it during exercise, possibly decreasing muscle fatigue and wasting, increasing recovery time, therefore, enhancing exercise performance.

1.5 Clinical Significance of Glutamine

Under normal dietary conditions, very little of the glutamine derived from dietary protein enters the bloodstream. The epithelial cells of the intestine will consume much of the dietary derived glutamine and utilize it as respiratory fuel (Newsholme, 2001).

Evidence that both parental and enteral glutamine feeding can have beneficial effects on gut function and/or the immune system in humans has been clinically demonstrated in at least 16 randomized, blind, controlled clinical trials. During periods of catabolic stress, plasma glutamine levels are depressed and muscle wasting occurs. After a high intense, prolonged bout of exercise, one can conclude that damage to the skeletal muscle occurs.

Exercise induced muscle damage to muscle fibers results in an inflammatory response.

During inflammatory states, the consumption of glutamine in immunologic tissues and cells increases. This increase in consumption, coupled with enhanced utilization by other tissues, results in a demand for glutamine that outstrips supply (Newsholme, E., 2001).

As a result, blood, immunologic tissue and muscle glutamine levels fall. It is possible that exogenous supplementation of glutamine may decrease the severity of the inflammatory response, resulting in less muscle damage and possibly enhancing muscle recovery.

1.6 Justification for Research

As previously mentioned, exhaustive exercise can cause significant physiological changes in the human body. One of major importance, especially to athletes who engage

in repeated bouts of exercise, is muscle damage and recovery. Whether glutamine can enhance recovery from an intense, prolonged bout of exercise in humans is yet to be determined. It is possible that exogenous supplementation of glutamine may decrease the severity of the inflammatory response by serving as a fuel source for cells of the immune system, resulting in less muscle damage and possibly enhancing muscle recovery.

1.7 Purpose of the Study

The purpose of the study is to examine the influence of glutamine supplementation on muscle recovery and exercise performance. Specifically:

- (1) To examine the influence of glutamine on time to exhaustion
- (2) To examine the influence of glutamine on peak power (PP)
- (3) To examine the influence of glutamine on recovery
- (4) To examine the influence of glutamine on cardiovascular parameters, specifically blood flow (BF) and heart rate variability (HRV)

1.8 Research Hypothesis

Based on the available data, the following hypotheses are derived:

- 1) Exercise performance as defined by Fatigue Index (FI) will not be further enhanced as a result of glutamine supplementation.
- 2) Peak Power, as defined by the highest power output generated during any 3-5 second period of the test will not be further enhanced as a result of L-glutamine supplementation.
- 3) Cardiovascular parameters, as defined by BF and HRV, will not be altered as a result of glutamine supplementation.

1.9 Limitations

This study is limited by the following:

- 1) Subjects will not all receive different dosages of glutamine based on g/kg of body weight;
- 2) Subjects ranging in age from 18-30;
- 3) Subjects are all male;
- 4) The training regimen among subjects will be different which may influence Peak power or Fatigue Index;
- 5) Subjects dietary intake will vary for carbohydrate, fat, and protein;
- 6) Past history of supplementation by subjects may influence changes in exercise performance and peak power;
- 7) Subjects were not controlled for caloric intake or energy balance during the 6 day supplementation program

1.10 Significance of Research

Dietary supplements are a popular aid in a variety of athletic sports. Competitive and recreational athletes have long been known to try nutritional supplements in an attempt to improve their performance. With the lack of data indicating whether or not glutamine supplementation has the potential to improve muscle recovery after an intense bout of aerobic exercise to fatigue and enhance cardiovascular performance, more research is needed to determine its potential

CHAPTER 2

REVIEW OF LITERATURE

2.1 Review of Glutamine Metabolism

Glutamine is the most abundant amino acid in plasma as well as skeletal muscle and accounts for more than 60% of the total intramuscular free amino acid pool (Antonio, J. and Street, C., 1999, Kreider, R., 1999). It is also a precursor for the synthesis of amino acids, proteins, nucleotides, and many other biological molecules (Smith, R., 1990). Glutamine can be synthesized from glutamate and ammonia by the enzyme glutamine synthetase, and it can be degraded by the enzyme glutaminase (Bernadette, A., Van Acker, C., Maarten, F., Von Meyenfledt, Rene, R., Van der Hulst, W., Karel, W., Hulsewe, E., Anton, J., Wagenmakers, M., Nicolaas, E., Deutz, P., Ivo de Blaauw, Cornelis, H., Dejong C., Bernard, K., Van Kreel, and Soeters, B., 1999, and Rowbottom, D. et al., 1995). Glutamate is formed from alpha-ketoglutarate, an intermediate of the Krebs cycle, and ammonia. The formation of glutamine requires energy (ATP), glutamate, ammonia, and a phosphate-dependent glutamine synthetase (Lacey, J. and Wilmore, M., 1990). Since skeletal muscle represents such a large mass of tissue, it is the most important site of glutamine synthesis despite the fact that glutamine synthetase activity is relatively low per unit mass in skeletal muscle (Antonio, J. and Street, C., 1999). Glutamine is a neutral amino acid with a plasma concentration of 500 to 600 mol/L and an intramuscular concentration of 15 to 20 mmol/L (Rennie, M., Ahmed, A., Khogli, S., Low, L., Hundal, H., and Taylor, P., 1996, and Rowbottom, D. et al., 1995). It is found in relatively high levels in many human tissues and contains two nitrogen atoms making it the most significant nontoxic nitrogen transporter in the body. In certain

conditions, glutamine accounts for over 80% of all amino acid nitrogen transported in the blood (Bernadette, A. et al., 1999). In addition, glutamine is the most important substrate for ammoniogenesis, not only in the gut but also in the kidney, allowing it to play an important role in the regulation of acid-base homeostasis (Bernadette, A. et al., 1999).

Glutamine synthesis involves many major organs in the body. The primary synthesizer is skeletal muscle, but other organs include the lungs, brain, and possibly adipose tissue. In addition to its involvement with these organs, glutamine is utilized primarily as fuel by tissues such as the small intestine, immune system (neutrophils, thymocytes, lymphocytes, and macrophages), and hair follicles. Also, the gastrointestinal tract accounts for 40% of the total glutamine utilized by the body (Antonio, J. and Street, C., 1999). In the liver, glutamine is used for glucose and urea synthesis whereas the brain utilizes glutamine as a precursor for neurotransmitter substances (Antonio, J. and Street, C., 1999).

2.2 Glutamine Metabolism and Transport in Skeletal Muscle

The store of free glutamine within the skeletal muscle is considerable, estimated at 20 mmol/L of intracellular water, which accounts for the majority of the body's total glutamine stores (Rennie, M. et al., 1994, and Rowbottom, D. et al., 1995). Skeletal muscle and possibly adipose tissue are the most significant sources of glutamine. Also, the skeletal muscle is the primary source of glutamine released to the bloodstream which may play an important role in delivering glutamine to the immune system (Keast, D., Cameron, K., and Morton, A., 1988, and Newsholme, E. and Parry-Billings, M., 1990). The ability of the skeletal muscle to control the conversion of amino acids into glutamine represents part of a physiological mechanism whereby glutamine is available for the

regulation of acid/base balance and as fuel for various cells (Antonio, J. and Street, C., 1999). Although the liver can oxidize most of the 20 amino acids, skeletal muscle has shown that it can only oxidize 6 amino acids, that is, the branched chain amino acids (leucine, isoleucine, valine) aspartate, asparagine, and glutamate (Antonio, J. and Street, C., 1999).

Not only does muscle tissue maintain a large intracellular store of free glutamine, skeletal muscle has very high glutamine transport and synthesis rates at physiological levels (Rowbottom et al. 1995). These rates of synthesis and transport of glutamine in the skeletal muscle are influenced by the glucocorticoids. For example, muscle glutamine synthetase activity is increased following glucocorticoid treatment. Glucocorticoids are known to be elevated during periods of catabolic stress and have been reported to mediate changes by: (a) increasing glutamine afflux from skeletal muscle in rats and humans; (Darmaun et al. 1994, Darmaun et al. 1988, Leighton et al. 1991, Rennie et al. 1989, Rowbottom et al. 1995) (b) increasing glutamine synthesis activity; (Hundal et al. 1991) and mRNA expression; (c) decreasing intramuscular glutamine stores; (Rowbottom et al. 1995) and (d) changing glutamine transporter kinetics such that glutamine afflux could still occur maximally at lowered intramuscular glutamine levels (Hundal et al. 1991, Rowbottom et al. 1995). These observations of glutamine transport and synthesis activity are both inducible under certain conditions, particularly catabolic stress, indicating that the skeletal muscle is capable of increasing the rate of release and synthesis of glutamine in response to increased demand from other organs and tissues of the body.

Not only do glucocorticoids influence the rate of synthesis and release of glutamine, a number of amino acids contribute a stimulatory effect on glutamine

synthesis and release. As shown in **Fig. 2.1**, the branched-chain amino acids (leucine, isoleucine, and valine), along with tyrosine, cystine, and lysine appear to stimulate the release of glutamine, whereas the amino acids threonine, proline, and ornithine have a stimulatory affect on the synthesis, but not the release of glutamine. It is likely that added amino acids must stimulate glutamine synthesis by mechanisms other than by influencing the supply of only a single precursor such as glutamate alone, because reciprocal decreases in the release of other precursors did not occur (Garber, 1980). These conclusions are based on studies with added glutamate alone, therefore exhibiting that added amino acids most likely stimulate glutamine synthesis by supplying both the amino groups as well as a portion of the carbon skeleton required for ongoing glutamine synthesis in the skeletal muscle.

As glucocorticoids and amino acids impose stimulatory effects on glutamine synthesis and release, glycogenolytic agents, such as epinephrine, inhibit glutamine output from the skeletal muscle. Shown in **Fig. 2.2** are the effects of epinephrine on glutamine, alanine, and glutamate release from skeletal muscle preparations in vitro (Garber, 1980). This effect was reproduced by isoproterenol (10^{-6} M), suggesting the participation of a (α -adrenergic receptor for this effect. Also, Garber (1980) concluded that (α -adrenergic antagonists such as phentolamine do not block these effects of epinephrine but that (α -adrenergic antagonists such as propranolol completely block adrenergic inhibition of muscle glutamine formation and release. The finding of (α -adrenergic receptor participation in adrenergic inhibition of glutamine output from skeletal muscle is similar to the receptor requirement for adrenergic regulation of muscle glycogenolysis (Garber, 1980). The skeletal muscle adenylyl cyclase-cAMP system may

also participate in this inhibition. Concentrations of epinephrine causing inhibition of glutamine production from skeletal muscle and activation of muscle glycogenolysis also activated skeletal muscle membrane adenylyl cyclase activity and caused the accumulation of increased intracellular levels of cAMP (Garber, 1980). Maximal stimulation of adenylyl cyclase activity was found at 10^{-5} M epinephrine, a concentration producing maximal inhibition of glutamine output from skeletal muscle (Garber, 1980). These observations support the concept that the (α -adrenergic mechanism regulating muscle glycogen homeostasis also participates in the mechanism of adrenergic inhibition of glutamine synthesis and release from skeletal muscle.

2.3 Glutamine and Enhanced Protein Synthesis/Decreased Protein Degradation

Since skeletal muscle accounts for most of the protein pool in the body, the regulation of protein metabolism in skeletal muscle is important for whole-body protein homeostasis (Antonio, J. and Street, C., 1999). Glutamine serves a significant role for the regulation of muscle protein level (MacLennan, P et. al., 1987) used an isolated perfused rat hindquarter model to examine the effect of glutamine on muscle metabolism. They found that increasing the concentration of glutamine significantly increased intracellular glutamine and protein synthesis in the absence of insulin. It was concluded that modulation of intracellular glutamine concentration could influence the protein balance in muscle, possibly through control of the membrane-transport process of glutamine. Also, an *in vitro* study showed that glutamine augments protein synthesis in myotubes that are under heat-stressed conditions, whereas there was no effect on myotubes under normal conditions (Antonio and Street, 1999). In addition, glutamine has an anti-proteolytic effect on the non-contractile protein component of skeletal muscle (MacLennan et. al).

When comparing the effects of glutamine versus glycine in humans, an enteral infusion of glutamine increased protein synthesis.

The hydration state of cells is a critical factor that may influence metabolic processes within a cell. An increase in cellular volume or hydration status acts as an anabolic signal while a decrease in cellular volume acts as a catabolic signal (Haussinger et al. 1994). Glutamine may exert an anti-catabolic effect by mediating increases in cellular volume.

Finally, the depletion of intramuscular glutamine is associated with increased muscle catabolism (Antonio and Street, 1999). In order to maintain skeletal muscle size, it is significant to maintain these stores. For example, during times of illness and stress, the release of muscle glutamine is accelerated by glucocorticoids. Falduto et al. (1992) showed that glucocorticoid administration for 4 days reduced plantaris and quadriceps muscle mass to 90% of control values. These values were accompanied by a threefold increase in glutamine synthetase mRNA and enzyme activity in the deep quadriceps (fast-twitch red) muscle. Exercise training resulted in a protective effect on skeletal muscle mass as well as a reduction in the effects of glucocorticoid treatment on glutamine synthetase mRNA and enzyme activity (Falduto et al., 1992).

2.4 Glutamine and the Immune System

As previously mentioned in Chapter 1, severe stressors such as burns, surgery, sepsis, prolonged exercise, and athletic overtraining cause a significant reduction in skeletal muscle and plasma glutamine concentration. Hence, glutamine serves as an important fuel source for lymphocytes macrophages, and possibly natural killer (NK) cells and it is thought that during times of stress or illness, glutamine metabolism is

increased in order to promote rapid cell division and antibody production (Antonio and Street, 1999, Rowbottom et al. 1995, Walsh et al., 1998). Also, glutamine is needed for the process of wound healing. Without adequate glutamine, lymphocyte proliferation diminishes, as does the synthesis of interleukin-1 by macrophages and interleukin-2 by lymphocytes (Newsholme and Calder, 1997). While lymphocytes have a high intracellular activity of glutaminase, they do not have any significant activity of glutamine synthetase (Rowbottom et al., 1995). Consequently, cells of the immune system rely on the supply of glutamine in the plasma to meet their metabolic needs, which are to be delivered by the release of glutamine from skeletal muscle.

Following intense exercise of more than 1 hour, the lymphocyte count, the NK cell activity, and the lymphokine activated killer (LAK) cell activity declines (Rhode et al., 1998). The mechanisms underlying exercise-induced immuno-modulation are probably multi-factorial and may include changes in adrenaline, noradrenaline, growth hormone, and cortisol. In addition, it has been shown that plasma glutamine concentration declines after intense exercise and a lack of glutamine has been suggested to play a role in the impaired immune function after sustained physical activity (Rhode et al., 1998). Parry Billings et al. (1992) have shown that reductions in glutamine levels below 600 mol/L are associated with reduced RNA synthesis, IL-2 production, immunoglobulin synthesis and proliferative responses to mitogens in lymphocytes, and a decreased rate of phagocytosis in macrophages (Walsh et al., 1998). The normal range of plasma glutamine in apparently healthy humans is 500 to 750 mol/L but values as low as 200 mol/L have been reported in cases caused by burns and sepsis. More recent studies on the glutamine requirements of leukocytes indicate that transcription of early activation

markers in lymphocytes occurs even in the absence of glutamine, but later events depend on the provision of exogenous glutamine in a dose dependent manner (Walsh et al., 1998). These findings provided some evidence that a decrease in plasma glutamine levels associated with catabolic stress states such as burns, sepsis, surgery, and trauma may be at least partly responsible for the associated impairment of immune function. Further studies and findings involving exercise will discuss specific effects on plasma glutamine levels and how prolonged exercise and over-training could be associated with the reduction of plasma glutamine and impairment of the immune system.

2.5 Glutamine and Exercise

The effects of exercise on glutamine metabolism are not well established. However, several studies do examine the effects of exercise on plasma glutamine level. Comparable exercise stress has been undertaken in exercise trials when attempting to relate the results from different studies. Without these studies, precaution comparisons cannot be drawn with regard to changes in plasma glutamine levels or any other physiological parameter. This requires that relative exercise intensity, the duration of the exercise, the mode of the exercise, and the active muscle mass involved all be taken into consideration. The studies in this review have been divided into five categories for comparative purposes: Acute duration, high intensity, prolonged duration, interval exercise, and very prolonged duration, all primarily aerobic.

2.5.1 Plasma and Tissue Glutamine Changes During Acute Exercise and Recovery from Exercise

Keast et al. (1995) induced over-training by having subjects exercise twice a day for 10 days, followed by a 6-day recovery period. It was found that plasma glutamine concentrations decreased in 4 of 5 subjects by the 6th day of over-training, with all of

them experiencing a significant decline by the 11th day. After the 6th day of recovery, two of the subjects had still not returned to normal plasma levels of glutamine. Castell et al. (1996) examined athletes who had consumed glutamine versus a placebo immediately after and 2 hours after a marathon or ultra-marathon running race. During the follow-up period 7 days post-exercise, the glutamine group had a greater percentage of individuals who reported no infections, 81% versus 49% for the placebo group.

2.5.2 Plasma Glutamine Levels During High Intensity Exercise

When compared to the data available for acute exercise, there is a lack of data for high intensity exercise effects on plasma glutamine levels. A few studies have shown an increase in plasma glutamine levels following brief (<1 hour) high intensity exercise in humans (**Table 2.1**). For example, Babij et al. (1983) observed increases from 575 mol/L at rest to 734 mol/L during exercise at 100% VO₂ max. Eriksson et al. (1985) found plasma glutamine levels increased from 538 to 666 mol/L during 45 minutes of incremental exercise at 80% VO₂max. These findings were supported by Katz et al. (1986) who reported elevation of plasma glutamine from 555 to 699 mol/L following 4 minutes of exercise at 100% VO₂ max. These increases in plasma glutamine levels during exercise suggest that glutamate acts as a sink for NH₃ in the formation of glutamine during enhanced NH₃ production in exercise. During a period of brief fatiguing high intensity exercise, it is likely that most of the increased ammonia production arises from increased breakdown of adenine nucleotides (Meyer and Terjung, 1979).

Table 2.1. Plasma Glutamine Concentration Following Exercise in Humans

Reference	Study participants	Exercise intensity and duration	Change in plasma glutamine level ^a (%)	
			<u>immediately after exercise</u>	<u>during recovery</u>
<i>Continuous or intermittent high intensity exercise</i>				
Parry-Billings et al.	10 RA	10 x 6 sec treadmill sprint	↑↓11	
Sewell et al.	9 RA (2F)	60 sec treadmill run At 20 km/h	↑ 5	return to baseline at 5min after
		20 km/h treadmill run to exhaustion	↑ 14	
Robson et al.	18 TM	Cycling to exhaustion at 80% VO ₂ max	↑3	↑2 at 5 h
Katz et al.	8 RA M	Cycling to exhaustion at 100% VO ₂ max	↑ 26	↑ 13 at 10 min
Van Hall et al.	8 T M	3 min cycling at 50% W _{max} and 6min at 80% W _{max} alternated to exhaustion	↓ 9	↓ 16 at 2h
Van der Schoor et al.	8 T M	2 min cycling at 90% W _{max} alternated with 2 min at 50% W _{max} until exhaustion	↓ 20	↓ 24 at 2h
Keast et al.	7 T M	15 x 1 min treadmill exercise at 90% VO ₂ max	↓ 44	
		15 x 1 min treadmill exercise at 120% VO ₂ max	↓ 55	
Walsh et al.	8 T M	20 x 1 min cycling at 100% VO ₂ max	↓ 2	↓ 16 at 5h
<i>Prolonged light-moderate intensity exercise</i>				
Parry Billings et al.	22 T	(20M) Marathon 150min	↓ 16	
	12 T M	30 km self-paced treadmill run	↑ 8	
	4 T M	Cycling to exhaustion at 73% VO ₂ max)	↑ 8	

(table continued)

Rennie et al.	4 RA M	3.75h cycling at 50% VO ₂ max	↓ 16	↓30 at 2h
Babij et al.	8 RA M	10 min incremental Cycling at 25, 50, and 75% VO ₂ max and to Exhaustion	↑ 28	returned to baseline at 10min
Eriksson et al.	11 RA M	45 min incremental Cycling to 75% VO ₂ max	↑ 24	↑ 12 at 1 h
Maughan & Glesson	5 RA M	90 min cycling at 70% VO ₂ max	↑3	
Robson et al.	18 T M	Cycling at 55% VO ₂ max for 180 min	↓11	↓23 at 1 h
Decombaz et al.	8 T M	100km run	↓ 16	↓ 7 at 24 h
Rohde et al.	8 T M	2500m swim 81 km cycle 19 km run	↓ 20	↓ 32 at 2h

Percentage difference from pre-exercise plasma glutamine level

F= female; **M**= male; **RA**= recreationally active but not specifically endurance trained; **T**= trained;

Wmax= maximal work rate attained during an incremental exercise test; = increased; = decreased

2.5.3 Plasma Glutamine Changes During Prolonged Exercise and Recovery from Exercise

In contrast to high intensity exercise, there is an abundance of evidence that plasma glutamine levels fall substantially during and/or after very prolonged exercise (**Table 2.1**). Rennie et al. (1994) monitored plasma glutamine for 4.5 hours following 3.75 hours of cycling at 50% VO₂max. A fall from 557 mol/L at rest to 470 mol/L immediately after exercise was reported. After 2 hours' recovery, plasma glutamine levels had fallen to 391 mol/L. After 4.5 hours of recovery, plasma glutamine had still not returned to its resting level while measuring at 482 mol/L. Parry-Billings et al. (1992) reported significant falls in plasma glutamine level following a marathon race from 592 mol/L (pre-race) to 495 mol/L (post-race) in 24 club standard athletes. However, both a 30 km treadmill run and a cycle ride to exhaustion at 73% VO₂max had no effect on plasma glutamine levels during exercise. Not only have plasma glutamine levels been examined, but Rennie et al. (1994) also discovered that muscle glutamine release is accelerated during prolonged exercise, where muscle glutamine levels fell from 21.6 to 14.3 mmol/kg wet weight following 3.75 hours of cycle exercise at 50% VO₂max. Two other studies have reported that human muscle glutamine levels fall during exercise (Dohm et al., 1981, and Felig, 1975), but there have also been reports of an increase (Bergstrom et al., 1975).

The majority of research supports a post-exercise fall in plasma glutamine following very long duration protocols (Brambilla et al., 1970, Hood and Terjung, 1990, Kipnis and Noall, 1958, Rhode et al., 1996, Sewell et al., 1994, and Walsh et al., 1998). Continuous cycling at 55% VO₂max for 3 hours in 18 healthy men led to a 23% fall in

plasma glutamine 1 hour post-exercise (580 mol/L pre-exercise compared with 447 mol/L after 1 hour's recovery). However, continuous cycling to exhaustion at 80% VO₂max (which occurred within 1 hour) in the same group, did not alter plasma glutamine level compared with the pre-exercise value (**Fig. 2.2**).

Plasma glutamine levels at any one time reflect a net balance between release and utilization of glutamine by various organs and body tissues. Therefore, glutamine levels in other tissues and rates of uptake and release must also be considered during and following exercise to fully understand exercise-induced changes in the plasma concentration (Walsh et al., 1998). One investigator showed a two-fold increase in the release of glutamine from leg muscles during cycling at 75% VO₂max. Two other studies have reported that human muscle glutamine levels fall during exercise (Dohm et al., 1981, and Felig, 1975), but there are also reports of an increase (Bergstrom et al., 1974) or no change (Eriksson et al., 1985). It was also reported that liver glutamine fell 43% during exercise when the concentrations of all other amino acids were elevated.

2.5.4 The Effect of Interval Exercise on Plasma Glutamine Levels

The effect of interval exercise on plasma glutamine has been a topic of investigation. 20 bouts of treadmill running for 1 minute (90 to 120% VO₂max), each separated by 2 minutes' recovery, led to a significant reduction in plasma glutamine level at 5 minutes post exercise. Keast et al. (1995) reported a mean fasted glutamine level of 1244 mol/L at rest, mean levels of 702 mol/L and 560 mol/L were recorded after the exercise protocol at 90% and 120% VO₂max, respectively. The very high plasma glutamine values raise a question over the validity of the method used to measure glutamine.

2.5.5 Why Do Plasma Glutamine Levels Fall After Very Prolonged Exercise?

After very prolonged exercise, the fall in plasma glutamine observed may result from an increased demand and uptake of glutamine by tissues of the body that require it. Also, it could be caused by a decreased production and/or altered transport kinetics of this amino acid, resulting in diminished release of glutamine by muscle (Walsh et al., 1998). The transport kinetics has been studied by Walsh et al. (1995), and the studies have revealed a maximum velocity (V_{max}) of 333 mol/kg/min for alanine uptake and 1156 mol/kg/min for glutamine uptake. Glutamine transport depends on the net electrochemical potentials of the amino acid plus Na^+ . Whenever intracellular Na^+ rises, glutamine efflux from muscle increases. The dependence of Na^+ on glutamine transport could have implications for glutamine turnover and whole-body nitrogen metabolism Newsholme, E. and Calder, P., (1997) Intracellular Na^+ concentrations have been shown to increase during injury, sepsis, and infection. In addition, Na^+ concentrations may be elevated by the action of corticosteroids (Sellers, T. et al., 1988). Over several hours, this could produce a substantial fall in the muscle glutamine level.

Prolonged exercise is known to cause an elevation in plasma cortisol concentration which stimulates not only protein catabolism and glutamine release, but also hepatic gastrointestinal and renal gluconeogenesis (Kipnis, D. and Noall, M., 1958, Rhode, T. et al., 1998, Sellers, T. et al., 1988). As liver glycogen becomes depleted and blood glucose concentration starts to fall, an increased rate of gluconeogenesis in the liver could place a significant drain on plasma glutamine availability (Walsh, N. et al., 1998). In humans, glutamine is a major gluconeogenic precursor and carbon transfer to glucose from glutamine is similar to that from alanine. During prolonged exercise, plasma levels

of glucagon, growth hormone, and cortisol rise (Galbo, H., 1983). Glucagon and cortisol increase uptake of glutamine by the liver whereas growth hormone stimulates glutamine uptake by the gut and kidneys (Walsh, N. et. al., 1998). Similar changes in plasma hormones occur after starvation, surgical trauma, and prolonged exercise and all of these states of catabolic stress are indicated by plasma glutamine depletion, immunodepression, and increased gluconeogenesis (Herberer, M. et al, 1996, Keast, D. et. al., 1995, Krebs, H, 1980, and Rhode, T. et. al., 1995).

In addition to the reasons for a fall in plasma glutamine following prolonged exercise, the postexercise falls following endurance exercise may also be due to an increased uptake of glutamine by the kidneys in an attempt to buffer metabolic acidosis (Walsh, N. et. al., 1998). During and after exercise, acidosis can arise from increased lactic acid production, accumulation of free fatty acids, and acetoacetate. The production of ammonia in the kidneys and the excretion of excess protons in the urine will protect against acidosis. During conditions of metabolic acidosis, the renal uptake of glutamine has been shown to increase to provide for ammoniogenesis (Damian, A. and Pitts, R, 1970). Greenhaff et al reported that diet-induced metabolic acidosis with a high protein (24%), high-fat (72%) diet for 4 days led to a 25% reduction in both plasma and muscle glutamine levels. Muscle glutamine release may have increased along with renal uptake in an attempt to maintain acid-base balance.

Increased ammoniogenesis and the effects of cortisol on muscle Na⁺ dependent glutamine transport may explain the raised glutamine level during exercise and immediately following acute high intensity exercise. However, during recovery from prolonged exercise, the observed falls in plasma glutamine level suggest an increased

uptake by other tissues (e.g. liver, kidneys) that are outstripping the rate of release of glutamine from muscle (Walsh, N. et. al., 1998). Several investigators assume a common mechanism may be responsible for the depletion of plasma glutamine after prolonged exercise, primarily increased liver and gastrointestinal uptake of glutamine for gluconeogenesis at a time when muscle release of glutamine remains constant or falls.

2.6 The Role of Glutamine during Over-training and Infection

The over-trained state or Over-training Syndrome (OTS) is characterized by recurrent infections, fatigue, impaired immune function, and reduced exercise performance. Also, evidence of recurrent infections during periods in the OTS suggest that increased susceptibility to infection may be part of the syndrome. There have been links between decreases in plasma glutamine and an increased risk of infection (Newsholme, E. and Parry Billings, M, 1990, and Rowbottom, D. et. al., 1995). The precise role of glutamine in the immuno-suppressed state of over-trained athletes is not yet completely understood.

Parry Billings et al reported mean plasma glutamine levels of 503 mol/L in a group of 40 athletes diagnosed as having the OTS, compared with a mean level of 550 mol/L in a group of controls (Budgett, R., 1990 and Parry Billings, M. et. al., 1992). Rowbottom et al have observed a lower mean plasma glutamine level in a group of 10 athletes classified as overtrained (703 mol/L) compared with sedentary (1030 mol/L) and athletic age-matched controls (1179 mol/L) using their bioassay technique for the estimation of plasma glutamine level (Rowbottom, D. et. al., 1995). After 2 weeks of intensified training in elite swimmers, plasma glutamine levels were 23% lower (Walsh, N. et. al., 1998). Kingsbury et al conducted a study involving a screening program of elite

athletes before and after the 1992 Olympic Games (Kingsbury, K. et. al., 1998). Three groups of athletes were studied, based on varying levels of training fatigue. Group A had no lasting fatigue, group B had heavy fatigue at night during a period of heavy training but recovered overnight to continue training, and group C had chronic fatigue and had been unable to train normally for several weeks. Group A exhibited normal amino acid patterns with a mean glutamine level of 554 mol/L, whereas group B and group C showed markedly lower plasma glutamine levels (group B: 356 mol/L, and group C: 383 mol/L). Following the Olympic competition during lighter training, there were no changes in plasma glutamine from group A. However, of the 12 athletes in group B, all but 2 showed increases in plasma glutamine levels above 450 mol/L. In group C, 53% of the athletes still had a plasma glutamine level below 450 mol/L (Kingbury, K. et. al., 1998).

Over-trained athletes and those experiencing chronic fatigue may be in a constant glutamine debt. This has important ramifications for (a) those cells and tissues that require glutamine, and (b) those cells and tissues that do not have the capability to produce this amino acid (Walsh, N. et. al., 1998). Such cells include lymphocytes and monocytes of the immune system. To date there have been a few studies linking low plasma glutamine levels with impaired immune function and increased susceptibility to infection in humans (Shewchuk, L. et. al., 1997). In addition, there have been reports of lower plasma glutamine levels in athletes with upper respiratory tract infection (URTI) (Lemon, P., 1992). Kingsbury et al have shown that in athletes presenting with infection, 73% had a plasma glutamine level below 450 mol/L (Kipnis, D. and Noall, N., 1958). Mackinnon and Hooper found no relationship between low plasma glutamine level and

the occurrence of UTRI in trained swimmers (Mackinnon, L. and Hooper, S., 1996). It was surprising that UTRI was more common among well-trained swimmers (with 23% higher plasma glutamine) than overtrained swimmers (Walsh, N. et. al., 1998).

2.7 Oral Glutamine Supplementation and Infection

Although there is no direct evidence of low plasma glutamine (either after exercise or in the OTS) with impaired immune function, epidemiological data showing low plasma glutamine and an increased occurrence of URTI in some groups of highly trained athletes has raised interest in glutamine supplementation. A drink containing 0.3 g/kg bodymass protein hydrolysate given at exhaustion following a prolonged cycling protocol can prevent the post-exercise fall in plasma glutamine (Schoor, P. et. al., 1997). Kingsbury et al also showed that 3 weeks of additional dietary protein intake (20g/day) increased plasma glutamine levels in 9 of 10 athletes exhibiting initial plasma glutamine levels below 500 mol/L (Walsh, N. et. al., 1998). Of these 10 athletes, all had presented signs of infection, although the authors gave no indication of the effect of dietary protein supplementation on recovery from infection. An adequate level of dietary protein intake in athletes has been estimated to be 1.2 to 1.8 g/kg body mass, compared with 0.8 g/kg for untrained individuals. Analysis of the diets that athletes usually consume indicate that a substantial proportion of them are not eating enough protein and that severe protein malnutrition has been associated with a depressed immune system and an increased susceptibility to infections (Walsh, N. et. al., 1998)

2.8 The Safety of Glutamine in Humans

Glutamine is safely absorbed in the jejunum (Antonio, J. and Street, C., 1999 and Dechelotte, P. et. al., 1991). Acute oral ingestion of glutamine at doses of 0.1 and 0.3

g/kg body weight showed no evidence of clinical toxicity (Antonio, J. and Street, C., 1999). Also, after a dosage of 0.285 and 0.570 g/kg body weight/day, glutamine had no harmful effects after 5 days of administration in normal subjects. In addition, glutamine was confirmed safe after several weeks of administration in patients.

As a dipeptide, glutamine was examined in polytrauma patients. Weingartmann et al. found no ill effects of glycyl-glutamine using doses equal to 14, 21, and 28 g of glutamine per day. He also indicated that glutamine is absorbed efficiently in the human jejunum and is demonstrably safe. The doses used to elicit a positive effect on nitrogen balance are considerably large (0.2-0.6 g/kg body weight per day).

2.9 Theoretical Basis for Glutamine Use by Athletes

Although there is minimal data on glutamine supplementation in the athletic population measuring strength and performance, based on the available data, it would seem reasonable to state that glutamine supplementation may provide beneficial effects for individuals engaged in chronic and intense exercise training. Muscle glutamine levels fall in a dose-dependent manner to the degree of stress. Furthermore, plasma glutamine levels decline during and after prolonged training. In addition, the amount of glutamine released by the skeletal muscle under stressful situations is greater than the amount found in the intracellular pool and incorporated into proteins. On the other hand, glutamine may improve the hydration status of the skeletal muscle, resulting in an increase in cellular volume. The increase in cell volume could be an anabolic signal for the muscle cell, which may increase muscular strength (Antonio, J. and Street, C., 1999 and Haussinger, D. et. al., 1994). Because intracellular glutamine concentrations decline in a dose-dependent manner (i.e. the greater the stress, the greater the decline), one could argue that

chronic exercise training would increase the requirements for glutamine such that exogenous self-administration may be necessary for top performance (Antonio, J. and Street, C., 1999).

Even though parenteral administration is the primary route of glutamine in clinical situations, the human gastrointestinal tract absorbs glutamine efficiently. Glutamine-enriched enteral and parenteral feeding results in similar amino acid profiles when given in identical doses (Fish, J. et. al., 1997), hence, oral supplementation would be an effective method of delivering exogenous glutamine. In addition, the dosage required by athletes would be far more less than required by post-surgical patients (i.e. burn, sepsis).

With regards to over-training, glutamine could benefit the athletic individual. A decrease in the testosterone:cortisol ratio is one indicator of an over-trained state (Hoogeveen, A. and Zonderland, M., 1996). Furthermore, the administration of glucocorticoids accelerates the release of intramuscular glutamine and that the subsequent provision of glutamine alleviates the loss of muscle glutamine and protein (Antonio, J. and Street, C., 1999). In other words, if an over-trained athlete supplemented with glutamine, he/she might prevent the loss of protein due to elevated cortisol levels. The additional glutamine may help maintain normal immune system function, which may be depressed in an over-trained athlete (Pedersen, B., 1991).

Finally, the role of glutamine in the regulation of glucose is interesting. Glutamine can provide substrate for glycogenesis and gluconeogenesis. It is unknown whether this is better than providing carbohydrate as a substrate in exercising individuals. However, the role of glutamine in lessening insulin resistance secondary to high fat consumption might

be important in the prevention of excess fat gain.

2.10 Conclusion

It is interesting to note that the evidence stating whether or not glutamine supplementation has the potential to increase muscular strength and performance is unknown. No known studies to this point have been conducted on humans to determine whether glutamine has this potential. Granchelli et al has proved that 10 mg/kg of glutamine and 10 mg/kg of glutamine plus alanine has improved whole body strength in the genetically dystrophin-deficient mdx mouse. As for athletes, the use of glutamine as a dietary supplement may have possible ergogenic benefits. The protein-sparing effect would be important for athletes engaged in strength-power activities, which call for a large amount of skeletal muscle mass. In all athletes, glutamine could serve to ameliorate the effects of over-training on the immune system.

Because glutamine is an important fuel for the gastrointestinal tract and cells of the immune system, self-administration of dietary glutamine could spare muscle protein while providing fuel for other cells and tissues (Antonio, J. and Street, C., 1999). It is speculated that athletes engaged in intense exercise training, the need for glutamine utilization might exceed the amount released by various organs/tissues, especially skeletal muscle and adipose tissue, to maintain normal plasma levels, which may result in the breakdown of muscle protein. As is the case for many athletes, the preservation of muscle mass is a critical factor that could have an impact on performance and recovery.

Using doses lower than those provided to post-surgical patients, glutamine supplementation could prevent the loss of lean body mass which result from over-training and promote gains in lean body mass in strength power athletes. Furthermore, glutamine

could have a beneficial effect on the immune system and thus decrease the incidence of infection or illness (Antonio, J. and Street, C., 1999).

Whether glutamine could be as effective as carbohydrate in the accumulation of muscle glycogen after prolonged exercise remains to be examined. One study did show that an infusion of glutamine promoted the accumulation of glycogen in skeletal muscle in the first 2 h of recovery from severe exercise (45 min at 75% VO₂max) (Varnier, M. and Leese, G., 1995). In addition, there is evidence supporting glutamine as a essential amino acid in patients who are critically ill. Furthermore, glutamine might also be an essential amino acid for athletes engaged in intense exercise training. This study will examine the potential of glutamine for muscular strength and performance and whether glutamine can be characterized as a safe ergogenic aid for athletes.

Fig 2.1 The Effect of Added, Exogenous Amino Acids on Glutamine Release from Rat Skeletal Muscle Preparations *in vitro*.

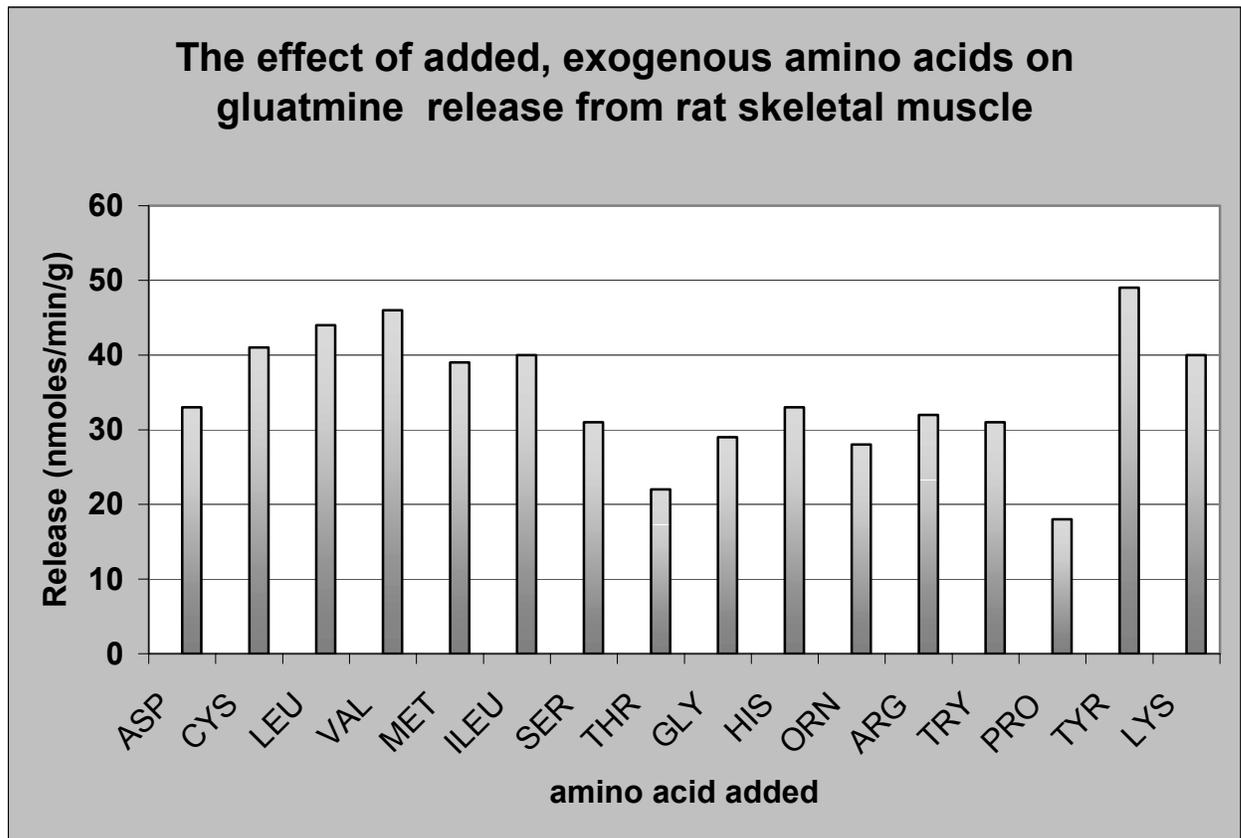
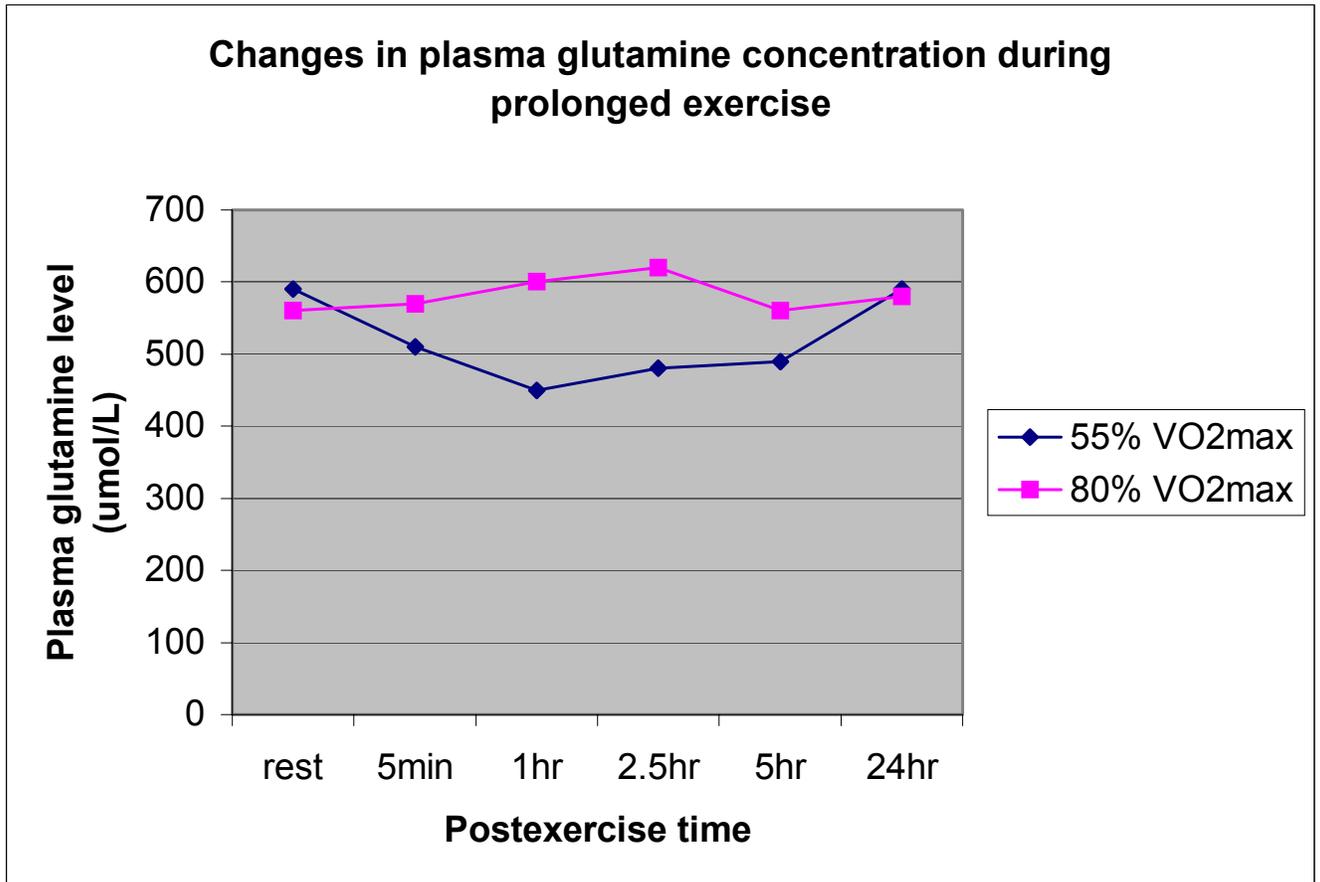


Fig. 2.2 Changes in Plasma Glutamine Concentration in 18 Healthy Male Volunteers after 3 Hours of Cycling at 55% VO₂max and after Exercise to Exhaustion at 80% VO₂max. The Mean (SEM) Endurance Time at 80% VO₂max was 38 ± 9 Minutes.



CHAPTER 3

MATERIALS AND METHODS

3.1 Subject Characteristics

Twelve subjects participated in the study. All were well-trained cyclists who were participating in a training regimen (spinning class of three or more days a week for a least an hour) or competitive cycling (road racing). Table 4.1 demonstrates descriptive characteristics of the subjects. Measurement of body composition was obtained prior to testing using the three-site skin-fold caliper method.

Table 3.1 Subject Baseline Descriptive Data

Group	Ht (in)	Wt (lbs)	BMI	BF%	SBP	DBP	RHR	VO2Pk	Peak Power (watts)	Total Revs	Time to Exhaustion (mins)
Glutamine	69.66 ± 2.94	171.56 ± 19.03	24.87 ± 2.69	10.9 ± 3.44	120.66 ± 12.81	71 ± 7.64	57.66 ± 5.46	44.53 ± 3.57	717.71 ± 118.89	38.5 ± 2.25	46.3 ± 10.80
Placebo	68.16 ± 4.26	163.33 ± 37.41	24.47 ± 3.03	13.01 ± 2.82	122.33 ± 2.94	74.66 ± 7.0	64.14 ± 3.12	41.9 ± 5.26	593.66 ± 117.08	35.5 ± 5.64	43.83 ± 3.81

Data are mean ± SD

A one-way ANOVA demonstrated a significant difference in resting heart rate (RHR) between groups ($p=.03$), but no difference was present within groups.

3.2 Experimental Protocol

This is a randomized; double-blind study with subjects assigned to either a glutamine supplementation or placebo group.

A washout phase of any supplementation two weeks prior to baseline testing was required before testing procedures begin. All subjects participated in 2 separate exercise trials (T1 and T2). T1 consisted of 3 days of baseline testing. On day 1, each subject reported to the Exercise Physiology laboratory in the morning where bodyweight and

resting heart rate and blood pressure were assessed before testing procedures begin.

3.3 Anaerobic Testing (Wingate)

The Wingate protocol has five distinct periods: (1) prior exercise, (2) recovery interval, (3) acceleration period, (4) Wingate Test, and (5) cool-down period. The Wingate Test will require 5 participants (4 assistants, 1 subject). Each assistant will have a significant role in determining the results of the test: (1) The *Timer* will officially begin the test itself as soon as the force setter finishes setting the prescribed resistance. The timer will initiate the test by yelling “Go!” or “Start!” when the prescribed force is set. The timer will begin the clock, and the performer begins pedaling as fast as possible for a 30s period while remaining on the ergometer. The timer will shout the time every 5s. At the end, the timer indicates to “Stop!” (2) The *Force Setter* maintains the prescribed setting on the ergometer. At the end of the 30s spring, the force setting lowers the force to a cool-down or recovery setting (usually between 10N or 20 N) while the performer continues pedaling (3) The *Counter* will shout the number of pedal revolutions for the respective 5s intervals by observing the number of times the left or right pedal makes complete rotation from the original pedal position at the “GO” signal. The counter will give the whole, not fractional, number of revolutions for each 5s interval (4) The *Recorder* records each 5s value onto Form 1.1, then circles the highest revolutions and sums the revolutions.

After each warm-up phase is completed, each subject will perform a Wingate Test and peak power (PP), mean power (MP), and fatigue index (FI) will be assessed. After completion of the Wingate test, subjects will be allowed a 2-3 minute cool-down period at low to moderate aerobic power (e.g. 25 W to 100 W) immediately followed by a 15

minute inactive recovery period. After the recovery period each subject will perform an exhaustive bout of exercise at 70% of their VO₂ max on the cycle ergometer until they reach a point of fatigue. Fatigue will be defined as the inability to maintain at least 50 revolutions per min (rpm). Immediately following the exhaustive bout of exercise, each subject will perform another Wingate test. Subjects will return at the same time on day 2 and day 3 (24 hour period), and perform Wingate test to determine if any change in power occurs.

3.4 Glutamine Administration

Immediately after baseline testing, subjects were randomly assigned to a glutamine (GL) or placebo (PL) group in a double-blind fashion. The GL supplement group received a 6-day supply of L-glutamine (EAS) in crystallized form. The PL group received a carbohydrate only solution (gatorade). Both the GL and PL group were not isocaloric in nature but each individual was administered 1 g/kg of carbohydrate. L-glutamine was also mixed with the same carbohydrate solution (gatorade) making both products indistinguishable by color, taste, and texture. All subjects received 0.3 g/kg/day in 2 separate doses throughout the day. Each subject received a 6-day prepackaged supply of glutamine. No additional nutrients (carbohydrate, fat, protein) or calories were contained in the GL or PL solution. Previous research utilizing oral GL supplementation has demonstrated that 0.1 g/kg body weight has increased plasma glutamine concentrations by at least 50% (Castell and Newsholme, 1997). During the supplementation period, all subjects were asked to maintain their normal levels of physical activity, caloric intake and report any unusual side effects. On the other-hand, participants were not required to maintain a food record to assess caloric intake during the 6 day supplementation period.

Recent research (Antonio et al., 2002) has indicated that high doses of oral glutamine supplementation (0.3 g/kg) did not cause any harmful side effects. Subjects will be contacted regularly during the study period to ensure adherence to the supplementation regimen.

Post-treatment testing (T2) was carried out on the fourth day after T1. T2 will consist of the same exercise protocol and recovery intervals as T1 for each subject. Body weight and resting heart rate and blood pressure were to be recorded before and after testing and subjects performed a warm-up similar to that during T1.

3.5 Data Analysis

Peak anaerobic power (Pk-AnP), mean anaerobic power (M-AnP), and fatigue index (FI) were assessed during the Wingate protocol to determine statistically significant differences between the glutamine and placebo groups.

Pk-AnP was expressed in watts. To facilitate this, it is best to use the newton (N) unit as the expression for force (F), the Newton meter (N·m) or joule (J) as the expression for work (w), and the Newton meter per minute or per second (N·m·min⁻¹; N·m·s⁻¹) because of the ease of converting them to the watts power unit. The formula for Pk-AnP (N·m·s⁻¹;W) = [F-setting (N) x (rev max x 6m)] / 5 s.

M-AnP measures the average anaerobic power (W) during the test and is calculated by M-AnP (W; J·s⁻¹) = total w (J)/ 30s.

FI indicates the decrease in power from the Pk-Anp to the lower anaerobic power (AnP). The higher the person's percentage value, the greater the decrease. The following formula will be used to calculate FI: FI (%) = [(Pk-AnP – lowest AnP)/ Pk-AnP] x 100.

3.6 Statistical Analysis

Independent t-tests were used on baseline measures to determine if differences between treatment groups (GL vs. PL) were present. A 4 X 2 analysis of variance with repeated measures was completed to assess statistically significant differences between the glutamine and placebo groups. Alpha levels were set ($p=0.01$).

CHAPTER 4

RESULTS

4.1 Trial 1 (Pre & Post Exhaustive Bout) – Day 1 (Baseline Testing)

Descriptive statistics for total revolutions and peak power at baseline are displayed in Table 4.1. Subjects performed a 30s Wingate test, followed by an endurance test to exhaustion, and then followed by a second 30s Wingate test. Subjects were allowed a 10 minute inactive recovery period before performing the next bout of exercise.

Table 4.1 Means for Total Revolutions and Peak Power (watts) in Pre and Post Exhaustive Bouts of Exercise to Fatigue.

Bout	5 sec	10 sec	15 sec	20 sec	25 sec	30 sec	Total revs	Peak Power (watts)
Pre-Exhaustive	8 ± .95	7.33 ± .88	6.58 ± .90	5.83 ± .83	4.91 ± .90	4.33 ± .65	37.00 ± 4.39	655.68 ±129.82
Post-Exhaustive	6.25 ± .86	5.33 ± .65	4.75 ± .62	4.16 ± .71	3.58 ± .51	3.0 ± .60	27.08 ± 3.36	513.71 ±114.84
Total	7.12 ± 1.26	6.33 ± 1.27	5.66 ± 1.20	5.0 ± 1.14	4.25 ± .98	3.66 ± .91	32.04 ± 6.34	584.69 ±140.09

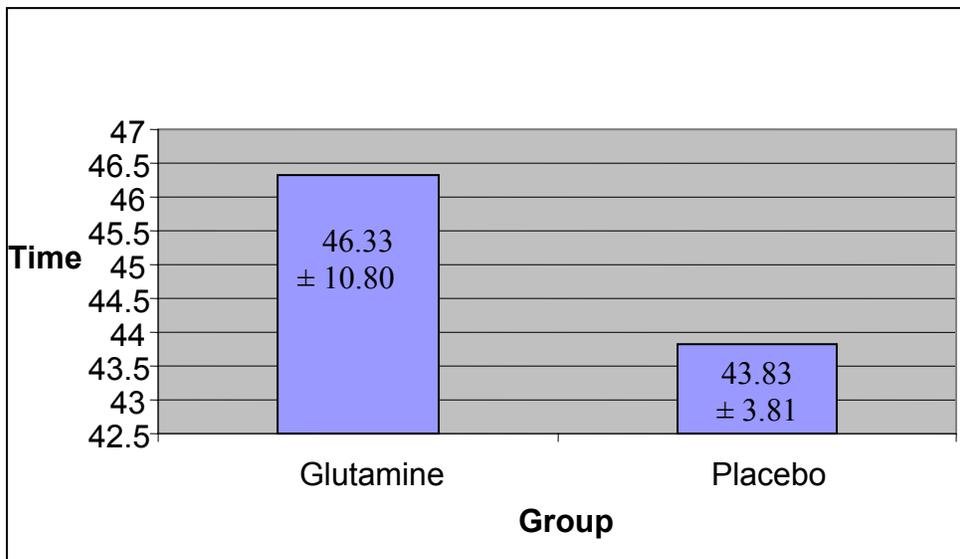
Evidence following an exhaustive bout of exercise shows a drop off in total revolutions (PRE - 37.00 ± 4.39 and POST – 27.08 ± 3.36) and peak power (PRE – 655.68 ± 129.82 and POST – 513.71 ± 114.84). The decrease in total revolutions during the post-exhaustive bout were significant at SEC 5 ($p= .024$), SEC 10 ($p= .040$), SEC 15 ($p= .018$), and SEC 30 ($p= .009$). Values for SEC 20 ($p= .059$) and SEC 25 ($p= .058$) were not significant. The drop in Peak Power following an exhaustive bout was

significant ($p = .008$). These results are consistent with a power related decrement following an exercise bout to fatigue (Beelen & Sargeant, 1991). Further analysis indicates no differences between groups in the magnitude of drop off.

4.2 Time to Exhaustion

Figure 4.1 demonstrates baseline values for time to exhaustion. There were no significant difference between groups ($p = .605$).

Figure 4.1 Time to Exhaustion ~ Baseline Values



4.3 Trial 1 (Bout 3) – Day 2 (24 hour recovery power test)

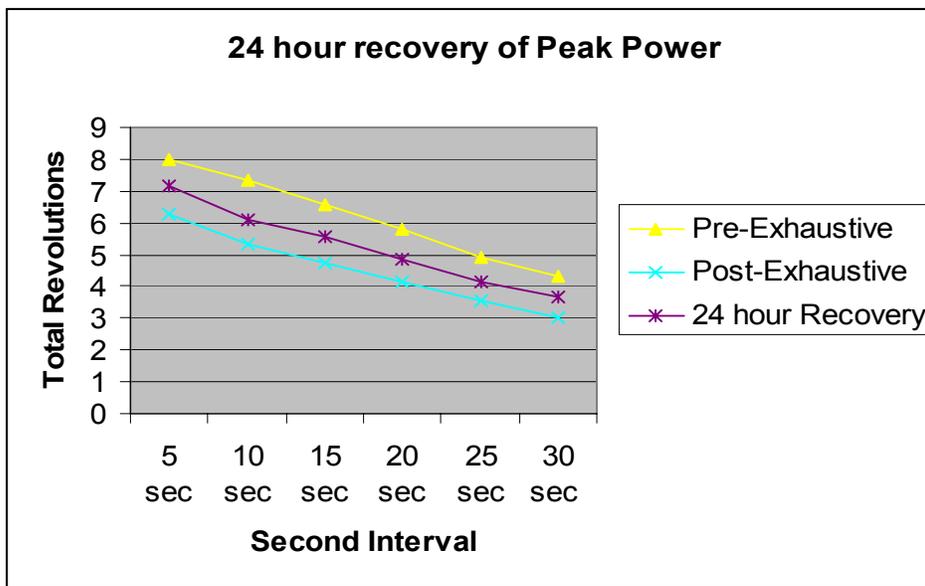
Table 4.2 – Means and Standard Deviations for Total Revolutions and Peak Power during a 24 hour Recovery Period.

Bout	5 sec	10 sec	15 sec	20 sec	25 sec	30 sec	Total revs	Peak Power (watts)
24 hour recovery	7.16	6.08	5.58	4.83	4.16	3.66	31.5	568.94
	± 1.02	± .99	± .90	± .93	± .71	± .49	± 4.56	±121.97

4.4 Total Revolutions and Peak Power

After a 24 hour rest period, subjects returned to perform another 30s Wingate test to assess recovery of peak power and total revolutions. As previously mentioned, total revolutions and peak power declined following an exhaustive bout of exercise. Figure 4.2 and 4.3 demonstrate the results of peak power and total revolutions after 24 hours of recovery from repeated bouts of exhaustive exercise. After 24 hours, total revolutions and peak power subjects showed signs of recovery compared to pre and post exhaustive exercise values, but did not return to baseline. See Table 4.3.

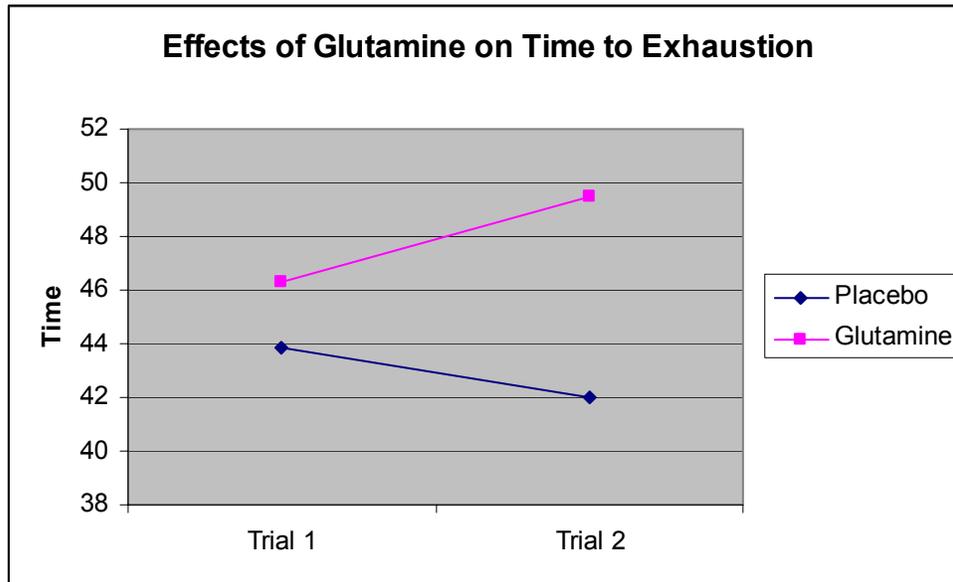
Figure 4.2 Twenty-Four Hour Recovery of Peak Power



4.5 The Effects of Glutamine on Time to Exhaustion

Following supplementation, time to exhaustion improved by 3.16 ± 0.75 min compared to baseline values (Baseline: 46.33; Glut: 49.50) whereas no change occurred in the placebo ($p=0.001$). See Figure 4.4.

Figure 4.3 Effects of Glutamine supplementation on Time to Exhaustion



4.6 The Effects of Glutamine Supplementation on Peak Power and Total Revolutions

As previously noted at baseline, both groups showed significant decreases in total revolutions and peak power during each time interval after performing a bout of exercise to exhaustion. On the other hand, the glutamine group demonstrated an improvement in total revolutions and peak power pre and post an exhaustive bout of exercise compared to placebo group (see Figures 4.5 and 4.6) indicating the glutamine appeared to recover from an exhaustive bout of exercise sooner than the placebo group. In addition, the glutamine demonstrated an increase in peak power from baseline (Baseline: 655.68 ± 129.82 vs. Glutamine: 719.00 ± 144.95)

Figure 4.4 Effects of Glutamine Supplementation on Total Revolutions before and after an Exhaustive Bout of Exercise.

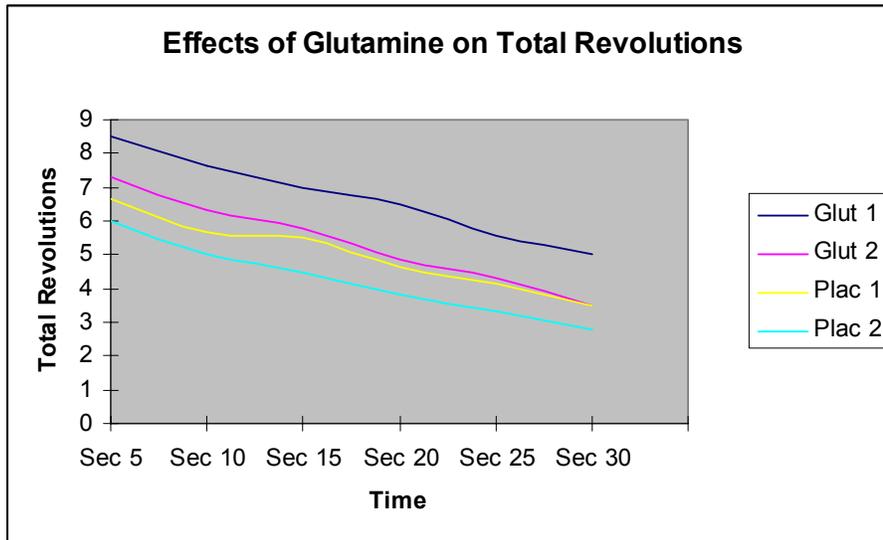
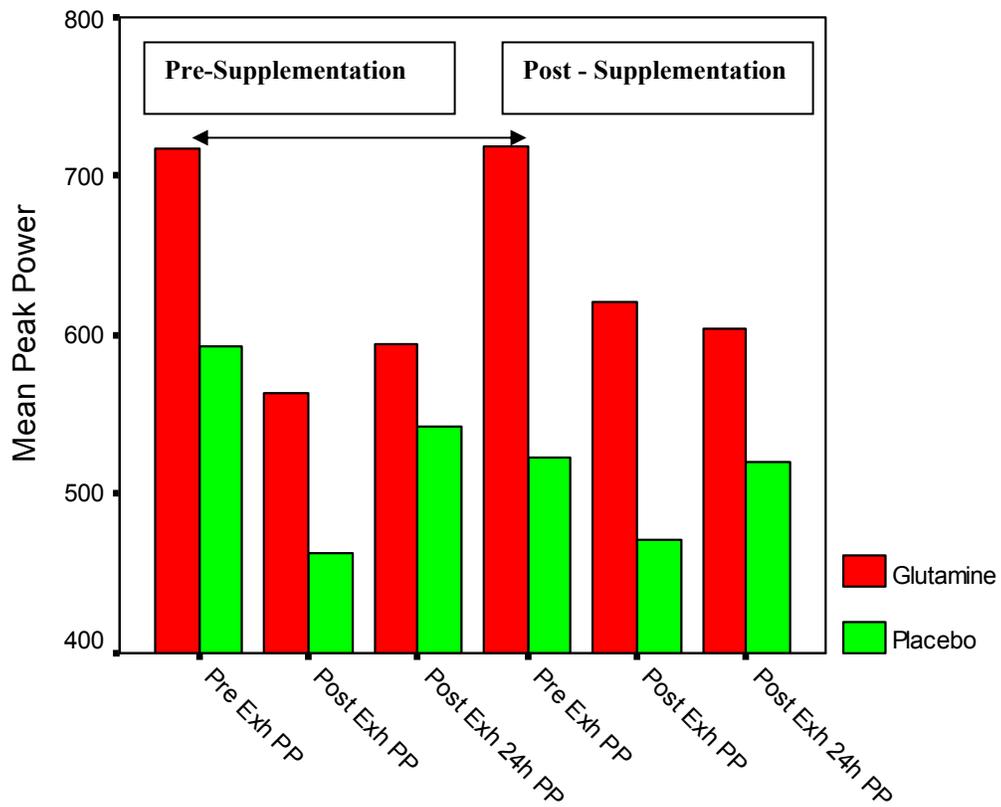


Figure 4.5 Effects of Glutamine Supplementation on Peak Power before and after an Exhaustive Bout of Exercise.



CHAPTER 5

DISCUSSION

The purpose of the present investigation was to examine the effect of glutamine supplementation on recovery from repeated bouts of intense exercise. Specifically, we measured 24 hour recovery of peak power after an exhaustive bout of exercise. Following six days of glutamine supplementation, there was a significant improvement in time to exhaustion compared to the placebo group (Baseline: Glut: 46.33 ± 10.80 to Post: Glut: 49.50 ± 10.74 min; $p = 0.0001$). In regards to recovery of peak power, there are no apparent differences between groups immediately after time to exhaustion and 24 hours post exercise (Pre exhaustion: Glut: 719.00 ± 144.95 watts; Plac: 523.12 ± 79.11 watts; Post exhaustion: Glut: 620.28 ± 129.86 watts; Plac: 471.90 ± 78.40 watts). An interesting note is that subjects receiving glutamine had an increase in baseline peak power values compared to visit one and visit four (V1: 655.68 ± 129.82 watts; V4: 719.00 ± 144.95) indicating an increase in anaerobic power.

5.1 Time to Exhaustion

Limited data exist examining the effects of glutamine supplementation on time to exhaustion, especially after an intense bout of exercise. Previous data reports glutamine supplementation showed no improvements in time to exhaustion after prolonged, exhaustive exercise (Castell, L., Poortmans, J., and Newsholme, E., 1996). One limiting factor in this study may have been a lower dose (0.1 g/kg) of glutamine supplementation.

The results of the present investigation are inconsistent than that reported by Haub et al (1998) where glutamine did not improve time to fatigue after a similar dose (0.3 g/kg) was administered. One explanation for the difference in results may be the short

supplementation period (1 day) and the time at which exercise followed supplementation (90 minutes). The present investigation supplemented for six consecutive days at 0.3 g/kg which may have increased muscle glutamine uptake and possibly glycogen storage. Increased or improved storage of muscle glutamine levels are critical for glutamine release into circulation. In addition, Newsholme et al. (1990) concluded that skeletal muscle provides the majority of glutamine required by other tissues. It is also known that improved glycogen storage has improved exercise performance. Previous studies by Varnier et al. (1995) and Bowtell et al. (1999) demonstrated that infusion and oral glutamine supplementation promotes storage of muscle glycogen.

The subjects in the present investigation demonstrated improved time to exhaustion compared to the study by Haub et al. (1998) although the subjects in the previous study had significantly higher VO₂ peak values ($53.6 \pm 1.9 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ vs. $43.21 \pm 7.01 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). One explanation for these differences may have been the exercise intensity where subjects in the previous studied cycled at 100% VO₂ vs. 70% VO₂.

5.2 Peak Power

Both groups (Glutamine and Placebo) demonstrated significant decreases in peak power following an exhaustive bout of exercise at 70% VO₂ peak ($22\% \pm 11$). These are similar decrements in peak power compared to those reported by Beelen and Sargeant (1991) which reported a $23\%-28\% \pm 19$ decrease in peak power following six minutes of cycling at 90% VO₂ peak.

Research examining 24 hour recovery of peak power after intense exercise has not been reported. Previous studies have examined recovery of peak power after 2 minute

recovery intervals (Bowtell et al., 1999), 3 and 6 minute recovery intervals (Bogdanis et al.), and no recovery intervals (Beelan and Sargeant, 1991) In the current study, we examined the effects of repeated bouts of intense exercise on recovery of peak power after 24 hours of passive recovery. As previously mentioned, the subjects experienced a significant drop off in peak power following an exhaustive bout of exercise. After a 24 hour recovery period, subjects demonstrated partial recovery of peak power (Pre-exhaustive: 655 ± 129.82 ; Post-exhaustive: 513.71 ± 114.84 watts; 24hr recovery: 568.94 ± 121.97 watts). Beelan and Sargeant (1991) reported decreases in peak power immediately after a fatiguing bout of exercise at different pedal frequencies of 90 ($25 \pm 19\%$), 105 ($28 \pm 11\%$), and 120 rpm ($25 \pm 11\%$). The reductions in peak power for their study were similar in magnitude to the current study. It would appear recovery of peak power in Beelan and Sargeant (1991) would be similar in comparison to the current study if measured after 24hours due to similar decreases in peak power.

5.3 Supplementation

Data from previous studies excluded the use of supplementation to assess recovery of peak power. We examined the effects of glutamine supplementation on recovery of peak power. Subjects receiving glutamine experienced similar drops in peak power after an intense bout of exercise compared to those receiving placebo (Pre exhaustion: Glut: 719.00 ± 144.95 watts; Plac: 523.12 ± 79.11 watts; Post exhaustion: Glut 620.28 ± 129.86 watts; Plac: 471.90 ± 78.40 watts). An interesting note is that subjects receiving glutamine had an increase in baseline peak power values compared to visit one (V1: 655.68 ± 129.82 watts; V2: 719.00 ± 144.95). One explanation for this may have been the glutamine group may have had higher baseline peak power values prior to

supplementation verses the placebo group. This could be due to a higher level of training to those randomized to glutamine. Another possible explanation for the increase in power is glutamine supplementation has been shown to increase muscle glycogen concentration (Bowtell et al., 1999; Varnier, Leese, Thompson, and Rennie, 1995). Restoring muscle glycogen after intense exercise is one of the most important components in facilitating recovery.

5.4 Future Considerations

Previous studies have demonstrated the benefits of glutamine supplementation for enhancing immune system function, decreasing inflammation, improved muscle mass, and in several clinical populations (HIV, sepsis, cancer, and trauma patients). In addition, it is quite apparent that decreases in plasma and muscle glutamine levels occur after In the present study, we demonstrated glutamine may be beneficial for improving recovery after repeated bouts of intense exercise.

The future direction of research involving glutamine supplementation may be of extreme benefit for those with symptoms of cardiovascular disease. As previously mentioned, glutamine has been shown to decrease inflammation. A high C-reactive protein (CRP) level is an indicator for inflammation of the myocardium. High CRP levels are correlated with increased incidence of heart attacks, although blood lipids such as cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) may be normal. It is suggested that oral glutamine supplementation may be beneficial in maintaining or possibly lowering CRP levels.

Future studies may be able to help clarify the role of glutamine by continuing to examine the effectiveness of glutamine supplementation in recovery

5.5 Conclusions

Theoretically, the current protocol put our subjects in an exhaustive state, mentally and physically. We felt the protocol was essential for examining recovery, especially after a 24 hour resting period. It is known that the nutritional composition of the diet can enhance muscle recovery, especially using a 4:1 ratio of carbohydrate to protein. On the other hand, it was previously unknown whether the addition of glutamine after intense exercise could improve recovery or time to exhaustion. As a result, we concluded that supplying a carbohydrate drink with glutamine versus carbohydrate alone immediately after repeated bouts of intense exercise, was appropriate to determine any performance enhancing benefit. This conclusion is based on evidence from previous studies supporting the use of glutamine supplementation for enhancing immune system function, decreasing inflammation, improved muscle mass, and its wide use and benefit in several clinical populations (HIV, sepsis, cancer, and trauma patients).

The increase in time to exhaustion following six days of glutamine supplementation presented in the current study represented a unique finding to our understanding. Participants in the glutamine group increased time to exhaustion following 6 days of supplementation. Both participants in the glutamine and placebo group experienced similar declines in performance immediately, and 24 hours after an exhaustive bout of exercise.

Furthermore, although there was a significant decrease in peak power following an exhaustive bout of exercise, it appears those receiving glutamine appeared to recover quicker after a 24 hour resting period. In addition, those receiving glutamine exhibited an increase in peak power from baseline measures indicating a possible increase in

anaerobic power and leg strength. Obviously, improvements in muscle recovery and strength can be of benefit to all exercising populations, specifically individuals participating in repeated bouts of exercise.

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APPENDIX

L-GLUTAMINE SUPPLEMENTATION: EFFECTS ON RECOVERY FROM EXERCISE

Department of Kinesiology

Subject Participation Questionnaire

1. Name _____
2. Date of Birth _____ Ht _____ Wt _____
3. How often do you train? _____
4. What type of exercise/s do you perform and do you train for strength gains, to increase performance or for health and wellness? (Briefly explain)

5. Do you perform cardiovascular training? (treadmill, jogging, stairmaster, elliptical training, stairclimber, stationary bicycling) yes no
How many days per week? (Circle One) Never 1 2 3 4 5 6 7
Duration of cardiovascular exercise? (Circle One)
Less than 30 minutes 30 minutes- 1hour 1-2 hours > than 1 hour
6. Have you previously taken dietary supplements? (protein powder, creatine, amino acids, ripped fuel, xenedrine, etc.) yes no
Please specify those that you have taken (This is optional) _____

7. Are you currently taking supplements? Yes no
8. This study requires no supplementation 2 weeks before the administration of glutamine and during the 6 day supplementation period. If selected as a subject, are you willing to discontinue supplementation until the end of the study?
Yes No
9. Do you have a family history of Heart Disease or High Blood Pressure? Yes No

10. Have you ever experienced any side-effects while taking supplements? (muscle cramps, chest pain, joint pain, rash, weakness, difficulty breathing) Please explain! If you have never taken supplements, please skip this question.

VITA

Tavis Piattoly was born and raised in New Orleans, Louisiana, where he attended and graduated from Holy Cross High School. Tavis attended Louisiana State University (LSU) in Baton Rouge and received a Bachelor of Science Degree in dietetics. Furthermore, Tavis was accepted into the Touro Infirmary Dietetic Internship program and was awarded the license to practice as a Dietitian. In 2001, Tavis married Lindsey Ware and currently lives in Covington, Louisiana. Tavis continued to pursue a master's degree in kinesiology from Louisiana State University, while working as a full-time dietitian. In August 2005, Tavis is expected to graduate from LSU with a master's degree in kinesiology.