EFFECT OF GLUTAMINE SUPPLEMENTATION COMBINED WITH RESISTANCE TRAINING ON MUSCULAR PERFORMANCE, BODY COMPOSITION AND PROTEIN DEGRADATION

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ABSTRACT

The purpose was to assess the effect of oral glutamine supplementation combined with resistance training in young adults. Thirty-one subjects, aged 18-24, were randomized (double blind) to receive glutamine (0.9g/kg lean tissue mass; n=17) or placebo (0.9g/kg lean tissue mass; n=14) during 6 weeks of total body resistance training. Exercises were performed for 4-5 sets of 6-12 repetitions at intensities ranging from 60-90% 1-RM. Before and after training, measures were taken for 1-repetition maximum squat and bench press strength, peak knee extension/ flexion torque (Biodex isokinetic dynamometer), lean tissue mass (dual energy X-ray absorptiometry) and muscle protein degradation (urinary 3-Methylhistidine by high performance liquid chromatography [HPLC]). Repeated measures ANOVA showed that strength, torque, lean tissue mass and 3-Methylhistidine increased with training ($p<0.05$), with no significant difference between groups. Both groups increased their 1-RM squat by 30% and 1-RM bench press by 13.5%. The glutamine group had increases of 6% for knee extension torque, 5% for knee flexion torque, 2% for lean tissue mass and 41% for 3-Methylhistidine excretion. The placebo group increased knee extension torque by 2%, lean tissue mass by 1.7% and 3-Methylhistidine by 56%. However, knee flexion torque decreased by 2%. We conclude that glutamine supplementation during resistance training has no significant effect on muscular performance, body composition or muscle protein degradation in young healthy adults.

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Finally, I need to thank my mother Jocelyn, father Glenn, brother Mark and all my friends for their continual support and encouragement. Without you, none of this would be possible.

DEDICATION

This thesis is dedicated to my parents, my mother Jocelyn and father Glenn. I have been blessed with two parents who have showed me the importance of having honor and pride in everything I do. Life is full of good times and bad times but how you handle each situation makes you the person you are. What comes from the heart truly matters and nothing can take that away. For that, you will always be perfect to me.

Life is a long journey filled with fear, excitement and anticipation. Dreams make life tolerable so make the most of it and never look back.

" Two roads diverged in a yellow wood and sorry I could not travel both but be one traveler long I stood and looked down one as far as I could to where it bent in the undergrowth"

Robert Frost

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Scientific Framework

1.1 Introduction

Success in the world of athletics is primarily dependent upon genetic endowment combined with specific physiological traits intrinsic to performance (Willams, 1995). The difference between success and failure usually involves a few seconds or points and although small to the layperson, this difference is critical to the athlete who falls short in relation to their competition. Success is what every athlete strives for and any action or product that may give them an advantage will become much more appealing. In today's high pressured society, more and more athletes are testing unfamiliar waters by experimenting with products that are proposed to help get them past their genetic limitations and attain their goals faster. These products are refereed to as ergogenic aids.

Ergogenic aids can be described as products that increase work capacity, athletic performance and muscle recovery (Bucci, 1996). Athletes often rely on theoretical or anecdotal support rather than documented literature when deciding to use ergogenic aids (Burke & Inge, 1999). Previously, pharmacological agents were the principal ergogenic aids experimented with but their banning by several

athletic bodies have caused more and more athletes to lean towards nutritional supplements in recent years (Burke & Inge, 1999). Ergogenic aids range from anabolic steroids which may have serious side effects to vitamin supplements (Burke & Heeley, 1999; Willams, 1995). Despite the recent widespread use of ergogenic aids in the athletic population, much of the documented research reports conflicting results (Willams, 1995). Athletes manipulate their diets and consume these nutritional aids without having scientific evidence regarding the beneficial or harmful side effects of their actions.

Amino acid supplementation has become popular due to the belief that it will enhance protein synthesis and muscle recovery (Curthoys & Watford, 1995). Amino acids are utilized at high rates during cell recovery and may serve as an energy source in times of need. They are also involved in the formation of enzymes and hormones (Burke & Inge, 1999; Willams et al. 1998). Of the 20 amino acids identified, nine are considered essential for the human due to the body's inability to endogenously produce them and thus, must be consumed in the diet (Wagenmakers, 1998). Progressive exercise causes a net breakdown of muscle protein stores, which releases various amino acids into free pools for various physiological processes (Antonio & Street, 1999; Burke & Heeley, 1999; Hickson et al. 1995; Wagenmakers, 1998).

Glutamine, the most common amino acid in the body, is considered a "conditionally" essential amino acid because the body's requirement for glutamine increases during times of physiological stress. Glutamine has become increasingly popular in recent years among athletes because it has been shown to

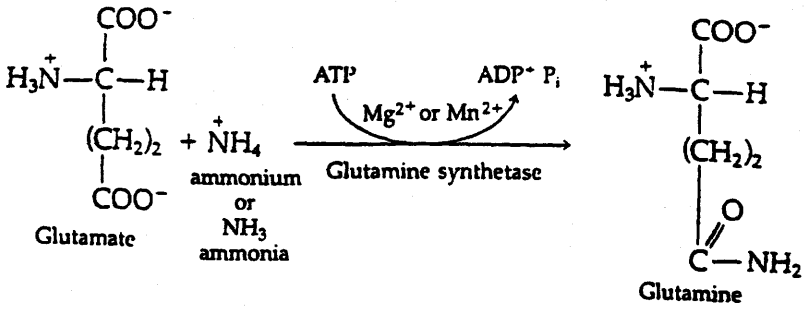
have a beneficial effect on muscle glycogen resynthesis, immune system response and muscle protein synthesis following physiological stress such as intense exercise or surgery (Antonio & Street, 1999). Glutamine supplementation appears to increase intramuscular glycogen concentrations (Bowtell et al. 1999; Varnier et al. 1998) following prolonged endurance exercise by acting as an additional substrate for gluconeogenesis in the liver. This increase in muscle glycogen could potentially attenuate amino acid release from skeletal muscle during extended exercise which may reduce muscle protein degradation. During periods of severe physiological stress, glutamine supplementation has been shown to reduce the severity of illness and infection by acting as an additional fuel source for lymphocytes, macrophages and fibroblast cells of the immune system (Antonio & Street, 1999). Exogenous glutamine may help maintain intramuscular glutamine concentrations which normally decrease during times of immune suppression (Biolo et al. 2000). Immune suppression is common following severe exercise (Castell et al. 1997); therefore, supplementing with glutamine could theoretically allow an athlete to return to training at a faster rate by increasing the rate of recovery following exercise. Glutamine supplementation has been shown to decrease muscle protein degradation by acting as a fuel source for various organs and cells of the immune system (Petersson et al. 1994). This sparing action would reduce the efflux of glutamine from skeletal muscle during times of physiological stress and may offset muscle protein catabolism. Glutamine infusion has been shown to decrease protein degradation and increase protein synthesis in animal models (Maclennan et al. 1987, 1988).

Given that strength training results in substantial glycogen depletion (Roy & Tarnopolsky, 1998) and elevated protein turnover (Macdougall et al. 1995), it has been suggested that glutamine supplementation may be of a benefit to strength trained athletes (Antonio & Street, 1999). It is somewhat surprising then that glutamine has never been evaluated as an ergogenic aid for strength training. Therefore, we propose to determine the effect of glutamine supplementation on muscular performance, body composition and muscle protein degradation during strength training in young, healthy adults.

1.2 Review of Literature

1.2.1 GLUTAMINE

Glutamine, the most versatile and abundant amino acid in plasma and skeletal muscle (Antonio & Street, 1999; Curthoys & Watford, 1995; Lacey & Wilmore, 1990; Lee et al. 1998), accounts for more than half the total intramuscular free amino acid pool (Antonio & Street, 1999; Lacey & Wilmore, 1990; Perrielo et al. 1997;Walsh et al. 1998). Glutamine is synthesized primarily in skeletal muscle, adipose tissue and the lungs through the conversion of L-glutamate to L-glutamine by glutamine synthetase through the following reaction:



During periods of physiological stress such as cancer, trauma, and over training which induce hypercatabolic states (Wagenmakers, 1998), the body's endogenous rate of glutamine synthesis falls below the concentrations required for homeostasis (Antonio & Street, 1999; Wagenmakers, 1998). Thus, glutamine is considered to be a "conditionally" essential amino acid (Antonio & Street, 1999; Curthoys & Watford, 1995; Labow et al. 1999; Wagenmakers, 1998). Glutamine is thought to play a key role in the production of pyrimidine and purine bases, which are involved in DNA and RNA synthesis (Antonio & Street, 1999; Labow et al. 1999; Lee et al. 1998). It is a nitrogen carrier between tissues and is considered the main fuel source for intestinal, endothelial and lymphocyte cells (Nieman, 1997) where it is oxidized after conversion to alpha-ketoglutarate and entry into the Kreb's cycle (Bowtell et al. 1999; Castell & Newsholme, 1998; Dechelotte et al. 1991;Rhode et al. 1995). Glutamine is also highly involved in protein synthesis (Antonio & Street, 1999; Bowtell et al. 1999; Wagenmakers, 1998), and degradation (Antonio & Street 1999; Bowtell et al. 1999; Hack et al. 1997).

The kidneys use glutamine to excrete toxic ammonium ions from peripheral tissues, which help protect against acidotic states (Antonio & Street, 1999; Burke

& Heeley, 1999; Lacey & Wilmore, 1990; Sourba, 1992; Wagenmakers, 1998). This process is initiated when ammonia binds to free hydrogen ions and enhances urinary excretion of excess protons (Curthoys & Watford, 1995; Lacey & Wilmore, 1990; Welbourne, 1995). The functions of the pancreas also seem to be enhanced by glutamine. Glutamine appears to increase total pancreatic trypsinogen and lipase content which helps filter free fatty acids to the liver (Sourba, 1992).

The liver plays a key role in glutamine synthesis and degradation by closely regulating the disposal of glutamine nitrogen through the process of ureagenesis (Antonio & Street, 1999; Labow et al. 1999; Lee et al. 1998; Williams & Haynes. 1998). Ammonia ions that escape the initial excretion process eventually become captured by high affinity hepatic cells. These "scavenger" cells contain glutamine synthetase and are highly efficient in excreting excess ammonia before they reach circulation (Sourba, 1992), thus reducing acidotic states (Curthoys & Watford, 1995; Lacey & Wilmore, 1990).

Glutamine also appears to play a significant role in neurotransmitter synthesis within the brain (Castell et al. 1996; Labow et al. 1999; Lee et al. 1998.). Glial cells, located in the lower stem region of the brain (Sourba, 1992), have high levels of glutamine synthetase and therefore have the capacity to synthesize glutamine. Glutamine is then catabolized into glutamate, a known neuroexcitatory substance (Sourba, 1992).

For over a century, the amino acid pool in skeletal muscle has been considered to be a reservoir for synthesis of contractile proteins and enzymes

(Wagenmakers, 1998). Glutamine, the end product of muscle amino acid metabolism, (Antonio & Street, 1999; Sourba, 1992;Wagenmakers, 1998) is thought to be important in the regulation of protein synthesis in skeletal muscle (Antonio & Street, 1999; Wagenmakers, 1998). Using an isolated rat hindquarter model, the perfusion of glutamine caused intracellular glutamine concentrations to increase by 30-200% and protein synthesis by 66-80% (MacLennan et al. 1987) while protein degradation decreased (Maclennan et al. 1988). In subjects supplementing with 2 grams of glutamine following extended endurance type exercise, plasma glutamine and growth hormone concentration were significantly elevated compared to a control group (Welbourne, 1995) Therefore, glutamine supplementation seems to be associated with increased potential for protein synthesis.

During periods of physiological stress such as disease, trauma and over-training, glutamine export from skeletal muscle increases in relation to glutamine consumption by non- skeletal muscle tissues (Antonio & Street, 1999; Wagenmakers, 1998). The glutamine that is exported is the end product of protein degradation. Therefore glutamine supplementation has the potential to take the place of glutamine exported from skeletal muscle, thus preventing protein degradation. The following are specific examples of physiologically stressful situations during which glutamine supplementation may prevent protein degradation.

In patients undergoing elective open cholecystectomy surgery, the absence of glutamine supplementation caused decreased nitrogen balance and increased

fatigue. Patients who supplemented with glutamine (20g/day) showed higher levels of protein synthesis as assessed by ribosome counts by the third day following surgery (Petersson et al. 1994). Ribosomes are involved in RNA translation within cells leading to muscle protein synthesis (Petersson et al. 1994). Therefore, glutamine supplementation may offset protein degradation by increasing protein synthesis.

In examining the prevention of muscle atrophy in glucocorticoid (cortisol acetate) treated female rats, glutamine supplementation for a seven-day period caused a significant increase in myosin heavy chain synthesis compared to non-supplemented rats and was an effective antagonist against glucocorticoid mediated muscle atrophy (Hickson et al. 1995, 1996).

Recent speculation has surfaced that an increase in exogenous glutamine consumption will increase muscle protein synthesis and athletic performance (Biolo et al.1995). If glutamine can enhance muscle protein synthesis following resistance type exercise, the possibility exists for an athlete to train at higher volumes on a consistent basis which could lead to increases in performance. Intense training can lead to elevations in cortisol levels, which may attenuate muscle hypertrophy (Kraemer et al. 1995). Given the ability of glutamine to offset glucocorticoid induced muscle atrophy in animals, glutamine supplementation may be beneficial for athletes engaged in heavy training. Glutamine may offset the catabolic effect of these hormones by inducing an anabolic signal by stimulating an increase in circulating growth hormone (Haussinger et al. 1994; Welbourne, 1995). By acting as a fuel to vital organs such as the liver and

kidneys, the provision of glutamine may directly spare its rate of decline when inadequate dietary intake occurs and thus, muscle protein levels are maintained (Antonio & Street, 1999). This sparing action would be important for athletes engaged in strength-power sports which require enhanced levels of muscle mass. With intense exercise, an athlete may utilize more glutamine than can be released by other organs and muscle catabolism may occur. Therefore, glutamine supplementation may preserve muscle protein during intense exercise. This would allow for enhanced muscle recovery, which over the long run could lead to increases in athletic performance.

Glutamine supplementation also increases the rate of recovery between exercise sessions by enhancing muscular glycogen stores (Bowtell et al. 1999; Varnier et al. 1998) and increasing the concentration of Krebs- cycle intermediates needed for ATP synthesis (Wagenmakers, 1998). This may lead to enhanced recovery needed for building muscular strength and power. Following extensive endurance exercise, immune system function diminishes and intramuscular glutamine concentrations are decreased when used as a fuel source by lymphocytes, macrophages and fibroblast cells. Supplementing with glutamine may prevent or decrease the severity of infection or illness after an intense bout of exercise, therefore, enhancing recovery from exercise.

1.2.2 Glutamine Metabolism at Rest

During times of rest, skeletal muscle is capable of oxidizing six amino acids. These six are leucine, isoleucine, valine, asparate, aspargine and glutamate (Sourba, 1992; Wagnemakers, 1998). In the reaction catalyzed by glutamine synthetase, glutamate combines with ammonia to form glutamine or it may donate its amino group to pyruvate to form alanine (Graham et al. 1997; Sourba, 1992;Wagnemakers, 1998). Both these reactions provide a mechanism for the transport of the amino groups from the muscle (Antonio & Street, 1999; Sourba, 1992;Wagenmakers, 1998).

With prolonged resting periods such as an overnight fast, there is a net breakdown of muscle protein as amino acids get released for various physiological processes (Wagenmakers, 1998). Glutamine and alanine account for 60-80% of the amino acids that get exported from skeletal muscle (Forslund et al. 2000). The various amino acids that do not get metabolized by the working muscles eventually get released in proportion to their concentration in the muscle (Burke & Heeley, 1999). Glutamine, having two nitrogen atoms/molecule, appears to be the dominant carrier of nitrogen from skeletal muscle (Sourba, 1992; Wagenmakers, 1998). Upon its release, glutamine is utilized by the liver, kidneys, gut, immune system and cells of the intestinal wall for processes such as ureagenesis, gluconeogenesis, and protein synthesis (Antonio & Street, 1999; Wagenmakers, 1998).

1.2.3 Glutamine Metabolism During Exercise

The initiation of exercise causes various amino acids to become released into free pools and exchanged between different tissues by specific physiological pathways (Antonio & Street, 1999). The primary amino acids that are released are the branched chain amino acids and alanine and glutamine (Muhlbacher et al. 1984; Wagenmakers, 1998; Willams & Haynes, 1998). This enables the branched chained amino acids leucine, valine and isoleucine to travel from the liver and stomach to the working muscles (Sourba, 1992; Wagenmakers, 1998; Willams & Haynes, 1998). Alanine and glutamine travel to the liver and gastrointestinal tract to synthesize glucose through the process of gluconeogenesis (Antonio & Street, 1999; Wagenmakers, 1998), which in turn allows blood glucose levels to remain constant during exercise.

As protein synthesis is suppressed during extended exercise, there is a net release of free amino acids to the tissues that are affected (Burke & Inge, 1999; Sourba, 1992; Wagenmakers, 1998). Tissues with high rates of turnover will be more affected than the ones with minimal change. Skeletal muscle is less affected during exercise due to its low rates of protein turnover and responds less to the flux of free amino acids (Sourba, 1992; Wagenmakers, 1998). Exercise stimulates amino acid oxidation and purine catabolism which produces an increase in nitrogen and ammonia (Graham et al. 1997; Willams et al. 1998). If ammonia

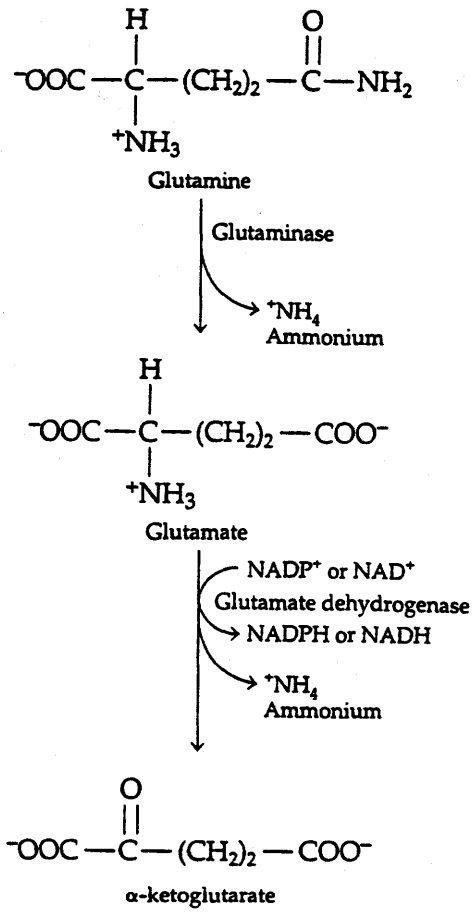
levels increase too much, muscular contraction becomes jeopardized. Thus the majority of ammonia is released from skeletal muscle through the formation and release of glutamine and alanine (Galassetti et al. 1998). Glutamine and alanine enter the blood and are converted to glucose via gluconeogenesis in the liver (Wagenmakers, 1998). This represents a fundamental link between amino acid and carbohydrate metabolism. In its role as a transporter of ammonia, glutamine is broken down to glutamate by glutaminase in the liver. Ammonia is then excreted by the formation of urea. The remaining glutamate is either metabolized in the gut and liver or transported back to the muscle for resynthesis of glutamine through the glutamine-glutamate cycle (Wagenmakers, 1998). While it is known that endurance type exercise causes an increase in glutamine production through the enhanced enzymatic action of glutamate dehydrogenase and glutamine synthetase (Graham et al. 1997), little is known about glutamine metabolism during resistance training.

1.2.4 Glutamine Synthesis and Degradation

The exact mechanism for glutamine synthesis is not fully known due to the lack of understanding as to the source of ammonia needed for synthesis (Wagenmakers, 1998). A small portion of ammonia is generated in the gastrointestinal tract from the bacterial breakdown of urea and amino acids (Groff & Gropper, 2000) as well as from the digestion and absorption of food. However, there is a lack of consensus as to where the majority of ammonia supply originates (Wagenmakers, 1998).

All of the deamination reactions within the body are capable of providing the ammonia source needed for the synthesis of glutamine but two enzymatic reactions have been proposed as the major sources for ammonia needed. Adenosine monophosphate (AMP) deaminase catalyses the deamination of AMP to inosine monophosphate (IMP) and ammonia (Graham et al. 1997; Wagenmakers, 1998). This mainly occurs during heavy exercise when the ATP/ADP ratio is compromised (Curthoys & Watford, 1995; Maclean et al. 1996; Sourba, 1992; Wagenmakers, 1998).

The second proposed source of ammonia is from the reversible reaction catalyzed by glutamate dehydrogenase:



Within the inner membrane of the mitochondria, glutamine degradation occurs when glutamine is hydrolyzed to form glutamate and ammonia from the enzymatic action of glutaminase (Graham et al. 1997). Tissues such as the gut mucosal and lymphocyte cells contain high concentrations of glutaminase which causes the breakdown of glutamine so it can be used as a fuel source (Antonio & Street, 1999; Burke & Heeley, 1999; Sourba, 1992; Wagenmakers, 1998). Glutaminase is located in the membrane of the mitochondria and it is thought that glutamine must travel across the inner and outer membranes before it can be hydrolyzed and become available as a fuel source for demanding cells (Sourba, 1992).

1.2.5 Glutamine Transport

Amino acid transport systems are present in the small intestine and plasma membrane of different cells (Sourba, 1992;Wagenmakers, 1998). The amino acid transport systems are essential for transport to tissues against a concentration gradient. Following a meal, transport "System B" actively transports glutamine and most of the di-polar amino acids(Sourba, 1992) to the portal blood stream for available uptake to other demanding tissues. This system is located primarily in the epithelial cell lining and small intestinal tract (Sourba, 1992). "System A" is responsible for transporting glutamine into the regions containing hepatocytes, fibroblasts and lymphocyte cells (Lee et al. 1998; Sourba, 1992; Wagenmakers, 1998) while "System ASC" regulates glutamine transport to the pulmonary endothelial cells and alveolar cells (Sourba, 1992). The large difference in

glutamine concentration between skeletal muscle and the blood stream is governed by the nitrogen transport system; (System- N) (Lee et al. 1998; Sourba, 1992). "System-N" was first discovered in hepatocytes and is unique in that it has a low affinity, high capacity, sodium dependent transporter that only takes asparagine, histidine and especially glutamine as major substituents (Wagenmakers, 1998). The process for glutamine transport by System- N is regulated by a sodium dependent carrier mediated component (Sourba, 1992; Wagenmakers, 1998). This system plays a major role in the controlled release of glutamine from muscle into the circulation. Its affinity has been shown to be elevated during times of physiological stress or trauma (Wagenmakers, 1998), and intense exercise may stimulate the rate of glutamine release from skeletal muscle into circulation.

The sodium dependent process involves the binding of glutamine to an active site on a protein complex with several spanning membrane domains (Sourba, 1992). For the coupling reaction to take place, sodium ions are released to form a high intracellular/extra cellular gradient differential (Sourba, 1992). Initial binding from the sodium ions to the active site allows for a glutamine-sodium co-transporter complex and thus, an electrochemical gradient is established across the plasma membrane (Sourba, 1992). The energy needed for glutamine to travel through the plasma membrane is produced by the sodium chemical gradient and membrane potential. The electrochemical membrane potential increases the driving forces for glutamine transport, which allows for an increase in the rate of absorption from circulation (Sourba, 1992). The energy

required for the continuous uptake of glutamine is maintained by the sodium-potassium ATPase ion transporter in the plasma membrane, which actively exchanges sodium with potassium (Lee et al. 1998; Sourba, 1992). This cycle is continuously repeated when glutamine release is high such as following glycogen depleting exercise or during times of physiological stress (Wagenmakers, 1998).

1.2.6 Glutamine and Glucose Regulation

During periods of starvation or extended exercise, muscle glycogen stores are depleted (Wagenmakers, 1998; Williams & Haynes, 1998) and protein degradation occurs for provision of energy. Previously, it was thought that alanine was the primary amino acid released into the bloodstream from muscle to become converted to glucose by the liver to refuel working muscles (i.e. glucose-alanine cycle) (Wagenmakers, 1998). Recently, the metabolic pathway for the glucose-alanine cycle has been challenged (Wagenmakers, 1998; Willams et al. 1998) due to the uncertainty as to the source of pyruvate needed for the production of alanine. Glutamine appears to be the end product in the pathway for protein catabolism during extended exercise or starvation and is released from muscle to blood (Wagenmakers, 1998). In this case, protein and amino acids in skeletal muscle are metabolized to generate carbon and nitrogen for synthesis of glutamine. Protein and amino acid derived carbon are then exported from muscle in the form of glutamine to the kidney and liver and may play more of a role in gluconeogenesis and muscle glycogen resynthesis than alanine (i.e. glucose-glutamine cycle) (Perriello et al. 1997; Wagenmakers, 1998). Supplementation of

exogenous glutamine may prevent this utilization by substituting for that which would have been released from muscle. This would in turn suppress protein degradation in skeletal muscle during prolonged exercise (Wagenmakers, 1998).

Following exhaustive endurance exercise, both muscle glycogen and plasma glutamine levels are decreased. Glutamine supplementation appears to promote the activation of glycogen synthetase, the enzyme that controls glycogen synthesis, resulting in an increase of whole-body carbohydrate storage (Bowtell et al, 1999). The activation of glycogen synthease may occur as a result of glutamine-induced cell swelling (Low et al. 1996). Therefore, glutamine supplementation seems to enhance glycogen resynthesis after high intensity prolonged exercise (Antonio & Street, 1999; Bowtell et al. 1999; Roy & Tarnopolsky, 1998).

Although glycogen depletion is thought to be limiting during prolonged endurance exercise, significant glycogen depletion has been observed after resistance training (Roy & Tarnopolsky, 1998). Ten young (19-21 yrs) males who were regularly participating in strength training performed two supervised bouts of total body resistance training. It was shown that a single training session caused substantial muscle glycogen depletion. This may limit performance of resistance exercise on subsequent days. Glutamine supplementation stimulates glycogen resynthesis following endurance type exercise (Bowtell et al. 1999; Varnier et al. 1995). It has therefore been proposed that glutamine supplementation can aid with glycogen resynthesis following resistance exercise,

allowing for enhanced recovery between training sessions (Antonio & Street, 1999).

1.2.7 Glutamine and Immune System Response

Physiological stressors such as burns, surgery, athletic over-training and prolonged exercise cause a significant reduction of glutamine in both plasma and skeletal muscle (Antonio & Street, 1999; Wagenmakers, 1998). To counteract these stressors, glutamine metabolism is thought to increase in order to promote antibody production, cell division, protein synthesis, (Maclennan et al. 1987) and wound healing (Antonio &Street, 1999; Bowtell et al. 1999; Castell et al. 1997). Glutamine is a major fuel source for macrophages and lymphocytes (Antonio & Street, 1999; Bowtell et al. 1999; Castell et al. 1996; Wallace & Keast, 1992) and without adequate supply, synthesis of various immune cells diminishes (Antonio & Street, 1999; Ardawi & Majzoub, 1991; Maclennan et al. 1987). Macrophages are terminally differentiated cells that have low rates of DNA synthesis and are unique in that they lack the ability of cell replication but maintain high rates of protein secretions and membrane cycling (Wallace & Keast, 1992). They act as the first line of defense in the immune system by ingesting, degrading and eliminating infectious agents that enter the body (Wallace & Keast, 1992). Macrophages appear to be dependent on extracellular sources of glutamine due to their inability to synthesize this amino acid especially during times of immune system stress such as trauma, sepsis and burns (Newsholme, 1994; Wallace & Keast, 1992). Lymphocytes resemble macrophages in that they are involved in

immune system response to infectious agents but are biologically diverse in that they have the ability to undergo cell division (Newsholme, 1994; Wallace & Keast, 1992).

Athletic over-training is a concept that relates to excessive exercise over an extended period of time without adequate recovery between training periods (Fry et al. 1998; Hiscock & Mackinnon, 1998; MacDougall et al. 1998). Symptoms include performance decrements, mood variations, fatigue, muscle soreness and impaired immune function (Hiscock & Mackinnon, 1998). Glutamine appears to be released from skeletal muscle and used as a fuel source by cells of the immune system at high rates during times of excessive training (Antonio & Street, 1999; Jackson et al. 2000; Rowbottom et al. 1996). This could lead to increased protein degradation and impaired muscle recovery following exercise.

Several studies have suggested that after extensive endurance exercise, immune response may be altered and plasma glutamine concentrations decreased (Castell et al.1996; Castell & Newsholme, 1998; Volek et al. 1997). Intense exercise appears to increase lymphocyte cell volume but regional distribution to areas most affected appear to decrease resulting in an impaired response to physiological stress (Newsholme, 1994). In studying the effects of glutamine supplementation on exercise-induced immune changes following a marathon, Rhode et al. (1998a) showed that plasma glutamine levels declined by 20% in a placebo trail whereas glutamine levels were maintained or enhanced above rest values with glutamine supplementation (0.1g/kg body weight) at 0, 30, 60 and 90 minutes post exercise. In a subsequent study, Rhode et al. (1998b) showed that

plasma glutamine levels were maintained with 0.9g/kg body weight of glutamine supplementation for two hours following submaximal exercise at 30%, 45% and 60% VO_2 max on a bicycle ergometer. Endurance athletes who consumed oral glutamine (0.3g/kg body weight) versus placebo reported a greater decrease in the rate of infection one week following a marathon (Castell & Newsholme, 1998). Glutamine was also found to enhance the activation of T-helper cells, which reduce the chance of infection or disease (Castell et al. 1996; Jackson et al. 1999; Rhode et al. 1998a). However, glutamine supplementation appeared to have no effect on lymphocyte function following intense endurance exercise (Rhode et al. 1998a).

1.2.8 Glutamine and 3-Methylhistidine

Measurement of 3-Methylhistidine was used as an indicator of muscle protein degradation in the current study; therefore, this measurement and the potential for glutamine supplementation to affect this measurement will be reviewed here. 3-Methylhistidine is an amino acid found in skeletal muscle and is considered to be an index of skeletal muscle protein catabolism (Frontera et al. 1988; Hickson & Hinkelman, 1985; Lukaski et al. 1981; Pivarnik et al. 1989; Welle et al. 1995). It is produced by the posttranslational methylation of specfic histidine residues in actin and myosin. During myofibrillar protein catabolism, 3-Methylhistidine is neither reutilized for protein synthesis nor metabolized oxidatively but is excreted quantitatively in urine (Lukaski et al. 1981). It is

usually expressed as a ratio to creatinine because creatinine excretion is proportional to muscle mass; the more muscle mass, the more 3-Methylhistidine will be excreted (Burke & Heeley, 1999). Over a 4-month resistance training program, daily excretion of urinary 3-Methylhistidine was shown to increase by 41% compared to baseline levels (Frontera et al. 1988). Extensive resistance training for eleven days resulted in a significant increase in 3-Methylhistidine by the third day of training (Pivarnik et al. 1989). However, other studies have failed to demonstrate increases in 3-Methylhistidine following exercise (Rennie & Millward, 1983). Urinary 3-Methylhistidine excretion was not significantly affected by 3 months of resistance training in young or old adults (Welle et al. 1995).

With these findings, we can only speculate that intense resistance training will elicit an increase in urinary 3-Methylhistidine excretion. A progressive increase in 3-Methylhistidine over time may be the result of muscle hypertrophy, which is accompanied by an increase in actin-myosin turnover (Frontera et al. 1998). Even though criticism exists as to the accuracy of 3-Methylhistidine for assessing myofibrillar degradation (Rennie & Millward, 1983), it still seems plausible that intense resistance training should result in muscle tissue damage, increased myofibrillar turnover and an increase in 3-Methylhistidine. Therefore, it is considered an adequate indicator for muscle protein breakdown during training (Welle et al. 1995).

Glutamine supplementation has been speculated to spare muscle proteins during times of physiological stress such as over training (Antonio & Street,

1999; Rowbottom et al. 1996; Wagenmakers, 1998) by increasing muscle protein synthesis and preventing degradation. Therefore, one could assume that subjects who supplement with glutamine would have decreased levels of 3-MH during a resistance-training program compared to subjects without supplementation.

1.2.9 Potential Of Glutamine As An Ergogenic Aid

Since glutamine supplementation helps offset muscle protein degradation during trauma or different disease states, it has been hypothesized that it may serve the same function during recovery from heavy exercise (Antonio & Street, 1999). In order to gain strength and power, a positive nitrogen balance is needed to enhance muscle mass and performance (Burke & Heeley, 1999). A positive nitrogen balance results from enhanced rates of protein synthesis and decreased rates of protein breakdown.

Glutamine supplementation in post-absorptive humans causes a significant increase in gluconeogenesis, which may allow for enhanced rates of glucose availability for energy or glycogen resynthesis (Bowtell et al. 1999; Dechelotte et al. 1991; Hankard et al. 1997; Peirrelo et al. 1997; Varnier et al. 1995). Studies have shown that extensive resistance training results in a significant reduction in both plasma glucose and muscle glycogen levels (Roy & Tarnopolsky, 1998). Glutamine may serve as a substrate for gluconeogenesis in the liver which could enhance muscle glycogen resynthesis. Glutamine from exogenous supplementation may substitute for glutamine that is released from skeletal

muscle for gluconeogenesis in the liver. This would prevent muscle protein breakdown and enhance recovery from resistance exercise.

Studies regarding immune system function seem to support glutamine supplementation as being beneficial. Glutamine is a major fuel source for cells of the immune system such as macrophages, fibroblasts and lymphocytes (Ardawi & Majzoub, 1991; Bowtell et al, 1999; Castell & Newsholme, 1998; Newsholme, 1994). It maintains skeletal muscle protein synthesis following times of physiological stress such as trauma and surgery (Griffths, 1997) and may enhance immune system response following prolonged, intense training (Antonio & Street, 1999). Glutamine also appears to have the ability to offset protein degradation caused by elevated glucocorticoid hormone levels (Falduto et al. 1992; Haussinger et al. 1994; Hickson et al. 1995; Mulbacher et al. 1984).

1.2.10 Glutamine Safety

Research regarding the safety of glutamine for human consumption suggests no sign of toxicity or harmful side effects. Lowe et al. (1990) studied the effect of glutamine doses of 0.3-0.6g/kg body weight for three 5 –day periods. Ammonia and glutamate levels, which may have harmful side effects, did not change significantly. At dosages of 0.1-0.3g/kg body weight, no evidence of clinical toxicity was observed in subjects over a 4-hour period (Ziegler et al.1990). In addition, at levels of 0.3-0.6g/kg body weight for 5 days, glutamine appeared to be well tolerated without any harmful clinical or biochemical side effects (Ziegler et al. 1990).

1.3 Statement of the Problem and Hypothesis

1.3.1 Statement of the Problem

No study to date has examined the effect of oral glutamine supplementation combined with resistance training on muscular performance and body composition. The majority of research has focused on (1) glucose regulation (2) immune system response and (3) muscle protein synthesis/degradation. With the proposed effects of glutamine on these areas, speculation has risen that glutamine may enhance glycogen resynthesis, muscle recovery, protein synthesis and prevent protein degradation following resistance training. This would enable an athlete to train harder, longer and at a more efficient rate, which may lead to increases in muscular performance.

The purpose of this study, therefore, was to determine the effect of oral glutamine supplementation combined with resistance training on muscular strength, torque output, bone mineral free lean tissue mass and protein degradation. Thirty-one subjects (seventeen males and fourteen females) were randomized into either glutamine (N=17) or placebo (N=14) groups. The study was double blind with neither subjects nor persons involved in the experiment aware of group assignment. All subjects participated in a periodized total body resistance-training program for six weeks. Exercises were performed for 4-5 sets of 6-12 repetitions ranging from 60-90% 1- repetition maximum (1-RM). The effect of glutamine on strength was investigated during 1-RM squat and bench press. Torque output was assessed on a Biodex isokinetic dynamometer for knee

extension/flexion. Bone mineral free lean tissue mass was analyzed by dual energy X-ray absorptiometry. Urine samples were assessed for muscle protein catabolism by measuring levels of 3-Methylhistidine, an amino acid residue of myofibrils. Food intake prior to each 72-hour meat free phase was analyzed as a potential confounding variable.

1.3.2 Hypotheses

The major hypotheses were that:

1. There would be an increase in strength, torque and bone mineral free lean tissue mass over six weeks of resistance training combined with glutamine supplementation that would be greater than resistance training alone.
2. There would be a decrease in urinary excretion of 3-Methylhistidine for subjects supplemented with glutamine compared to subjects receiving placebo during six weeks of resistance training.

1.3.3 Limitations

1. Results obtained from the study could only be applied to the specific population that subjects were drawn from.
2. The primary measure of strength and power was dependent on subject motivation, time of testing, 6-week training compliance and supplementation adherence.

3. Dietary habits were not completely controlled since the subjects were free-living people. Food intake estimation relies upon the ability of the subjects to report accurate portion sizes as well as their loyalty in filling out the 3-day food record.

4. Urine samples were based on subject adherence to the designated procedure. Human error may have occurred during sample collection.

5. Without controlling for diet, the 3-day meat free protocol for urine collection may not have been adhered to completely. Meat-based products may elevate 3-Methylhistidine levels above normal and may produce inaccurate measures of muscle catabolism (Lukaski et al.1981).

1.3.4 Delimitations

1. Results from this study apply to the age range of subjects, their training status and their glutamine intake since all variables may affect the results.

2. Since this study was conducted under a laboratory setting, it is difficult to predict if glutamine would have an effect in a field setting.

Chapter 2

Methods

2.1 Research Design

The study used a double blind repeated measures design in which every subject participated in strength training and was randomized to a glutamine or placebo treatment condition. All subjects were required to come into the laboratory on two occasions, at the beginning of the 6-week study for initial data collection and familiarization of resistance equipment and Biodex ioskinetic dynamometer and again at the end of the study for post-testing .The treatment was either glutamine or placebo powder and the dependent variables were (1) strength, (2) peak torque, (3) bone mineral free lean tissue mass, (4) body fat percentage and (5) urinary 3-Methylhistidine excretion. In addition, individual dietary records for three days prior to each 72-hour meat free phase before testing trials were completed to assess for nutrient differences between groups. This study was approved by the Ethics Review Board of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

2.2 Participants

Forty participants, male (N=21) and female (N=19), were involved in the study. Subjects were recruited from an active population whereby they participated in resistance training at least twice a week but no more than 4 times a week. Individuals were free from other ergogenic aids for at least 6 weeks prior to initial testing to eliminate any effects from other supplementation. For example, creatine monohydrate has been shown to elicit enhancements to muscular performance for up to 4 weeks post supplementation (Vanderberghe et al. 1997). They were also instructed not to consume any additional ergogenic aids during the six-week study. By limiting all additional ergogenic aids, a more valid estimate of the effect of glutamine supplementation on athletic performance could be made. All participants were instructed not to change their diets before or during the resistance program so that the treatment could provide a more efficient and controlled effect on performance.

2.3 Procedures

2.3.1 Test Protocol

Participants were recruited from the College of Kinesiology, University of Saskatchewan, on a volunteer basis by a circulating sign up sheet, posters and word of mouth. Participants were informed as to the nature of the study and what would be required of them and written consent was obtained (Appendix A). Prior

to the first visit to the laboratory for initial testing and data collection, all participants were instructed to refrain from resistance training for 24 hours. They were also instructed to collect urine samples at least 24 hours before the initial testing period after a 72-hour meat free diet where no fish, poultry, beef, pork or processed meat products could be consumed. A meat free diet was implemented to reduce exogenous 3-Methylhistidine intake which would facilitate the analysis of muscle protein degradation (Hickson & Hinkelman, 1985). It has been determined that meat consumption affects 3-Methylhistidine levels and that three days of a meat-free diet are required to return 3-Methylhistidine levels to baseline (Lukanski et al. 1981). For the present study, by limiting all meat products for three days prior to urine collection, a more concise conclusion regarding 3-Methylhistidine can be made with regards to glutamine supplementation. Following the initial testing day, subjects were permitted to go back to their normal diet until the final urination collection at the end of week 6.

Each participant attended the laboratory for initial testing and again immediately following the 6-week resistance-training period. During the initial and final testing day participants were assessed on two strength measures of 1-RM squat and bench press. Peak torque was assessed by 3 sets of 1-repetition maximal flexion and extension with the dominant leg on Biodex isokinetic dynamometer.

2.3.2 Strength Measures

To measure the 1-RM squat, a squat rack and an Olympic barbell was used. Each subject positioned his/her feet approximately shoulder width apart inside the squat rack and in front of a full body mirror. Subjects were instructed to lower the squat bar until an internal angle at the knees of 80-90 degrees was achieved before returning to the upright position. A warm-up consisted of the modified hurdler's stretch held twice on each leg for 20 seconds followed by 10 repetitions of squat using a weight determined by each subject as an appropriate warm-up weight.

For bench press, subjects were positioned on the bench with both feet flat on the floor. Subjects were not allowed to lift their buttocks off the bench or arch their backs during a lift. A complete repetition went from the top straight-arm position, down until the bar touched the chest, and then ended with the bar returning to the top straight-arm position. A warm-up consisted of 20 push-ups; 2 static stretches of the chest musculature against a wall, held for 8 seconds each; and 10 repetitions with a comfortable starting weight as determined by each subject.

Following warm-up, for both squat and bench press testing, subjects selected a weight they felt they could complete 3 repetitions with. At this weight, they only performed one repetition. Subjects then selected a weight they felt would be their 1-RM for their next attempt. Weight was then progressively increased by 2-5 kg for subsequent attempts. This procedure was repeated, with each subject performing only one repetition until his/her 1-RM was reached (Kraemer et al.

1998). The 1-RM was usually reached in 4- 6 sets, including the warm-up set. There was 3-minutes rest between sets and two assistants changed the weight on the bar between sets. Reproducibility of squat and bench press was assessed by testing 1-RM strength on 12 subjects at two occasions, one week apart. Coefficient of variation was 5.97% for squat and 1.89% for bench press.

2.3.3 Torque

Torque was measured in the right knee extensors and flexors, using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley NY). The dynamometer was set in the concentric mode for knee extension/flexion, at an angular velocity of 60 degrees per second. Range of motion consisted of movement from 90 to 170 degrees of knee flexion (internal angle). Subjects sat against a back support, producing an angle of 85 degrees of hip flexion. Stabilizing belts were placed over the lap, across the chest, and across the distal one-third of the tested leg thigh. The rotational axis of the dynamometer was positioned to be coaxial with the knee axis (lateral condyle) during testing. One repetition of knee extension, followed by knee flexion, at maximal effort was repeated 3 times with 1-minute rest between sets. The highest peak torque obtained during the three repetitions was recorded for extension and flexion. Torque measures were corrected for the effects of gravity on the lower leg and the dynamometer's resistance pad. The torque output on the dynamometer was checked with a calibration weight on a weekly basis

throughout the study duration. Reproducibility was assessed by testing maximal knee extension/flexion on 8 subjects, one week apart. Coefficent of variation for knee extension was 2.43% and 6.16% for flexion.

The order of tests was the same each time: squat, bench press, and isokinetic knee extension/flexion, with at least 10 minutes of rest between tests.

2.3.4 Body Composition

Bone mineral free lean tissue mass and body fat percentage was assessed by dual energy x-ray absorptiometry (DEXA) (Appendix D) at the beginning of the study and following 6 weeks of supplementation. Whole-body (headless) DEXA scans were performed on a Hologic QDR-2000 in array mode and analyzed using system software version 7.01. The same technician conducted all DEXA scans and regional body analysis. Reproducibility was determined on 10 subjects on two separate occasions. The coefficient of variation for lean tissue mass was 0.54% (Wallace, 1995). Body weight was measured on a Toledo scale, accurate to the nearest 0.1 kilogram.

2.3.5 Treatment Dosage

Each participant was randomly assigned to receive either glutamine or placebo powder based on individual bone mineral free lean tissue mass which was determined by dual energy X-ray absorptometry (DEXA). The dose was 0.9g/kg

supplementation compliance, all eight individual Ziploc bags had to be returned before additional supplements were provided.

2.3.6 Urine Analysis

Individual urine collection was carried out at two stages, week 0 and 6 (Appendix B). The designated urine collection procedure was to miss the first urination upon waking up in the morning and then collect every sample for that day, including the first one upon waking up the following morning. Urine samples were brought into the researcher where individual urine volume and subject identification number were recorded. Aliquots of each urine sample were drawn off from the twenty-four hour collection and stored at – 20 degrees Celsius until analyzed. 3-Methylhistidine was assessed with the methods of Long et al. (1975). To summarize, 3-Methylhistidine was measured by high performance liquid chromatography (HPLC) on a Varian 5000 system following derivatization with o-phthalaldehyde (OPA) reagent at absorbance detection of 338 nm. To prepare samples for analysis, 500 uL of urine and 500 uL of acetonitrile were mixed, vortexed and centrifuged at 5000 rpm for twelve minutes. Following centrifugation, 100 uL of this solution was then mixed with 100uL OPA reagent. To create the OPA reagent, 50 mg OPA was combined with 1ml methanol which was then added to 9ml 0.4 M borate buffer and 50 uL 2-mercaptoethanol. Buffer A was 50mM phosphate at a pH of 7.2 (7.1 g Na_2HPO_4/L, 6.9 g $NaH_2PO_4.H_2O$/L) and buffer B was methanol. A flow rate of 1ml/min and

gradients of 18-25% methanol for the first 15 minutes, 25% methanol for minutes 15 – 20, 25-70% methanol for minutes 25-30 and 70-18% methanol for minutes 30-35 were used to isolate 3-Methylhistidine. An Econosol [250 mm x 4.6mm, 10um (Alltech Associates)] column was used as the stationary phase with an injection loop of 10 uL. Retention time for 3-Methylhistidine was seventeen minutes and was quantified by peak height comparison to a standard curve of 3-Methylhistidine treated in the same manner as the urine samples. 3-Methylhistidine measurements were expressed in absolute units relative to bone mineral free lean tissue mass (umol/kg bone mineral free lean tissue mass).

2.3.7 Dietary Assessment

Dietary intake was recorded three days prior to each 72-hour meat free phase to assess whether there were differences in caloric intake and macronutrient composition between groups. Participants used a three-day food booklet (Appendix E) to record what they ate for two weekdays and one weekend day. They were instructed to record all food items and serving sizes they consumed for the three designated days. The Fuel 2.1 dietary analysis program (Nutrition Software, Logiform Nutrition Sport, 1999) was used to analyze the food records (Appendix F). Each item was entered and the program provided the total number of calories consumed on average over the three days as well as the amount of energy from carbohydrates, protein, lipids and alcohol. Carbohydrates and protein provide approximately 4 kcal/g to total energy volume while lipids and alcohol

provide 9 kcal/g and 7 kcal/g respectively. This information was used to determine whether diet might be a confounding factor in the study.

2.3.8 Resistance Program

All subjects followed the same high volume, heavy load, periodized, free-weight resistance-training program for 6 weeks (Appendix G) which has been shown to elicit significant increases in strength and torque (Burke et al. 2000). Weight training started on the first day of supplementation and consisted of a 4-day split routine involving whole body musculature (Appendix H). Day 1 involved chest and triceps musculature and included the following exercises in order: bench press, incline bench press, flat bench dumbbell flys, incline dumbbell flys, cable triceps extensions, rope reverse triceps extensions, and French curls. Day 2 involved back and biceps musculature and included the following exercises in order: chin-ups, low row, "lat" pull-downs, alternate dumbbell row, standing EZ-curls, preacher curls, and alternate dumbbell curls. Day 3 involved leg, shoulder, and abdominal musculature and included the following exercises in order: squats, leg extensions, hamstring curls, standing calve raises, military dumbbell press, upright row, shrugs, deltoid flys, and abdominal crunches. Day 4 was a rest day. This 4-day cycle was repeated continuously throughout the duration of the study. The periodized program was broken into five blocks of two cycles or eight days for a total of 40 days at an intensity level ranging from 60-90% 1-RM. Block one consisted of four sets of 10-12 repetitions, with 1-minute

rest between sets. Block two (days 9-16) consisted of 4 sets of 8-10 repetitions, with 1.5-minutes rest between sets. Block three (days 17-24) consisted of 5 sets of 6-8 repetitions, with 2-minutes rest between sets. Block four (days 25-32) consisted of 4 sets of 8-10 repetitions, with 2-minutes rest between sets. Block five (days 33-40) consisted of 4 sets of 10-12 repetitions; with 1.5-minutes rest between sets.

2.3.9 Training Logs

Each participant was instructed to record training logs (Appendix I) for each workout day. The workout date, number of repetitions and sets were all filled out for each of the three different working days of the program. All training logs for the eight-day cycle were completed and brought into the researcher for program compliance and verification. Total training volume / session over the six-week resistance program was calculated by multiplying the weight used with the number of sets and repetitions for each exercise.

2.4 Statistical Analysis

A 2 (groups) x 2 (time) ANOVA with repeated measures on the second factor was used to determine whether there were any differences between the glutamine and placebo groups over time for the dependent variables of strength, torque, bone mineral free lean tissue mass, body fat percentage, 3-Methylhistidine and diet (energy and macronutrients). An independent sample t-test was used to determine whether there was a difference in training volume between glutamine and placebo group. An independent sample t-test was also used to assess any baseline differences in subject characteristics between groups. All results were expressed as means± standard error. Statistical significance was set at $P<0.05$.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results

The purpose of this study was to determine the effect of oral glutamine supplementation on muscular performance, body composition and muscle protein degradation. It was hypothesized that muscular strength, torque and bone mineral free lean tissue mass would increase and secondarily, that 3-Methylhistidine excretion would be decreased in the glutamine group compared to placebo.

3.1.1 Participant Characteristics

Forty [(Gln=21; 12 male / 9 female), (Pla=19; 10 male / 9 female)] subjects, aged 18-24, were recruited for this study. Seven (Gln=3, Pla=4) of the original participants were excluded from data analysis due to lack of compliance with the resistance-training program. Compliance was set so that each subject could not miss more than one exercise day per cycle. Two participants (Gln=1, Pla=1) withdrew from the study due to medical reasons. One male participant in the glutamine group who completed initial 1-RM bench press could not perform post testing because of injury. Therefore, of the forty original participants, thirty were used for 1-RM bench press data analysis while thirty-one [(Gln=17; 9 male / 8 female), (Pla=14; 8 male / 6 female)] were used for data analysis of squat, torque,

bone mineral free lean tissue mass and body fat percentage. Twenty- one subjects [(Gln=10; 6 male / 4 female), (Pla=11; 6 male / 5 female)] were able to provide urine samples at week 0 and week 6 for analysis of 3-Methylhistidine. Two male subjects (Gln=1) and (Pla=1) were able to correctly identify the treatment they were consuming.

There were no significant differences between the glutamine and placebo group for any baseline measures, shown in Table 3.1.

Table 3.1 Subject characteristics (mean ± standard error) at week 0 for glutamine and placebo group.

Group	WT (kg)	HT (cm)	SQ (kg)	BP (kg)	KE (Nm)	KF (Nm)	LTM (kg)	BF (%)
Glutamine (N=17)	72.7 ±2.7	175.7 ±2.6	91.2 ±14.6	56.1 ±11.7	227.8 ±53.6	114.7 ±7.9	50.1 ±2.9	22.2 ±2.3
Placebo (N=14)	77.3 ±4.14	173.4 ±2.4	83.5 ±11.8	55.8 ±16.1	208.3 ±12.9	116.1 ±9.5	49.4 ±3.2	26.5 ±3.2

note: no significant differences between groups. (P> 0.05)

WT = weight (kg)

HT = height (cm)

SQ = 1-Rm squat (kg)

BP = 1-RM bench press (kg)

KE = knee extension torque (Nm)

KF = knee flexion torque (Nm)

LTM = lean tissue mass (kg)

BF = body fat percentage (%)

3.1.2 Strength

There was a significant time main effect, [F (1,29)=54.128, p=0.01] for 1-RM squat. The glutamine group had a relative increase of 31%, going from 91.2 ± 14.6 kg to 118.4± 11.0 kg. The placebo group increased by 30%, going from 83.5± 11.8 kg to 108.1± 14.3 kg. However, there were no significant differences between the groups over time, [F (1,29)=0.144; p=0.707], shown in Figure 3.1.

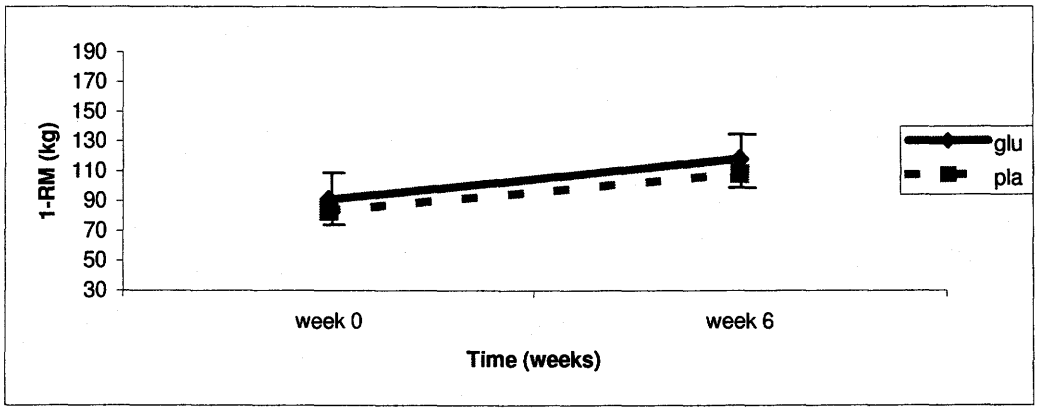


Figure 3.1 Mean 1-RM squat value (± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6 of resistance training.

There was a significant time main effect [F (1,29)=41.23; p=0.01], for 1-RM bench press. The glutamine group had a relative increase of 14%, going from 56.1 ± 11.7 kg to 63.8 ± 6.47 kg while the placebo group increased by 13%, going from 55.8 ± 7.3kg to 63 ± 7.3 kg. There were no significant differences between the groups over time, [F (1,29)=0.038; p=0.85], shown in Figure 3.2.

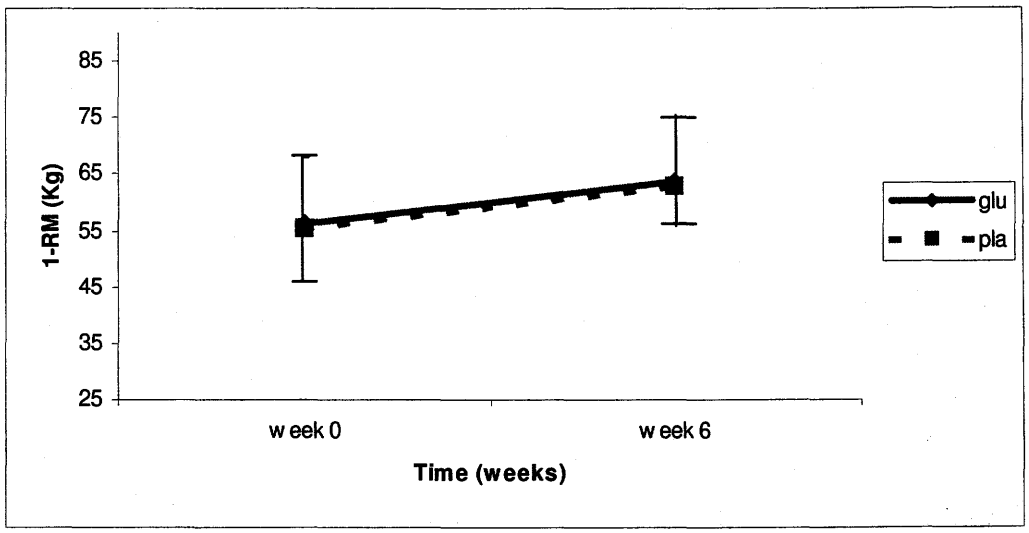


Figure 3.2 Mean 1-RM bench press (± standard error) for glutamine (N=16) and placebo (N=14) at week 0 and week 6 of resistance training.

3.1.2 Torque

There was a significant time main effect, [F (1,29)= 7.7, p=0.01], for knee extension torque. The glutamine group increased their torque output by 6%, going from 227 ± 13.0 Nm to 241 ± 12.7 Nm while the placebo group had a slight increase of 2%, going from 208 ± 12.1 Nm to 218 ± 12.9 Nm. There were no significant differences between the groups over time, [F (1,29)=0.18, p=0.68], shown in Figure 3.3.

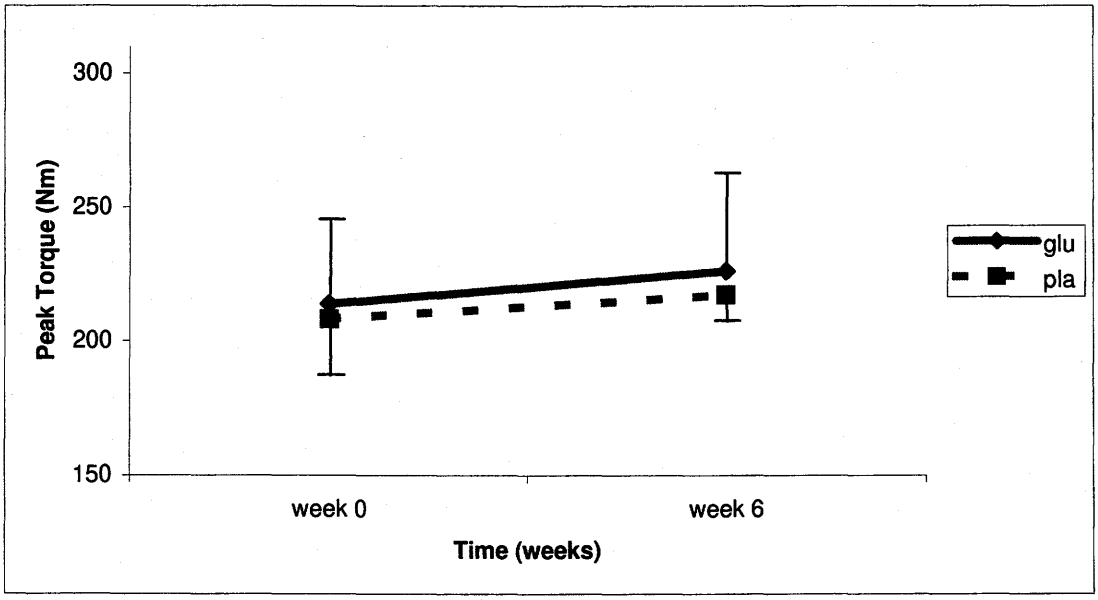


Figure 3.3 Mean leg extension torque (± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6 of resistance training.

Knee flexion torque did not significantly change in either group over time. Flexion measures for the glutamine group increased by 5%, going from 114.7 ± 7.93 Nm to 121 ± 10.2 Nm while the placebo group decreased over time, going from 116 ± 8.61 Nm to 114 ± 9.5 Nm; shown in figure 3.4. This was the only

performance measure where a group x time interaction approached significance, [F (1,29)= 2.76, p=0.11].

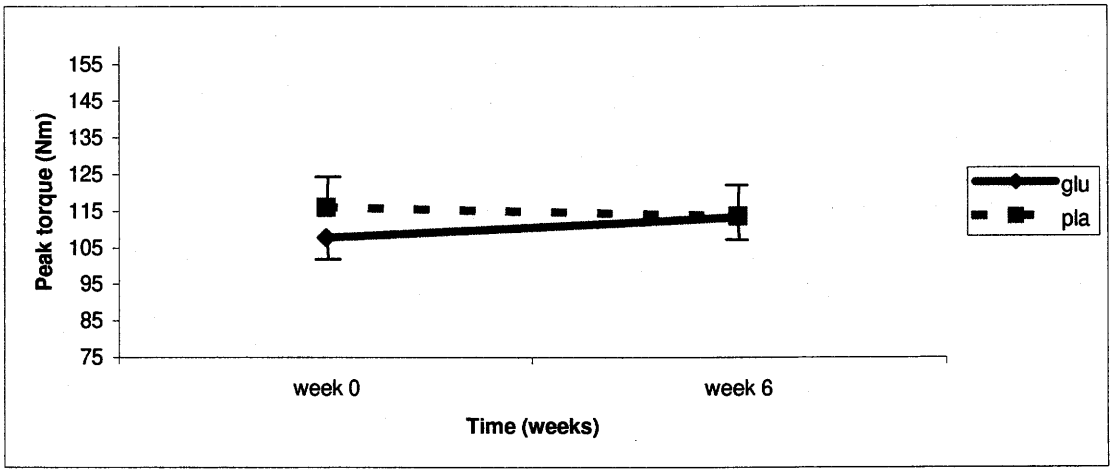


Figure 3.4 Mean leg flexion torque (± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6 of resistance training.

3.1.4 Lean Tissue Mass

There was a significant time main effect, [F (1,28)=7.87; p=0.05] for lean tissue mass. The glutamine group increased their lean tissue mass from 50.1 ± 2.93 kg to 51.1± 2.93 kg while the placebo group increased from 49.4 ± 3.26 kg to 50.2 ± 2.86 kg, as shown in Figure 3.5. There were no differences between groups over time, [F (1,28)=1.61; p=0.21].

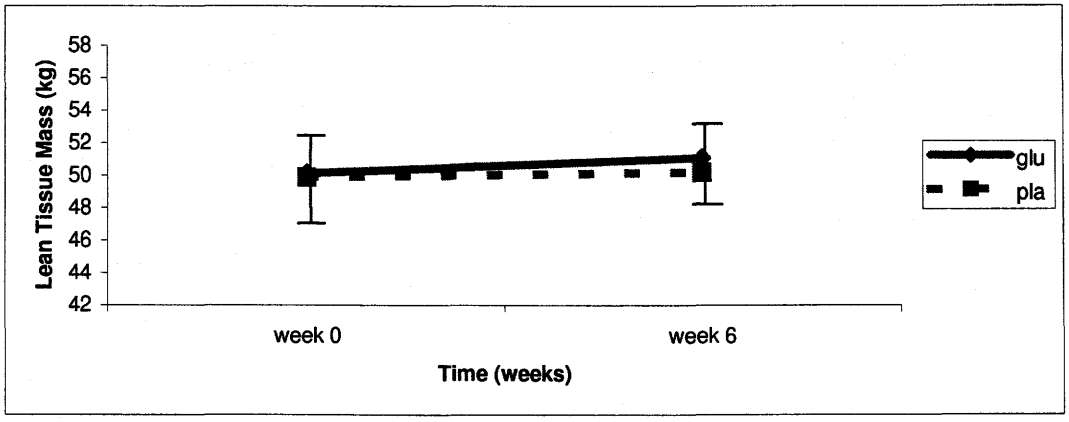


Figure 3.5 Mean lean tissue mass (± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6 of resistance training.

3.1.5 Body Fat

There was no effect of glutamine on percent body fat, [F (1,28)=0.91; p=0.35]. The glutamine group had a slight decrease in percent body fat (22.2 ± 2.3 to 22.0 ± 2.3) while the placebo group increased (26.5 ± 3.23 to 27.1 ± 2.96), as shown in Figure 3.6.

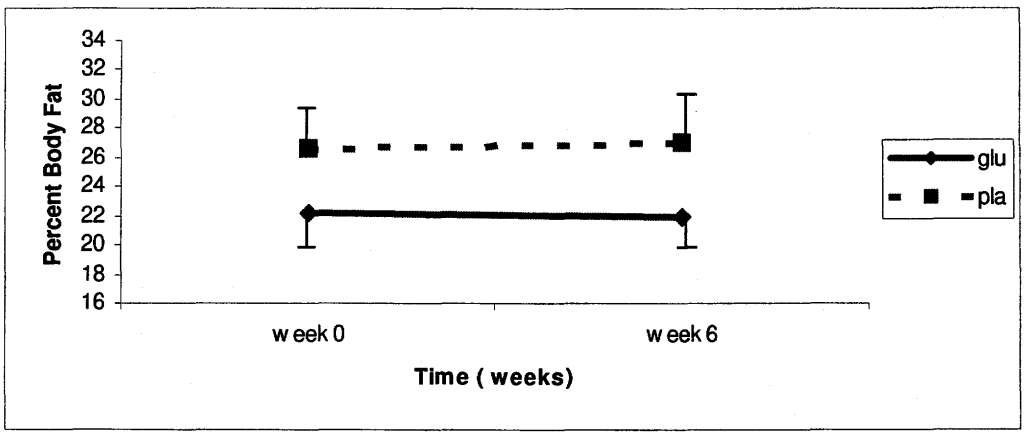


Figure 3.6 Mean total body fat percentage (± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6 of resistance training.

3.1.6 Training Volume

An independent sample T-test showed no difference in training volume for glutamine or placebo group, [T (29)=0.056; p=0.96]. The glutamine group had an average volume of 2367.42 ± 237.8 (kg) per exercise session while the placebo group had an average total of 2389.1 ± 283.9 (kg) per session for the six weeks of resistance training, shown in Figure 3.7.

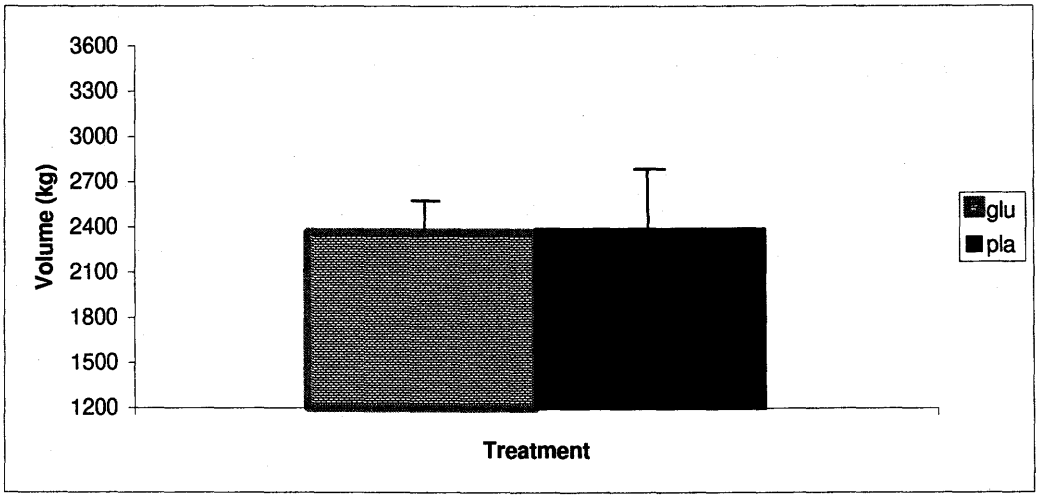


Figure 3.7 Mean training volume (± standard error) for glutamine (N=17) and placebo (N=14) group during 6 weeks of resistance training.

3.1.7 Dietary Intake

Dietary intake did not differ significantly between glutamine and placebo groups, [F (1,29)=1.534; p=0.23], and did not differ significantly from before to the end of training, as shown in Table 3.2.

Table 3.2.Dietary variables (mean ± standard error) for glutamine (N=17) and placebo (N=14) at week 0 and week 6.

	Placebo		Glutamine	
	Week 0	Week 6	Week 0	Week 6
Ave. Kcal/Day	3112±195	2910±227	3018±195	3023±215
% CHO	54.6±2.1	53.4±1.5	51.9±1.8	54.6±1.7
% Pro	16.5±1.2	18.9±1.1	18.3±1.1	21.5±1.2
% Fat	28.9±1.6	27.7 ±13.2	29.8±1.6	23.9±2.3

note: no significant differences between groups. (P>0.05)

% CHO = carbohydrate

% PRO = protein

% FAT = fat

3.1.8 3-Methylhistidine

There was a significant time main effect, [F (1,19)=48.82, p=0.01] for 3 -Methylhistidine excretion. There was no significant difference between the groups over time, [F (1,19)=0.97, p=0.34]. The glutamine group had a relative increase of 41%, going from 4.2 ± 1.64 to 6.1 ± 1.74 umol/day. The placebo group had an increase of 56%, going from 5.2 ± 1.41 umol/day to 7.9 ± 2.13 umol/day; shown in Figure 3.8.

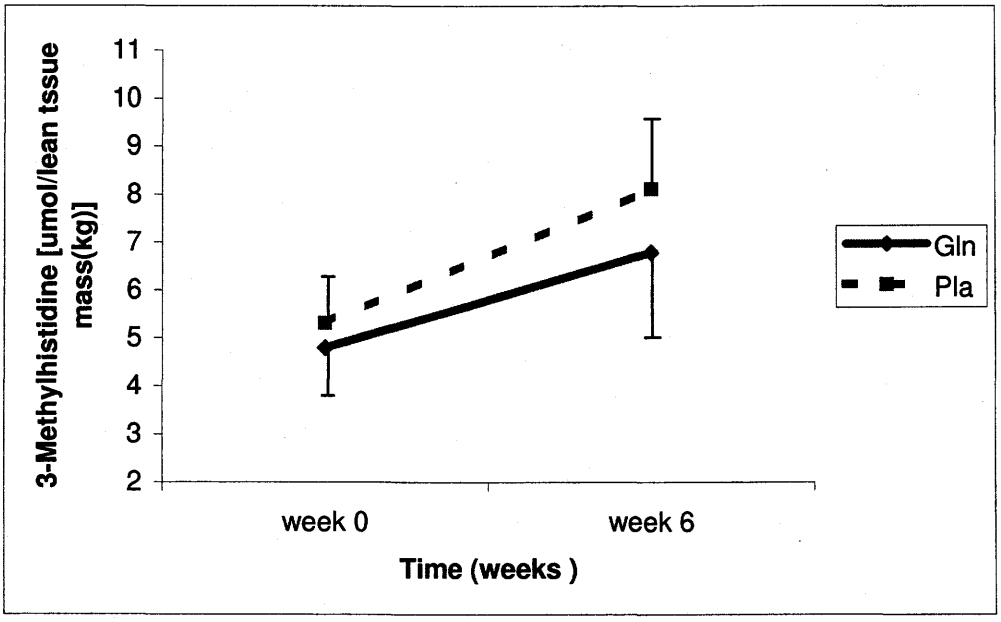


Figure 3.8 Mean 3-Methylhistidine (umol/kg bone mineral free lean tissue mass) excretion (± standard error) for glutamine (N=10) and placebo (N=11) at week 0 and week 6 of resistance training.

3.2 Discussion

The purpose of this study was to investigate the effect of oral glutamine supplementation on muscular strength, torque, lean tissue mass, body fat percentage and 3-Methylhistidine excretion. Thirty-one participants (Gln=17, Pla=14) performed two experimental testing regiments before and following six weeks of resistance training combined with double blind ingestion of glutamine or placebo. Participants completed 1-repetition maximum (1-RM) squat and bench press protocols with progressingly increasing weight separated by two minute rest intervals until a given load could not be completed. Following strength testing, participants completed three maximal 1-repetition sets of leg extension and flexion on a Biodex isokinetic dynamometer. Bone mineral free lean tissue mass and body fat percentage were assessed by dual energy X-ray absorptiometry (DEXA) while 3-Methylhistidine was measured by individual urine sample at two stages throughout the study using high performance liquid chromatography (HPLC).

Previous research regarding glutamine supplementation and athletic performance has primarily focused on endurance type exercise. The general consensus from these studies is that glutamine appears to have the ability to enhance muscle glycogen resynthesis (Bowtell et al. 1999; Varnier et al. 1995) and improve immune system response (Castell et al. 1996, 1997,1998) following endurance type exercise. Experiments with animals demonstrate that glutamine administration increases protein synthesis and decreases degradation (Hickson et al. 1995,1996; Maclennan et al. 1987, 1988). In humans recovering

from surgery, glutamine supplementation also attenuates muscle atrophy (Petersson et al. 1994). Based on the fact that resistance training results in substantial glycogen depletion (Roy & Tarnopolsky, 1998) and protein degradation and resynthesis (Biolo et al. 1995; MacDougall et al. 1995, Phillips et al. 1997), it has been speculated that resistance training may benefit from glutamine supplementation (Antonio & Street, 1999). To date, this is the first experimental study to examine the effect of glutamine supplementation on muscular performance when combined with resistance training. We hypothesized that strength, torque and bone mineral free lean tissue mass would increase more in our glutamine group than in the placebo group. However, the results of the present study found no effect of glutamine on any of the dependent variables measured.

Glutamine may be more beneficial to athletes who are under greater stress from training such as those involved in a combined aerobic and resistance training program or those involved in a weight-loss training program. Also, glutamine supplementation may be more beneficial to those using exercise rehabilitation to recover from diseased conditions such as surgery or corticosteroid use.

Glutamine and placebo groups significantly increased 1-RM squat and bench press over the six week resistance training program but there were no significant differences between the groups. Both glutamine and placebo groups adhered fully to the resistance training program which incorporated total body training and has been shown to produce significant gains in 1-RM squat and

bench press (Burke et al. 2000). The six week resistance training program was chosen because this duration had previously been used to demonstrate differences between groups with other ergogenic aids such as whey protein and creatine (Burke et al. 2000). It is important to mention that one of the proposed ergogenic effects of glutamine is to provide an energy source for cells involved in immune system response. Exhaustive endurance exercise has been shown to suppress the immune system and glutamine supplementation has been shown to improve immune system response and prevent infection or flu like symptoms following exercise (Castell et al. 1996, 1997, 1998). Taking these findings into account, it has been proposed that glutamine may promote recovery between bouts of heavy resistance exercise (Antonio & Street, 1999) and therefore promote muscular adaptation. However, there appears to be no evidence that heavy resistance exercise has a detrimental effect on the immune system (Flynn et al. 1999; Rall et al. 1996). Therefore, resistance training may not be stressful enough to benefit from glutamine supplementation.

The present study used individual bone mineral free lean tissue mass as the basis for administering supplementation dosage. Glutamine is the most abundant amino acid in skeletal muscle and a dosage based on bone mineral free lean tissue mass would provide a more efficient result regarding muscular performance. Our dosage was the upper limit found to be safe with no toxicity (Zielger et al. 1990) and it was greater that the doses shown to be effective for promoting glycogen resynthesis (Bowtell et al. 1999; Varnier et al. 1995) and preventing protein degradation following surgery (Petersson et al. 1994).

During times of physiological stress such as heavy resistance training or trauma, glutamine is utilized at high rates to satisfy demand (Antonio & Street, 1999; Maclennan et al. 1987). If the dosage provided was insufficient, the ability to stimulate an enhanced rate of protein synthesis in the glutamine group may have been diminished.

Torque measures for knee extension and flexion all appeared to increase in the glutamine group but there was no significant difference when compared to the placebo group. However, the group x time interaction for knee flexion approached significance. Both groups completed all leg exercises equally and there was no difference in total body training volume which could have affected performance. Torque measures were taken shortly after 1-RM squat and bench press. As exercise continued, the possibility of having enhanced muscle glycogen stores from glutamine supplementation may have enabled the glutamine group to perform at a more efficient level.

The fact that the group x time interaction for knee flexion exercise approached statistical significance, while the squat 1-RM did not could relate to the amount of learning that takes place over time for these two exercises. A difference in the amount of learning between the Biodex isokinetic dynamometer and squat exercise could also provide an explanation for the relatively small increase in Biodex knee extension and flexion compared to the large increases in the squat 1-RM with training. A large learning effect may have occurred with the squat exercise which requires substantial skill, as both groups showed relatively large increases in strength over time. This may have

masked any differences due to glutamine between groups. A simple exercise such as knee flexion would be influenced less by a learning component, especially since the Biodex isokinetic dynamometer was not used in training. Therefore, this type of test would be more affected by actual changes in skeletal muscle.

It was hypothesized that bone mineral free lean tissue mass would be increased in the glutamine group compared to placebo. As glutamine has been shown to increase muscle protein synthesis and attenuate protein degradation in animal models or following surgery in humans (Hickson et al. 1995, 1996; Maclennan et al. 1987, 1988; Petersson et al. 1994), an enhanced rate of muscle protein turnover should have caused a greater increase in bone mineral free lean tissue mass in the glutamine group compared to the placebo group. There was however no difference in lean tissue mass between the groups but each group did significantly increase over the six weeks of training.

A possible mediating factor that may have affected bone mineral free lean tissue mass was the intensity and duration of each training session. In order to find a real difference between the glutamine and placebo group, perhaps longer, more intense training periods could have been implemented. The higher the intensity, the more catabolic each working session would be (Kraemer et al. 1995). As a result, muscle protein turnover should become enhanced and subjects who supplemented with glutamine may have an accelerated rate of protein turnover leading to increases in muscle recovery. This would potentially allow for an increase in muscle mass as the participants who supplement with

glutamine would be able to continue training at a higher volume than their placebo counterparts.

Individual urine analysis showed that the majority of subjects in the glutamine group had slightly reduced rates of 3-Methylhistidine excretion when compared to placebo but the differences were not significant. It was hypothesized that subjects consuming glutamine would have decreased 3-Methylhistidine excretion rates due to glutamine's ability to increase protein synthesis and decrease protein degradation (Hickson et al. 1995, 1996; Maclennan et al. 1987, 1988; Petersson et al. 1994). Our results are in agreement with Maclennan et al. (1988) and Fang et al. (1995) who showed that glutamine infusion to septic rats did not significantly affect 3-Methylhistidine levels.

Other possible mediating factors that may have affected 3-Methylhistidine excretion was total training volume, individual lean tissue mass and average protein consumption. It was hypothesized that glutamine may enhance muscle protein synthesis following exercise leading to increased rates of muscle recovery. Therefore, we would expect that the supplemented group would select heavier loads as they progressed with training and this would increase training volume. We have previously found this to be the case during supplementation with creatine monohydrate which led to differences in adaptation to training in supplemented and non-supplemented groups (Chrusch et al. 2000). However, analysis of data showed that glutamine supplementation did not significantly decrease muscle protein degradation which may explain the lack of difference in

training volume between groups. Since glutamine did not offset muscle protein degradation, training status between groups remained relatively unchanged. As for individual bone mineral free lean tissue mass, 3-Methylhistidine is often measured as a ratio to bone mineral free lean tissue mass because the more bone mineral free lean tissue mass an individual has, the more 3-Methylhistidine should be excreted (Burke & Heeley, 1999). By basing our supplement dosage on bone mineral free lean tissue mass, the study was better suited to make a valid conclusion as to the effect of glutamine on protein degradation as glutamine is the most abundant amino acid in plasma and skeletal muscle. Analysis of 3-Methylhistidine as a ratio to bone mineral free lean tissue mass showed no real difference between groups prior to and six weeks following glutamine supplementation. A third factor that may have influenced 3-Methylhistidine was average protein consumption. It has previously been shown that a diet high in protein will cause an increase in 3-Methylhistidine excretion and this may inhibit our ability to determine the impact that glutamine had on muscle protein degradation (Hickson & Hinkelman, 1985). Analysis of data showed that there was no difference in average protein consumption for glutamine or placebo groups during the six weeks of training. It is important to note that a possible mediating factor that may have affected performance was total energy balance during the 72-hour meat free period that preceeded each testing trail. Food records were not filled out during these phases and if subjects were in caloric deficit, it may have had a negative impact on performance. It is

important to mention that no individual side effects were reported throughout the six-week study.

It appears that resistance training does not have a differing effect on gender. The present study incorporated a mixed gender design (Gln= 17; 9 male/ 8 female, Pla= 14; 8 male / 6 female) and found no differences in the relative changes between males and females over the six weeks of resistance training. Resistance training appears to elicit the same level of response in males and females regarding muscle damage (Stupka et al. 2000), muscle morphology (Staron et al.1994) and strength changes (Abe et al. 2000).

In conclusion, the ingestion of glutamine supplementation throughout resistance training seems to have little effect on muscular performance, body composition or muscle protein degradation.

CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1 Summary

Nutritional supplementation has become more publicized and analyzed in recent years. A large number of research studies are being performed to determine what ergogenic supplements are beneficial to the athletic population. Glutamine is one of the many nutritional aids available to the athletic population and speculation has risen that glutamine may benefit athletes engaged in intense training as it has previously been shown to enhance muscle glycogen resynthesis, immune system response following aerobic type exercise and muscle protein synthesis in animal models.

This study was performed to determine the effect of glutamine supplementation on strength, torque, bone mineral free lean tissue mass, body fat and urinary 3-Methylhistidine excretion. It was hypothesized that glutamine supplementation would cause an increase in 1-RM squat and bench press, maximal leg flexion and extension, bone mineral free lean tissue mass and decrease body fat percentage and muscle protein degradation.

Thirty-one young adults (Gln=17, Pla=14) received glutamine or placebo supplementation during six weeks of total body resistance training in a double

blind randomized order. Dietary food intake for three days prior to each 72-hour meat free phase were assessed by questionnaire. Individual urine samples were collected twenty-four hours before the initial and final testing periods in compliance with a seventy-two hours meat free diet.

Prior to the initiation of the six week training period, all participants were provided with 0.9g/kg bone mineral free lean tissue mass of glutamine or placebo rounded to the nearest 100mg (average dose of 47.3g for glutamine and 49.1g for placebo).

Repeated measures ANOVA did not show any significant differences between the glutamine or placebo groups over the six weeks of training. These results do not support any of the stated hypotheses.

4.2 Conclusions

According to the results from this study, we conclude that glutamine supplementation had no effect on muscular strength, torque output, body composition or protein turnover during resistance training.

4.3 Recommendations for Future Research

This study monitored participants during the initial and post testing trails. Based on the findings from this study, we recommend that glutamine supplementation should be considered by athletes who participate in prolonged, intense exercise. Training programs which combine cardiovascular and

resistance type exercise have been shown to elevate cortisol levels (Kraemer et al. 1995) which may increase muscle protein degradation and decrease immune system response. Glutamine supplementation has been shown to offset the catabolic effect of glucocorticoids (Hickson et al. 1995, 1996) as well as enhance immune system response following prolonged endurance exercise and trauma (Castell & Newsholme, 1998). Glutamine may also benefit populations that are more easily taxed following physiological stress such as they elderly or individuals with disease conditions. Glutamine supplementation has been shown to increase muscle glycogen resynthesis following exercise (Bowtell et al. 1999; Varnier et al. 1998) which may enhance the rate of substrate metabolism and recovery. Following periods of severe physiological stress such as trauma or extended endurance type exercise, immune system response to stressors is increased with glutamine supplementation (Castell & Newsholme, 1998; Rhode et al. 1998a, 1998b). Glutamine appears to act as an additional fuel source for cells of the immune system and may attenuate amino acid release from skeletal muscle. Therefore, supplementation may offset muscle protein degradation usually observed during physiological stress.

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APPENDICES

Appendix A Consent Form

Consent Form

Title of the Study: Effect of Glutamine Supplementation combined with resistance training on Changes in Muscular Strength, Endurance and Body Composition.

Names of Researchers:
Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, 966-6469.
Darren G. Candow, M.Sc., College of Kinesiology, University of Saskatchewan, 966-2604.

Purpose of the Study: The purpose of the study is to determine the effects of glutamine supplementation on muscular strength, endurance and body composition.

Possible Benefits of the Study: Benefits include the possibility that your muscle mass and strength will increase. These benefits are not guaranteed.

Procedures: You will be randomized into one of two groups. One group will receive a glutamine supplement (0.3g/kg body weight) on a daily basis for 6 weeks. The other group will receive a placebo for 6 weeks. The study will be double blinded, that is, neither you nor the principle researchers will know whether you are taking the glutamine supplement or the placebo. You will be required to participate in a weight-training program during the 6-week period that you are receiving a supplement or placebo. This will involve exercises designed to target different muscle groups each day. Prior to and following 6 weeks of training, your body composition will be assessed by dual energy x-ray absorptiometry, a technique that allows measurement of muscle, fat and bone mass. Also, you will be required to provide 24-hour urine samples prior to the study, and during weekly intervals throughout the study. Prior to and following the 6 weeks of training, your muscular strength will also be assessed in three different exercises. This involves a determination of the maximum amount of weight you can lift during a single lift.

Possible Risks:
There is some radiation exposure during the body composition measurement with the dual energy x-ray absorptiometry, but it is small. The amount of radiation you will be exposed to is equivalent to the amount of radiation you would be exposed to during a return flight to Toronto at 30,000 feet and is about one eighteenth the amount of radiation you would be exposed to during a chest X-ray.

While it remains possible that the supplementation or procedures used in this study may involve side effects or risks that are unknown and currently unforeseeable, none have thus far been associated with this formulation at this dose being supplemented in this study.

Possible risks with the strength training include muscle pulls and strains. An adequate warm up prior to the strength training sessions should avoid this.

You will be free to withdraw from the study at any time and this withdrawal will not affect your academic status or access to services at the university.

All documents or computer flies relating to the study will be stored in a locked office in the College of Kinesiology, so that your anonymity is protected. Data collected from this study will be used as part of a graduate thesis and may be published in a journal article. Only aggregate data will be reported in any publication.

If you have any questions with regard to the research project, you can call Philip Chilibeck at 966-6469 or 343-6577, or Darren Candow at 966-2604.

We will advise you on any new information that will have a bearing on your decision to continue in the study.

We will give you feedback on your individual results and the results of the study at the completion of the research.

I acknowledge that the study and the contents of the consent have been explained to me, that I understand the contents, and that I have received a copy of the consent for my own records.

Date:_____

Signature: _____

Researcher: _____

Witness: _____

Appendix B Subject Information Form

Effect of Glutamine Supplementation Combined with Resistance Training

Study Characteristics

Duration: 6 weeks (March 1- April 11)

Pre- testing measures will take place during Feb 15-21

Testing measures will be:

1. 1- repetition maximum squat
2. 1-repetition maximum bench press
3. Maximal knee extension/flexion contraction on a Biodex isokinetic dynamometer.
4. Lean tissue mass and body fat percentage assessed by dual energy X-ray absorptiometery (DEXA)

Urine Collection

3-day "meat free" urine collections will take place throughout the study at:

1. **Baseline collection**- this collection will take place before the beginning of the six-week resistance-training program. **Protocol**: Skip the first urination in the morning of the collection day and then collect every other sample including the first one when you wake up the following morning.

2. **Post-test collection**- same procedure as baseline protocol but at the end of the six week resistance training program.

Training Program

Each training day must be filled out in designated training log with the weight and number of repetitions used for each exercise. Training logs must be brought into researcher for program compliance and verification on a weekly basis.

Supplementation

Supplementation can be picked up at the R.J.D. Williams building in Room # 467. You will be assigned an identification number which will be listed on the supplement package. The supplement should be consumed twice daily, preferably following a workout and before you go to bed. On rest days, take when desired.

Supplement can be mixed in cold water or fruit juice. Each bag will contain your supplement for the entire day so it is up to you to divide tem evenly upon your desire.

Contact: Call Darren at 374-6890 or 966-2604

Appendix C Study Protocol
Visit at Week 0 and Week 6

Study Protocol

Visit at Week 0

1. Consent Form
2. Warm up on stationary ergometer for 5 minutes
3. Lower limb stretching for 5 minutes
4. Adjust squat bar to suit individual
5. Demonstrate proper squat technique:
    a. Position bar behind head so that there is an even distribution on both sides
    b. Tilt head upwards approximately 15 degrees
    c. Place feet slightly more than shoulder width apart
    d. Extend hip region outwards so that it creates a 90 degree angle with the chest and knees
    e. Lower body until there is a 80-90 degree angle from the knees to the floor
    f. Raise upwards to starting position
6. Rest for 10 minutes
7. Light static pectoral stretching
8. Perform 10-15 push-ups
9. Demonstrate bench press:
    a. Position hands evenly on horizontal bar slightly more than shoulder width apart.

b. Un-rack bar from safety frame

c. Lower bar until it slightly touches the chest region

d. Extend arms back to starting position

e. Re-rack bar on safety frame

10. Rest for 10 minutes

11. Record height (cm) and weight (kg)

12. Demonstrate Biodex isokinetic dynamometer

a. Place individual in chair

b. Adjust chair setting

c. Perform two practice sets of knee extension and flexion

d. Perform 3 sets of maximal knee extension and flexion at 180 degrees/ second separated by 60 second rest interval.

Study Protocol

Visit at Week 6

1. Warm up on stationary ergometer for 5 minutes
2. Lower limb stretching for 5 minutes
3. Adjust squat bar to suit individual
4. Demonstrate proper squat technique:
    a. Position bar behind head so that there is an even distribution on both sides
    b. Tilt head upwards approximately 15 degrees
    c. Place feet slightly more than shoulder width apart
    d. Extend hip region outwards so that it creates a 90 degree angle with the chest and knees
    e. Lower body until there is a 80-90 degree angle from the knees to the floor
    f. Raise upwards to starting position
5. Rest for 10 minutes
6. Light static pectoral stretching
7. Perform 10-15 push-ups
8. Demonstrate bench press:
    a. Position hands evenly on horizontal bar slightly more than shoulder width apart.
    b. Un-rack bar from safety frame

c. Lower bar until it slightly touches the chest region

d. Extend arms back to starting position

e. Re-rack bar on safety frame

9. Rest for 10 minutes

10. Record height (cm) and weight (kg)

11. Demonstrate Biodex isokinetic dynamometer

a. Place individual in chair

b. Adjust chair setting

c. Perform two practice sets of knee extension and flexion

d. Perform 3 sets of maximal knee extension and flexion at 180 degrees/ second separated by 60 second rest interval.

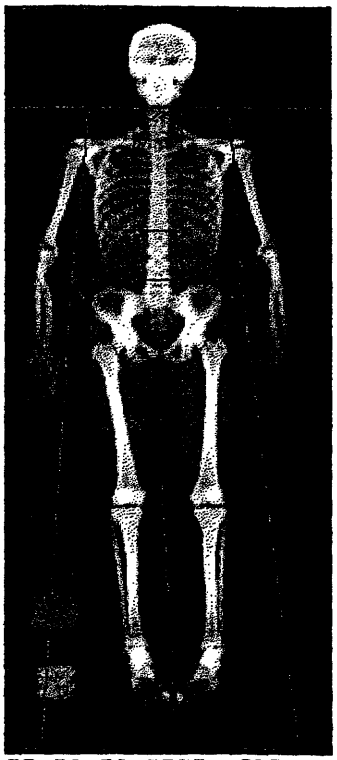
Study Protocol

Visit at Week 0

1. Consent Form
2. Warm up on stationary ergometer for 5 minutes
3. Lower limb stretching for 5 minutes
4. Adjust squat bar to suit individual
5. Demonstrate proper squat technique:
   a. Position bar behind head so that there is an even distribution on both sides
   b. Tilt head upwards approximately 15 degrees
   c. Place feet slightly more than shoulder width apart
   d. Extend hip region outwards so that it creates a 90 degree angle with the chest and knees
   e. Lower body until there is a 80-90 degree angle from the knees to the floor
   f. Raise upwards to starting position
6. Rest for 10 minutes
7. Light static pectoral stretching
8. Perform 10-15 push-ups
9. Demonstrate bench press:

Appendix D DEXA Scan Print Out

Nuclear Medicine Dept - RUH - Saskatoon



·Apr 29 06:06 2000 [333 x 152]
Hologic QDR-2000 (S/N 2124)
Enhanced Array Whole Body V5.68A

A0401001F Sun Apr 1 12:38 1900
Name:
Comment: Glutamine Study
I.D.: NM-69273 Sex:
S.S.#: 830-22-3304 Ethnic:
ZIPCode: Height: 172.72 cm
Scan Code: 113 Weight: 63.64 kg
BirthDate: 03/06/79 Age: 21
Physician: WLKNSN,DUDZC
Image not for diagnostic use

TOTAL BMC and BMD CV is < 1.0%

	C.F. 1.004	1.045	1.000
Region	Area (cm2)	BMC (grams)	BMD (gms/cm2)
L Arm	184.86	131.33	0.710
R Arm	195.64	150.85	0.771
L Ribs	109.80	71.81	0.654
R Ribs	114.19	74.78	0.655
T Spine	164.10	111.90	0.682
L Spine	61.09	61.04	0.999
Pelvis	305.84	317.77	1.039
L Leg	431.21	521.90	1.210
R Leg	408.85	477.01	1.167
SubTot	1975.60	1918.40	0.971
Head	260.72	404.85	1.553
TOTAL	2236.32	2323.25	1.039

HOLOGIC

Nuclear Medicine Dept - RUH - Saskatoon

Hologic QDR-2000 (S/N 2124)
Enhanced Array Whole Body V5.68A
·Apr 29 06:06 2000

TBAR254
F.S. 68.00% 0(10.00)%

A0401001F Sun Apr 1 12:38 1900
Name:
Comment: Glutamine Study
I.D.: NM-69273 Sex:
S.S.#: 830-22-3304 Ethnic:
ZIPCode: Height: 172.72 cm
Scan Code: 113 Weight: 63.64 kg
BirthDate: 03/06/79 Age: 21
Physician: WLKNSN,DUDZC

Region	BMC (grams)	Fat (grams)	Lean (grams)	Lean+BMC (grams)	Total (grams)	% Fat (%)
L Arm	131.3	475.3	2647.8	2779.2	3254.5	14.6
R Arm	150.9	432.1	3038.4	3189.2	3621.3	11.9
Trunk	637.3	1674.5	25426.1	26063.4	27738.0	6.0
L Leg	521.9	1597.8	8833.0	9354.9	10952.7	14.6
R Leg	477.0	1751.4	8385.6	8862.6	10614.0	16.5
SubTot	1918.4	5931.2	48330.9	50249.3	56180.5	10.6
~Head	404.8	851.3	3334.2	3739.1	4590.3	18.5
TOTAL	2323.2	6782.5	51665.1	53988.3	60770.9	11.2

~assumes 17.0% brain fat
LBM 73.2% water

HOLOGIC

Appendix E 3-Day Food Record

College of Kinesiology
University of Saskatchewan

THREE-DAY FOOD RECORD

NAME: _____

DATES: _____

SPORT: _____

AGE: _____

HEIGHT: _____

WEIGHT: _____

INTRODUCTION

This booklet is used to record your detailed daily food intake. It is meant to give the researchers some idea of your usual dietary intake. Therefore, it is very important that you do not alter your eating habits while taking part in this study. In other words, do not let the fact that you are writing down what you eat influence your choice of foods. The names of the participants in this study will be kept confidential.

The usefulness of the results of this study depends on the accuracy with which you record your daily food intake. Please write down full details on all the food and drink that you consume each day.

INSTRUCTIONS

1) The purpose of this diary is to record all the food (including drinks) which you eat for a three day period. The three day period should include 2 weekdays and 1 weekend day.

2) Two pages are provided for each day of the three day period.

3) After each meal or snack that you eat, please write down in detail each separate food item you consumed - including the type of food (e.g. processed cheese) and the amount of food in household measures (e.g. 1 cup of cooked spaghetti). A meal will have to be listed by its separate parts (e.g. fried steak - 8 oz., french fries - 1 cup, coleslaw - 3 tbsp.)

4) The best way to record the information is by carrying this diary around with you wherever you go. Before going to sleep, you should look over the diary to check that you have not missed anything. Remember to include snacks!

5) If you eat fast food, you can just list the type of food you ate (e.g. 1 Big Mac, 1 large fires, 1 chocolate milkshake).

6) The following pages explain the use of household measures, and the description of foods. A sample day's diet sheet is given. Please take the time to read these pages as it will help to make your diet record more accurate.

RECORDING IN THE DIARY

1) Please use household measures. For example:

cup: vegetables, cereal, fruit, milk, beverages
tablespoon: sauces, fats
teaspoon: sugar, honey, drink mix
slices: bread, bacon
fractions: 1/6 pie.

2) State the <u>type</u> of food eaten. For example:

Milk: homo, 2%, 1%, skim, goat's
Cheese: processed, Swiss, spread
Bread: enriched white, 60% whole wheat, sweet cinnamon bun, bran muffin
Cereal: Sugar Pops, Miniwheats, granola
Meat: hamburger, fried chicken - breasts, scrambled eggs, cod fillets
Others: strawberry jam, Becel margarine, Caesar dressing, oatmeal cookies.

3) State the <u>amount</u> of food eaten. For example:

Cheese: 1" cube cheddar
3 tbsp lite cream cheese
1/4 cup 2% creamed cottage cheese
Fruit: 1/2 cup canned peaches (in heavy syrup)
12 grapes
1 medium banana
Bread: 2 slices 100% whole wheat
1 large kaiser
Cereal: 3/4 cup corn flakes
1 shredded wheat biscuit

Meat: 1 cup baked beans with pork
2 cups tuna casserole (tuna, cream of mushroom soup, noodles, peas)
4 thin slices roast beef

Vegetables: 2 slices cucumber
1/2 cup boiled cabbage

4) Include manner of cooking: fried, boiled, raw.

5) Remember all alcoholic drinks.

Here is a Sample: Date: Sat., Dec. 14th (Day 3)

Time	Food Description	Amount	Code
9:30a.m.	Waffles-white flour	3, 8"x4"	ea.
	syrup-Aunt Jemima	1/2 cup	
	yogurt-peach	125ml	
	coffee, 1 tsp. sugar	1 cup	
	milk (2%)	1/4 cup	
10:30	Chocolate chip cookies	3	
	coffee, 1 tsp. sugar	1 cup	
	milk (Half & Half-10%)	1/4 cup	
12:30	Sandwich		
	-2 slices whole wheat bread	2 slices	
	-mozarella cheese (3"x1/4"x2")	2 slices	
	-salami	4 slices	
	-lettuce	1 leaf	
	-butter	1 tsp.	
	-mayonaise	1 tsp.	
5:30	Spaghetti	1 cup	
	meat sauce	1/2 cup	
	garlic toast	2 slices	
	(Continue on the next page if your need		
	it) Leave Code column blank.		

Date: _____

Time	Food Description	Amount	Code

Appendix F Diet Analysis Print Out

Energy

Calories	3025 Kcal		Energy expenditure : 2680 kcal
Carbohydrate	427.0 g	59%	
Monosaccharides :	10.4g	2%	(39% available data)
Disaccharides :	31.3g	7%	(39%)
Other sugars :	0.5g	0%	(25%)
Protein	120.7 g	16%	
Fat	83.3 g	25%	
Saturated :	37.3g	45%	(97% available data)
Mono-unsaturated :	24.3g	29%	(97%)
Poly-unsaturated :	8.7g	10%	(97%)
Alcohol	0.0 g	0%	

Vitamins

			R.N.I.	Mini / maxi	% R.D.I.
Vitamin A	585.80 RE	!	800.00	640.00 / 8000.00	58.58 %
Vitamin D	8.40 µg		2.50	1.75 / 12.50	168.00 %
Vitamin E	1.84 mg	!	7.00	4.20 / 1000.00	18.40 %
Vitamin C	68.34 mg		30.00	21.00 / ...	113.90 %
Thiamin (B1)	2.87 mg		1.30	0.98 / ...	220.77 %
Riboflavin (B2)	3.09 mg		1.50	1.13 / ...	193.13 %
Niacin (B3)	49.68 NE		15.00	11.25 / ...	216.00 %
Pyridoxin (B6)	1.59 mg		2.00	1.50 / ...	88.33 %
Folic acid (B9)	232.39 µg		180.00	135.00 / ...	105.63 %
Cobalamin (B12)	10.65 µg		1.00	0.75 / ...	532.50 %

Minerals and trace elements

		R.N.I.	Mini / maxi	% R.D.I.
Magnesium	326.53 mg	200.00	170.00 / 700.00	130.61 %
Calcium	1546.02 mg	700.00	560.00 / 2800.00	140.55 %
Potassium	3507.08 mg	2000.00	1400.00 / 8000.00	
Sodium	3693.69 mg	5000.00	3500.00 / 25000.00	
Phosphorus	1957.87 mg	850.00	722.50 / 5100.00	177.99 %
Iron	18.05 mg	13.00	9.10 / 65.00	128.93 %

For this subject, D vitamin intake doesn't include the amount synthetized by sun exposure.

For this subject, sodium intake doesn't include the amount added during or after cooking.

! deficiency !!! excess

Appendix G Resistance Training Program

Appendix H Resistance Training Exercises

Resistance Training Exercises

Day 1. Chest/ Triceps

1. Flat bench press
2. Incline bench/ dumbbell press
3. Flat bench dumbbell press
4. Flat bench dumbbell fly's/ peck deck
5. EZ bar skull crushers
6. Dumbbell tricep extensions
7. Tricep cable extensions.

Day 2. Back / Biceps

1. Wide grip chin ups
2. T-bar rows
3. Lat pull downs
4. Seated rows
5. Straight bar bicep curls
6. Preacher curls
7. Seated dumbbell curls.

Day 3. Legs / Shoulders/ Abdominals

1. Full squats/ leg press
2. Leg extensions
3. Hamstring curls
4. Seated/ standing calf raises
5. Military press
6. Standing lateral raises
7. Up-right rows
8. Seated crunches

Day 4- Rest.

Appendix I Training Log

weight (lbs)	reps	weight (lbs)	reps	weight (lbs)	reps	weight (lbs)	reps
weight (lbs)	reps						
205,205,210,205	10,10,8,9						