THE EFFECTS OF ORAL SUPPLEMENTATION OF THE AMINO ACID ARGININE ON BODY COMPOSITION AND MUSCLE FUNCTION DURING ENERGY RESTRICTION IN MALE WEIGHT LIFTERS

by

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(ABSTRACT)

Manufacturers of amino acid supplements claim that they can act as natural steroids. Eighteen experienced male weight lifters were studied to test this hypothesis for the amino acid arginine. All subjects followed a prescribed weight maintenance diet for one week (MAINT) and weight lifting program (6d/wk) for the entire experiment. Then, 12 of the subjects were given a low calorie formula diet (22 kcal/kg/d) to follow for the next 10 days (EXPER). Half of these subjects (n=6) were given arginine supplements while the other half were given casein as a placebo to be taken twice per day at 0.1 g/kg/d. The control subjects (n=6) continued their normal diet and did not consume any supplement. Body fat was assessed with hydrodensitometry just before and at the end of EXPER. Daily twenty-four hour urine collections were made and analyzed for urinary nitrogen (UN) (Kjehdahl method) and urinary creatinine (UC) during EXPER. Nitrogen balance (NBAL) was determined by total nitrogen consumed minus urinary nitrogen, estimated fecal nitrogen and estimated sweat nitrogen. Several aspects of muscle function, including peak torque (5 reps,

60°/sec) and endurance (30 reps, elbow flexion 120°/sec, knee extension 180°/sec) were performed using the Cybex II at the beginning and ending of MAINT and at the end of EXPER. Repeated measures ANOVA indicated a significant decrease in body weight ($\bar{x}$=3.2 kg) and body fat ($\bar{x}$=2.0%) when the dieting groups were combined with no effect of the supplement consumed. No significant effects were found for NBAL. However, UN/UC ratio was significantly different between groups and for group x time interaction. A reduction in peak torque ($p<.05$) for groups over time occured for elbow flexion and knee extension and an increase in elbow flexion endurance was observed. Type of supplement did not influence these effects. In conclusion, this study identified a reduction in muscle peak torque with short term weight loss but did not support any value of oral arginine supplements.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS.....iv
LIST OF TABLES.....viii
LIST OF FIGURES..... ix

I: INTRODUCTION
Statement of the Problem.....3
Research Hypothesis.....4
Significance of the Study.....5
Delimitations.....6
Limitations.....7
Definitions and Symbols.....8
Basic Assumptions.....11
Summary.....12

II: REVIEW OF THE LITERATURE
Introduction.....14
Protein Needs of Body Builders.....14
Muscle Function and Diet.....25
Amino Acid Supplementation.....27
Human Growth Hormone.....40
Response of Growth Hormone to Exercise.....45
Summary.....46

III: JOURNAL MANUSCRIPT
Introduction.....51
Methods.....53
Results.....60
Discussion.....63
References.....85

IV. SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH
Summary.....90
Research Implications.....94
Recommendations for Future Research.....100

APPENDIX A: Methodology.....103

APPENDIX B: Instructions to Subjects, Diet and Weight Lifting Protocols.....119

APPENDIX C: Informed Consent and Human Subject Forms.....143

APPENDIX D: Raw Data and Statistical Analysis....150

BIBLIOGRAPHY.....182

VITA.....188

LIST OF TABLES

Table Page

1: Age and Initial Weight of Subjects.....76

2: Alternating Weightlifting Routine During Maintenance and Hypoenergy Phase.....77

3: Hydrostatic Weighing Data.....78

4: Muscle Performance.....79

LIST OF FIGURES

Figure Page

1: Plot of nitrogen balance over time during the hypocaloric diet.....81

2: Plot of urine creatinine over hypoenergy phase.....82

3: Plot of total urinary nitrogen over hypoenergy phase.....83

4: Plot of urinary nitrogen excretion/urinary creatinine excretion during the hypoenergy phase.....84

Chapter I

INTRODUCTION

In an attempt to improve physical performance or appearance, many athletes engage in fad dietary practices which manipulate macronutrient as well as micronutrient consumption. For those athletes for whom aesthetic appeal is considered important (e.g. ballet dancers, figure skaters, gymnasts) the maintenance of low weight and low percentage of body fat is critical. For distance runners, low body weight is believed to enhance performance. In other sports such as wrestling or light-weight boxing, regulation of body weight is crucial because the athlete must meet a certain weight classification in order to compete.

Body builders are another classification of athlete who engage in severe diet manipulations prior to competition. Body building is a sport that dictates both muscle size and definition for success. As a result, not only are these athletes interested in reducing their body fat but they are also interested in maintaining, if not increasing, their muscle mass to achieve maximum muscle definition for competition.

When preparing for competition, body builders suffer through extreme energy restriction. During energy restriction, when metabolic fuels are low, the body responds

by shunting calories away from growth related processes which may not be critical for survival (Phillips, 1986). When the body does not receive adequate calories, it enters a glycogen depleted state. When this occurs, the body's carbohydrate demands are met through gluconeogenesis from amino acids obtained from protein catabolism. It has been shown that body builders, in spite of a regular weight-lifting schedule, lose body protein during times of energy restriction although their protein intake was in the RDA range (Walberg et al., 1988).

Not only have body builders experimented with diet manipulation in an effort to achieve an optimal physique but they have also experimented with anabolic steroids in an effort to increase muscle hypertrophy as well as decrease the percentage of body fat. However, with the advent of negative press toward anabolic steroid use and more stringent testing, many of these athletes are experimenting with other ergogenic aids, such as amino acid supplementation. Manufacturers often suggest that amino acids act as a "natural" release stimulator of the anabolic hormone, human growth hormone (HGH), from the pituitary gland (Slavin et al., 1988). Endogenous HGH is known to stimulate muscle growth by stimulating protein synthesis in both muscle and liver and at the same time decrease body fat by stimulating lipolysis (Macintyre, 1987). If the theory

holds true that orally ingested arginine will stimulate the pituitary gland to release HGH, then perhaps, muscle tissue integrity and function may be spared during times of energy restriction. This could be very important for body builders, as well as other athletes, who attempt to maximize fat loss while minimizing lean tissue loss. If there is no effect of oral arginine on body composition or muscle functioning during energy restriction, there will be direct evidence to refute the claims being made by manufacturers of these supplements.

Statement of the Problem

It is well known that intravenously administered amino acids will stimulate HGH release from the anterior pituitary (Merimee et al., 1969), with arginine being documented as one of the most potent stimulators (Casanueva et al., 1983; Knopf et al., 1965; Merimee et al., 1969). However, since athletes are taking these supplements orally, it is possible that the high arginase levels in the liver will prevent any increase in blood arginine or HGH.

Some evidence exists that oral arginine can reduce lean tissue loss in rats (Seifter et al., 1978) and humans (Daly et al., 1988) subjected to surgical trauma. Major surgery and injury results in weight loss, primarily due to catabolism of lean tissue (Daly et al., 1988), thus oral arginine may be most effective in catabolizing conditions.

However, the research on the effectiveness of oral arginine supplementation in healthy populations has been equivocal (Barr et al., 1987; Elam 1988; Elam et al., 1989).

Since male body builders may be at risk of catabolizing lean mass during energy restriction practiced prior to competition, the intent of this study was to examine the effects of oral arginine supplementation on body composition and muscle strength and endurance during energy restriction in male body builders.

Research Hypothesis

The following null hypothesis were tested in this study:

H_{01}: There were no differences in percent body fat or fat free mass measured by hydrostatic weighing between the arginine supplemented group and placebo group during the ten day hypoenergy phase.

H_{02}: There were no differences in muscle strength (peak torque) and muscle endurance of elbow flexion and knee extension between the arginine, placebo, or control groups as determined by pre and post experimental phase scores.

H_{03}: There were no differences between the arginine and placebo groups in nitrogen balance, urinary creatinine, total urinary nitrogen, and urinary

nitrogen excretion/urinary creatinine excretion during the ten day experimental phase.

Significance of the Study

Body building has become an extremely popular sport with enthusiasts involvement ranging from novice weight lifter to professional competitor. Since success in the sport of body building is dictated by muscle definition and size, many of these athletes experiment with their diets to maximize the training results of their weight-lifting programs. That is, they suffer through low energy diets with the intent of shedding unwanted fat. From anecdotal accounts, the information that they receive in regards to diet manipulation is based on recommendations of other athletes which is often a mixture of tradition and folklore, with some scientific fact thrown in.

Not only do these athletes experiment with diet manipulation but they also experiment with ergogenic aids to achieve the "quick fix" for the ideal physique. One type of ergogenic aid which has become popular in the gym is amino acid supplementation. Manufacturers of amino acid supplements claim that amino acids will stimulate protein synthesis, decrease body fat and increase endurance. Research has indicated that the amino acid arginine will cause an increase in serum levels of HGH and that in traumatized humans and animals oral and infused arginine

will spare protein mass as determined by nitrogen balance studies. However, research to date has been equivocal as it relates to healthy human subjects. That is, the question "will oral supplementation of arginine increase protein synthesis, increase lipolysis, or improve muscle performance?" has not been adequately answered.

The purpose of this study is to investigate the possible positive effects of oral supplementation of the amino acid arginine on lean body mass maintenance (as measured by nitrogen balance), percent body fat and muscle performance in male body builders on a hypoenergy diet. If a relationship between oral ingestion of the amino acid arginine can be documented, then not only will athletes benefit but possibly obese populations as well.

Delimitations

The following delimitations were made:

1. The subjects were trained male body builders age 20 - 25.
2. The mode of training utilized was weight lifting.
3. A maintenance week where diet and activity were controlled was completed by all subjects prior to the experimental treatment.
4. The hypoenergy diet consumed by both the arginine and placebo groups was a formula diet (Exceed, Ross Laboratories) and powdered milk which

provided 22 kcal/kg/day with protein intake controlled at 1.0 g/kg/d.

5. The independent variable was oral arginine (0.1 g/kg body weight) supplementation given twice daily.

6. The dependent criterion scores were: change in percent body fat by hydrostatic weighing; change in dynamic muscular endurance in percent by endurance ratio; change in dynamic muscular strength (torque); and nitrogen balance as determined by the amount of nitrogen consumed compared with nitrogen lost through urine, sweat and feces.

Limitations

The following limitations of the study were noted:

1. The small sample size may limit this study's findings to the subject sample studied.

2. Dermal nitrogen measures were estimated based on recent FAO/WHO/UNO 1985 committee recommendations of 8 mg N/kg body weight per day (WHO, 1985).

3. Fecal nitrogen measures were estimated from Ross Laboratories recommendations (5.85 % of ingested nitrogen in Exceed).

4. Subjects were free living (not in a metabolic ward).

5. Due to the use of a liquid diet versus solid food, the results of this experiment may be limited.
6. The results obtained from this study are limited only to the amino acid arginine at the specified dose of 0.1 g/kg/d.

Definitions and Symbols

The following definitions and symbols will be utilized:

1. Body Composition: The major components of the body which include muscle, fat, organs and bone (McArdle, Katch and Katch, 1981)
2. Percent Body Fat (%BF): Percentage of total body composition which includes essential and nonessential lipid stores (Brooks and Fahey, 1984).
3. Fat Free Mass (FFM): All the nonfat tissues including skeleton, water, muscle, connective tissue, organ tissue and teeth (Brooks & Fahey, 1984).
4. Body Builder: Subjects who have engaged in weight lifting for physique development on a consistent basis (3 - 7 times per week) for the past two years.
5. Muscle Function: Measured by Cybex II isokinetic dynamometer and delimited to maximum dynamic

muscle torque and dynamic muscle endurance of elbow flexion and knee extension.

6. Maximum dynamic muscle torque: Maximum peak torque measured from 5 maximum efforts at the angular velocity of 60 degrees per second for both elbow flexion and knee extension.

7. Dynamic muscle endurance: The ability to perform a physical activity or resist muscular fatigue measured from 30 maximal efforts at the angular velocity of 120 degrees per second for elbow flexion and 180 degrees per second for knee extension.

8. Endurance Ratio: The ratio calculated from the area under the torque curve during the last five contractions divided by that area during the first five contractions.

9. Hydrostatic weighing: The procedure of weighing subjects underwater in a special tank, to determine body density using weight in air, weight underwater, water density, and residual lung volume.

10. Residual Lung Volume (RLV): The volume of gas remaining in the lungs at the end of a maximal expiration (Brooks & Fahey, 1984) as measured by oxygen dilution technique (Wilmore et al., 1980)

11. Maintenance Diet: Diet prescribed during a seven day maintenance phase. An exchange list diet was utilized and calorie intake was set at 35 kcal/kg/d (protein: 1.0 g/kg/d) for those subjects in the arginine and placebo groups.
12. Hypocaloric Diet: Diet prescribed during experimental phase. Subjects in the arginine and placebo groups consumed a formula diet (Exceed) approximately 22 kcal/kg/d, plus enough skim milk (provided as powder) to provide the same amount of protein (1.0 g/kg/d) to all subjects.
13. Maintenance Phase (MAINT): Days 1 - 7 of the study.
14. Experimental Phase (EXPER): Ten days duration. Days 8 - 18 of total study time period.
15. One repetition max (1RM): The maximum amount of weight that can be lifted for one repetition.
16. Arginine Group (ARG): The group of subjects (n=6) who received an arginine supplement consisting of purified arginine hydrochloride (Sigma Chemical Company) in a gelatin capsule. The arginine supplement dose was calculated to be 0.1 g/kg body weight given twice a day.
17. Placebo Group (PLA): The group of subjects (n=6) who received a placebo in the form of powdered

casein (Sigma Chemical Company) in the same type gelatin capsule and dose as utilized by the ARG during the experimental phase.

18. Control Group (CON): The group of subjects (n=6) who received neither the amino acid nor placebo. Also, this group did not participate in the hypoenergy diet but consumed food ad libitum in an effort to keep their weight stable. This group followed the same exercise prescription as the arginine and placebo group and participated in muscle function tests. Thus, the primary purpose of this group was to control for exercise effects on muscle function tests.

19. Nitrogen Balance (NBAL): Dietary intake of nitrogen (grams) equals excretion of nitrogen (Brooks & Fahey, 1984).

20. Protein degradation: Catabolism of protein content into amino acids.

21. Protein synthesis: Anabolism of amino acids into lean body tissue.

22. Human Growth Hormone (HGH): Stored within the anterior pituitary and causes growth of almost all cells and tissues of the body (Guyton, 1991).

Basic Assumptions

The following basic assumptions were made:

1. It was assumed that for the muscle functioning tests, all subjects gave a maximum effort.
2. It was assumed that maximum expirations were given by all subjects to achieve the correct residual volume.
3. It was assumed that maximum stability was achieved during each trial of hydrostatic weighing procedures.
4. It was assumed that all subjects refrained from ingesting amino acid supplements other than those prescribed at least two weeks prior to and during participation in the study.
5. It was assumed that subjects had taken no anabolic steroids at least 3 months prior to and during participation in the study.
6. It was assumed that subjects twenty-four hour urine collections were complete in volume.
7. It was assumed that all subjects complied with diet, supplement and exercise prescriptions.

Summary

Body building is a sport that dictates muscle size and low body fat for success. In an effort to achieve a low body fat percentage, many body builders engage in diets that are severely low in calories and micronutrient content. As a result, the body responds by shunting calories away from

growth related processes as well as depleting glycogen stores. This process is experienced by the athlete as general fatigue and impaired muscle functioning.

In an effort to counteract these negative effects of a hypocaloric diet, many body builders have begun to experiment with oral amino acids as a dietary supplement. Amino acids have been advertised by their manufacturers as being natural stimulators of the anterior pituitary to release growth hormone. Endogenous growth hormone is known to stimulate muscle growth and at the same time increase the rate of lipolysis.

Although there has been some data to suggest that amino acids may influence the release of HGH, there has been insufficient evidence documenting body composition changes nor performance enhancement from the oral ingestion of these amino acids. However, many athletes continue to purchase these supplements with the hope that they well enhance their performance and/or physique development. Therefore, it would be advantageous to determine from controlled research, what potential oral supplementation of amino acids may have, if any, on physique development and performance.

Chapter II

REVIEW OF LITERATURE

In an attempt to gain a "leading edge" in competition, many athletes have turned to various fad diet manipulations as well as ergogenic aids such as amino acid supplements. From the consumption of these supplements, body builders hope to experience an increased reduction of their fat and an increase in their muscle mass so as to enhance their performance. The following is a review of the literature as it relates to the present study. This review will be divided into five sections: 1) Protein needs of body builders; 2) Muscle Function and Diet 3) Amino acid supplementation; 4) Human Growth Hormone; and 5) Response of growth hormone to exercise.

Protein Needs Of Body Builders

When preparing for competition, body builders suffer through extreme energy restriction in an effort to shed subcutaneous fat so that muscle definition may be more fully accentuated (Bazzarre et al., 1990). Unlike endurance activities where the primary fuel sources are derived from glucose and free fatty acids, heavy-resistance exercise (e.g., weight training) is primarily protein anabolic in nature, especially when the exercise is of low repetition

and heavy resistance. In the human muscle, this increase in protein synthesis is manifested as increased muscle size (MacDougall, 1980) and this hypertrophy has been observed even when energy intake is inadequate (Lemon and Chaney, 1988). However, in these instances it is believed that inactive tissues are degraded to compensate (Goldberg et al., 1975). That is, during energy restriction, when metabolic fuels are low, the body responds by "decreasing the rate of calorie consumption, and by shunting calories away from growth related processes which may not be critical for survival" (Phillips, 1986).

The ancient Greek athletes have been attributed with endorsing the practice of eating large quantities of meat in an effort to replace muscle tissue that was "spent" during exercise (Cooper, 1966). Surfeit protein feeding continues to be practiced today by many engaged in heavy physical activity. Bazarre et al. (1990) noted that body builders mean protein intake from diet and supplements was more than 300% of RDA and represented 34$\pm$ 12% of total kilocalories for males. Although surfeit protein feeding continues, the theory that protein is the main source of energy for muscular contraction is generally considered incorrect (Hedman, R., 1958; Gontzea et al., 1984). In the isocaloric state, the general consensus is that glycogen stores usually fuel the initial stages of an exercise bout giving way to

free fatty acids as duration increases (Lemon & Nagle, 1981). It has been estimated that protein accounts for only 5.5 - 15% of total available energy during exercise depending on the type of exercise program and the energy substrates available at the onset of exercise (Rosenek & Stone, 1984). Limitations placed on protein when considered as a fuel source are due to the fact that each protein molecule serves another physiological role (i.e., contractile or structured protein, enzymes, etc.) (Felig & Warren, 1975).

The present RDA recommendation for protein intake of an adult male age 19 years or older is 0.8 g/kg of body weight/day of mixed protein (RDA, 1989). However, how much protein that is required to maintain or increase muscle mass in exercising individuals is presently controversial especially as the literature applies to body builders (Marable, 1979; Tarnopolsky, 1988).

There are many factors that could have an influence on the protein requirement for an athlete, including the state of training, training type and volume, energy density of the diet and carbohydrate content of the diet (Tarnopolsky et al., 1988; Lemon et al., 1984). In an attempt to determine these various effects, researchers often rely on nitrogen balance studies, a much better index then body weight change (Chiang, 1988; Goranzom, 1985; Todd et al., 1984) to test

whether a specific intake of protein is sufficient to maintain body protein homeostasis and organ function (Rand, 1981). Normally, the body's protein stores are continuously broken down to amino acids and resynthesized. The body is able to maintain homeostasis by maintaining a dynamic equilibrium between the absorbed amino acids in the digestive tract and those which originate from the splitting of endogenous proteins. It is this combination of ingested protein and endogenous protein that makes up the body's amino acid pool from which contractile protein, hormones, enzymes, antibodies etc. are built from (Garza 1974). Because of the increased metabolism that occurs during muscular activity, it would be expected that this increase in activity would affect nitrogen equilibrium (Garza 1974) which can be demonstrated by nitrogen balance.

Nitrogen balance is an index of the difference between nitrogen ingested and nitrogen excreted. For nitrogen balance to be used as an effective measure of nitrogen equilibrium, the total nitrogen content of the diet must be measured (Lemon et al., 1984). In regards to nitrogen excretion, not only is it important to measure urinary nitrogen content but fecal and sweat nitrogen as well (Garza, 1974).

In a research review presented by Lemon et al. (1984), it was suggested that urinary urea excretion may not

accurately reflect protein breakdown because blood flow to the kidneys is reduced during exercise. Thus, other protein degradation end-products become more important with exercise. Decreases in blood flow to the kidneys and increases to the skin during the exercise, especially in prolonged exercise, suggest that the skin (sweat) becomes an important organ of urea excretion during exercise. Gontzea et al. (1974) reported that nitrogen loss in sweat during muscular activity (cycle ergometer) accounted for 22-28% of total nitrogen lost. Walberg et al. (1988) compared body builders involved in a controlled weight lifting regimen but placed on two different macronutrient hypocaloric diets lost .17% to .20% of total nitrogen loss in sweat. The World Health Organization (1985) presently recommends that an estimated 8 mg N/kg/d/ of sweat be utilized in nitrogen balance studies when nitrogen loss in sweat is not measured directly.

Many studies have been conducted to determine the effect of protein content of diet and of exercise on urinary nitrogen. The question here is: Does an exercise bout increase muscle protein degradation (catabolism) as demonstrated by an increase in urinary nitrogen and thus increase the need for dietary protein? Gontzea et al. (1974) looked at the protein needs of 30 untrained males who were placed on a cycle ergometer exercise regimen. All

subjects were fed 1.0 g protein/kg/d during a two week maintenance phase and were in positive nitrogen balance before the exercise phase commenced. Although the caloric value of the diet was 10% higher than the energy expense, the nitrogen balance became negative. They concluded that during intense activities, increased expenditure of energy resulting from the bout of exercise is accompanied by intensification of protein metabolism. This increase in protein metabolism was demonstrated not only by an increase in urea nitrogen but sweat nitrogen as well.

Gontzea et al. (1974) also observed that by increasing protein intake from 1.0 g to 1.5 g protein/kg/d while subjects performed the same exertion, nitrogen balance was achieved. Thus, this supports the belief that muscular work increases amino acid metabolism and consequently increases the need for protein. However, as was observed by Garza et al. (1975) and others (Calloway, 1975) exercising subjects who experience an initial excess nitrogen loss during the first 4 - 6 days of effort will achieve equilibrium over time. This effect was observed in a later study by Gontzea et al. (1975) in which subjects, after 3 weeks of the same muscular work (cycle ergometer), controlled diet (kcal 10% above estimated energy expenditure) and controlled protein intake (1 g/kg/d) demonstrated negative nitrogen balance during the first 4 days of exercise. This negative nitrogen

balance gradually decreased to where by the end of 3 weeks it was lower by 90% compared to the first 2-4 days. Since the caloric consumption decreased by only 18%, the authors stated that the ". . . diminution of protein catabolism is not due to reduction of energetic metabolism alone, following training, but also a complex process of adaptation of the organism to a lower protein intake" (Gontzea et al. 1975). This observed pattern of initial increased protein catabolism followed by protein conservation is similar in time course to the events during starvation (Lemon and Nagle, 1981) when the body adapts and adjusts to conserve protein stores. It has been suggested (Rand et al., 1981) that 9 - 12 days are necessary for the body to adjust (achieve a steady state) to an altered protein intake and that the precise length of period is dependent upon the magnitude of change.

It should be noted that the studies by Gontzea et al. (1974 and 1975) utilized cycle ergometry as the mode of exercise. However, the physiological responses to cycle ergometry (an endurance-building activity) and body building (a strength-building activity) are different (Hickson et al., 1990) and as a result, protein metabolism may not be affected similarly. Also, the training regimens of the two types of exercise are different as well. Body building requires brief (30 sec), high intensity sets of interval

work (Keul et al., 1972) whereas, endurance exercise requires prolonged, continuous, submaximal work. Thus, the fuel sources utilized to provide ATP to accomplish work may be different.

Hickson (1986) studied the acute effects of a standard weight training program in selected urinary indices of protein metabolism in ten trained males. The experimental design consisted of subjects consuming a 41 kcal/kg/d lacto-ovo-vegetarian diet with protein intake approximating .93 g/kg/d. A weight training exercise regime was performed on two non-consecutive days with the two days immediately following the exercise bout utilized for collection of urinary metabolite excretion data which included total urinary nitrogen, urea and creatinine. These researchers found that an acute bout of strength training had no significant effect on any of the urinary indices measured over the two day period, suggesting that an acute bout of weight lifting does not stimulate skeletal muscle catabolism. This was a contradiction of a study documented by Dohm et al. (1982) who studied the acute effects of a power lift routine on urinary indices (one day sample) in four experienced weight lifters. Unlike the Hickson (1986) study, diet was not controlled in the Dohm study other than instructing subjects to consume their normal diets. Dohm et al. (1982) observed that with an acute power lift routine an

increase in total and urea nitrogen excretions were observed. It was thus concluded by Dohm et al. (1982) that exercise increases amino acid catabolism. The differences in the results of Hickson (1986) and Dohm et al. (1982) may be attributed to the lack of diet control in the Dohm study and the difference in volume and intensity of the weight lifting protocols. Regardless, both of these studies looked at only acute effects, neglecting compensatory mechanisms during recovery from exercise which may alter the overall response.

Not only have the acute effects of a weight lifting routine been researched, but these same effects have been studied utilizing short term (< 30 days) nitrogen balance data. Marable (1979) looked at bodybuilders versus sedentary subjects and the effect of two protein intake levels (0.8 and 2.4 g/kg/d) over a 28 day period on nitrogen balance. They found that the exercising subjects excreted significantly less urinary nitrogen relative to intake nitrogen for both protein intake levels than did non-exercising subjects. However, the exercising group received more kcal than the sedentaries (62 kcal/d vs. 41 kcal/d), they gained weight (X=3.2 kg), and the exercising group sweated more (nitrogen content of sweat was not accounted for). These three factors may have accounted for the

decrease in urinary nitrogen observed thus confounding the comparisons.

Hickson et al. (1990), utilized eleven untrained males in a study to examine the effects of a weight lifting regimen on urinary nitrogen excretions over a 12 day experimental period. Energy intake was based on body weight (50 kcal/kg/d) and was lacto-vegetarian in nature. Protein intake level was set at 0.8 g/kg/d. Also, an extra 250 kcal/d of a carbohydrate source was fed to offset the energy cost of weight training exercise. They found no effect of exercise to increase or decrease group mean urinary total or urea nitrogen excretions during the experimental period for untrained subjects. Thus, their conclusion that a bodybuilding regimen does not cause an exercise induced catabolism of amino acids when subjects are fed an adequate diet.

Tarnopolsky et al. (1988) compared endurance athletes, bodybuilders and sedentary subjects in an attempt to determine protein intake requirements for male athletes consuming a high energy and high carbohydrate diet and who are in steady state training. The nitrogen balance data revealed that body builders required 1.12 times and endurance athletes required 1.67 times more daily protein then sedentary controls. As a result, the authors recommended that a body builders extrapolated protein intake

for a zero nitrogen balance would be 1.2 g/kg/d. This guideline falls in line with others (Consolazio et al., 1975; Goranzon and Forsum, 1985; Todd et al., 1984) who have concluded that bodybuilders require 0.8 - 1.4 g/kg/d of protein when not on an energy restricted diet.

The above studies incorporated adequate to high levels of energy intake for their subjects while adjusting protein intakes during the experimental phases. However, it has been shown that energy intake above (Chiang, 1988; Inoue et al., 1973) as well as below (Munro & Naismith, 1953; Garza, 1976) maintenance requirements may influence nitrogen utilization when protein intake is maintained at a set level. That is, deficient dietary energy intakes decrease the efficiency of nitrogen utilization. In a study by Garza et al. (1976) it was found that energy deficiency caused by increased activity or reduced energy intake both increase urinary nitrogen output and decrease nitrogen balance in male subjects consuming 0.57 g protein/kg/d. These researchers further hypothesized that it is labile proteins (i.e., those easily mobilized from liver, pancreas and intestinal mucosa) that are utilized in the beginning stages of a hypoenergy phase to cause a greater increase in urinary nitrogen that is often initially observed in these type studies.

Calloway (1975) also observed that total energy intake appears to have a much greater effect on nitrogen balance than does protein intake when both are close to estimated energy maintenance levels. As a result, the equivocal results of nitrogen balance studies may be a result of the differences in the energy content of the diets utilized for study.

Thus, body builders who are attempting to decrease their body fat by engaging in hypocaloric diets may be sacrificing lean body mass if their protein intake is inadequate or they may be compromising their energy stores if their carbohydrate intake is inadequate. The optimal protein and carbohydrate intake, under low calorie intake conditions, has yet to be determined to ensure that muscle integrity, as well as energy stores are maintained. It may be that by the nature of hypocaloric intake, that this balance may not be possible.

Muscle Function and Diet

Not only will energy content of the diet effect nitrogen balance, but it may effect muscle performance as well. Walberg et al. (1988) demonstrated that body builders, in spite of a regular weight-lifting schedule, lose body protein during times of energy restriction (18 kcal/kg/d), although their protein intake was in the RDA range (0.8 g/kg/d) indicating that body builders may require

more protein in their diet then the RDA recommended levels. Walberg et al. (1988) also found that those subjects consuming a high protein/moderate carbohydrate diet maintained positive nitrogen balance but these same subjects demonstrated decreased muscle endurance in the quadriceps as measured by the Cybex II dynamometer. This decrement in endurance may have been due to "a progressive glycogen depletion in this group, which could not be replaced due to the moderate carbohydrate intake" (Walberg et al, 1988). Others have confirmed that a weight-lifting routine relies on anaerobic glycolysis and therefore muscle glycogen for ATP production, as well as the creatine-phosphogen system (Guezennac et al., 1986) If the diet is insufficient in supplying adequate energy, then the glycogen stores cannot be repleted. This decrement in fuel supply is often experienced by the athlete as general fatigue and impaired muscle functioning.

In a study utilizing wrestlers in which caloric intake was restricted to 33% of baseline for two days, Houston et al. (1981) reported a significant decrease in quadriceps peak torque measured isokinetically. As in the Walberg study, the decrease in strength was found to be concurrent with a decrease in muscle glycogen.

Krotkiewski et al. (1988) studied the effects of a very low calorie (544 kcal/d) high protein diet for four weeks in

32 obese women on isokinetic (dynamic) muscle strength and endurance of knee extension at three different angular velocities (30, 60 and 180°/sec). After two weeks, the subjects indicated a significant decrease in muscle strength that corresponded with a decrease in glycogen. However, an improvement in muscle endurance was observed. It was speculated that the improvement in endurance may have been the result of improved muscle metabolism of glucose caused by increased insulin sensitivity or an improved capacity to oxidize fatty acids.

As the above studies indicate, muscle performance may also be affected by macronutrient content of the diet as well as by caloric density. If the caloric intake of the exerciser is reduced or energy expenditure increased sufficient to produce a caloric deficit then muscle performance may be compromised.

Amino Acid Supplementation

Not only have body builders experimented with diet manipulation in an effort to achieve an optimal physique, but they have also experimented with anabolic androgenic steroids in an effort to increase muscle hypertrophy as well as decrease body fat percentage. However, with the advent of negative press toward anabolic androgenic steroid use and more stringent testing, many of these athletes are choosing to supplement their diets with amino acids. Many body

builders, from anecdotal accounts, are convinced that supplement manufacturer claims are true that amino acids are beneficial in increasing muscle hypertrophy, strength, endurance and decreasing body fat. The mechanism that is believed to be involved is that amino acids act on the pituitary to release human growth hormone. If these claims are true, by supplementing their diets, athletes may be able to counteract some of the losses experienced with hypocaloric diets (i.e., decrease in strength, loss of LBM) However, as will be discussed in the following sections, the research has yielded equivocal results as it relates to healthy human adults.

Human growth hormone secretion is controlled by the hypothalamus which contains growth hormone-releasing hormone (GH-RH) and growth hormone-inhibiting hormone (somatostatin). Release of hypothalamic hormones are regulated by a number of neurotransmitters (e.g, dopamine, serotonin, norepinephrine) (Kraemer, 1988). As a result, the release of HGH from the anterior pituitary may occur in response to various stimuli such as exercise, sleep, stress, and drugs (Macintyre 1987). Another secretagogue of HGH from the pituitary are amino acids. There has been some evidence to suggest that when injected intravenously certain amino acids (arginine, methionine, phenylalanine, lysine and histidine) promote HGH secretion (Knopf et al., 1965). The

mechanism involved is not completely understood but it is believed that amino acids stimulate hypothalamic HGH releasing factors (perhaps mediated by insulin secretion) which subsequently cause HGH release from the pituitary gland (Lemon & Chaney, 1988). This response appears to decrease with age, especially in men, and there appears to be a great deal of individual variability (Buckler, 1969).

One amino acid that has been studied for its effect on HGH secretion is arginine. Arginine, is unique in that it is considered essential (i.e., required in the diet) during growth but not for the adult. Although other amino acids have been found to stimulate HGH release upon infusion (e.g., ornithine, lysine, methionine), arginine appears to be one of the most potent (Jacobson, 1990) next to methionine which is very toxic (Lemon & Chaney, 1988). In fact, arginine infusion is routinely used as a clinical test of pituitary function in children (Lemon & Chaney, 1988). Arginine has many roles within the human body other than as HGH release stimulator. Arginine is used in synthesis of body proteins, is essential for ammonia detoxification via urea synthesis which prevents metabolic derangements caused by elevations in tissue ammonia (Visek, 1986) and it is also a precursor in the creatine synthetic pathway (Crim et al., 1975).

There have been several studies to document a rise in HGH from intravenously administered arginine. Merimee et al. (1969) administered arginine intravenously to male and female healthy subjects at three dose levels (0.08 g/kg, 0.17 g/kg and 0.25 g/kg) to test their effectiveness in increasing HGH response when compared to baseline. The minimum arginine load that was found to be effective in stimulating HGH release was found to be different for females and males. A significant HGH response for females was identified at the 0.08 g/kg dose after 60 minutes whereas for males, a significant increase in HGH was not noted after 60 minutes until a 0.17 g/ kg arginine load was infused. Similar response patterns of HGH to intravenous administration of arginine has been reported by others. Knopf et al. (1965) found that a 30 g (.43 g/kg body weight for a 70 kg subject) infused dose of arginine increased HGH by 1,084.6% by 90 minutes. Casanueva et al. (1983), also utilizing a 30 g dose, found that in a group composed of two women and four men HGH increased significantly at 60 minutes (16.6$\pm$5mg/ml) compared to baseline (0.9$\pm$0.1 mg/ml)

Not only has research been aimed at the secretagogue effects of arginine, but the corresponding metabolic effects of the arginine-HGH relationship. Evidence has been documented to support the use of intravenous arginine as a method of attenuating the catabolism of protein and immune

suppression during times of trauma and in burn patients. Typically, during these situations, the body catabolizes dramatic amounts of protein which is exemplified by elevated urinary nitrogen loss.

Elsair et al. (1978) tested the effects of infused arginine (7.5 g twice a day; 0.11 g/kg for a 70 kg subject), on nitrogen balance disturbed by surgical shock. The supplements were given for 3 consecutive days following routine surgery (cholecystectomy) in normal human adults, These researchers documented a 60% improvement in nitrogen balance for the arginine supplemented group when compared to isonitrogenous controls. This dose per day was 1/2 of the doses given adults (30 g) in studies evaluating HGH responses to an arginine infused bulbus.

There have been numerous animal model studies demonstrating the efficacy of arginine supplemented diets in reducing the catabolic response to major trauma. In the case of traumatized rats (i.e., bilateral femural fracture), Barbul et al. (1981) compared three supplemented groups: A: control which received infused D5W; B: dextrose supplemented with 1.55 g/L of arginine; C: dextrose supplemented with 4.05 g/L arginine. The increase in arginine of group C was done at the expense of glycine content. Thus, Group B and C were isonitrogenous. All three groups lost weight over the 5 day study period with

Group C losing significantly less weight than those in the other two groups. Also, Group C had a positive nitrogen balance during all five days with a cumulative balance of 492±117 mg, whereas Group A had a negative nitrogen balance throughout the study period (cumulative = -1,057±89 mg) and Group B had a cumulative balance of -8±129 mg. Although nitrogen retention was greater in Group C, no differences were found in liver, splenic or adrenal weights of the three groups. Thus, the authors speculated that the increase in nitrogen retention of Group C occurred in muscle mass and/or other viscera and the weight loss that occurred may have reflected GI tract losses or body fat.

Previous to Barbul (1981), Pui and Fisher (1979) studied the effects of four different diets for six days following femur fracture in rats. The four diets prescribed were: control diet which contained 25% crude casein; a diet supplemented with 2.4% arginine HCl and 1% glycine; a diet supplemented with 2.4% arginine HCl alone and; a diet with 1% glycine alone. The group fed a 25% base casein diet supplemented with 2% arginine and 1% glycine indicated better growth after 3 days and retained 113% as much nitrogen over the other three groups. The rats given arginine only (25% casein + 2% arginine and aspartic acid) also showed a significant increase in weight on Day 1 and Days 3 - 6 post trauma. However, this increase in weight

was not accompanied by a parallel increase in nitrogen retention. Thus, unlike Elsair et al. (1978) and Barbul et al. (1981) which sacrificed glycine to increase arginine, Pui and Fisher showed that arginine alone did not have a nitrogen sparing effect but required the addition of glycine.

The findings of Pui and Fisher supported the previous work of Sitren and Fisher (1976) who demonstrated that a 200 g casein/kg body weight based diet supplemented with 20 g/kg arginine and 10 g/kg glycine increased nitrogen retention 60 - 70% for the first 5 days post bilateral femoral fracture in rats. However, Sitren and Fisher also found that when dietary casein, as well as the supplemental arginine and glycine were reduced by half that nitrogen retention did not improve. As a result, it was suggested that both protein quality and quantity are important following injury.

Another factor that may be important in determining the possible benefits of arginine supplementation is the physiological state of the animal Based on their previous work involving injured rats, Barbul et al. (1984) studied the metabolic and immune effects of increased intravenous arginine administration in non injured, mildly energy depleted rats. All the animals received hypertonic dextrose (20%) supplemented with 1.55 g, 4.05 g or 7.5 g arginine/liter. No significant difference was found in

weight gain or cumulative nitrogen retention among the different groups. However, based on thalmic weight from autopsy, the arginine infused supplements showed a significant effect indicating the positive attributes of arginine infusion on immune function.

The difference in results between the two Barbul studies may be attributed to the differences in physiological state. Traumatic shock modifies protein metabolism which may result in alteration of the amino acid requirements of the body. Trauma is associated with an increase in the catabolism of amino acids due to increased muscle protein breakdown or decreased muscle protein synthesis (Sitren & Fisher, 1976). As a result, the demands for arginine might be increased not only for the significant role it plays in the repair of muscle tissue, but also for the role arginine plays in the urea cycle where it is essential for the detoxification of ammonia arising from the catabolism of other amino acids (Sitren & Fisher, 1976).

Although evidence supports the positive effect of infused amino acids on lean muscle mass retention in traumatized rats and humans, the results of research investigating effects of oral amino acid supplementation has been equivocal. The discrepancies found in the research literature may be due to intrinsic differences associated with delivery system of the amino acid (i.e., direct blood

infusion vs oral ingestion with gastrointestinal involvement). When an amino acid enters the system through the digestive tract, it is absorbed into the liver via the portal vein and its fate is determined by the amount of amino acid catabolizing enzyme which is present in the liver. In the case of arginine, the liver has very high arginase activity which may blunt the change in plasma levels of arginine following consumption. Also, the metabolic state of the animal, as demonstrated by the two studies conducted by Barbul et al. (1981 & 1983) may be a determining factor when discussing the positive effects of arginine on body composition.

To expand on the ideas that liver arginase involvment with oral supplementation and the metabolic condition of the animal may be contributing factors in determining the potential benefits of arginine supplementation, two related studies are presented for discussion. In a study by Daly et al. (1988), the immune and metabolic effects of enteral supplementation of arginine were tested in 30 cancer patients undergoing major surgery. Part of the subject sample received 25 g/d of L-arginine (0.36 g/kg for 70 kg subject) (n=16) and the other part received 43 g/d (0.61 g/kg for 70 kg subject) (n=14) of L-glycine for 7 days. Measurement of plasma somatomedin C levels, a monitor of the overall effects of arginine on growth hormone secretion,

indicated a level of significance by day 7 in the arginine group compared with the glycine group, suggesting a stimulatory effect of arginine on the pituitary. In regards to nitrogen balance, although there was no significant difference between the two groups, after Day 5 mean nitrogen balance became positive in the arginine group, whereas it remained negative in the glycine group throughout the seven day experimental period. Daly et al. also reported a significant impact of arginine on increasing the mean T-lymphocyte and CD4 phenotype (% T-cells) response compared to the glycine control group.

The nitrogen balance response found in the Daly et al. study was not in agreement with an earlier study by Seifter et al. (1978). Seifter et al. (1978) reported from a study of rats subjected to surgical trauma and oral amino acid supplementation (rat chow supplemented with 1.8% arginine), that those rats receiving the oral supplementation had reduced nitrogen loss. They also found that the arginine supplemented group had an increase in wound breaking strength and increase rate of collagen deposition. Although Daly et al. (1988) and Seifter et al. (1978) reported conflicting results in regards to nitrogen balance, they both support distinct immunological benefits from arginine supplementation.

Unfortunately, many manufacturers of amino acids have made the leap between the results obtained from infused and oral amino acid research in traumatized humans and animals to the effectiveness of oral supplementation in healthy humans, with little published evidence. There have been a few studies concerned with the effects on HGH of oral administration of amino acids. In healthy subjects, Isidori et al. (1981) reported that a single oral dose of 1200 mg (17 mg/kg in 70 kg adult) each of arginine and lysine stimulated an increase of up to 794% in HGH. However, when given separately, only a minimal rise in HGH was observed. This study lends weight to the belief that gastrointestinal involvement may not be a mitigating factor in regards to HGH release. However, these researchers performed no measure of muscle function, conducted no measure of changes in muscle mass, nor did they measure nitrogen balance. As a result, it is difficult to state what the physiological relevance the observed increase in HGH production produced.

In another study, Barr et al. (1987) indicated a lack of effect of supplemental arginine (2% of diet, 1600 mg/kg body weight) and ornithine (1% of diet, 800 mg/kg body weight) in regards to muscle morphological changes as well as fat loss in healthy adult rats after 4 weeks of supplementation and access to an activity wheel. Although a decrease in weight was observed in some rats, this loss was

determined to be associated with wheel-running activity since no change in growth hormone was revealed at autopsy. This finding is in contrast to the studies involving traumatized rats (e.g., Seifter, 1978) and humans (Daly et al., 1988) and may lend credence to the theory (as outlined above in regards to infused supplementation) that the anabolic value of arginine may depend on the metabolic condition of the animal or individual as well as the intensity, frequency, duration and type of exercise.

Only two studies have been reported in regards to the effect of oral arginine supplementation plus exercise on muscle function. Elam (1988) conducted a study using 18 adult males to determine the effects of weight training (3 days per week) and dietary supplementation of arginine (1 g/day; 0.014 g/kg for 70 kg subject) and ornithine (1 g/day; 0.014 mg/kg for a 70 kg subject) or a placebo. Those subjects receiving the supplement significantly reduced their body weight and body fat as measured by skin fold thickness when compared to the placebo group. Thus, he concluded that the oral supplements combined with resistance exercise will reduce total body mass and body fat in adult males. However, he did not control for diet, assuming that the subjects maintained their established dietary habits. As a result, it may be that the changes in body weight were due to individual dietary changes. It is interesting to

note that subjects in the placebo, as well as the supplement group, decreased their body weight over the 5 week study. Elam also did not discuss lean body mass changes but did note that of 4 composite body girth measurements (obtained from circumference measurement technique), both groups increased with no significant difference between groups. This could lead one to speculate whether the weight training alone or the amino acid was the mitigating factor involved here since weight training alone is anabolic. Although Elam claimed that muscle strength increased significantly in the supplemented group, muscle function tests were a post test only design making it difficult to compare changes over time between the two groups. Elam also claimed that the subjects rate of metabolism changed as a result of the oral supplements and from the influence of exercise. However, he did not test for this directly by means of resting metabolic rate measurement.

During a follow-up study, Elam et al. (1989) tested arginine and ornithine effect in 22 adult males who participated in a 5 week progressive strength training program. Half the subjects received 500 mg doses of L-arginine and L-ornithine and the other half received a placebo (Vitamin C and oyster shell calcium in 500 mg and 300 mg doses respectively) The subjects were instructed to take their prescribed doses twice a day except on non-

workout days when the entire 4 tabs were to be taken before going to bed. Results indicated that the arginine plus ornithine group scored significantly higher in measures of total strength (one-repetition max performance) and lean body mass levels (skinfold technique). As in the previous documented research (Elam et al., 1988), diet was not controlled which could have a confounding effect. In this more recent study (Elam et al., 1989), subjects were also tested for urinary hydroxyproline in an attempt to assess tissue breakdown. The supplement group indicated lower levels of urinary hydroxyproline than the placebo group. Again, due to lack of diet control, as well as due to the fact that the amino acid and placebo groups received non isonitrogenous supplements, any lean mass indices may be suspect.

Human Growth Hormone:

Oral supplementation of amino acids has been promoted by manufacturers as being a "natural" release stimulator of the anabolic hormone, growth hormone, from the pituitary gland (Slavin et al., 1988). Endogenous human growth hormone (HGH), a powerful protein anabolic hormone, is known to stimulate muscle growth by stimulating protein synthesis in both muscle and liver and at the same time stimulate lipolysis of fat cell triglyceride to decrease body fat (Macintyre, 1987).

The study of muscle growth is complicated by the dual influences of work and hormonal factors. Whether growth is occurring longitudinally (childhood) or circumferentially, the formation of new proteins has many cellular level requirements (Macintyre, 1987). The mRNA template is produced from nuclear DNA from an RNA polymerase catalysed reaction (Macintyre, 1987). In the cell cytosol, mRNA attaches to ribosomes that subsequently interact with the tRNA which carries the specific amino acids necessary to be incorporated into the final protein (Macintyre, 1987). Thus, an adequate supply of amino acids, ribosomes and enzymes are required for this process to proceed. Work-induced muscle growth is different from hormone-induced growth because the synthesis of new RNA is required for the work induced growth whereas HGH enhances the rate and translation of existing RNA (Kostyo and Reagan, 1979).

The importance of HGH as a regulator of linear growth in childhood has been known for over 50 years. However, it is now known that HGH has a wide variety of biological actions related to maintaining the body's normal structure and metabolic function (Kraemer, 1988). The type of metabolic effect produced (anabolic vs diabetogenic) is determined by time of effect (acute vs delayed). The effects observed after a single injection of HGH are biphasic (Frohman, 1987) and may occur in hypophysectomized

animals, HGH deficient humans and to a lesser extent normal person. The initial effects are a decrease in concentrations of blood glucose, free fatty acids and amino acids (Frohman, 1987) After a few hours, blood glucose and amino acids rise above normal. Thus, the acute effects are insulin-like in that HGH increases amino acid uptake and incorporation into protein in muscle and liver. This process is independent of new RNA synthesis. In muscle and adipose tissue, HGH stimulates glucose uptake and utilization, antagonizes the lipolytic effect of catecholamines in adipose tissue and stimulates RNA and mitochondrial protein synthesis. These effects disappear within 3 to 4 hours and then there is a refractory period (Frohman, 1987). After the disappearance of these acute effects, a series of delayed HGH effects appear which form the basis of the diabetogenic effects. These effects are demonstrated by increased free fatty acids, increased lipolytic effects of catecholamines and inhibition of both glucose uptake and glucose utilization. (Frohman, 1987). All the late effects persist for many hours and are additive with additional exposure to HGH (Frohman, 1987).

A few studies have been reported assessing the anabolic effects of exogenous HGH in healthy human subjects. Crist et al. (1988) studied the effects of exogenous HGH on body composition in eight subjects experienced in progressive

resistive exercise. The dose utilized was 2.67 mg given three times a week. They found, after 6 weeks, a significant decrease in percent fat and an increase in free fat weight as measured from hydrodensiometry. These changes were significantly greater than those produced in the exercise alone group.

Snyder and his colleagues (1990) utilized an obese female subject sample to test the anabolic effects of exogenous HGH. A dose of 0.9 mg/kg ideal body weight (IBW) was infused daily for fourteen weeks. Since these researchers were interested in finding a method for conserving lean tissue and accelerating loss of fat during weight reduction, the subjects were prescribed a 12 kcal/kg IBW with 1.0 g/kg IBW protein diet. Although no difference in weight loss was observed between HGH and placebo group, only the HGH group achieved an immediate positive nitrogen balance. This study as well as the study by Crist et al., lends credence to the theory that HGH will alter body composition in the direction desired by body builders (i.e., a decrease in fat and an increase in muscle bulk). However, muscle functioning was not measured which may have given insight as to whether the increase in free fat weight was due to increases in connective tissue or contractile elements.

A potential side effect of increased HGH secretion is acromegaly. This is a condition that leads to gradual thickening of bones and soft tissues which may lead to deformities of the skull, face, hands and feet (Macintyre, 1987). Although muscles often appear to be outwardly hypertrophied in acromegaliacs, they are "actually functionally impaired, with the victims complaining of muscle weakness, easy fatigability, and decreased exercise tolerance" (Macintyre, 1987). As a result of this potentiality, it would appear that a measurement needs to be included within the research design aimed at measuring HGH effects in an attempt to differentiate LBM that is being retained or increased. That is, is the enhancement of muscle mass due to increases in connective tissue alone and not contractile tissue.

Another potential negative effect of exogenous HGH administration that was observed by Crist et al. (1988) was that exogenous treatments of HGH may suppress endogenous release of HGH in some individuals. Other possible side effects include glucose intolerance and disruption of thyroid hormone metabolism (Cowart, 1988). In light of these possible side effects, researchers have investigated the use of amino acids as natural stimulators of endogenous HGH.

Response Of Growth Hormone To Exercise

Not only is the release of HGH been shown to be related to arginine, but exercise also has an effect on HGH release. Sutton and Lazarus (1976) looked at the effect on serum growth hormone of cycle ergometry in 8 healthy male subjects. They found that the response was correlated to intensity. Exercise at 900 kpm/min (75 - 90% of subject masimum oxygen consumption) elicited a significant increase in serum growth hormone and compared favorably with insulin hypoglycemia test but was found to be a better stimulator of HGH release than sleep, arginine or L-Dopa. Similar effects were found by Casanueva et al. (1984) when they exercised a mixed group of men and women at 475 kpm/min for 20 minutes on a cycle ergometer. The mediation of exercise-induced HGH release is believed to be due to cholinergic neurotransmission (Casanueva et al., 1984; Nabil & Jacobs, 1986).

Only limited data are available regarding the response of HGH to resistance exercise. VanHelder et al. (1984) and Lukaszewoka (1976) both showed that it is possible to stimulate increased serum concentrations of HGH following an acute resistance training exercise session. Both studies found that increased serum concentration of HGH was dependant upon intensity of the exercise. Loads ranging from 85% of a 7 RM (VanHelder et al., 1984) to 70- 85 % of a

1RM (Lukaszewoka, 1976) in an Olympic lift were required before an increase in serum concentrations of HGH was observed following exercise (1.8 baseline to 4.5+0.4 ug.1-1 and 6.2+3.9 to 44.0+29.0 ug.1-1 respectively) The effects of higher volumes (i.e., repititions x sets x load) of exercise and/or higher intensities (ie., percent 1RM) has yet to be elucidated.

There are other factors that may influence HGH responses to resistance training that may have a confounding effect on study results. Some of these factors are lactate and/or oxygen deficit, hypoxia, changes in body temperature resulting from an exercise session, fitness, clearance rate changes and hemoconcentrations (VanHeler et al., 1984). It should also be noted that serum levels of HGH are usually sporadic over a 24 hour period and normally total serum levels of HGH are lower in adult males (although they tend to have higher peak levels) than females or adolescents (Macintyre, 1987).

Summary

Body building is a sport that dictates muscle size and low body fat for success. In an effort to achieve a low body fat percentage, many body builders engage in diets that are severely low in calories and micronutrient content. As a result, the body responds by shunting calories away from

growth related processes as well as depleting glycogen stores.

In an effort to counteract these negative effects of a hypocaloric diet, many body builders have begun to experiment with oral amino acids as a dietary supplement. Amino acids have been advertised by their manufacturers as being natural stimulators of the anterior pituitary to release growth hormone. Endogenous growth hormone is known to stimulate muscle growth and at the same time increase the rate of lipolysis.

Although there has been some data to suggest that amino acids may influence the release of HGH and that amino acids may prevent lean mass catabolism when the body is in a traumatized state, there has been insufficient evidence documenting body composition changes nor performance enhancement from the oral ingestion of these amino acids. However, many athletes continue to purchase these supplements at great financial expense with the hope that they well enhance their performance and/or physique development. Therefore, it would be advantageous to determine from controlled research, what potential oral supplementation of amino acids may have, if any.

Chapter III

JOURNAL MANUSCRIPT

The Effect of Oral Supplementation of the Amino Acid Arginine on Body Composition and Muscle Function During Energy Restriction in Male Weight Lifters

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The Effect of Oral Supplementation of the Amino Acid Arginine on Body Composition and Muscle Function During Energy Restriction in Male Weight Lifters

Body building is a sport in which success is dictated by both muscle size and definition. As a result, these athletes are interested in maximizing their muscle mass as well as reducing their body fat to achieve peak muscle definition. To accomplish this end, body builders often suffer through extreme energy restriction in preparation for a contest often at the expense of lean tissue. Walberg et al. found that in spite of a regular weight-lifting schedule, body builders may lose body protein during times of energy restriction although their protein intake is in the RDA range (37). This loss has been demonstrated by an increase in urinary nitrogen output and a decrease in nitrogen balance (4)(14)(37). Not only will a hypoenergy diet affect nitrogen balance, but it may affect muscle performance as well (19)(22)(37).

In an effort to achieve an optimal physique, body builders have also experimented with anabolic androgenic steroids. However, with the advent of negative press toward anabolic steroid use and more stringent testing, many body builders (both competitive and recreational) are experimenting with other ergogenic aids, such as amino acid supplements, to achieve a "competitive edge". Manufacturers often suggest that amino acids act as a "natural" release stimulator of the anabolic hormone, human growth hormone (HGH). Endogenous HGH is known to stimulate muscle growth

by stimulating protein synthesis in both muscle and liver and at the same time decrease body fat by stimulating lipolysis (23). If these claims are true, by supplementing their diets, athletes may be able to counteract some of the losses experienced with hypocaloric diets (i.e., decrease in strength, loss of lean body mass).

It is well known that intravenously administered amino acids will stimulate HGH release from the anterior pituitary (25) with arginine being documented as one of the most potent stimulators (5)(25)(28). However, since athletes are taking these supplements orally, it is possible that the high arginase levels in the liver will prevent any increase in blood arginine or HGH.

Some evidence exists that oral arginine can reduce lean tissue loss in rats (31) and humans (10) subjected to surgical trauma. Major surgery and injury results in weight loss, primarily due to catabolism of lean tissue (10), thus oral arginine may be most effective in catabolic conditions. However, the research on the effectiveness of oral arginine supplementation in healthy populations has been equivocal (3)(11)(12)(20).

Since male body builders may be at risk of catabolizing lean mass during energy restriction practiced prior to competition, a research project was designed to test whether oral supplementation of the amino acid arginine would affect

lean tissue maintenance, body fat loss, and muscle function in male body builders placed on a hypoenergy diet.

Methods

Subjects

Twenty male body builders were randomly selected from a group of volunteers who were recruited from weight lifting clubs located in the Blacksburg, Virginia area. All subjects had been weight lifting for a minimum of 2 years, 3 - 6 times a week, for the purpose of physique development. All subjects denied the use of steroids during their lifting history and all denied competing in a body building competition. The subjects were asked not to use amino acid supplements at least two weeks prior to the study or during the study. The subjects were randomly assigned to either the placebo (PLA), n=7, arginine (ARG), n=7, or control (CON), n=6. Because of the range of body weights, the subjects for ARG and PLA were ranked from highest to lowest body weight and assigned to a group consecutively to ensure that body weights were not different between groups (Table 1). Experimental design was double-blind. During the study, one of the PLA subjects dropped out and one of the ARG subjects data was dropped due to non compliance of urine collection procedures. Thus, for the analysis of all indices PLA n = 6 and the ARG n = 6. The study was approved by the Virginia Tech Institutional Review Board for Research

Involving Human Subjects. All subjects were informed of risks and signed informed consents.

Experimental Procedures

Only PLA and ARG participated in diet manipulations. CON was utilized to control for possible effects of the exercise regimen and repeated testing on muscle function tests. A seven-day maintenance period (MAINT) was established to ensure that PLA and ARG subjects were consuming similar composition diets and familiarize subjects with the weight training routine. Prior to MAINT, subjects of all three groups were instructed in the correct weight lifting techniques for each lift and the repetition maximum (1RM) for each lift was measured either directly or estimated from a 5RM (dumbell exercises) for each subject.

The MAINT diet was prescribed to ARG and PLA subjects using an exchange list diet and energy intake was prescribed as 35 kcal/kg body weight (BW)/d (26). Adjustments were made when necessary to ensure body weight maintenance occurred. Protein intake was calculated to be 1.0 g protein/kg BW/d (approximately 15% protein) with carbohydrates accounting for approximately 55% and fats 30% of the total diet. The CON was advised to eat ad libitum throughout the experiment to maintain a stable body weight, within a 5% daily variation.

All three groups participated in the weight lifting sessions which were also regimented during this week to normalize all subjects to a similar exercise routine. The weight lifting sessions, with an average duration of 60 minutes, consisted of exercises for the back/biceps/abdominals, chest/triceps/abdominals and legs/shoulder/abdominals, performed on alternating days for six days and then one day rest during MAINT (Table 2). Three to five sets (number of sets were dependent upon what exercise was being performed) of 8 - 10 repetitions at 70% 1RM for upper body and 85% 1RM for legs was prescribed. Individual adjustments were made, however, to achieve required repetitions and sets. As a result, actual intensities ranged 60 - 85% of 1RM. Weight lifted, repetitions and sets were recorded for each session. Subject exercise volume was calculated (weight lifted x number of repetitions for each set of each exercise. These volumes were total to determine a daily exercise volume. For purposes of analysis, cumulative volumes of the three day cycles were utilized. That is volume for one cycle equaled volume of Day 1: Back/Biceps plus Day 2: Chest/Triceps plus Day 3: Legs/Shoulders. This allowed for comparison of two cycles in MAINT and two complete cycles in EXPER. To ensure compliance, subjects worked in pairs and weight lifting sessions were supervised by the

investigators. Subjects were instructed to limit their exercise to the weight lifting sessions only.

The ten day experimental (EXPER) period began immediately following MAINT. All three groups continued their weight lifting schedule of 6 days work, one day rest, then 3 days of work. Subjects in PLA and ARG consumed a 22 kcal/kg BW/day liquid diet (Exceed, Ross Laboratories), plus enough powdered skim milk to provide the same amount of protein (1.0 g/kg BW/d) to all subjects. An increase in nonprotein calories was provided as needed depending on body weight by a powedered carbohydrate beverage (Wyler's). To ensure adequate sodium, subjects were allowed two bullion cubes a day. All subjects were allowed noncaloric beverages as desired. CON continued with their ad libitum diet during EXPER.

During EXPER, PLA and ARG took either a placebo or arginine supplement twice daily. The arginine supplement consisted of purified arginine HCL (Sigma Chemical Company) in a gelatin capsule. The placebo was powdered casein (Sigma Chemical Company) also in a gelatin capsule. The dose for both arginine and casein were 0.1 g/kg BW twice a day. From an unpublished pilot study conducted in our lab, this dose was sufficient to invoke a significant increase in serum HGH in 1 of 2 subjects tested at 0.02, 0.06, 0.1 and 0.2 g/kg BW. As recommended by supplement companies, one

dose was consumed in the morning after an overnight fast and another dose was consumed before bed.

Subjects in the PLA and ARG collected 24 hour urine specimens daily (in 1-liter polyethylene containers with 2 ml 50% HCl as a preservative) beginning with the second voiding of the day and ending with and including the first voiding of the next day. This collection continued for the entire 10 days of EXPER. Urine volume was measured and recorded for each day's sample and 150 ml aliquotes were frozen for later analysis of nitrogen excretion. A separate aliquote was frozen for analysis of urine creatinine.

Apparent nitrogen balance (NBAL) was calculated using the known values in the Exceed nutritional beverage, nonfat milk and supplements (arginine HCl or casein). Fecal nitrogen was estimated from the digestibility estimates for Exceed (i.e., 5.8% lost in feces) as provided by the manufacturers (Ross Laboratories). Dermal and sweat loss was also estimated at 8 mg/kg BW/d, the present WHO recommendation (38). Total urine nitrogen was determined by Kjehldahl method (4) in duplicate.

The difference between nitrogen consumption and urinary nitrogen excretion plus estimated fecal and dermal nitrogen loss determined the NBAL for each 24 hour period. Urine creatinine was measured as an index of the completeness of the daily urine collections (24). Urine creatinine was

assayed in duplicate using Sigma Diagnostics Procedure No. 555.

Percent body fat (%BF) was calculated by hydrostatic weighing technique, which was performed on day 6 of MAINT and day 9 of EXPER. The three highest values of 8 trials were averaged to determine underwater weight. Body density was calculated using the equation by Keys and Brozek (21) and taking into account residual volume and intestinal factors (100ml). Residual lung volume (RLV) was determined using the oxygen-dilution nitrogen equilibration method of Wilmore et al. (39) using Applied Electrochemistry oxygen and carbon dioxide analyzers. %BF was determined from the Siri equation (32) where (4.95/BD - 4.50)*100 was equal to %BF. Fat free mass (FFM) was calculated as the difference between total weight and fat weight.

Muscle function tests of elbow flexion and knee extension were performed two days prior to MAINT, day 6 of MAINT, and day 9 of EXPER for all three groups. All muscle function tests were dynamic isokinetic tests performed on the Cybex II dynamometer (Lumex, Ronkonkoma, NY). All test data was measured by the Cybex Data Reduction Computer (CDRC) and weight of the limb tested and weight of the dynamometer lever arm were compensated for. The sequence of testing for the first day of testing was randomly assigned with subsequent test sequence being controlled based on

initial. All subjects received proper orientation prior to any testing, all tests were performed utilizing the subjects preferred limb and a standard warm-up protocol was followed. The Cybex II was appropriately calibrated prior to each test day according to manufacturer recommendations (9).

Two measurements to determine muscle function were performed during each test session for both elbow flexion and knee extension. The first was a torque test of dynamic strength measured in foot pounds to determine peak maximum torque. An angular velocity of 60°/sec and a 5 maximum contraction sample was utilized for both the elbow and knee. The second test was a work test of dynamic muscle endurance. This endurance test consisted of 30 maximum dynamic repetitions exerted at an angular velocity of 120°/sec for elbow and 180°/sec for the knee. The test speed for the elbow extension test was determined from previous pilot work in which it was found that 120°/sec closely matched the speed that resistance trainers utilize when engaging in a preachers bench bicep curl. From this same pilot work it was determined that 30 repetitions was sufficient in eliciting a 50% fatigue rate at 120°/sec. for bicep flexion and 180°/sec. for knee extension. Endurance was determined from an endurance ratio which was calculated from the area under the torque curve during the last 5 contractions

divided by that area under the curve during the first five contractions.

The elbow flexion test was performed following a protocol developed in our lab from a previous pilot study (18). This protocol involved a mock preachers bench which was attached to the Cybex II Upper Body Exercise Testing Table (UBXT). Measurement of elbow flexion occurred while the subject was sitting with the shoulder flexed at 45°. A damp setting of 1 and chart speed of 25 mm/sec was utilized. All elbow flexion tests started in full flexion with anatomical zero determined from full extension. Test-retest reliability was determined from Spearman-Rho correlation (torque test: p=.88; endurance test: p=.84). The knee extension test followed Cybex II manufacturer suggestions (9).

All data is expressed as mean (±SEM). The experimental design was a two-way factorial design for all tests except for muscle function which utilized a three-way factorial design. All dependent measures were analyzed by repeated measures analysis of variance (ANOVA), with the alpha level set apriori at 0.05. A test of the simple main effects was conducted to determine significance when there was a significant group x time interaction identified.

Results

Changes in body weight, %BF, and FFM as determined by hydrodensiometry for ARG and PLA are depicted in Table 3. CON group did not significantly change their body weight over the experment (MAINT day -2: 86.37 (4.39) kg; MAINT day 6: 86.20 (4.14) kg; EXPER day 9: 86.3 (4.19)kg). Weight loss was significant for both ARG and PLA over time but the two groups did not differ significantly from one another. Weight for ARG decreased from 80.37 kg (2.25) to 77.13 kg (2.08) signifying a 4% change from initial weight. For PLA weight decreased from 79.4 kg (4.13) to 76.25 (4.27) also a 4% change. Change in %BF was also significant for both ARG and PLA over time. ARG decreased from 12.52% (2.74) to 10.51% (2.89) a 16% change and PLA decreased from 10.48 (0.80) to 8.39% (1.02) a 20% change. As with weight change, there was no significant interaction between the groups over time for %BF. Lean mass showed no significant change over time nor was there an interaction of group with time.

The data for daily nitrogen balance is graphically depicted in Figure 1 as mg of nitrogen per kg of daily body weight per day. There was no significant difference in nitrogen balance for either group over time or interaction between groups over time. However, the PLA group showed a trend towards a more positive nitrogen balance than the ARG beginning on Day 4. This same trend was observed when comparing cumulative nitrogen balance (mg/kg/10 days).

Cumulative nitrogen balance for ARG was -4.49 (93.38) and for PLA was 327.18 (134.4). However, two sample T-test results indicated no significant difference between the two groups.

The data for urinary creatinine excretion (mg/kg daily body wt/d) depicted in Figure 2, and data for total nitrogen excreted over the 10 days (Figure 3) indicated no difference over time nor any significant interaction between groups over time. However, there was a trend towards a higher mean total urinary nitrogen excretion for ARG.

Urinary nitrogen excretion/urinary creatinine excretion ratio (UNE/UCE) (Figure 4) between the groups over time was significantly different. Test of simple effects indicated significance on Days 4, 5, 6, 7, & 9 with PLA showing a significantly higher ratio then ARG on these days.

Total Volume for four cycles when analyzed by Repeated Measures ANOVA indicated no significance between groups, over time, or interaction thus indicating that Volume was not a confounding factor in the analysis of muscle function tests. Volume means were: ARG Cycle 2: 85473 (11560), Cycle 4: 87677 (11909); PLA Cycle 2: 77033 (4595) and Cycle 4: 75901 (5469); and CON Cycle 2: 74618 (4192) and Cycle 4: 74738 (4423).

Muscle performance results are shown in Table 4. Muscle peak torque tests indicated a significant time effect

when groups were combined which reflected a reduction for both elbow flexion and knee extension. However, there was no significant difference between groups or interaction.

In regards to elbow flexion endurance, a significant time effect was noted towards increased endurance. However, the knee extension endurance test indicated no difference over time nor significant interaction between groups over time.

Discussion

In the present study, an oral 0.1 g/kg/d dose of arginine given twice a day, appeared to give no added benefit to weight lifting subjects during a 22 kcal/kg BW/d diet. The purpose of the reduced caloric intake was to mimic a diet of a competitive body builder who is preparing for a competition. Walberg et al. demonstrated that body builders, in spite of a regular weight-lifting schedule, lose body protein during times of energy restriction, although their protein intake was in the RDA range (37). This loss of body protein was documented by a decrease in NBAL and this decrease is similar to that observed in trauma victims. That is, in both situtations, there is an associated increase in the catabolism of amino acids due to increased muscle protein breakdown or decreased muscle protein synthesis. This catabolism may be documented by a

decrease in nitrogen balance a much better index than weight loss (7)(18)(40).

During trauma, it has been hypothesized that the demands for arginine may be increased for muscle protein synthesis (31) (13), as well as because of its role in the urea cycle, where it is essential for the detoxification of ammonia arising from the catabolism of other amino acids (33) It may also be that arginine becomes essential in traumatized animals and humans due to its role in wound healing by nutritional mechanisms as well as by its influencing hormone mediated reactions that are specific to trauma (31). The benefits of infused arginine, whether used alone (2)(13) or in combination with glycine (29)(33), have been well documented for traumatized rats and humans as it relates to nitrogen balance studies. Specifically, these studies indicate that infused arginine increases nitrogen retention and decreases weight loss. However, the results from oral supplementation studies have been equivocal as they relate to NBAL.

There have been only a few studies documenting the effectiveness of oral arginine in traumatized humans and animals. In a study by Daly et al. (10), the immune and metabolic effects of enteral supplementation of arginine (.36 g/kg for 70 kg subject dose) were tested in 30 cancer patients undergoing major surgery. Measurement of plasma

somatomedin C levels, a monitor of the overall effects of arginine on growth hormone secretion, indicated a significantly higher concentration by day 7 in the arginine group compared to controls (isonitrogenous glycine), suggesting a stimulatory effect of arginine on the pituitary. However, in regards to nitrogen balance, there was no significance noted, even though after Day 5 mean nitrogen balance became positive in the arginine group, whereas it remained negative in the control group throughout the seven day experimental period.

In an earlier study, Seifter et al. (31) reported that rats subjected to surgical trauma and oral amino acid supplementation (rat chow supplemented with 1.8% arginine), indicated significant reduced nitrogen loss. They also found that the arginine supplemented group had an increase in wound breaking strength and increase rate of collagen deposition. Although the Seifter et al. study suggests positive metabolic effects of oral arginine in traumatized rats, Daly et al. found only moderate effects on nitrogen balance.

In the present study no treatment effects were noted in our healthy subjects in regards to NBAL. There is little reported research in regards to the possible positive benefits of oral supplementation of arginine in healthy animals and humans from which to make a comparison to our

study. Barr et al. (3) indicated a lack of effect of supplemental arginine (2% of diet, 1600 mg/kg body weight) and ornithine (1% of diet, 800 mg/kg body weight) combined with access to an activity wheel on muscle morphological changes or fat loss in adult healthy rats after 4 weeks. Although a decrease in weight was observed in some rats, this loss was determined to be associated with wheel-running activity since no change in growth hormone was revealed at autopsy.

The findings of Barr and associates were in line with what was found here but in contrast to a study by Elam (1) who looked at morphological changes in male weight lifters who consumed an amino acid supplement twice a day composed of 1.0 g arginine and 1.0 g ornithine. After a 5 week period, the amino acid supplemented group lost a significant amount of body weight and body fat (skin fold technique) when compared to controls. Thus, the author concluded that the oral supplements combined with resistance exercise will reduce total body mass and body fat in adult males. However, Elam did not control for diet and assumed that the subjects maintained their established dietary habits. As a result, it may be that the changes in body weight were due to individual dietary changes. It is interesting to note that subjects in the placebo, as well as the supplement group, decreased their body weight over the 5 week study.

Elam also did not discuss lean body mass changes but did note that of 4 composite body girth measurements (obtained from circumference measurement technique), both groups increased with no significant difference between groups. This could lead one to speculate whether the weight training alone or the amino acid was the mitigating factor involved here since weight training alone is anabolic. It is also important to note that the diffences in results obtained by Elam from ours as well as Barr et al. cannot be explained by an amino acid dose relationship. The Elam dose (0.014 g/kg for 70 kg subject of both arginine and ornithine taken twice a day) was less then the dose administered by our group (0.1 g/kg for a 70 kg subject taken twice a day) and less than that administered by Barr et al. (1.6 g/kg arginine plus 0.8 g/kg ornithine).

Although no benefit was determined to occur from arginine treatment in this study, changes did occur which may be explained by nutritional mechanisms. Although both ARG and PLA showed similar trends in their NBAL with no significant difference indicated between the two groups, only the ARG group was in negative nitrogen balance (Days 4 - 6, 8, 10). This difference may have been attributed to a lower initial mean NBAL demonstrated by the ARG on Day 1. Unfortunately, NBAL was not assessed during the maintenance week so it is not known whether a trend for a lower mean

NBAL occurred for ARG prior to EXPER. Also, arginine per gram has a higher nitrogen content then casein (17), which may have accounted for the greater total urine nitrogen content observed in ARG. Although this increase was not sufficient to make a significant difference when comparing total urinary nitrogen content alone, it was sufficient to cause a significant difference between groups for urinary nitrogen/urinary creatinine ratio.

It is interesting to note that although both ARG and PLA lost a significant amount of weight, no significant change in their NBAL occurred. It has been noted by others that a deficient dietary energy intake as well as increased muscular work may cause a decrease in nitrogen balance (14)(15) and often times in cases of weight loss a significant negative nitrogen balance occurs (27)(37). However, how much decrement, if any, is observed may be dependent upon the macronutrient content of the diet (37). The protein requirements of weight lifters has been suggested to be 0.8 - 1.4 g/kg/d (7)(15)(30)(36) when athletes are consuming adequate energy diets. However, whether weight lifters require more protein than the RDA recommends is controversial. In addition, the amount of required protein to ensure maintenance of lean mass when consuming a hypoenergy diet has not been well documented.

Walberg et al. (37) studied the affects of macronutrient content of a hypoenergy diet (18 kcal/kg/d) on nitrogen retention in exercising male weight lifters. One diet was moderate-protein (.8 g/kg/d) high-carbohydrate and the other was a high-protein (1.6 g/kg/d), moderate-carbohydrate hypoenergy diet. Both groups lost a significant amount of weight and percent body fat with no difference noted between the groups. However, only the high-protein group was able to maintain positive nitrogen balance throughout the experimental phase. The protein dose utilized in this study was intermediate to the two protein doses utilized in the Walberg study and from analysis of nitrogen balance, appeared to be effective in maintaining lean mass in exercising body builders.

Another possible contributing factor associated with the maintenance of lean mass during weight loss, as demonstrated by this study, was the exercise protocol utilized. It has been hypothesized that if a caloric deficit is caused by exercising it may have a positive effect on lean mass compared to the effect of a caloric deficit developed through food restriction (26). McMurray et al. (26) tested this hypothesis by examining the effects of 7 days of caloric deficit (1,000 kcal/d) induced by diet or exercise on weight loss and nitrogen balance in 6 endurance trained males. They found that when subjects

dieted alone they had a higher cumulative nitrogen loss then when the same subjects incurred a caloric deficit from exercise alone.

The caloric deficit established by the diet in the McMurray study (20 kcal/kg/d) was similar to the diet protocol used in the present study (22 kcal/kg/d) as well as the protein dose (1.0 g/kg/d). However, although the amount of weight lost was similar between the McMurray diet subjects and the subjects in our study, our subjects did not experience similar negative nitrogen balance during weight loss. Instead, the nitrogen balance results of the present study matched more closely those of the endurance exercising group. Although the McMurray group utilized endurance activities others have noted the anabolic effects of weight lifting (1) and the process involved is believed to be due to an interaction of work and hormonal influences (23) on contractile tissue. The results from our study reinforce the idea that weight lifting exercise may have a positive effect on conserving lean tissue when a caloric deficit is present.

If arginine supplementation at the dose utilized in this study was to stimulate the release of HGH then it might be possible to detect differences in muscle function between the two treatment groups with the ARG group demonstrating greater positive changes. However, there were no positive

effects from arginine supplementation noted from any of the muscle performance tests. This finding is not in line with studies by Elam (1) and Elam et al. (2) who found that their arginine/ornithine supplemented groups performed significantly better then controls after a five week weight training regimen. Although Elam claimed that muscle strength increased significantly in the supplemented group, muscle function tests were only done at the end of the study making it impossible to compare changes over time between the two groups. Regardless, as discussed earlier, in neither Elam study was diet controlled which may again have had a confounding effect on the results.

The amount of work a muscle is able to perform may be limited by nutritional mechanisms. The decrease in peak muscle torque observed in this study has been observed by other researchers when studying the effects of a hypoenergy diet. Krotkiewski et al. (22) studied the effects of a very low calorie (544 kcal/d) high protein diet for four weeks in 32 obese women on isokinetic muscle strength and endurance of knee extension at three different angular velocities (30, 60 and 180°/sec). After two weeks, the subjects indicated a significant decrease in muscle strength that corresponded with a decrease in glycogen. This finding correlates with that of Houston et al. (19) who studied wrestlers in which caloric intake was restricted to 33% of baseline for two

days. That is, a significant decrease in quadriceps peak torque measured isokinetically was observed concurrent with a decrease in muscle glycogen.

The results of both the Krotkiewski and Houston et al. studies have validity for body builders as well. A weight-lifting routine relies on anaerobic glycolysis and therefore muscle glycogen for ATP production, as well as the creatine-phosphogen system (16). If the diet is insufficient in supplying adequate energy then the glycogen stores cannot be replenished and a decrement in strength may occur such as occurred in our ARG and PLA subjects.

Another possible explanation for the observed decrement in maximum peak torque may be due to a reduction in ATP-generating enzymes and muscle cell minerals that are a function of a hypoenergy diet (37). However, as Walberg indicated in a review article, although there has been some documented research in the obesity literature in regards to electrolyte and other mineral disturbances involved in impaired muscle function in energy-restricted obese individuals there has been very little research in this area utilizing resistance exercise during energy restriction.

Statistical analysis of the muscle endurance tests indicated a significant time effect towards increased elbow flexion endurance for all groups. Although this phenomenon is difficult to explain in light of the observed decrement

in maximum peak torque of these same energy restrictec subjects, others have noted increases in isokinetic endurance tests in subjects exposed to a hypoenergy diet. Krotkiewski et al. (22) noted an increase in dynamic quadriceps endurance, as measured from Cybex II isokinetic dynamometer, after 32 women were placed on a very low calorie diet (544 kcal/d) for two weeks, even though glycogen stores were reduced. Obese individuals have also been found to have a greater percentage of Type IIA fibers, which these authors speculated would not be as limited by the fall in glycogen levels (22). Although athletes who have trained in resistance exercise, especially those involved in high volume training such as our subjects, have been noted to possess a high ratio of Type IIA compared to Type I and Type IIB muscle fibers (34) (8), it is difficult to accept the hypothesis that this fiber ratio would be a contributing factor in an endurance test that was of only 30 seconds duration.

Our results, as well as Krotkiewski et al., are in conflict with Walberg et al. (41) who found that those subjects consuming a high protein/moderate carbohydrate hypoenergy diet demonstrated decreased muscle isometric endurance in the quadriceps as measured by the Cybex II dynamometer. They speculated that the observed decrement was due to depleted glycogen stores. However, the

discrepancy in the results of this study and ours, may have been due to a difference in the macronutrient diet content (low carbohydrate) as well as the test protocols. Walberg et al. utilized an isometric test whereas we used, as well as Krotkiewski, a dynamic muscle endurance test. During an isometric contraction blood flow to the working muscles is occluded thus causing more of a reliance on immediate ATP and creatine-phosphate stores then might occur during successive dynamic contractions.

In conclusion, the results of this study give no support to the use of arginine by healthy male body builders on a hypoenergy diet as an ergogenic aid in improving physique development over a ten day period. This hypocaloric diet in combination with weight lifting appeared to have a protein sparing effect. This suggests that 1.0 g/kg/d of protein is adequate to maintain lean mass for weight lifters even during weight loss. As has been documented by others, the caloric deficit caused a decrement in muscle peak torque which may have been due to a change in muscle enzyme activity and/or a depletion of the muscle glycogen stores. Since the only amino acid tested was arginine and the experimental phase was only ten days in duration, more research is indicated in regards to possible synergistic effects of amino acid combinations as well as long term effects of supplementation in healthy populations.

Until then, it appears that hard work and proper nutrition remain the keys to improvement for body builders.

Table 1. Age and Initial Weight of Subjects

	ARG	PLA	CON
n	6	6	6
Age (yrs)	21.8 (0.7)	20.8 (0.5)	22.0 (0.4)
Weight (kg)	80.4 (0.3)	79.4 (4.1)	86.5 (4.4)

Values are means (SEM).
ARG = Arginine supplemented group
PLA = Placebo supplemented group
CON = Control

Table 2. Alternating Weightlifting Routine During Maintenance and Hypoenergy Phase

Day 1 & 4: Back, biceps, abdomen			
	Sets		Sets
Lat Pulldowns	5	Dumbell curls	3
Pull-ups	1	E-Z curls	3
Seated rows	4	hyperextensions	1
Bentover DB rows	3	Crunches	2
Preacher Curl	3	Knee up crunces	2
Day 2 & 5: Chest, triceps, abdomen			
Supine Bench Press	5	Cable Pushdowns	3
Incline Bench Press	4	Tricep Extensions	3
Decline Bench Press	4	Crunches	2
Flat Bench Flies	3	Knee up crunches	2
Dips	2		
Day 3 & 6: Legs, shoulders, abdomen			
Leg extensions	4	Side lateral raise	3
Leg press	4	Bent over lat raise	3
Leg curls	4	Shoulder shrug	3
Calf raises	4	Crunches	2
Military press	3	Knee up crunches	2
Day 7: Rest			

Table 3. Hydrostatic weighing data

	ARG		PLA	
n	6		6	
	Pre	Post	Pre	Post
Weight (kg)	80.4 (2.3)	77.1* (2.1)	79.4 (4.1)	76.2* (4.3)
%BF	12.5 (2.7)	10.5* (2.9)	10.5 (0.8)	8.4* (1.0)
FFM (kg)	69.7 (2.1)	69.4 (2.6)	69.5 (3.1)	69.5 (3.0)

Values are means (SEM)
*indicates significant difference from pre value.

Table 4. Muscle Performance

	ARG	PLA	CON
n	6	6	6
Peak Torque (ft.lbs.)			
Elbow Flexion*			
MAINT1	49.17 (4.0)	44.83 (3.49)	39.5 (2.36)
MAINT2	46.5 (2.53)	45.83 (2.73)	41.33 (1.12)
EXPER1	41.00 (3.18)	43.17 (2.90)	40.50 (1.86)
Knee Extension*			
MAINT1	185.17 (8.48)	179.33 (12.00)	170.83 (9.02)
MAINT2	175.83 (6.09)	178.17 (10.80)	174.17 (8.84)
EXPER1	163.50 (6.78)	161.00 (6.64)	170.00 (7.29)
Endurance (%)			
Elbow Flexion*			
MAINT1	40.17 (3.22)	42.83 (3.95)	40.50 (2.64)
MAINT2	43.00 (4.19)	44.5 (4.51)	40.5 (3.36)
EXPER1	48.33 (4.96)	45.17 (7.03)	48.00 (4.65)
Knee Extension			
MAINT1	49.83 (1.92)	48.17 (3.56)	50.67 4.04
MAINT2	46.83 (3.32)	48.00 (2.84)	45.83 (2.82)
EXPER1	46.17 (2.41)	52.83 (4.22)	47.83 (2.54)

Values are means (SEM)
*Indicates significant time effect for groups.

FIGURE LEGEND

Figure 1. Plot of nitrogen balance over hypoenergy phase of experiment for the arginine and placebo supplement groups.

ARG - Arginine supplemented group
PLA - Casein supplemented group

Figure 2. Plot of urine creatinine over hypoenergy phase of experiment for the arginine and placebo supplement groups.

ARG - Arginine supplemented group
PLA - Casein supplemented group

Figure 3. Plot of total urinary nitrogen over hypoenergy phase of experiment for the arginine and placebo supplement groups.

ARG - Arginine supplemented group
PLA - Casein supplemented group

Figure 4. Plot of mg of urinary nitrogen excretion/mg urinary creatinine excretion over time during the hypocaloric diet. Significant difference between groups. Significant interaction between groups over time (*).

ARG - Arginine supplemented group
PLA - Casein supplemented group

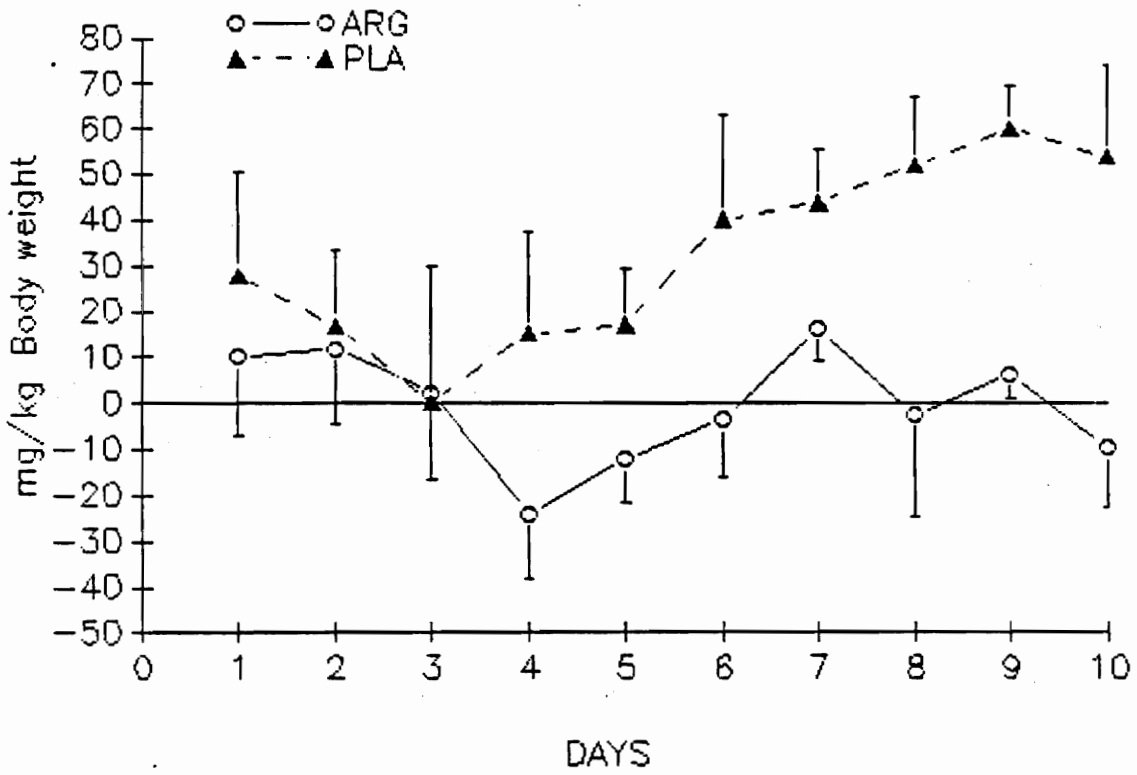

Figure 1: Plot of nitrogen balance over time during the hypocaloric diet.

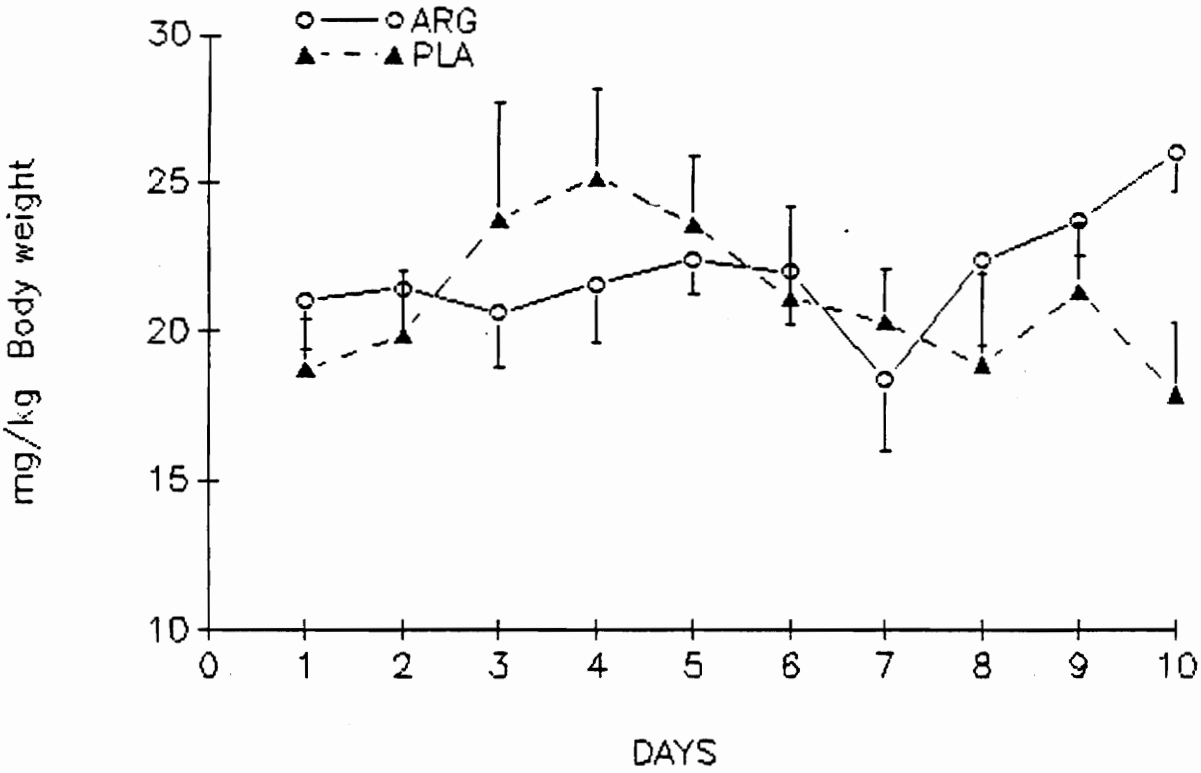

Figure 2: Plot of urine creatinine over hypoenergy phase.

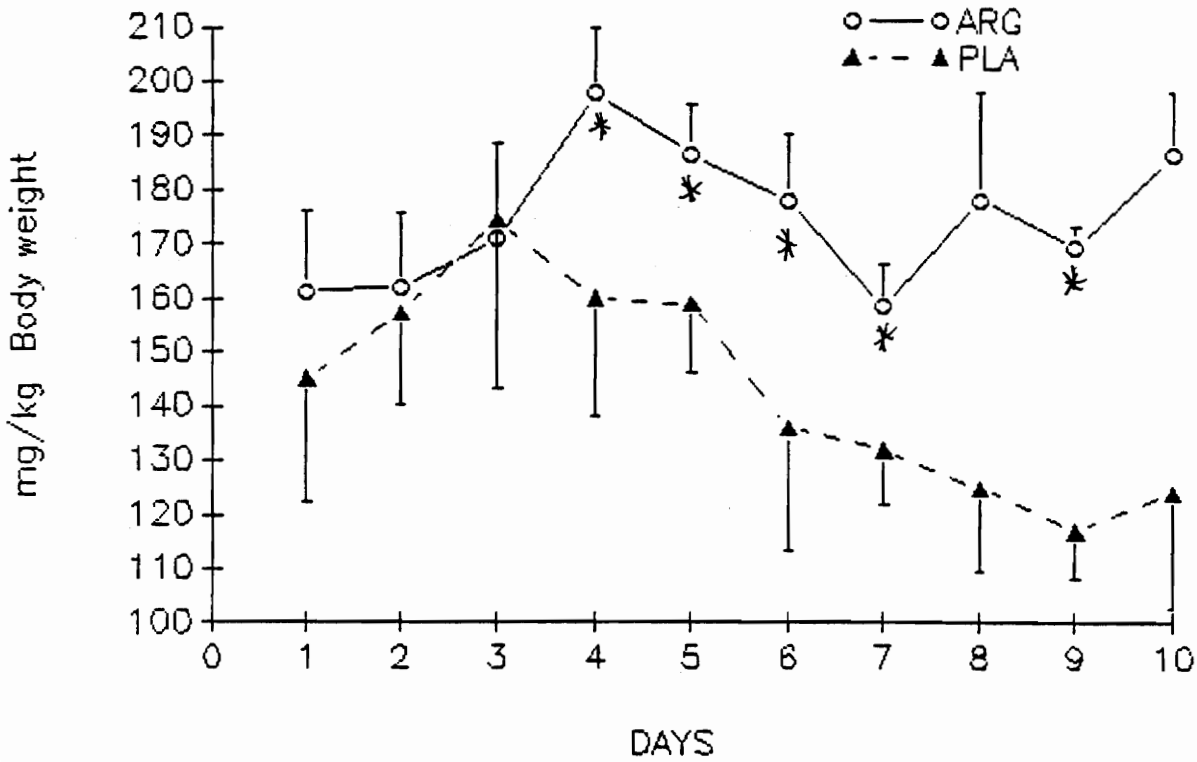

Figure 3: Plot of total urinary nitrogen over hypoenergy phase.

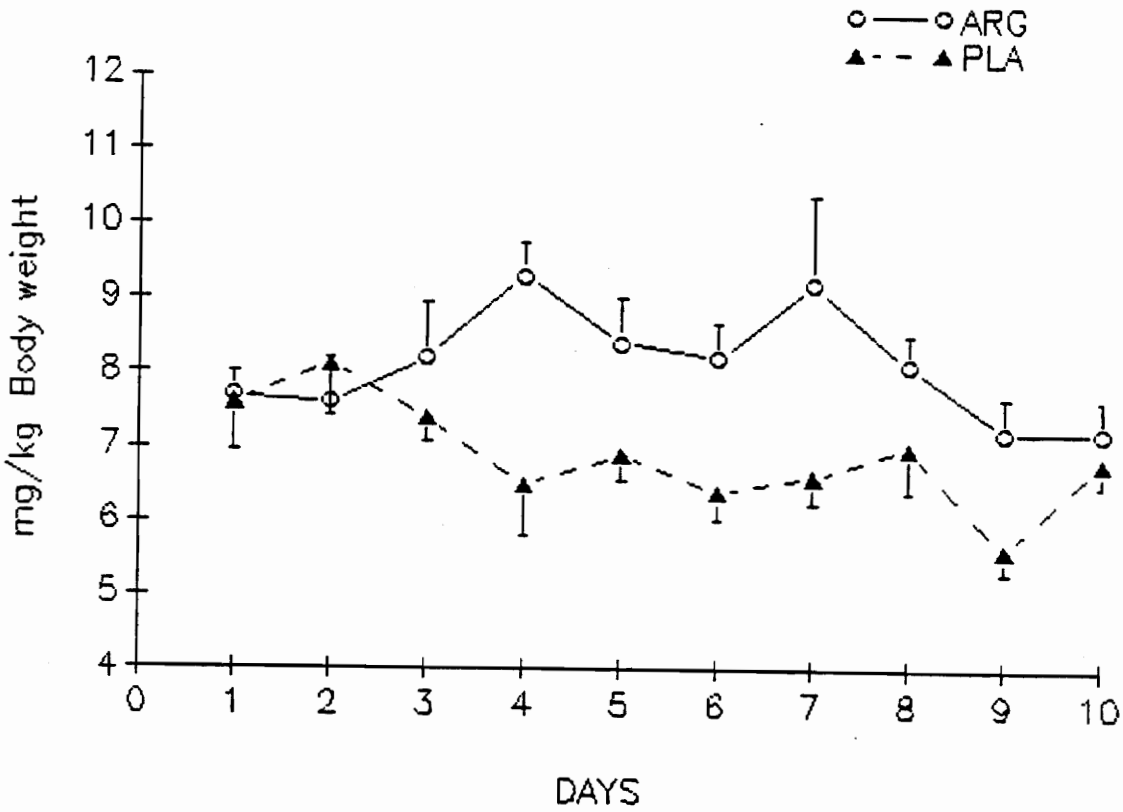

Figure 4: Plot of urinary nitrogen excretion/urinary creatinine excretion over time during hypoenergy phase. Significant difference between groups. Significant interaction between groups over time.

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Chapter IV

SUMMARY

Body building is a sport in which success is dictated by both muscle size and definition. As a result, these athletes are interested in maximizing their muscle mass as well as reducing their body fat to achieve peak muscle definition. To accomplish this end, body builders often suffer through extreme energy restriction in preparation for a contest often at the expense of lean tissue. Walberg et al. (1988) found that in spite of a regular weight-lifting schedule, body builders may lose body protein during times of energy restriction although their protein intake is in the RDA range. This loss has been demonstrated by an increase in urinary nitrogen output and a decrease in nitrogen balance (Calloway et al., 1971; Garza et al., 1976; Walberg et al., 1988). Not only will a hypoenergy diet affect nitrogen balance, but it may affect muscle performance as well (Houston et al., 1981; Krotkiewski et al., 1990; Walberg et al., 1988).

Not only have body builders experimented with diet manipulation in an effort to achieve an optimal physique, but they have also experimented with anabolic androgenic steroids. However, with the advent of negative press toward anabolic steroid use and more stringent testing, many body

builders (both competitive and recreational) are experimenting with other ergogenic aids, such as amino acid supplements, to achieve a "competitive edge". Manufacturers often suggest that amino acids act as a "natural" release stimulator of the anabolic hormone, human growth hormone (HGH). Endogenous HGH is known to stimulate muscle growth by stimulating protein synthesis in both muscle and liver and at the same time decrease body fat by stimulating lipolysis (Macintyre, 1987). If these claims are true, by supplementing their diets, athletes may be able to counteract some of the losses experienced with hypocaloric diets (i.e., decrease in strength, loss of lean body mass).

It is well known that intravenously administered amino acids will stimulate HGH release from the anterior pituitary (Merimee et al., 1969) with arginine being documented as one of the most potent stimulators (Casanueva et al., 1983; Merimee et al., 1969; Knopf, 1965). However, since athletes are taking these supplements orally, it is possible that the high arginase levels in the liver will prevent any increase in blood arginine or HGH.

Some evidence exists that oral arginine can reduce lean tissue loss in rats (Seifter et al., 1978) and humans (Daly et al., 1988) subjected to surgical trauma. Major surgery and injury results in weight loss, primarily due to catabolism of lean tissue (Daly et al., 1988), thus oral

arginine may be most effective in catabolizing conditions. However, the research on the effectiveness of oral arginine supplementation in healthy populations has been equivocal (Barr et al., 1987; Elam, 1988; Elam et al., 1989).

Since male body builders may be at risk of catabolizing lean mass during energy restriction practiced prior to competition, a research project was designed to test whether oral supplementation of the amino acid arginine would affect lean tissue maintenance, body fat loss, protein metabolism as measured by nitrogen balance and muscle function in male body builders placed on a hypoenergy diet.

Twenty male body builders were randomly selected from a group of volunteers and assigned to one of three groups: Arginine supplemented group (ARG), casein supplemented group (PLA), and a group to control for the muscle performance tests (CON). For one week prior to the experimental week, all subjects consumed a similar maintenance diet and followed the daily weight-lifting regimen.

During the experimental week (EXPER) subjects in ARG and PLA consumed a hypocaloric liquid diet (22 kcal/kg body wt/d, Exceed) with protein intake maintained at 1.0 g/kg/d. ARG received arginine HCl at a dose of 0.1 g/kg body weight twice a day. This same dose was maintained for the PLA which received powdered casein instead. CON received no

supplements and continued on an ad libitum diet to maintain body weight.

All subjects continued the supervised weight training program through EXPER. Subjects in ARG and PLA turned in daily 24-hour urine samples. Urine was analyzed for nitrogen excretion and creatinine. An estimation of fecal nitrogen and sweat nitrogen were made (WHO, 1985).

All subjects were tested for muscle performance of elbow flexion and knee extension utilizing the Cybex II isokinetic dynamometer. Tests were conducted two days prior to MAINT, day 7 of MAINT and day 9 of EXPER.

Hydrostatic weighing was utilized to assess changes in percent body fat (%BF) and fat free mass (FFM) in the ARG and PLA groups. Hydrostatic weighing occurred on day 6 of MAINT and day 9 of EXPER.

Both ARG and PLA lost a significant amount of body weight and %BF over time but were not different from one another. There was no significant change in FFM for either group.

Results of the repeated measures ANOVA showed no significant difference between ARG and PLA for nitrogen balance, cumulative nitrogen balance, creatinine or total urinary nitrogen. However, ratio of urinary nitrogen/urinary creatinine was significantly different between groups and indicated a group*time interaction. This

ratio outcome was believed to be due to the fact that arginine is a very nitrogen rich amino acid (i.e., 4 nitrogen molecules per amino acid) as compared to casein which is a mixture of amino acids which is likely to fit the average of 16% nitrogen value used for mixed amino acid proteins.

In regards to muscle performance tests, a significant time effect for groups combined was noted for both elbow flexion and knee extension. However, there were no treatment effects noted between groups nor group x time interaction. A significant increase over time was noted for all groups when combined in elbow flexion. Again no significant interaction between groups over time occurred. For the knee extension endurance test statistical analysis indicated no difference between groups, no difference over time nor any significant interaction between groups over time.

Research Implications

From anecdotal accounts, body builders, as well as other athletes, have been consuming amino acids, at great financial expense, with the expectation that these supplements will assist them in decreasing body fat as well as increasing muscle mass. The mechanism involved in achieving these results, as promoted by manufacturers of amino acid supplements, is that certain amino acids

stimulate the release of human growth hormone. Arginine in particular has been shown to increase serum levels of human growth hormone when infused. Arginine has also been proven to improve nitrogen retention in traumatized animals and humans when infused as well as when given orally. However, few healthy human studies which incorporate tests of arginine effectiveness toward conserving the body's protein pool or improving muscular performance have been documented. The few studies that have been conducted to explore these relationships have been equivocal.

In the present study, subjects consuming the arginine supplements indicated no positive effects on the indices measured. All subjects participating in the hypoenergy diet indicated a significant decrease in body weight and body fat over time with no change in fat free mass. Although no significant difference was found between the two groups in regards to nitrogen balance, the ARG group showed a trend toward a higher total urinary nitrogen excreted and a lower nitrogen balance.

One possible explanation for the trends observed in nitrogen balance may have been due to the quality of the protein content consumed. For a 70 kg man in our study, the base diet provided 70 grams of protein. The supplements for this same 70 kg man would provide an additional 14 grams, accounting for 17% of the total protein intake. Although

the base diets were the same, the PLA group received casein as their supplement. Casein is a complete protein which contains all 20 amino acids. Although there was no significant difference between the groups, the casein group showed a trend toward more positive nitrogen balance throughout the experimental phase which may have been due to the added 17% of a complete protein to the PLA diet. This leads to the speculation that a diet sufficient in complete protein is more then adequate to conserve protein mass during times of energy restriction in male body builders.

As was discussed earlier, it should also be noted that arginine per gram has a higher nitrogen content then casein. As a result, this increase in nitrogen content may have accounted for the increase in urine nitrogen content observed in the arginine group (Hamilton & Gropper, 1987). Amino acids are oxidized to carbon dioxide, water and urinary metabolites containing nitrogen. As a result, biological energy content will be greatest when the weight proportion of nitrogen is least (May and Hill, 1990). What was observed here in the nitrogen balance studies may just be an example of how metabolically efficient the human body is at disposing of nitrogen when confronted with a large pool of one specific amino acid. Specifically, in the case of arginine, arginase activity in the liver is very high and

break down of arginine to ornithine and urea occurs rapidly (Hamilton & Gropper, 1987).

Another factor that may affect the positive benefits of amino acid supplementation on nitrogen balance is the quantity of supplement given. The dose utilized in this study was in line with others (Elam, 1988; Elam et al., 1989) and was sufficient in initiating an increase in serum growth hormone as documented by a pilot study conducted in our lab. However, as stated earlier, there was no significance noted between the two supplemented groups in regards to positive protein sparing effects.

In regards to muscle performance tests, there were no treatment effects noted. This was in contradiction to the Elam (1988) and the Elam et al. (1989) studies. These two studies are the only other documented research studies that utilized arginine to test its effects on healthy weight lifters. Although both Elam studies indicated the positive effects of arginine when combined with ornithine on body composition and muscle performance, it is believed that these studies have many weaknesses. First, these researchers did not control for diet as we did. As a result, it is difficult to tease out what were the true contributing factors to the observed changes--was it the supplements or was it the diet? Also, the placebo was not isonitrogenous. This fact combined with the lack of dietary

control makes it difficult to determine if the macronutrient content of the diets were the same for both groups. As Walberg et al. (1988) documented from their research, macronutrient content of a diet is a contributing factor to muscle performance.

Another factor that may be involved in the differing results between the Elam studies and ours is that arginine may become effective only when combined with another amino acid such as ornithine. However, Barr et al. (1987), who also used an arginine and ornithine mix to supplement healthy rats, found no significance when compared to controls in regards to muscle morphological changes as well as fat loss.

Both ARG and PLA decreased elbow flexion and knee extension peak torque over time. This decrease in muscle strength was believed to be due to a depletion of glycogen stores caused by the nature of the diet and weight-lifting regimen. A weight-lifting routine relies on anaerobic glycolysis and therefore muscle glycogen for ATP production (Guezennec et al., 1986). If the diet is insufficient in supplying adequate energy, then the glycogen stores cannot be restored.

Statistical analysis of endurance tests for knee extension and elbow flexion indicated no treatment effects as well. However, a significant time effect was noted

towards increased elbow flexion endurance. The muscle performance results may be explained by the type of muscle fiber recruitment which occurs for a torque versus an endurance test. The torque test is dependent upon rapid high tension development which would call upon Type IIB fibers. Type IIb fibers are well-supplied with glycolytic enzymes but are poorly endowed with oxidative enzymes (Bandy, 1990). As a result, these muscle units generate a large amount of tension in a short time, but fatigue rapidly. The endurance tests are of a longer duration (approximately 30 seconds each) and as a result, recruitment of Type IIA fibers may be expected to occur. Type IIA fibers possess intermediate amounts of oxidative and glycolytic enzymes which indicates the use of both anaerobic and aerobic metabolism (Bandy, 1990).

It has been indicated by others (Staron et al., 1984; Costill et al., 1979) that resistance training increases the proportion of Type IIA:I and IIA:IIB fiber area ratios. Although the subjects participated in the prescribed weight-lifting regimen for only seventeen days, a training effect appears to have occurred and may have been related to Type IIA muscle fiber type. Since muscles involved in knee flexion tend to have a higher Type IIA to IIB and I ratio then elbow flexion muscles, then the training effect would be more apparent in the elbow flexion tests.

The results of our muscle endurance tests were not in agreement with Walberg et al. (1988) who found a decrease in knee extension and elbow flexion endurance. This difference may be attributed to the different testing protocols utilized on the Cybex II. That is, Walberg et al. used an isometric test whereas we used a dynamic test. During an isometric contraction, blood flow to the working muscles is occluded thus causing more of a reliance on immediate ATP and creatine-phosphate stores.

Bodybuilding is becoming extremely popular not just as a competitive sport but as a leisure activity as well. Success as a bodybuilder is determined by muscle definition. It appears from this investigation that an athlete who wants to decrease body fat while maintaining protein stores can do so utilizing a hypocaloric diet, but the diet must provide more than the RDA for protein and adequate carbohydrates to enhance protein retention. This can be achieved through normal diet manipulation, and as the results of this study indicate, the addition of amino acids is not warranted.

Recommendations for Future Research

The results of this investigation indicate possibilities for future research. In the present study, nitrogen balance was not measured during the maintenance phase. Although the groups would not be expected to be different during maintenance, the placebo group began the

experimental phase at a higher nitrogen balance than ARG. Since nitrogen balance was not assessed prior to experimental phase, it is unknown whether this was a normal fluctuation phenomenon or whether the placebo group had a higher nitrogen maintenance from the beginning.

Many of the trauma studies infuse arginine in combination with glycine. Elam and Barr looked at arginine and ornithine. As a result of this inconsistency in type of amino acid utilized for study, makes it difficult to compare research results. It would be interesting to use the same experimental protocols established here but supplement subjects with arginine plus glycine and/or arginine plus ornithine to determine whether there are synergistic effects.

Not only are there inconsistencies in the type as well as dosages of amino acids utilized but training regimens differ as well. A consistent training regimen should be developed, such as the one utilized here, as well as a consistent measurement of change in muscle performance. The utilization of Cybex II isokinetic dynamometer has its limitations when the training regimen is a different mode (e.g., isotonic exercises). Measurement of 1RM during short term studies are also limited due to their lack of sensitivity. As a result, it may be best to either use isokinetic training as the exercise protocol and then test

for changes and make comparisons using the Cybex, or use a longer training period after which a 1RM measurement would be adequate to assess changes.

The experimental phase of this study was of a short duration (10 days). It would be beneficial to study possible long term effects of arginine in male body builders on muscle mass and percent body fat. Also, the newer techniques available with magnetic resonance imaging are making it possible to differentiate these changes more accurately. These newer techniques should be pursued to determine their superiority over the more traditional methods of assessing lean mass and body fat.

In conclusion there is room for further study of amino acids being consumed by body builders. Future research needs to take the direction of establishing controlled diet and exercise protocols so that the efficacy of these various supplements may be established.

Appendix A

METHODOLOGY

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SELECTION OF SUBJECTS

Twenty male body builders between the ages of 20 - 25 were randomly selected from a group of volunteers who were recruited from weight lifting clubs located in the Blacksburg area. All subjects had been weight lifting for a minimum of 2 years, 3 - 6 times a week for the purpose of physique development. Weight range for subjects was 64.6 kg - 107.7 kg (x=81.7). All subjects denied the use of steroids during the lifting history. The subjects were asked not to have used amino acid supplements two weeks prior to the study or during the study. All claimed that they had followed these instructions.

Health screening was used to eliminate subjects with a history of liver or kidney problems, diabetes, hypertension or orthopedic limitations. All subjects signed an informed consent before participating.

Sampling Procedures

The subjects were randomly assigned to either the placebo (PLA) (n=7), arginine (ARG) (n=7) or control group (CON) (n=6). During the course of the study, one of the PLA subjects dropped out for personal reasons and one of the ARG subjects data was dropped due to non compliance of urine collection procedures. This left PLA n = 6 and ARG n = 6. The key to subject group assignment for the PLA and ARG was

kept confidential by a selected staff member who was not involved in subject testing. The CON group was not on a controlled solution diet as were ARG and PLA during EXPER. As a result, urine studies were not considered for this group. The only purpose of CON was to control for effects of exercise regimen on muscle function tests.

GENERAL METHODOLOGY

Prior to the experiment, a general meeting was held for interested potential subjects. Detailed explanations were made concerning the study and the necessity of strict adherence to the prescribed diet and exercise prescription as well as urine collections. For those subjects involved in energy restriction, detailed diet instructions (written as well as oral) were given on an individual basis. Prior to the hypocaloric phase, written instructions were given in regards to proper urine collection.

Written instructions were given to each subject prior to specific test days in regards to hydrostatic weighing and Cybex testing. The importance of following the prescribed instructions were emphasized verbally throughout the study.

Selection of Appropriate Criterion Scores

The dependent measures investigated were body weight, urinary nitrogen loss (in relation to nitrogen intake and urinary creatinine), changes in %BF and lean muscle mass determined from hydrostatic weighing technique, and muscle

function changes (elbow flexion and knee extension) as determined by Cybex II peak torque and endurance tests.

In regards to nitrogen balance, nitrogen intake was calculated for each subject from the values provided by the manufacturers of the diet products. The nitrogen content of the supplements were calculated by assuming that the amino acids were 16% nitrogen. Urine was analyzed for nitrogen by using the Micro-Kheldahl method. Fecal nitrogen excretion values were estimated utilizing digestibility estimates for Exceed (Ross Laboratories). Sweat excretion was estimated utilizing 8 mg N/kg/d (WHO, 1985). The criterion scores were determined by taking the difference between nitrogen intake and nitrogen excretion for each of the ten days of the experiment and calculating the nitrogen balance. Urine creatinine was assayed in duplicate using Sigma Diagnostics Procedure No. 555. To determine the completeness of the daily urine collections daily creatinine fluctuations were analyzed as well as a total daily urinary nitrogen to creatinine ratio.

Reliability and Validity Estimates

Reliability estimates for hydrostatic weighing were determined from a pilot study in which sixteen subjects were tested and then retested. The Spearman-Rho correlation was used. The calculated p value for hydrostatic weighing was

.96. The hydrostatic weighing procedure is a valid estimation of body density (Pollock & Jackson, 1983).

Reliability estimates were determined for elbow flexion criterion during a pilot study utilizing 10 subjects who were tested and then retested. The test/retest score for endurance ratio for elbow flexion was p = .84. The test/retest score for maximum torque was p = .88. The muscle function tests for knee extension have been researched in depth by others in regards to validity and reliability (Montgomery et al., 1989).

Experimental Procedures

The entire study lasted a total of 17 days. Days 1-7 were labeled the maintenance phase (MAINT) with days 8 - 17 referred to as the experimental phase (EXPER). Only those subjects in the PLA and ARG participated in diet manipulations. MAINT served to ensure that PLA and ARG subjects were in energy balance, were consuming similar composition diets, and were participating in a similar exercise program so as to provide a baseline for analysis. During MAINT a diet was prescribed to each athlete using an exchange list diet and energy intake was prescribed as 35 kcal/kg/d (McMurray et al., 1985). Adjustments were made when necessary (manipulation of carbohydrate and fats) to ensure body weight maintenance occurred. Protein intake was calculated to be 1.0 gm/kg/d (approximately 15% protein)

with carbohydrates accounting for approximately 55% and fats 30% of the diet for each subject. Subjects were counseled prior to the diet and sample diets were given to all subjects. In addition, cards were distributed to all subjects with the number of servings that should be consumed each day. Cards were completed by subjects and turned in daily.

The diet during EXPER was a formula diet (approximately 22 kcal/kg/d) which consisted of at least 4 cans of Exceed (Ross Laboratories), plus enough skim milk (provided as powder) to provide the same amount of protein (1.0 gm/kg/d) to all subjects. An increase in nonprotein calories were provided as needed depending on body weight by a carbohydrate beverage (Wyler's). To ensure adequate sodium, subjects were allowed two bullion cubes a day. All diet foods were weighed and provided to the subjects each morning when they reported for their daily weigh-ins.

An example calculation of the diet for a 70 kg male was as follows:

	4 cans Exceed	1 1/2 Cup Skim milk	Total
kcal	1440	120	1560
protein (g)	60	12	72
carbohydrate	188	18	206
fat	48	---	48

Thus the macronutrient content of the diet was approximately 18.5% protein, 52.8% carbohydrate and 27.7% fat.

During EXPER all PLA and ARG subjects underwent a hypoenergy diet with subjects taking either a placebo or arginine supplement twice daily. The arginine supplement consisted of purified arginine HCL (Sigma Chemical Company) in a gelatin capsule. The arginine supplement dose was calculated to be 0.1 mg/kg body weight. Thus, for a 70 kg male the dose would be 7 mg twice a day. As recommended by supplement companies, one dose was consumed in the morning after an overnight fast and another dose was consumed before bed. The placebo was the same quantity (.1 mg/kg body weight) of powdered casein (Sigma Chemical Company) also in a gelatin capsule. Thus, supplements provided equal amounts of nitrogen to the entire diet.

Subjects in the PLA and ARG collected 24 hour urine specimens commencing the first day of EXPER with the collection of the second voiding of the day. Urine collections continued through the entire EXPER with the first voiding of day 18 being the final specimen collected. Urine was collected in 1 liter polyethyline bottles containing 2 ml of 50% HCl as a preservative. The 24 hour collections began with the second voiding of the respective day and ended with (and included) the first voiding of the following day. Daily urine samples were collected each morning when the subjects reported for their daily weigh-ins as well as to collect their diets for the day.

For each subject, urine volume was measured and recorded for each day's sample and a 150 ml sample was frozen for later analysis of nitrogen excretion. A separate aliquote from each day's sample was utilized for the analysis of urine creatinine.

Nitrogen consumption was calculated using the manufacturers values in the Exceed nutritional beverage, nonfat milk and supplements (arginine HCl or casein). Fecal nitrogen was estimated from the digestibility estimates for Exceed (Ross Laboratories)--94.2% digested, 5.8% lost in feces. Dermal and sweat loss was also estimated at 8 mg N/kg/d (WHO, 1985). Total urine nitrogen was determined by the Department of Human Nutrition and Foods Lab, VA Tech, utilizing a Buchi Kjeldahl Nitrogen Analyzing System in duplicate (Calloway, 1971).

The difference between nitrogen consumption and nitrogen excretion determined the nitrogen balance for each 24 hour period during EXPER. Urine volume and urine creatinine were measured as indices of the completeness of the daily urine collections (Marable, 1979). Urine creatinine was assayed in duplicate using Sigma Diagnostics Procedure No. 555.

Muscle function tests were performed two days prior to MAINT, on day 6 of MAINT and on day 9 of EXPER. Hydrostatic weighing was performed on day 6 of MAINT and on day 9 of

EXPER. Subjects from the PLA and ARG participated in hydrostatic weighing whereas all three groups (PLA, ARG, and CON) participated in muscle function testing.

Prior to MAINT, subjects were required to submit a volume recall of their usual training regimen for a week from which a standard weight training protocol was determined for all subjects. Each subject was given a written description of their exercise prescription after each subjects repetition maximum (1RM) was determined for each lift using either a true maximum or a predicted maximum from a submaximal lift of five repetitions (see sample in Appendix B). Each subject was given exercise logs to keep track of their workouts.

The resistance weight training program was the same for all 18 subjects. This program was controlled for volume (i.e., sets x repetitions x load) and was followed during MAINT as well as EXPER (see Appendix C for analysis data in regards to Volume). The training sessions lasted approximately one hour a day and were performed on a 6 day on 1 day off rotation. The following protocol was prescribed and followed:

Day 1: back/biceps/abdominals

Day 2: chest/triceps/abdominals

Day 3: legs/shoulders/abdominals

This three day rotation was then repeated with a day of rest allowed on day 7. This entire cycle was then repeated during EXPER. Three to five sets (number of sets were dependent upon what exercise was being performed) of 8 - 10 repetitions at 70 - 85% 1 RM were performed each exercise session (see sample in Appendix B). Since the number of participants and the limitation of University Gym facilities made it difficult to work all subjects at the same time, participants were encouraged to work with a partner to monitor one anothers compliance to the exercise protocol. Also, researchers involved in the study monitored these workouts as well.

Percent body fat (%BF) was calculated by hydrostatic weighing. A metal tank filled with water, containing a chair suspended from one load cell, was used for the procedure to determine body density (BD). The load cell was connected to an amplifier which connected to an analog to digital converter within an Apple IIE computer. The system was appropriately calibrated prior to each test using a 10.8 kg weight suspended from the load cell as well as by weighing 0 weight. Water temperature was recorded prior to each test. After being weighed in air, the subjects were instructed to enter the tank and remove air bubbles from their bathing suits and hair. In a sitting position, after expelling as much air as possible and with nose clips in

place, subjects bent forward so as to totally immerse their bodies in the water. When no air bubbles could be seen, a reading was achieved on the Apple IIE computer. Eight trials were conducted and the three highest values were averaged and used to determine underwater weight (pounds). This number was divided by 2.2 kg/lb and the resulting number was used as the underwater weight. Body density was calculated using the equation by Keys and Brozek (1953) and %BF was determined from the Siri equation (1961) where (4.95/BD - 4.50)X100 was equal to %BF.

Residual lung volume (RLV) was determined using the oxygen-dilution nitrogen equilibration method by Wilmore et al. (1980) An evacuated 5 liter anaesthesia bag was filled with 100% oxygen to 85% of the subject's vital capacity. One end of the bag was clamped shut and the open end was fitted with a 3 way valve. A mouthpiece was attached to the valve. Subjects assumed a sitting position and a noseclip was placed on the nose. The mouthpiece was positioned in the mouth and subjects were instructed to breath at a rate of 1 breath every 2 seconds. After 2 minutes of normal breathing, with the valve open to room air, the subjects were instructed to exhale as much air as possible. When this was completed the valve was opened to the bag (100% oxygen) and 5 - 7 breaths were taken at the rate of 1 breath every 2 seconds. A maximal exhalation was made again after

the 5 - 7 breaths and the valve was turned back to room air. The nose clips and mouthpiece were removed and the contents of the bag were analyzed for oxygen and carbon dioxide concentrations with the Applied Electrochemistry (Model numbers 5-3A, CD-3A) gas analyzers. Residual volume was calculated using the equation RLV=VO2 X (b-a)/(c-d) where RLV=Residual lung volume; VO2=volume of oxygen in bag at beginning of test; a=%nitrogen impurity of original O2; b=%nitrogen in the mixed air in the bag at point of equilibrium (100%-(%O2+%CO2); c=%nitrogen in the alveolar air at the beginning of the test (assumed to be 80.0%); and d=%nitrogen in alveolar air during the last maximal breath (assumed to be 0.2% nitrogen higher than the equilibrium % i.e. b+0.2% nitrogen).

Muscle function tests of elbow flexion and knee extension were performed at three different times on the Cybex II isokinetic machine which was appropriately calibrated according to manufacturers suggestion, prior to each testing day. The sequence of testing for the first muscle function tests was randomly assigned with subsequent test sequence being controlled. Testing occurred two days prior to MAINT, once on day 6 (MAINT) and on day 9 (EXPER), All subjects received proper orientation prior to any testing. All tests were performed utilizing the subjects preferred limb. Two measurements to determine muscle

function were performed during each test session for both elbow flexion and knee extension. The first was a torque test of dynamic strength measured in foot pounds to determine peak maximum torque as measured by the Cybex Data Reduction Computer (CDRC). An angular velocity of 60°/sec and a 5 maximum contraction sample was utilized for both the elbow and knee. The second test was a work test of dynamic muscle endurance. This endurance test consisted of 30 maximum dynamic repetitions exerted at an angular velocity of 120°/sec for elbow and 180°/sec for the knee. During pilot studies 30 repetitions was determined to be sufficient to induce a 50% fatigue value at the prescribed speeds. The angular velocity of 120°/sec. was found also during pilot work, to closely match the speed of a normal preachers curl. The 180°/sec. angular velocity was utilized for knee extension due to its frequent use by other researchers so as to provide a means of comparison. Endurance was determined from an endurance ratio which was calculated from the area under the torque curve during the last five contractions divided by that area under the curve during the first five contractions. As in the test for peak torque, calculations for endurance determination were obtained from the CDRC.

Both tests were conducted during the same session for each subject after an appropriate warm-up as follows:

1. 3 minutes on cycle ergometer
2. 4 submaximal contractions at appropriate

test speed
3. 30 seconds rest
4. 2 maximal contractions at appropriate test speed
5. 1 minute rest
6. First Test
7. 1 minute rest
8. Second test

Prior to the initial test, the subjects were familiarized with the positions and movements required. Consistent verbal encouragement was given for all tests. Again, during pilot studies the method of verbal encouragement utilized had been practiced by this experimenter to ensure the maintenance of consistency.

During the elbow flexion tests, subjects were seated before a mock "preachers bench" designed to allow for 45° shoulder flexion. This bench was attached to the UBXT by a universal adaptor positioned in tube #4 of UBXT. The UBXT backrest was down flat with the seat in the highest position. A rotating hand grip was attached to the shoulder extension accessory bar which was inserted into the input shaft of the dynamometer. The hand was supinated and subjects were instructed to keep the wrist locked. The rotational axis of the elbow joint was aligned with the input shaft of the dynamometer.

Each subject sat with a straight back such that the bench fit snugly under the subjects armpit and the upper arm was maintained and stabilized against the bench via a velcro strap. To prevent interference from the contralateral limb

as well as to maintain shoulder alignment subjects were instructed to cross the contralateral arm across the bench with the contralateral hand resting on the shoulder of the working side. Subjects were instructed to remain seated and to maintain both feet flat on the floor throughout the test.

A damp setting of 1 and chart speed of 25 mm/sec was utilized for elbow flexion. All elbow tests were started in full flexion as suggested by Cybex II protocols with anatomical 0 determined from full elbow extension. Measurements for height of bar, dynamometer, hand grip and preachers bench were recorded and maintained for all trials.

Procedures to test knee extension were closely matched to those used for elbow flexion. The subject was seated on the appropriate S-H-D table of Cybex II isokinetic system. The subjects were moved back on the seat as far as possible to allow the heel of the working limb to touch the padded table leg while keeping the seat resting posterior of the knee. When necessary back pads were provided for solid back support. The shin pad was placed proximal to the malleoli below the bulk of calf musculature. Vertical and horizontal alignment was made between the knee axis of rotation and the dynamometer. A thigh stabilization strap, torso stabilization strap and pelvic stabilization strap were utilized for each subject. Subjects held to the sides of the table during all tests. Anatomical zero was set with

the knee fully extended and locked. Test starting position was with the knee flexed and the heel in contact with the padded table leg. Subjects were instructed to make heel contact at the end of each stage of knee flexion.

The length of the long input adapter with adjusting arm, position of dynamometer in relation to knee axis, and use of pads were all recorded for duplication purposes in all tests. Consistent verbal encouragement was given to all subjects to give a maximal effort during all trials.

Research Design and Statistical Procedures

The experimental design was a two-way factorial design for all tests except for muscle function which utilized a three-way factorial design. All dependent measures were analyzed by repeated measures analysis of variance (ANOVA), with the alpha level set apriori at 0.05. A test of the simple main effects was conducted to determine where significance occurred when a significant group x time interaction was identified.

Appendix B

INSTRUCTIONS TO SUBJECTS

DIET AND WEIGHT LIFTING PROTOCOLS

PERSONAL DATA SHEET

LAST NAME FIRST NAME MI

DATE OF BIRTH AGE SS#

ADDRESS CITY,STATE ZIP

HOME PHONE WEIGHT HEIGHT

PHYSICIAN ADDRESS PHONE#

DATE OF LAST PHYSICAL EXAM

In Case of Emergency, call:

NAME RELATIONSHIP PHONE#

Are you a Va Tech Student?_____ If yes, please indicate your class schedule for July 1990:

Are you presently working? _____ If yes, please indicate:

Place of employment:_____

Phone#:_____

Schedule:_____

Is there a time during the month of July when you plan to be out of town?_____ When?_____

DIET HISTORY

Have you taken steroids over the past six months?_____

If yes, are you currently taking steroids?_____

Have you taken amino acid supplements over the past 6 months? _____ If yes, what type and how often did you take them?_____

Are you presently using amino acid supplements?_____

Has your weight fluctuated more than a few pounds over the past year?_____ Did you attempt to bring about this weight change through diet and/or exercise?_____

Please specify:_____

Do you presently follow a specific diet regimen?_____

If yes, please specify:_____

Do you have any food allergies that you are aware of?_____

If yes, please specify:_____

MEDICAL HISTORY

Have you ever been told you have one of the following:

_____ High blood pressure	_____ High Cholesterol
_____ Diabetes	_____ hypoglycemia (low blood sugar)
_____ Kidney problems	_____ Thyroid problems
_____ Anemia	_____ Heart disease
_____ Ear infections	_____ Allergies
_____ Arteriosclerosis	_____ Heart murmur
_____ Rheumatic Fever	_____ aneurysm

If yes to any of the above, please elaborate: _____

_____

Have you given blood in the past? _____

Do you have problems giving blood?_____ Please

explain:_____

Have you ever experienced dizziness, nausea or fainting when

working out? _____ Please explain:_____

_____

Are you presently taking any form of medication?

(prescription as well as nonprescription) _____

If yes, please specify type and what it is for:

_____

Have you ever broken a bone? _____ If yes, which

bone(s) and when?_____

Have you ever incurred another form of injury (strain,

sprain, displacement, fluid retention, etc.) to

shoulders, elbows, wrists, hips, knees, ankles, etc.?

_____ If yes, please specify site and date:

_____

Do you have any other condition that might limit you while

exercising?_____ Please explain:_____

_____

EXERCISE HISTORY/HABITS

Are you currently weightlifting? _____

How long have you been weightlifting? _____

How many days per week do you lift? _____

What type of rotation schedule do you presently adhere to?

_____

Where do you work out?_____

What type of equipment do you usually use? _____

_____

Do you engage in any other form of exercise? _____

Please specify: _____

In the space provided below, please specify your present schedule for a week. Please be as specific as possible. This information will help us to determine what volume you have been lifting so that we can match, as closely as possible, what you've been doing with what we need you to do during the study period.

Type of Exercise	No. Repetitions	No. Sets

MAINTENANCE WEEK

The PURPOSE of the Maintenance week is to:

1. Control your diet so that:

   a. you don't lose or gain body weight

   b. everyone eats about the same amount of carbohydrate, protein, and fat

2. Control the exercise you do so that:

   a. everyone is doing the same amount of exercise

We will prescribe a DIET and an EXERCISE program for you to follow during this week.

MAINTENANCE DIET

The diet during this week is an "exchange system" diet. It will probably not be much different from your normal diet. "Exchange system" means that all foods are divided into 6 categories. Rather than counting calories, you count the number of exchanges to match them to your prescription. This will insure the appropriate amount of fat, carbohydrate, and protein. Your prescription is based on your body weight. However, if it is not enough food or too much food I will alter it for you. AGAIN, we would like it to be enough food for you to maintain your body weight during this week. Also, let me know if you have any other questions or problems with it. During this week (and through the rest of the experiment) do not consume any supplements unless we give them to you. You can consume as much water and other noncaloric beverages (e.g., diet coke, etc.) as desired.

Everyone will start the Maintenance Diet on Monday July 9. You should continue on this same diet through (including) Sunday July 15. Keep track of the number of exchanges you consume throughout the day on the notecards I give you so that we can check to see how close you come to your prescription. I will collect these at the end of the week.

YOUR DIET PRESCRIPTION IS:

_____	meat	_____	milk
_____	bread	_____	fruit
_____	vegetable	_____	fat

YOUR EXERCISE PRESCRIPTION IS:

Everyone will be lifting weights 6 days per week throughout the rest of the experiment. Please do not do ANY OTHER EXERCISE during the rest of the experiment since this could affect our results. An appointment will be made with you so as to determine your 1RM for the 21 exercises that will be utilized in your exercise routine. From this 1RM your exercise prescription will be calculated. Exercises will be prescribed at 70 - 85% of 1RM depending upon which exercise is involved. Repetitions are set at 8 - 10, and sets are prescribed as 3 - 5 sets, also depending upon which exercise is involved. You will receive a written prescription detailing how much weight, how many repetitions and how many sets are required for each exercise. Exercises are split into 3 day rotations: back/biceps/abdominals, chest/triceps/abdominals, legs/shoulders/abdominals. This three day rotation will then be repeated. A day of rest is then allowed before the rotation is begun again (low calorie phase).

Everyone will start the Maintenance Exercise program on Monday July 9.

The following is an example of the maintenance diet:

Food Group	Servings	Calories/Serving	Kcal/meal
Breakfast			
Fruit	2	40	80
Bread	4	68	272
Meat	3	73	219
Milk	1	170	170
Fat	2	45	90
Coffee/Tea	As much as desired		
Lunch			
Fruit	2	40	80
Bread	4	68	272
Meat	3	73	219
Milk	1	170	170
Fat	2	45	90
Coffee/Tea	As much as desired		
Raw veg.	As much as desired		
Vegetables	1	36	36
Dinner			
Fruit	2	40	80
Bread	5	68	340
Meat	3	73	219
Milk	1	170	170
Fat	2	45	90
Coffee/Tea	As much as desired		
Raw veg.	As much as desired		
Vegetables	1	36	36

LOW CALORIE DIET

WHY? The purpose of the diet is to:

1. Control calorie intake to decrease your body weight.

The diet will supply from about 1440 kcal/d to 2000 kcal/d depending on your body weight. Thus, it is not a starvation diet. You can expect to lose about 3 pounds of body fat. Your total weight loss (maybe up to 10 pounds) will be greater because of body water loss. You are likely to quickly regain most of the weight you lose on the diet once the experiment is over.

2. Control nutrient intake.

The amount of protein, fat, and carbohydrate are controlled in this diet. Everyone will be consuming about 18% protein, 52% carbohydrate, and 30% fat. It is VERY IMPORTANT that you consume all the diet you are given and that you DON'T CONSUME ANY OTHER CALORIES.

OK List

Water
diet soft drinks
coffee (no cream, artificial sweeteners only)
tea (artificial sweeteners only)
bouillon (1 cup per day)
sugarless gum (up to 3 sticks per day)

If you think there is something else that could be okay to eat during this week. . . check with me first!

The diet will be providing you with all the recommended amounts of vitamins and minerals. So, don't take any types of supplements except for the ones we give you. PLEASE DRINK LOTS OF WATER. . . AT LEAST 6 FULL GLASSES PER DAY AND MORE IF YOU LIKE. Dieting can be dehydrating and you need to drink lots of water. Consuming 1 cup of BOUILLON per day is a good idea to give you added salt. This will probably make you feel better since it helps to prevent dehydration (dehydration will make you feel tired and dizzy).

Expect to feel some fatigue on the diet but LET US KNOW IF YOU EXPERIENCE ANY UNUSUAL SYMPTOMS. You also may experience a change in your bowel habits due to the diet change, this is normal.

WHEN?

The controlled diet begins when you get up on Monday July 16 and continues through the end of the day on Wednesday July 25.

WHAT is the diet?

Your daily diet consists of:

The diet is better cold so put it in the refrigerator. You could also heat it in the microwave before consuming it if you want. We have three flavors of the EXCEED: strawberry, vanilla, chocolate. We will try to give you your choice.

WHEN do I take the supplement?

You should take one full dose of your supplement when you first get up in the MORNING before you eat anything and another dose in the EVENING before you go to bed.

The supplement dose is based on your body weight.

YOUR DOSE IS:

Content of Hypocaloric Diets

65 kg Weight Class:

	Exceed 4 cans	Milk	Carbohydrate	Total
kcal	1440			1440
Protein	60			60
Carbohydrate	188			188
Fat	48			48

70 kg Weight Class:

	Exceed 4 cans	Milk 34.0 g	Carbohydrate	Total
kcal	1440	120		1560
Protein	60	12		72
Carbohydrate	188	18		206
Fat	48			48

75 Kg Weight Class

	Exceed 4 cans	Milk 42.6 g	Carbohydrate 15.4 g	Total
kcal	1440	150	60	1650
Protein	60	15		75
Carbohydrate	188	22.5	14.2	224.7
Fat	48			48

80 Kg Weight Class

	Exceed 5 cans	Milk	Carbohydrate	Total
kcal	1800			1800
Protein	75			75
Carbohydrate	235			235
Fat	60			60

85 kg Weight Class

	Exceed 5 cans	Milk 28.4 g	Carbohydrate	Total
kcal	1800	100		1900
Protein	75	10		85
Carbohydrate	235	15		250
Fat	60	-		60

90 Kg Weight Class

	Exceed 5 cans	Milk 42.6 g	Carbohydrate	Total
kcal	1800	150		1950
Protein	75	15		90
Carbohydrate	235	22.5		235
Fat	60			60

95 Kg Weight Class

	Exceed 5 cans	Milk 56.7 g	Carbohydrate 15.4 g	Total
kcal	1800	225	65	2090
Protein	75	20		95
Carbohydrate	235	30	15	280
Fat	60			60

Name: _____ Time: _____

Date: _____

INSTRUCTIONS FOR TESTING

Please report to the lab (Room 230, War Memorial Hall) at the above designated time and date. You will be involved in three different tests in the following order:

1) Cybex muscle functioning test,

2) skinfold measurement for the estimation of body fat, and

3) hydrostatic weighing for the estimation of body fat.

Do not exercise prior to testing. Save your workout until after.

Do not eat two hours prior to testing. Do not eat foods that are known to be gaseous 24 hours prior to testing.

Do bring a bathing suit and towel.

INSTRUCTIONS FOR CYBEX MEASUREMENTS

Report to lab (Gym 230) at assigned time and check in with technician. Be sure to wear clothing that will allow free movement of the knee and elbow.

If testing quad sit on lower extremity machine and if testing biceps sit on chair in front of preachers bench.

Technician will adjust machine to proper position. Straps will be tightened around body.

Two speeds will be used for testing of both quadracep and biceps. A torque test will be administered first for each limb tested. During the torque test, 60 deg/sec is the speed utilized and 5 maximum repetitions will be analyzed. Prior to the torque test a warm-up will be given. The warm-up consists of five submaximal contractions followed by three maximal contractions. An endurance test will be administered immediately following the torque test for each limb tested. During the endurance test, 120 deg/sec (biceps) and 180 deg/sec (quad) will be utilized and the subject will be required to perform 30 maximal efforts. Prior to the performance of the endurance test you will be requested to perform 5 submaximal contractions at the specified endurance test speed and 3 maximal contractions. You will be given one (1) minute rest between the endurance warm-up and the endurance test.

INSTRUCTIONS FOR HYDROSTATIC WEIGHING

After skinfold testing you will be instructed as to where hydrostatic weighing will occur within the lab.

Change into bathing suit and have technician record body weight.

The technician will explain the procedures prior to the underwater weighing.

Upon entering the tank you will be instructed to totally immerse yourself in the water, removing air bubbles from hair and suit.

You will be requested to wear a weight belt that will help hold you down into the chair.

With head out of the water begin exhaling and submerging until you are sitting in the chair with head totally submerged.

Continue exhaling until air is completely out of lungs.

When the technician can no longer see air bubbles escaping the computer will be keyed to take a reading. Two seconds later the technician will knock on the side of the tank indicating that the trial is finished.

The procedure will be repeated 8 times.

HYDROSTATIC WEIGHING WORKSHEET

Name: _____ Date: _____
Time: _____

I. Body Density:

Wt of subject in air (kg) _____(Ma)

Wt of subject in water (lb)

1. _____ 5. _____

2. _____ 6. _____

3. _____ 7. _____

4. _____ 8. _____

Three highest wts:
_____, _____, _____

Average: _____(lbs)/2.2 = Average (kg) _____(Mw)

Temp. of water _____ Density of water _____(Dw)

$$BD = \frac{Ma}{\frac{Ma - Mw}{Dw} - (RV + 100ml)}$$

BD = _____

II. $$\%BF = \frac{4.95}{BD} - 4.5 \times 100$$

%BF = _____

Name: _____ Date: _____
Time: _____

Residual Volume:

VO2 in bag before rebreathing procedure _____Liters

a = % N2 impurity in O2 rebreathing gas: _____% N2

b = % N2 in mixed air in bag at the point of equilibrium: _____N2

c = % N2 in alveolar air at beginning of test: ____80_____ % N2

d = % N2 in alveolar air during last maximal breath (assumed to be 0.2% N2 + value of b)

RV = VO2 X (b-a)/(c-d)

RV = _____liters

_____

Analyzer Data:

% O2: _____ % CO2: _____

% N2 = 1 - (%O2 + %CO2) = _____

Volume of air in bag at end of rebreathing: _____

INSTRUCTIONS FOR DAILY URINE COLLECTION

All urine voided during the experimental period (solution diet phase) is to be collected and turned in.

You will be given two to three collection bottles each day. Each bottle contains a small amount of hydrochloric acid.

Collection bottles are to be turned in to the lab (Room 230 War Memorial Hall) each morning.

Daily collection will begin with the second (2nd) voiding of the day and will end with, and include, the first (1st) voiding of the next day.

All urine should be voided directly into the collection bottle.

Fill one collection bottle before using another bottle.

Urine collection will begin with the second (2nd) voiding of the first (1st) day of the experiment (July 16th) and will end with, and include, the first (1st) voiding of the eleventh (11th) day (July 26th).

Name: _____ Date: _____

Weight: _____ Time: _____

Cybex Testing

Biceps:

Dominant Arm: _____ Handgrip: _____

Long Input Adaptor Ext.: _____ holes

Universal Adaptor Extension: _____horizontal

_____vertical

Dynamometer Height: _____ distance: _____

Quadraceps:

Dominant Leg: _____

Dynamometer Height: _____ distance: _____

Shin Pad height: _____ Seat cushions: _____

INSTRUCTION SHEET FOR 1RM DETERMINATION

1. The subject should warm up with one set of 5 - 10 reps with something light (40% - 60% of perceived maximum).

2. After approximately a minute of rest and some light stretching, the subject should perform one set of 3 - 5 reps with something a little heavier (60 - 80% of perceived maximum).

3. After another minute of rest and some additional stretching, the subject should perform 3 - 4 1 rep attempts spaced by 30 - 60 second rest with increments of increasingly heavier weights.

4. The last successfully executed lift weight is recorded as the individual's 1 RM in that lift.

DATA FOR 1RM LIFTS

Subject: _____ Date: _____

	1 RM	75% 1RM
SUPINE BENCH PRESS	1RM	_____
INCLINE BENCH PRESS	1RM	_____
FLAT BENCH FLIES	5RM	_____
DIPS	BW	_____
CABLE PUSHDOWNS	1RM	_____
TRICEP EXTENSIONS (CURL BAR)	1RM	_____
LAT PULLDOWNS	1RM	_____
SEATED ROWS	1RM	_____
BENTOVER DB ROWS	1RM	_____
PREACHER CURL	1RM	_____
DUMBELL CURLS	1RM	_____
E-Z CURLS	1RM	_____
HYPEREXTENSIONS	BW	_____
MILITARY PRESS	1RM	_____
SIDE LATERAL RAISE	1RM	_____
BENT OVER LATERAL RAISE	1RM	_____
SHOULDER SHRUG	5RM	_____
LEG EXTENSIONS	1RM	_____
LEG PRESS	1RM	_____
LEG CURLS	1RM	_____
CALF RAISES	5RM	_____

EXERCISE PROTOCOL

Day 1

Back/biceps	Weight	Reps	Sets
Lat Pulldowns	75%	8-10	5
Pull-ups	BW	8-10	1
Seated rows	75%	8-10	4
Bentover DB rows	75%	8-10	3
Preacher Curl	75%	8-10	3
Dumbell curls	75%	8-10	3
E-Z curls	75%	8-10	3
hyperextensions	BW	EXH	1
Crunches		25+	2
Knee up crunches		25+	2

Day 2

Chest/triceps	Weight	Reps	Sets
Supine Bench Press	75%	8-10	5
Incline Bench Press	75%	8-10	4
Decline Bench Press	75%	8-10	4
Flat Bench Flies	75%	8-10	3
Dips	BW	EXH	2
Cable Pushdowns	75%	8-10	3
Tricep Extensions (Curl Bar)	75%	8-10	3
Crunches		25+	2
Knee up crunches		25+	2

Day 3

Legs/shoulders	Weight	Reps	Sets
Leg extensions	85%	8-10	4
Leg press	85%	8-10	4
Leg curls	75%	8-10	4
Calf raises	75%	8-10	4
Military press	75%	8-10	3
Side lateral raise	75%	8-10	3
Bent over lateral raise	75%	8-10	3
Shoulder shrug	75%	8-10	3
Crunches		25+	2
Knee up crunches		25+	2

Days 4, 5, and 6 are a repeat of the Days 1, 2, & 3 cycle. Day 7 is a rest day. The above cycle is then repeated for 6 days and then another rest day. This cycling of the workout will continue until the end of the study. It is important that you follow this workout and document on the form provided what you are doing. Please do not do any additional exercises other than those prescribed above.

NAME: _____

EXERCISES	Date: _____ Body Weight: _____				
	load / reps				
	set #1	set #2	set #3	set #4	set #5
	/	/	/	/	/
	/	/	/	/	/
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Comments →					

EXERCISES	Date: _____ Body Weight: _____				
	load / reps				
	set #1	set #2	set #3	set #4	set #5
	/	/	/	/	/
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Appendix C

INFORMED CONSENT AND HUMAN SUBJECT FORMS

HUMAN PERFORMANCE LABORATORY

Division of Health, Physical Education and Recreation
Virginia Polytechnic Institute and State University

INFORMED CONSENT

I, _____, do hereby voluntarily agree and consent to participate in a testing program conducted by the personnel of the Human Performance Laboratory of the Division of Health, Physical Education and Recreation of Virginia Polytechnic Institute and State University.

Title of Study:
The effect of oral amino acid supplementation of growth hormone, body composition, and muscle function during energy restriction in male weight lifters.

The purposes of this experiment include:

1) To determine the immediate effect of oral arginine consumption on blood growth hormone concentration. 2) To examine the effect of daily oral arginine supplementation on growth hormone and body composition changes during 10 days of calorie restriction.

I voluntarily agree to participate in this testing program. It is my understanding that my participation will include:

During the first week you will be asked to maintain your body weight and participate in a prescribed weight lifting session 6 days per week. At the end of this week we will do three tests: 1) body composition (underwater weighing), 2) muscle function (30 maximal repetitions on a Cybex II dynamometer), 3) acute response to amino acid (arginine) supplement. The latter test will include insertion of a catheter, a slender flexible tube, into a forearm vein. This will remain in place for the next 1 1/2 to 2 hours so that 6 different blood samples can be sporadically removed. Shortly after the initial insertion of the catheter, you will consume a capsule containing an amino acid mixture. You will remain seated during this entire procedure.

The next ten days will require you to consume a low calorie (approximately 1200 - 1800 kcal, depending on your body weight) formula diet (a commercial product for athletes called Exceed and skim milk), continue the prescribed weight lifting routine (no other exercise activity), consume a daily amino acid supplement and collect all urine daily (for determination of body protein changes). The three above

tests will be repeated at the end of the 10 day low calorie phase.

I understand that participation in this experiment may produce certain discomforts and risks. These discomforts and risks include:

1) Slight discomfort associated with placement of the catheter.
2) Slight risks of infection or blood clot associated with indwelling catheter.
3) Temporary fatigue associated with the low calorie diet.

Certain personal benefits may be expected from participation in this experiment. These include:

1) Determination of maximum muscle strength and muscle endurance.
2) Analysis of body composition.
3) Weight reduction
4) Knowledge of your body's response to amino acid supplements.

Appropriate alternative procedures that might be advantageous to you include:

I understand that any data of a personal nature will be held confidential and will be used for research purposes only. I also understand that these data may only be used when not identifiable with me.

I understand that I may abstain from participation in any part of the experiment or withdraw from the experiment should I feel the activities might be injurious to my health. The experimenter may also terminate my participation should he feel the activities might be injurious to my health.

I understand that it is my personal responsibility to advise the researchers of any preexisting medical problem that may affect my participation or of any medical problems that might arise in the course of this experiment an that no medical treatment or compensation is available if injury is suffered as a result of this research. A telephone is available which would be used to call the local hospital for emergency service.

I have read the above statements and have had the opportunity to ask questions. I understand that the researchers will, at any time, answer my inquiries concerning the procedures used in this experiment.

Scientific inquiry is indispensable to the advancement of knowledge. Your participation in this experiment provides the investigator the opportunity to conduct meaningful scientific observations designed to make significant educational contribution.

If you would like to receive the results of this investigation, please indicate this choice by marking in the appropriate space provided below. A copy will then be distributed to you as soon as the results are made available by the investigator. Thank you for making this important contribution.

_____ I request a copy of the results of this study.

Date_____ Time_____a.m./p.m.

Participant Signature_____

Witness_____
HPL Personnel

Project Director Janet L. Walberg, Ph.D. Telephone 231-6355

HPER Human Subjects Chairman Dr. Charles Baffi

Telephone 231-8284

Dr. Charles Waring, Chairman, Institutional Review Board for Research Involving Human Subjects. Phone 961-5283.

CERTIFICATE
OF
APPROVAL FOR RESEARCH
INVOLVING HUMAN SUBJECTS

Division of HPER

The Human Subjects Committee of the Division of Health, Physical Education and Recreation has reviewed the research proposal of

Janet L. Walberg, Ph.D.

entitled The effect of oral arginine supplementation on growth hormone, body composition, and muscle function during energy restriction in male weight lifters

The members have judged the subjects participating in the related experiment (not to be at risk) as a result of their participation.

(If a risk proposal) Procedures have been adopted to control the risks at acceptably low levels. The potential scientific benefits justify the level of risk to be imposed.

Members of Divisional
Human Subjects Committee

Chairman	4/10/89 Date
Douglas R. Southard	4/7/89 Date
	Date
	Date

Submitted to

Charles Baffi

Chairman, Division Human Subjects Committee and/or
Chairman, Institutional Review Board

by

Janet L. Walberg, Ph.D.

Principal Investigator

TITLE: The effect of oral arginine supplementation on growth hormone, body composition, and muscle function during energy restriction in male weight lifters.

BACKGROUND/SCIENTIFIC JUSTIFICATION: Many athletes today, particularly weight training athletes, are taking amino acid supplements. Claims are made by the manufacturers that these supplements stimulate the release of growth hormone which will subsequently reduce fat mass and increase muscle mass. These claims are based on tests of intraveneous amino acid administration (from research on burn and trauma patients) and thus may not apply to oral consumption. Since various diet and exercise factors influence growth hormone, a study testing these supplements must control both of these factors to isolate the effect of the amino acid.

PURPOSE(S): The purposes of this study are: 1) To determine the acute effect of oral arginine consumption on blood growth hormone concentration. 2) To examine the effect of daily oral arginine supplementation on growth hormone and body composition changes during 10 days of energy restriction.

EXPERIMENTAL METHODS & PROCEDURES: Sixteen male weight lifters will participate in a "maintenance week" when they will be asked to maintain their body weight and begin a weight training program prescribed by the experimentors. At the end of this week the subjects will undergo three tests: body composition (hydrostatic weighing), muscle performance and acute response to arginine supplement. The latter test will include insertion of a catheter (kept patent with heparin), removal of two 5 ml samples of blood prior to consumption of the supplement, consumption of 1 gm of arginine or placebo (casein), and 4 more 5 ml samples of blood over the subsequent hour. The muscle performance test will consist of 30 maximal contractions on a Cybex II dynamometer. (Continued on attached page)

STATEMENT DESCRIBING LEVEL OF RISK TO SUBJECTS: Risks associated with this study include: 1) slight discomfort associated with placement of the catheter, 2) slight risk of infection and blood clot formation associated with the presence of an indwelling catheter, 3) fatigue associated with a low calorie diet. Note that the arginine supplement at this dose is not considered a risk. Arginine is considered one of the least toxic of the amino acids. Others have fed arginine in 30 g daily quantities without evidence of negative side effects.

PROCEDURES TO MINIMIZE SUBJECT RISK (IF APPLICABLE): An experienced phlebotomist will do the catheter insertion and conduct the entire blood drawing procedure. The phlebotomist will use gloves when handling blood samples. The heparin solution used in the catheter will decrease risk of clot formation. The subjects will be seen daily during the low calorie phase (when they bring urine collection) and will be questioned concerning any discomfort or unusual symptoms associated with the low calorie diet.

RISK/BENEFIT RATIO (IF RISK PROJECT): These athletes will have the benefit of having their body composition, and muscle strength analyzed as well as determining their body's metabolic reaction to arginine supplementation. The risk of blood collection is minimized since an experienced phlebotomist will administer the test.

EXPERIMENTAL METHODS & PROCEDURES (cont.):

The subjects will at this point be assigned to one of two groups: arginine or placebo. Both groups will consume the same controlled hypoenergy formula diet (3 cans Exceed, Ross Laboratories, and 3 cups skim milk) for the next 10 days (19kcal/kg/d, 1.0 g protein/kd/d). All will be given a supplement containing 100% of the RDA for vitamins. Prescribed weight lifting sessions will continue for all athletes as during the first week. Each subject will consume either the 1 gram placebo or arginine supplement daily. All subjects will also collect their urine to be turned into the experimentors daily (for measurement of body protein loss). The three previously listed tests will be administered for a second time at the end of the hypoenergy phase.

Appendix D

RAW DATA AND STATISTICAL ANALYSES

Summary ANOVA for Body Weight (Kg) During EXPER

Source	SS	df	MS	F
Group	23.52	1	23.52	0.44
Error 1	538.20	10	53.82	
Day	24.04	10	24.04	32.29*
Interaction (Day x Group)	0.0004	1	0.0004	0.00
Error 2	7.45	10	0.75	

*F .05, 10, 10 = 2.98

Summary ANOVA for Percent Body Fat During EXPER

Source	SS	df	MS	F
Group	23.52	1	23.52	0.44
Error 1	538.20	10	53.82	
Day	24.04	1	24.04	32.29*
Interaction (Day x Group)	4.16^{-04}	1	4.16^{-04}	0.00
Error 2	7.45	10	0.75	

*F .05, 1, 10 = 4.96

Summary ANOVA for Fat Free Mass During EXPER

Source	SS	df	MS	F
Group	1.41	1	1.41	0.01
Error 1	1082.45	10	108.25	
Day	0.24	1	0.24	0.27
Interaction (Day x Group)	0.17	1	0.17	0.19
Error 2	8.90	10	0.89	

Summary ANOVA for Body Weight Change
For Three Groups*

Source	SS	df	MS	F
Group	705.22	2	352.61	1.47
Error 1	3587.39	15	239.16	
Day	47.95	2	23.97	50.25**
Interaction (Day x Group)	25.76	4	6.44	13.50***
Error 2	14.31	30	0.48	

*This analysis utilized body weight at MAINT Day -2, MAINT Day 6 and EXPER Day 9.

**F .05, 2, 30 = 3.32
***F .05, 4, 30 = 2.69

Cumulative Nitrogen Balance During EXPER
Two-Sample T-Test Results

	Group	Mean	SEM	T	Prob
Mg/Kg/10 days:	ARG	- 4.79	93.38	-2.03	0.07
	PLA	327.18	134.4	-2.03	0.07
G/10 days:	ARG	- 0.18	7.63	-1.94	0.08
	PLA	25.32	10.72	-1.94	0.08

Summary ANOVA for Nitrogen Balance During EXPER
(mg/kg body weight/d)

Source	SS	df	MS	F
Group	32971.3	1	32971.3	4.1
Error 1	80419.54	10	8041.95	
Day	17422.89	9	1935.88	1.87
Interaction (Day x Group)	12803.65	9	1422.63	1.37
Error 2	93262.41	90	1036.25	

Summary ANOVA for Urinary Creatinine During EXPER
(mg/kg body weight/d)

Source	SS	df	MS	F
Group	24.75	1	24.75	0.20
Error 1	1265.72	10	126.57	
Day	196.21	9	21.80	1.04
Interacation (Day x Group)	341.03	9	37.89	1.81
Error 2	1888.66	90	20.99	

Summary ANOVA for Total Urinary Nitrogen/Urinary Creatinine Ratio During EXPER (mg/kg body weight/d)

Source	SS	df	MS	F
Group	46.63	1	46.63	10.29*
Error 1	45.30	10	4.53	
Day	24.52	9	2.72	1.78
Interaction (Day x Group)	29.66	9	3.29	2.15**
Error 2	137.94	90	1.53	

*F .05, 1, 10 = 4.96
**F .05, 9, 90 = 2.06

Summary of Simple Main Effects for Urinary Nitrogen/Urinary Creatinine Ratio (mg/kg/d)

Source	SS	df	MS	F
Day				
Day at Group 1	29.55	9	3.28	2.14*
Day at Group 2	24.61	9	2.73	1.79
Group				
Group at Day 1	0.04	1	0.04	0.03
Group at Day 2	0.50	1	0.50	0.33
Group at Day 3	1.87	1	1.87	1.22
Group at Day 4	23.46	1	23.46	15.33**
Group at Day 5	7.32	1	7.32	4.78**
Group at Day 6	9.79	1	9.79	6.40**
Group at Day 7	21.28	1	21.79	13.91**
Group at Day 8	3.70	1	3.70	2.42
Group at Day 9	7.79	1	7.79	5.09**
Group at Day 10	0.54	1	0.54	0.35
Error	137.94	90	1.53	

*F .05, 9, 90 = 2.0
**F .05, 1, 90 = 3.96

Summary ANOVA Tables for Peak Torque (ft.lbs.)

Elbow Flexion (60°)

Source	SS	df	MS	F
Group	266.27	2	133.136	1.20
Error 1	1659.16	15	110.61	
Day	106.04	2	53.02	3.41*
Interaction (Day x Group)	133.96	4	33.49	2.16
Error 2	465.99	30	15.53	

*F 0.05, 2, 30 = 3.32

Knee Extension (60°)

Source	SS	df	MS	F
Group	92.03	2	46.02	0.04
Error 1	16291.31	15	1086.09	
Day	1901.44	2	950.72	7.36*
Interaction (Day x Group)	838.56	4	209.64	1.62
Error 2	3875.99	30	129.2	

*F .05, 2, 30 = 3.32

Summary ANOVA Tables For Muscle Endurance (%)

Elbow Flexion (120°)

Source	SS	df	MS	F
Group	13.00	2	6.50	0.02
Error 1	4060.33	15	270.69	
Day	351.00	2	175.50	4.13*
Interaction (Day x Group)	97.67	4	24.42	0.57
Error 2	1274.00	30	42.47	

*F .05, 2, 30 = 3.32

Knee Extension (180°)

Source	SS	df	MS	F
Group	41.37	2	20.69	0.16
Error 1	1930.06	15	128.67	
Day	70.26	2	35.13	1.38
Interaction (Day x Group)	136.63	4	34.16	1.34
Error 2	765.11	30	25.50	

Summary ANOVA for Exercise Volumes
4 Three Day Cycles Utilized For Comparison

Source	SS	df	MS	F
Group	1.66E09	2	8.28E08	0.76
Error 1	1.53E10	14	1.09E09	
Day	6.18E07	3	2.06E07	1.85
Interaction (Day x Group)	6.86E07	6	1.14E07	1.03
Error 2	4.67E08	42	1.11E07	

Wilcoxon Signed Ranks Test For Three Groups
Initial Body Weight (Kg)

Test Direction	z-value	Prob>|z|
ARG<PLA	-0.52	0.60
ARG<CON	1.15	0.25
PLA<CON	1.15	0.25

Wilcoxon Signed Ranks Test For Three Groups
Initial Elbow Flexion Peak Torque (60°)

Test Direction	z-value	Prob>|z|
ARG<PLA	-0.94	0.35
PLA<CON	-1.21	0.23
ARG<CON	-1.57	0.12

Wilcoxon Signed Ranks Test For Three Groups
Initial Elbow Flexion Endurance (120°)

Test Direction	z-value	Prob>|z|
ARG<PLA	0.11	0.92
PLA<CON	-0.11	0.92
ARG<PLA	0.11	0.92

Wilcoxon Signed Ranks Test For Three Groups
Initial Knee Extension Peak Torque (60°)

Test Direction	z-value	Prob>|z|
ARG<PLA	-0.94	0.35
PLA<CON	-0.11	0.92
ARG<CON	-0.73	0.46

Wilcoxon Signed Ranks Test For Three Groups
Initial Knee Extension Endurance (180°)

Test Direction	z-value	Prob>|z|
ARG<PLA	-0.55	0.58
PLA<CON	0.31	0.75
ARG<CON	0.73	0.46

RAW DATA FOR
PERCENT BODY FAT

SUBJ	GROUP	PRETEST	POST TEST
1	ARG	14.8	11.7
2	ARG	8.9	8.5
3	ARG	18.3	18.3
4	ARG	21.6	18.8
5	ARG	4.7	2.5
6	ARG	7.0	3.4
7	PLA	9.7	7.9
8	PLA	9.4	8.2
9	PLA	11.7	9.6
10	PLA	11.8	8.8
11	PLA	13.9	12.9
12	PLA	7.1	3.8

RAW DATA FOR
LEAN BODY MASS

SUBJ	GROUP	PRETEST	POST TEST
1	ARG	63.5	63.4
2	ARG	70.3	67.1
3	ARG	64.3	62.4
4	ARG	69.4	69.8
5	ARG	75.8	76.9
6	ARG	75.2	76.6
7	PLA	78.6	78.1
8	PLA	71.6	71.0
9	PLA	56.3	56.5
10	PLA	66.4	67.0
11	PLA	80.4	80.0
12	PLA	67.1	67.5

ARG = ARGININE
PLA = PLACEBO

RAW DATA FOR BODY WEIGHTS
DURING MAINTENANCE AND END OF EXPERIMENTAL

SUBJECT	GROUP	PRE MAINT	END MAINT	END EXPER
1	ARG	74.5	73.6	72.0
2	ARG	76.8	77.0	72.4
3	ARG	79.4	79.8	75.7
4	ARG	89.7	90.0	85.8
5	ARG	80.2	80.0	78.2
6	ARG	80.7	81.8	78.7
7	PLA	86.3	87.2	84.5
8	PLA	79.6	79.8	76.6
9	PLA	64.6	66.5	62.1
10	PLA	75.3	75.4	74.2
11	PLA	93.2	94.5	91.1
12	PLA	73.0	73.4	69.0
13	CON	78.2	79.0	78.2
14	CON	107.7	106.4	106.4
15	CON	80.9	80.9	81.8
16	CON	81.8	81.8	80.5
17	CON	84.1	83.6	84.5
18	CON	85.5	85.5	86.4

ARG = Arginine
PLA = Placebo
CON = Control

RAW DATA FOR
BODY WEIGHT DURING EXPER

GROUP	1	2	3	4	5	6	7	8	9	10	11
ARG	73.6	73.5	73.6	73.5	72.8	72.5	72.5	72.5	71.8	72.7	72.0
ARG	77.0	75.3	72.1	74.4	74.0	73.4	73.4	73.9	73.3	72.5	72.4
ARG	79.8	78.5	78.1	78.1	77.7	77.8	77.5	77.1	76.4	76.4	75.7
ARG	90.0	88.5	87.0	86.5	86.3	85.8	86.1	86.2	85.9	85.9	85.8
ARG	80.0	80.0	80.0	79.3	79.7	79.7	78.8	78.6	78.8	78.7	78.2
ARG	81.8	80.9	80.2	80.0	79.7	79.7	80.0	79.4	79.3	79.2	78.7
PLA	87.2	86.4	85.6	85.7	85.0	84.8	84.9	85.4	84.8	84.6	84.5
PLA	79.8	79.0	78.9	78.9	78.3	78.0	78.1	78.0	77.3	77.0	76.6
PLA	66.5	64.7	63.5	62.8	63.5	62.0	62.5	62.7	62.5	62.4	62.1
PLA	75.4	74.7	74.3	74.5	73.2	73.9	74.3	75.0	73.5	73.6	74.2
PLA	94.5	92.4	92.1	92.5	92.1	92.6	91.6	91.3	91.8	91.0	91.1
PLA	73.0	72.1	72.1	72.0	71.8	71.0	70.6	71.1	70.2	70.7	69.0

ARG = ARGININE
PLA = PLACEBO

RAW DATA FOR
DAILY URINE VOLUME

SUBJ	GROUP	1	2	3	4	5	6	7	8	9	10
1	ARG	550	1839	2260	1525	1680	1584	1061	840	1250	1252
2	ARG	1030	1075	1055	1080	897	1027	740	1080	773	775
3	ARG	1977	1060	1050	1245	935	812	1725	900	1039	1030
4	ARG	1439	957	680	1445	1079	1125	1254	825	992	1075
5	ARG	5840	4465	4540	5025	5175	5583	3590	5025	2695	5000
6	ARG	1279	984	1710	1832	1067	1653	727	1420	1140	1784
7	PLA	2285	1808	2284	2100	2051	2010	2052	2100	1020	2295
8	PLA	4615	3340	2055	1695	1305	2374	1685	2244	2228	2230
9	PLA	260	1145	1033	400	840	995	825	904	749	1525
10	PLA	1245	1095	1749	2616	2017	2870	920	870	653	2170
11	PLA	1882	2240	2063	1680	1899	1715	1110	1850	1178	1975
12	PLA	3775	4855	4945	1570	1936	2165	1880	2086	884	10720

RAW DATA FOR
NITROGEN BALANCE (G/DAY)

SUBJ	GROUP	1	2	3	4	5	6	7	8	9	10
1	ARG	3.71	-1.44	-1.48	0.39	0.39	0.41	1.7	-0.12	1.69	-0.05
2	ARG	4	3.92	0.01	-2.64	-3.53	-2.72	2.37	3.84	0.95	-1.48
3	ARG	0.86	2.03	3.24	-2.11	-2.44	2.23	-0.18	-0.04	-0.21	-1.5
4	ARG	1.56	4.93	4.96	1.95	0.81	1.45	0.47	4.89	0.69	3.24
5	ARG	-1.39	-1.34	0.42	-2.65	-0.12	0.93	-0.07	-2.54	0.76	0.44
6	ARG	-4.41	-2.43	-5.63	-6.08	-0.49	-3.74	3.27	-7.05	-1.03	-4.69
7	PLA	5.29	-0.88	3.16	-0.23	0.18	2.55	0.85	3.05	4.22	2.3
8	PLA	-3.77	-0.68	-0.3	-1.95	-1.65	3.37	2.29	2.2	2.41	5.14
9	PLA	4.34	5.13	5.64	3.43	4.04	3.59	1.57	6.14	5.4	6.47
10	PLA	-0.03	-1.78	-1.24	-4.6	1.19	-4.25	3.04	-0.04	3.19	-0.12
11	PLA	9.05	4.57	1.97	6.95	3.53	10.54	7.81	5.54	6.02	10.59
12	PLA	-0.78	0.95	-9.15	3.77	0.11	3.732	5.12	6.24	5.97	0.72

ARG = ARGININE
PLA = PLACEBO

RAW DATA FOR
URINARY CREATININE (G/DAY)

SUBJ	GROUP	1	2	3	4	5	6	7	8	9	10
1	ARG	1.497	1.567	1.319	1.378	1.711	1.627	1.489	1.891	1.72	1.857
2	ARG	1.153	1.129	1.676	1.647	1.881	1.974	1.236	1.12	1.972	2.158
3	ARG	1.457	1.412	1.249	1.466	1.434	1.095	1.506	1.592	1.671	1.68
4	ARG	1.877	2.132	1.419	1.324	1.858	1.773	1.056	1.159	1.717	1.927
5	ARG	1.952	2.067	2.069	2.128	2.007	1.893	2.225	2.344	2.113	2.224
6	ARG	2.138	1.878	1.985	2.196	1.606	1.927	1.035	2.299	1.89	2.195
7	PLA	1.29	2.141	1.416	2.173	2.424	2.044	2.207	1.805	1.839	1.724
8	PLA	1.876	1.799	2.007	1.882	1.922	1.277	1.436	1.476	2.032	1.25
9	PLA	0.175	0.687	0.65	2.119	0.871	1.093	1.275	0.462	0.764	0.738
10	PLA	1.792	1.595	1.949	2.476	2.152	2.401	1.844	2.247	1.857	1.714
11	PLA	1.408	2.137	2.372	1.544	1.876	1.037	1.22	1.663	2.325	0.943
12	PLA	1.47	1.177	2.774	1.257	1.782	1.785	1.39	1.193	1.208	1.749

ARG = ARGININE
PLA = PLACEBO

RAW DATA FOR
URINARY NITROGEN/URINARY CREATININE (G/DAY)

SUBJ	GROUP	1	2	3	4	5	6	7	8	9	10
1	ARG	6.27	9.28	11.05	9.22	7.43	7.81	7.66	6.996	6.64	7.09
2	ARG	7.94	8.27	7.86	9.6	8.88	8.05	8.74	8.33	6.2	6.79
3	ARG	8.48	7.93	7.99	10.46	10.92	10.04	8.9	8.34	8.31	8.77
4	ARG	7.54	5.07	7.6	10.42	7.93	8.07	14.47	9.37	8.77	6.49
5	ARG	7.49	7.05	6.19	7.47	6.65	6.5	5.98	6.73	5.91	5.76
6	ARG	8.25	8.34	9.51	8.8	8.56	8.82	9.64	8.83	7.56	8.17
7	PLA	7.43	7.36	8.28	6.96	6.07	6.04	6.36	6.56	5.81	7.31
8	PLA	9.04	7.73	6.74	8.06	7.74	7.72	7.62	7.47	5.33	6.48
9	PLA	7.66	7.95	7.63	3.39	7.53	6.42	7.09	9.68	6.82	5.61
10	PLA	7.33	9.34	7.37	7.16	5.55	7.23	5.47	5.85	5.35	7.72
11	PLA	5.32	5.61	6.15	6.23	6.95	5.81	7.18	6.63	4.54	6.34
12	PLA	9.42	10.29	8.01	7.39	7.27	5.23	5.73	5.73	5.89	7.06

ARG = ARGININE
PLA = PLACEBO

Raw Data for Total Urinary Nitrogen
(mg/kg body weight/day

Subject	Group	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10
1	ARG	127.76	197.55	198.37	174.59	175.45	175.17	157.38	184.26	157.08	182.78
2	ARG	121.51	129.54	177.15	213.65	227.52	216.49	147.14	127.29	168.69	202.49
3	ARG	157.32	143.28	127.78	197.30	201.29	141.81	173.80	173.69	175.92	194.58
4	ARG	160.00	124.14	124.62	159.79	174.13	166.09	177.26	126.43	175.32	145.80
5	ARG	182.75	182.13	161.54	199.37	167.50	156.09	169.34	200.25	158.58	163.68
6	ARG	218.81	195.39	235.88	242.53	172.40	212.38	125.69	255.99	180.30	227.95
7	PLA	111.00	184.11	136.87	177.88	173.47	145.47	164.40	139.62	126.24	149.11
8	PLA	214.94	176.17	171.36	193.74	190.64	126.25	140.64	142.69	140.52	105.74
9	PLA	96.29	85.98	78.98	113.07	105.81	112.32	144.18	71.52	83.49	66.67
10	PLA	175.90	200.54	192.75	242.08	161.57	233.78	134.40	178.91	135.05	178.44
11	PLA	81.06	130.18	157.73	104.34	140.70	65.72	95.95	120.15	115.93	65.64
12	PLA	191.96	167.96	308.47	129.39	182.54	132.29	111.96	97.29	100.57	178.99

Raw Data for Nitrogen Balance
(mg/kg body weight/day)

Subject	Group	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10
1	ARG	50.48	-19.57	-20.14	5.36	5.38	5.66	23.45	-1.67	23.25	- 0.69
2	ARG	53.12	54.37	0.13	-35.68	-48.09	-37.06	32.07	52.39	13.10	-20.44
3	ARG	10.96	25.99	41.49	-27.16	-31.36	28.77	- 2.34	-0.52	- 2.75	-19.82
4	ARG	17.63	56.67	57.34	22.60	9.44	16.84	5.45	56.93	8.03	37.76
5	ARG	-17.38	-16.75	5.30	-33.25	- 1.51	11.8	- 0.89	-32.23	9.66	5.63
6	ARG	-54.50	-30.30	-70.38	-76.29	- 6.15	-46.75	41.18	-88.90	-13.00	-59.59
7	PLA	61.23	-10.28	36.87	- 2.71	2.12	30.04	9.95	35.97	49.88	27.22
8	PLA	-47.72	- 8.62	- 3.80	-24.90	-21.15	43.15	29.36	28.46	31.30	67.10
9	PLA	67.08	80.79	89.81	54.02	65.16	57.44	25.04	98.24	86.54	104.19
10	PLA	0.40	-23.96	-16.64	-62.84	16.10	-57.20	40.53	- 0.54	43.34	- 1.62
11	PLA	97.94	49.62	21.30	75.46	38.12	115.07	85.54	60.35	66.15	116.25
12	PLA	-10.82	13.18	-127.08	52.51	1.55	52.83	72.01	88.89	84.44	10.43

Raw Data for Creatinine
(mg/kg body weight/day

Subject	Group	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10
1	ARG	20.37	21.29	17.95	18.93	23.60	22.44	20.54	26.34	23.66	25.79
2	ARG	15.32	15.66	22.53	22.26	25.63	26.89	16.73	15.28	27.20	29.81
3	ARG	18.56	18.08	15.99	18.87	18.43	14.13	19.53	20.84	21.16	22.19
4	ARG	21.21	24.51	16.41	15.34	21.66	20.59	12.25	13.49	19.99	22.46
5	ARG	24.40	25.84	26.09	26.70	25.18	24.02	28.31	29.75	26.85	28.44
6	ARG	26.43	23.42	24.81	27.55	20.15	24.09	13.04	28.99	23.86	27.89
7	PLA	14.93	25.01	16.52	25.56	28.59	24.08	25.84	21.29	21.74	20.40
8	PLA	23.75	22.80	25.44	24.04	24.64	16.35	18.41	19.09	26.39	16.32
9	PLA	14.87	10.82	10.35	33.37	14.05	17.49	20.33	7.39	12.24	11.88
10	PLA	23.03	21.47	26.16	33.83	29.13	32.32	24.59	30.57	25.23	23.1
11	PLA	15.24	23.20	25.64	16.76	20.26	11.32	13.36	18.12	25.55	10.35
12	PLA	20.39	16.33	38.53	17.51	25.10	25.28	19.55	16.99	17.09	25.35

Raw Data for Urinary Nitrogen/Urinary Creatinine Ratio
(mg/kg body weight/day)

Subject	Group	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10
1	ARG	6.27	9.19	11.05	9.22	7.43	7.81	7.66	7.00	6.64	7.09
2	ARG	7.94	8.27	7.86	9.60	8.88	8.05	8.80	8.33	6.20	6.79
3	ARG	8.48	7.92	6.73	10.46	10.92	10.04	8.90	8.33	8.31	8.77
4	ARG	7.54	5.06	7.59	10.42	8.04	8.07	14.47	9.37	8.77	6.49
5	ARG	7.49	7.05	6.19	7.47	6.65	6.50	5.98	6.73	5.91	5.76
6	ARG	8.28	8.34	9.51	8.80	8.56	8.82	9.64	8.83	7.56	8.17
7	PLA	7.43	7.36	8.29	6.96	6.07	6.04	6.36	6.56	5.81	7.31
8	PLA	9.05	7.73	6.74	8.06	7.74	7.72	7.64	7.48	5.32	6.48
9	PLA	6.48	7.95	7.63	3.39	7.53	6.42	7.09	9.68	6.82	5.61
10	PLA	7.64	9.34	7.37	7.16	5.55	7.23	5.47	5.85	5.35	7.73
11	PLA	5.32	5.61	6.15	6.23	6.95	5.81	7.18	6.63	4.54	6.34
12	PLA	9.41	10.29	8.01	7.39	7.27	5.23	5.73	5.73	5.88	7.06

Raw Data For Cumulative Nitrogen Balance During EXPER
(mg/kg/10 days)

SUBJECT	GROUP	TOTALS
1	ARG	71.51
2	ARG	63.91
3	ARG	23.26
4	ARG	288.69
5	ARG	- 69.62
6	ARG	-404.68
7	PLA	240.29
8	PLA	93.18
9	PLA	728.31
10	PLA	- 62.43
11	PLA	725.80
12	PLA	237.94

Raw Data For Cumulative Nitrogen Balance During EXPER
(g/10 days)

SUBJECT	GROUP	TOTALS
1	ARG	5.20
2	ARG	4.72
3	ARG	1.88
4	ARG	24.95
5	ARG	- 5.56
6	ARG	-32.28
7	PLA	20.49
8	PLA	7.06
9	PLA	45.75
10	PLA	- 4.64
11	PLA	66.57
12	PLA	16.68

RAW DATA FOR
MUSCLE FUNCTION: ELBOW FLEXION 60 DEGREES

SUBJECT	GROUP	PRE MAINT	END MAINT	END EXPER
1	ARG	42	40	32
2	ARG	44	54	36
3	ARG	39	40	38
4	ARG	52	44	39
5	ARG	52	53	49
6	ARG	66	48	52
7	PLA	54	47	46
8	PLA	48	46	39
9	PLA	33	35	36
10	PLA	52	53	50
11	PLA	36	42	36
12	PLA	46	52	52
13	CON	34	38	38
14	CON	46	42	44
15	CON	44	44	42
16	CON	44	44	47
17	CON	36	38	36
18	CON	33	42	36

ARG = ARGININE
PLA = PLACEBO
CON = CONTROL

RAW DATA FOR
MUSCLE FUNCTION: ELBOW FLEXION 120 DEGREES

SUBJECT	GROUP	PRE MAINT	END MAINT	END EXPER
1	ARG	39	43	45
2	ARG	45	51	48
3	ARG	44	46	48
4	ARG	35	45	69
5	ARG	28	23	31
6	ARG	50	50	49
7	PLA	27	33	12
8	PLA	42	35	55
9	PLA	57	63	59
10	PLA	46	49	42
11	PLA	44	40	50
12	PLA	41	47	53
13	CON	36	36	37
14	CON	40	40	51
15	CON	35	38	54
16	CON	48	45	49
17	CON	49	54	64
18	CON	35	30	33

ARG = ARGININE
PLA = PLACEBO
CON = CONTROL

RAW DATA FOR
MUSCLE FUNCTION: KNEE EXTENSION 60 DEGREES

SUBJECT	GROUP	PRE MAINT	END MAINT	END EXPER
1	ARG	176	167	157
2	ARG	205	194	173
3	ARG	162	160	155
4	ARG	164	161	140
5	ARG	194	185	188
6	ARG	210	188	168
7	PLA	175	205	171
8	PLA	176	185	171
9	PLA	143	133	131
10	PLA	159	165	175
11	PLA	227	201	162
12	PLA	196	180	156
13	CON	179	176	180
14	CON	153	174	148
15	CON	194	196	181
16	CON	167	167	176
17	CON	139	137	147
18	CON	193	195	188

ARG = ARGININE
PLA = PLACEBO
CON = CONTROL

RAW DATA FOR
MUSCLE FUNCTION: KNEE EXTENSION 180 DEGREES

SUBJECT	GROUP	PRE MAINT	END MAINT	END EXPER
1	ARG	49	47	42
2	ARG	43	51	43
3	ARG	54	38	40
4	ARG	56	60	53
5	ARG	50	46	45
6	ARG	47	39	54
7	PLA	60	56	65
8	PLA	43	45	43
9	PLA	57	48	57
10	PLA	45	52	54
11	PLA	37	36	38
12	PLA	47	51	60
13	CON	56	47	45
14	CON	51	55	58
15	CON	35	44	43
16	CON	64	52	51
17	CON	53	40	49
18	CON	45	37	41

ARG = ARGININE
PLA = PLACEBO
CON = CONTROL

Raw Data For Weight Lifting Volumes
(Pounds lifted * repetitions)

SUBJECT*	GROUP	CYCLE 1	CYCLE 2	CYCLE 3	CYCLE4
1	ARG	95500	91395	96060	96425
2	ARG	59375	61455	60290	61120
3	ARG	127120	127440	125365	129630
4	ARG	68315	76240	74905	76950
5	ARG	71865	70835	77532	74260
6	PLA	92030	91540	95490	98500
7	PLA	65165	65265	65323	64162
8	PLA	82640	86620	73480	71005
9	PLA	74235	73260	73580	82095
10	PLA	63225	64330	65670	62325
11	PLA	81690	81180	83970	77320
12	CON	61417	65480	68480	66905
13	CON	61235	63395	71065	62678
14	CON	69840	77065	71350	74670
15	CON	85610	92270	92600	93800
16	CON	74930	74485	76552	78045
17	CON	75365	75010	81915	72330

Cycles indicate three day totals (Day 1 Back/Biceps + Day 2 Chest/Triceps + Day 3 Legs/Shoulders).
*One subject data not available for analysis.

Subject Sample of Daily Weightlifting Volumes

Subject 9

7/9/90 BICEPS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1500	1500	1500	1500	1500	1500.00	7500
1600	1600	1600	1600		1600.00	6400
950	800	800			850.00	2550
735	950	760			815.00	2445
950	950	950			950.00	2850
420	420	420			420.00	1260
TOTAL					1022.50	23005

7/10/90 CHEST

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1640	1640	1640	1640	1640	1640.00	8200
1360	1360	1280	1240		1310.00	5240
1640	1230	1560	1480		1477.50	5910
400	400	400			400.00	1200
810	700	640			716.67	2150
800	800	760			786.67	2360
TOTAL					1055.14	25060

7/11/90 LEGS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1120	950	1120	1120	920	1046.00	5230
200	200	200			200.00	600
200	200	200			200.00	600
700	700	700			700.00	2100
2920	2920	2920			2920.00	8760
450	450	450	450		450.00	1800
840	960	1080			960.00	2880
1200	1400	1600			1400.00	4200
TOTAL					984.50	26170

CYCLE 1 TOTAL: 74235

7/12/90 BICEPS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1500	1500	1500	1500	1500	1500.00	7500
800	800	800			800.00	2400
1600	1600	1600	1600		1600.00	6400
950	850	850			883.33	2650
950	950	950			950.00	2850
350	350	350			350.00	1050
TOTAL					1013.89	22850

7/13/90 CHEST

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1640	1640	1640	1640	1640	1640.00	8200
1360	1360	1360	1360		1360.00	5440
1200	1520	1330	1140		1297.50	5190
400	400	400			400.00	1200
900	900	900			900.00	2700
720	720	720			720.00	2160
TOTAL					1052.92	24890

7/14/90 LEGS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
950	1120	1120	1120		1077.50	4310
200	200	200			200.00	600
200	200	200			200.00	600
700	700	700			700.00	2100
2960	2960	2960			2960.00	8880
450	450	450			450.00	1350
1600	1600	1600			1600.00	4800
840	960	1080			960.00	2880
TOTAL					1018.44	25520

Cycle 2 TOTAL: 73260

7/16/90 BICEPS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1500	1500	1500	1500	1500	1500.00	7500
1000	1750	1750	1750		1562.50	6250
800	800	800			800.00	2400
900	900	850			883.33	2650
350	350	350			350.00	1050
950	950	950			950.00	2850
TOTAL					1007.64	22700

7/17/90 CHEST

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1680	1680	1470	1670	1435	1587.00	7935
1360	1360	1360	1190		1317.50	5270
1560	1365	1365	1330		1405.00	5620
480	480	480			480.00	1440
810	810	810			810.00	2430
760	760	760			760.00	2280
TOTAL					1059.92	24975

7/18/90 LEGS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1120	1120	840			1026.67	3080
250	250	225			241.67	725
200	200	200			200.00	600
840	840	840			840.00	2520
3240	2920	2920			3026.67	9080
540	540	540			540.00	1620
1680	1920	2160			1920.00	5760
720	840	960			840.00	2520
TOTAL					1079.38	25905

CYCLE 3 TOTAL: 73580

7/19/90 BICEPS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1500	1500	1500	1500	1500	1500.00	7500
1750	1750	1750	1750		1750.00	7000
960	960	960			960.00	2880
900	900	900			900.00	2700
350	350	350			350.00	1050
1050	1050	840			980.00	2940
TOTAL					1073.33	24070

7/20/90 CHEST

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
1680	1680	1470	1435	1400	1533.00	7665
1360	1360	1190	1530		1360.00	5440
1600	1600	1400	1365		1491.25	5965
420	420	420			420.00	1260
900	900	900			900.00	2700
760	760	760			760.00	2280
TOTAL					1077.38	25310

7/21/90 LEGS

SET 1	SET 2	SET 3	SET 4	SET 5	AVG	TOTVOL
2160	2160	2160			2160.00	6480
840	960	1080			960.00	2880
4050	3645	6240			4645.00	13935
540	540	540			540.00	1620
1260	1260	1260			1260.00	3780
200	200	200			200.00	600
300	300	300			300.00	900
840	840	840			840.00	2520
TOTAL					1363.13	32715

CYCLE 4 TOTAL: 82095

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VITA

Colleen Elizabeth (Ray) Hawkins was born March 20, 1953 in Vallejo, California and was the middle child of 5 children. Her father was in the Submarine Service of the U. S. Navy and as a result her family moved every two years resulting in Colleen attending a total of 10 different schools before graduating from Stafford High School, Stafford, Virginia in 1971.

After graduating with a degree in Social Work from Virginia Commonwealth University, Colleen moved to Galax, Virginia where she worked as Director of Social Services for Twin County Community Hospital. It was also in Galax where she met and married her husband Jim Hawkins in 1977, a third generation Railroad Cross Tie buyer from Huntington, West Virginia. In December 1980 Jim and Colleen had their first child Elizabeth and twenty months later their second child Marshall was born.

After the birth of Marshall Colleen began teaching aerobics. In January 1987, she became a co-manager of Twin County Nautilus Fitness Center, Galax, Virginia. It was during this time that she began to seriously consider furthering her education in the area of physical fitness especially as it related to special populations. As a result, in the summer of 1988, Colleen and her family moved to Blacksburg so that Colleen could attend graduate school in Exercise Physiology at Virginia Tech. She expects to graduate in the summer of 1991.

Colleen E. Hawkins