# THE EFFECT OF EXERCISE TRAINING ON PROTEIN CATABOLISM IN YOUNG ADULT WOMEN

A THESIS

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# INTRODUCTION

The effect of exercise on total body protein requirements has been studied extensively but the results have been conflicting. Feeding large quantities of protein to athletes is not uncommon because of a belief that surfeit protein feeding is beneficial for building and replacing muscle tissue (Marable, Hickson, Korslund, Herbert, Desjardins and Thye, 1979). Some studies support the need for extra dietary protein with athletic training (Conzolazio, Johnson, Dramise and Skala, 1975) while other studies indicate that because there is no excessive loss of protein with exercise there is no need for excess dietary protein (Marable et al., 1979).

Gontzea, Sutzescu and Dumitrache (1974) strongly suggest that, as proteins constitute the most important part of cellular protoplasm and are also in active substances (enzymes, hormones, etc.), the increased metabolism during muscular activity may affect nitrogen equilibrium. These researchers state that the protein ration considered adequate for humans at rest will not suffice for those in activity (Gontzea et al., 1974). Haralambie and Berg (1976) found a significant correlation between an increase in serum urea and a decrease in amino nitrogen with exercise. These

changes, according to the researchers, strongly suggest an increased breakdown of nitrogen-containing compounds during prolonged exercise. Dohm, Kasperek, Tapscott and Beecher (1980) state that amino acid utilization is increased during exercise.

The studies referenced above and others will often measure serum and urinary creatinine and urea when studying protein metabolism with exercise. Urea and creatinine are studied because both are metabolic by-products of protein metabolism. Creatinine is the excretion form of creatine which is present in all muscle tissue (Bohmer, 1975). Urea is the chief nitrogenous constituent of urine and the final product of amino acid catabolism (Katanuma, Okada and Nishii, 1966).

Poortmans (1975) states that usually the urea level remains stable in short term exercise, but some studies have indicated an increase in urea in muscle after prolonged work. Decombaz, Reinhardt, Anantharaman, von Glutz and Poortmans (1979) found urea production increased while creatinine production tended to decrease after a 100 kilometer (km) run in trained male subjects. Lemon and Mullin (1980) found that serum urea increased and urinary urea decreased after one hour cycling on a bicycle ergometer. These researchers concluded protein is utilized during exercise to a greater extent than is generally

assumed. In a study by Cerny (1975), five normal untrained medical students exercised on a bicycle ergometer. Cerny (1975) found that serum urea increased by 20 percent ($p<0.025$) during exercise but showed no significant change until after one hour. Also, the kidney creatinine and urea elimination rates decreased by 40 percent and 50 percent respectively during exercise.

This current study proposed to examine urinary creatinine and urea excretion in a group of athletically untrained females undergoing a training program to determine if protein catabolism increased with exercise training. Results indicated that protein metabolism may change during an exercise training program.

## Definition of Terms

Training- For this study training was defined as an improvement in the efficiency of the cardiopulmonary system accomplished by a program of regular aerobic exercise.

Aerobic exercise- Exercise that requires oxygen but does not produce an excess oxygen debt so exercise can be maintained for long periods of time.

## STATEMENT OF PROBLEM

The problem being investigated is: Does exercise in untrained women promote increased body protein catabolism as indicated by urinary urea and creatinine?

# REVIEW OF LITERATURE

## History

In the past few years there has been a tremendous increase in the popularity of physical fitness (American College of Sports Medicine, 1978). As a result, interrelationships between nutrition and physical activity have become a topic of increasing interest because of the important role of both nutrition and activity in the fitness and health of an individual (American Dietetic Association, 1980). Nutrition for athletes is a topic which is currently being given increased attention by sport medicine doctors, coaches and trainers (Lane, 1974).

For several thousand years, there have existed dietary recommendations for athletes that were assumed to ensure the optimal level of performance (Williams, 1974). For instance, the tradition of consuming large quantities of protein probably began with the ancient Greek athletes who were reported to have eaten high meat diets in an effort to replace muscle tissue that was "spent" during exercise (Marable et al., 1979).

Protein is one nutrient that has been studied frequently in relation to its metabolism during muscular activity. Durnin (1975) stated that there has existed,

possibly for hundreds of years, a widespread belief that violent muscular exercise requires the eating of a large amount of meat. Liebig, in the nineteenth century, believed that muscular work caused the metabolism of protein (Conzolazio and Johnson, 1972). Playfair, in 1865, found hard-working laborers had high intakes of protein, so he set down protein requirements in the diet ranging from 57 grams (g) per day for a subsistence diet to 187g per day for heavy laborers (Durnin, 1975). Voit, also in the nineteenth century, examined the theory that protein metabolism was affected by muscular activity (Pike and Brown, 1975). From a series of calorimetric and balance studies, Voit disproved the theory proposed by Liebig by demonstrating that metabolism is not affected by muscular work.

Many investigators have studied protein metabolism and protein requirements during both high and long term physical loading (Laritcheva, Yalovaya, Shubin and Smirnov, 1978). Christensen and Crampton (1965) stated that during the past century numerous experiments have been conducted to determine the effect of exercise on dietary protein requirements. Contradictory data in this field has made it difficult to ascertain exactly whether the protein needs of the organism do change during higher physical activity (Laritcheva et al., 1978). Rasch and Pierson (1962) state that emphasis has been placed on the importance of protein

supplements to the diet of those persons seeking increased muscular strength and hypertrophy even though the actual value of this has been seriously questioned by nutritionists. Also, Bergstrom and Hultman (1972) found that the enormous gorging of beef, often supplemented with protein pills and anabolic steroids, is a well known phenomenon among weight lifters, shot putters and discus throwers. Marable et al.(1979) stated that it is still unclear whether or not the practice of surfeit protein feeding has any beneficial effects for persons engaged in heavy physical activity or physical conditioning programs.

## Protein

The name protein is derived from a Greek word meaning "first". This name is not inappropriate because proteins are of vital importance to the continuing function of a cell (Kendrew, 1961). Poortmans (1975) states that proteins are the most remarkable chemical substances within living organisms. Proteins provide many structural elements of a cell and they help bind cells together into tissues. Also, enzymes, which are proteins, catalyze most of the chemical reactions that occur in the cell. Some proteins act as contractile elements to make movement possible; other proteins control the activity of genes, transport needed material across membranes and carry certain substances from

one part of an animal to another. Proteins, in the form of antibodies, protect humans from disease. Some hormones are also proteins (Kendrew, 1961).

Proteins vary in size and shape as well as function. Protein molecules are polymers of amino acids joined in peptide linkages (Klotz and Darnell, 1969). There are twenty different amino acids which primarily differ from one another by their side chain, designated R in Figure 1 (Hess and Rupley, 1971):

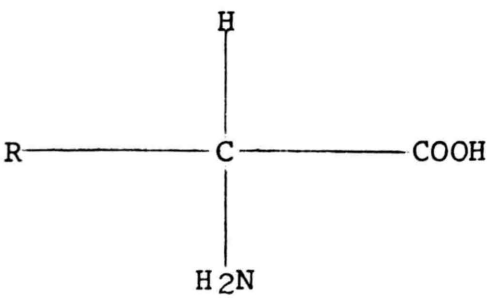


Fig. 1. General amino acid structure

Proteins contain carbon, hydrogen and oxygen, as do carbohydrates and fats, but proteins also contain nitrogen. Protein is almost the sole source of nitrogen in the diet. Most body protein is found in muscle tissue with the remainder in soft tissues, bones, teeth, blood and other body fluids (Kendrew, 1961).

Protein cannot be totally synthesized from endogenous amino acids in the body. There are amino acids that must be

supplied by the diet. Without an adequate supply of these essential amino acids, protein cannot be synthesized nor body tissue maintained (Harper and Kumta, 1959).

Body protein stores in normal man are approximately 10-11 kilograms (kg), of which one half to one third is in muscle, the body's principle nitrogen reservoir. In muscle, the bulk of protein is as actin and myosin, the two major proteins involved in the contractile process (Cahill, 1971). Collagen, another protein, is probably the most abundant of the mammalian proteins. In man it accounts for about one third of body proteins. Although the turnover of muscle protein is not rapid because of the great mass of this tissue, it can be assumed that muscle must play a significant role in total protein metabolism (Poortmans, 1975).

Poortmans (1975) states that proteins are the most abundant organic molecules within cells, contributing 50 percent or more of their dry cell weight. The potential modification of this large protein pool by exercise and training should be the subject of analysis, according to Poortmans.

Cahill (1971) states that protein is either integrated into the body machinery or, if the amino acid intake is in excess, it is metabolized, the nitrogen excreted and the caloric equivalents consumed or stored as adipose tissue.

Cahill further states that increasing dietary protein to a level above the Recommended Dietary Allowance does not increase muscle mass. Gonyea, Ericson and Bonde-Petersen (1977) state that during training there is an increase in muscle mass; the total amount in the muscle increases with training and decreases with inactivity. Goldberg, Etlinger, Goldspink and Jeblecki (1975) state that an increase in muscle weight seems to be the result of greater protein synthesis and reduced protein breakdown. Also, Poortmans (1975) states that collagen content in tendon and ligaments is high and may be changed by exercise.

## Urea and Creatinine

Urinary urea and creatinine are two of the major excretory products of protein catabolism. Urea (see Figure 2) is derived both from the breakdown of tissue proteins and the breakdown of dietary proteins. The major pathway of nitrogen excretion in humans is urea (Katanuma et al., 1966). Urea is synthesized in the liver from ammonia, carbon dioxide and amino acids, released into the blood, and cleared by the kidneys (Holt, Halac and Kajdi, 1962).

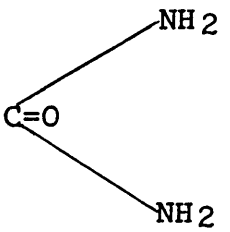


Fig. 2. Urea (Marshall, 1974)

Creatinine is the excretion form of creatine (Bloch, Schoenheimer and Rittenberg, 1941). Creatine is derived from the amino acids glycine, arginine and methionine and is found primarily in striated muscle tissues. Creatine is mainly produced in the kidneys, liver and pancreas and is absorbed from plasma by the muscular system (Bohmer, 1975). In the cell, about two thirds of creatine combines with phosphorus and is converted to phosphorylcreatine acid (Ennor and Morrison, 1958). Phosphorylcreatine acid is the source of energy for immediate use for muscular contractions. Creatinine is the anhydride of creatine and is excreted by the kidneys. Creatinine comes mainly from dephosphorylation of phosphorylcreatine during muscular contractions (Bohmer, 1975). Creatinine excretion is related to muscle mass and is more or less constant (Albanese, 1959). Most researchers feel that creatinine excretion is not influenced to any large degree by nutrition (Addis, 1975, Bohmer, 1975).

Creatinine excretion represents the endogenous

metabolism which takes place in the muscles while urea excretion represents exogenous metabolism (Albanese, 1959). Both of these elements are often tested in studies conducted on protein metabolism with exercise.

## Nitrogen Balance

Determination of nitrogen balance, which is the sum of the gains and losses from the various compartments of the body, (Allison, 1950) provides a method to study the effect of exercise on protein metabolism. Nitrogen balance is the simplest and oldest chemical method used to evaluate the nutritional properties of proteins in an animal (Albanese, 1959).

The amount of nitrogen in a given sample of food is an accurate index of the amount of protein in that sample. Most proteins contain approximately 16 percent nitrogen; and thus, nitrogen analysis can be used to determine the amount of protein in foods or body substances (Wallace, 1959). If the amount of nitrogen that goes into the body and the amount lost in excreta are determined, the portion used by the body can be calculated (Allison, 1950). If the nitrogen intake and output are equal, the individual is considered to be in nitrogen balance. If the intake of nitrogen is greater than the loss of nitrogen, an individual would be in positive nitrogen balance. In a positive

balance, the anabolism of tissue proteins is greater than the catabolism. There is a net gain of protein in the body. Should the excretion of nitrogen be more than that consumed, a negative nitrogen balance exists and the rate of protein breakdown is exceeding the rate of protein synthesis (Allison, 1950).

Gontzea et al.(1974) conducted a study on the influence of muscular exercise on nitrogen balance in man. Thirty normal healthy young men were hospitalized for a period of 28 to 52 days and fed a four day test diet each week during the study. The diet supplied 1.0g protein per kg body weight. The caloric value of the diet was 10 percent higher than the amount of energy expenditure. Energy expenditure was determined several times a day, both on days of sedentary activity as well as in the period of muscular activity, for two weeks. A bicycle ergometer was used daily for six to seven intervals of 20 minutes each, separated by breaks of half an hour; the activity lasted four days and was followed by another period of four or eight sedentary days. Four days later, six persons received an increase in protein in the diet to 1.5g per kg body weight. After eight days, these subjects performed the same physical protocol.

Although in this study, caloric intake was at a maximum, in 29 of 30 cases the muscular activity caused a

decrease in nitrogen balance from an average value of 0.46 $\pm$ 0.07g to -1.60 $\pm$ 0.07g. This difference was highly significant ($p<0.001$). The nitrogen imbalance was measured by the increase in urea excretion and nitrogen loss in sweat. When nitrogen intake was increased to 1.5g protein per kg body weight, the balance became negative in two of six subjects but the loss was not higher than 0.5g nitrogen per day, while in the other four subjects nitrogen balance was maintained within normal limits. The researchers state that the results show that the intensification of processes liberating energy during muscular activity are accompanied by an increase in amino acid metabolism, and consequently there is an increase in the need for protein during exercise.

In 1975, Gontzea et al.(1975) conducted another study on nitrogen balance and exercise. The bicycle ergometer was again used. Exercise by 12 healthy young men was repeated for three weeks. They were fed a diet containing 1.0g protein per kg body weight prescribed four days for each of the three weeks.

Although the caloric content of the diet was 10 percent higher than the energy requirements as established by repeated determination of oxygen consumption every two days, the nitrogen balance became negative in all 12 cases from the first day of effort. During the first few days of the

study the nitrogen excretion ranged from 0.45 to 3.25g, but excretion decreased as the study continued. At the end of the three week study, nitrogen loss had decreased by 90 percent and the subjects almost achieved nitrogen balance. These researchers concluded that the decrease of protein catabolism was not due to a reduction of energetic metabolism alone, but also to a lower protein intake than the researchers believe is required for intense physical activity.

Dohm, Hecker, Brown, Klain, Puente, Askew and Beecher (1977) conducted a study in protein metabolism with endurance training in male Holtzman rats. The rats were trained by a progressive six week treadmill running program which culminated in a workload of one hour per day, six days per week at 35 meters per minute up an eight degree incline. A control group of rats was not trained. Untrained and trained rats were allowed food ad libitum. Urine and feces were collected during week six of training and were analyzed for total nitrogen. The researchers found that urea nitrogen excretion was increased as a result of training. Nitrogen balance was positive in both the trained and untrained groups, which the researchers state was expected since all the rats were increasing in body weight as a result of growth. Trained rats did have a less positive balance than the untrained rats.

The researchers felt that the results demonstrate that protein catabolism is increased during exercise. The researchers calculated that approximately 2.0 kilocalories (kcal) of energy could have been derived from protein catabolism that resulted in the excess urea nitrogen excretion. The formula used for the calculation was:

(0.08g urea/0.16g nitrogen) x 4.0 kcal

This amounted to approximately 37 percent of the calculated energy expenditure during an exercise bout (5.4 kcal expended in excess of resting metabolism). The researchers further state that although these calculations do not prove conclusively that protein catabolism is a significant energy source, they suggest it may be unwise to dismiss protein as an energy source during exercise in trained animals (Dohm et al., 1977).

Protein Requirements

The protein recommendation for normal cell maintenance follows the estimates of the Food and Agricultural Organization (FAO)/World Health Organization (WHO) report of 1973 and is based on a recommendation of 0.45g of protein per kg of body weight per day to meet the needs of almost all members of the population. The 0.45g recommendation was estimated from nitrogen balance studies. This recommendation has been increased by 30 percent to take into

account individual variability in protein utilization. This provides a recommendation of 0.6g per kg body weight per day of high quality protein to cover the needs of almost all healthy individuals in the population. After correcting for 75 percent efficiency of utilization of the protein in a mixed diet compared with the reference proteins (the proteins in whole egg and milk) the allowance for the mixed proteins of the United States diet becomes 0.8g per kg body weight per day. The allowance for a 70 kg man is 56g protein per day and for a 55 kg woman, 44g protein (National Research Council, 1980). According to Hegsted (1959) there is no virtue in feeding protein beyond the minimum adequate quantity. If the body has special needs, such as in illness or stress, the time to consume extra protein beyond normal needs is at the time of the illness or stress (Levenson and Watkin, 1959). During growth, needs for protein are higher; illness and surgery are also times of increased protein need (Holt et al., 1962).

Holt et al.(1962) state that physical activity requires no extra protein. The most important dietary component for exercise is calories. A person expends primarily energy during exercise beyond basic body needs. Carbohydrates and fats (especially carbohydrates) help provide the calories to meet the energy needs. These calories allow protein to be used for more important functions in the body. If

sufficient calories are not available in the diet for energy needs, protein is metabolized to compensate for the dietary energy inadequacy. Inadequate dietary carbohydrate can cause a negative nitrogen balance (Ross, 1959).

Prior Studies

In 1962, Rasch and Pierson (1962) conducted a study on the effect of a protein dietary supplement on muscular strength and hypertrophy. The purpose of their study was to determine whether a given protein supplement contributes to the achievement of strength and muscular mass when taken in conjunction with a program of progressive resistance exercise. Thirty healthy adult male third and fourth year medical students served as subjects. Their weights were recorded, isometric strength was recorded, the girth of the upper arm was measured with a steel tape and the volume of the arm was measured.

The subjects were divided into two groups of fifteen each. Three training sessions (one week) were devoted to teaching the techniques and determining the amount each man could handle in fairly strict style in the following exercises with a barbell: two hand press, two hand curl, supine bench press and two hand reverse curl. The subjects then exercised three days a week for six weeks. During the training period the subjects increased the weight whenever

they felt more could be handled, but the number of repetitions remained the same. During the six weeks of exercise the experimental group received ten tablets per day of a dietary protein supplement. Each tablet contained 2.5g of protein. The control group ingested a similar number of tablets per day of a placebo. Basal protein intake was 1.8g per kg body weight. At the end of training, all measurements were repeated and a statistical analysis was completed.

The results showed that neither training program produced significant changes in body weight, arm volume or arm girth for those receiving the protein supplement or the placebo. The amount of weight handled in the four exercises showed a significant increase for both groups; there was no significant difference between the two groups. The researchers concluded that the addition of protein supplements to the food intake of male college students presumably subsisting on a normal American diet does not increase the amount of body weight, muscular hypertrophy or strength resulting from training with progressive resistance exercise at the level utilized in their study (Rasch et al., 1962).

Srivastava, Mani, Soni and Bhati (1967) studied the effect of muscular exercises on urinary excretion of creatine and creatinine. These researchers conducted this

study because of conflicting reports for creatinine output during exercise. In this study, four series of experiments were carried out using clinically normal army personnel (designated ORs - Official Recruits - in this study) as subjects. In series I, using four ORs as test subjects, normal values for 24 hour urinary nitrogen excretion of creatine and creatinine were determined on three consecutive days. The subjects were then given a daily exercise of marching on sandy terrain at a speed of three miles per hour (mph) for three hours on four consecutive days. Urine was collected and analyzed during each of these days before exercise, during exercise, three hours after exercise and for the remaining part of 24 hours. In series II, using two ORs, the effect of walking on a treadmill at three mph for two hours was studied on three consecutive days. Creatine and creatinine values were determined as in series I for the three remaining series. In series III and IV the effect of two degrees of work load was studied. In series III the subjects were made to march on sandy terrain at three mph for two hours on two days and three hours on two subsequent days. In series IV the subjects marched at two different speeds for the same amount of time - three hours for two days at 2.9-3.0 mph and three hours for two days at 3.8-4.0 mph. Estimation of creatine and creatinine was done according to Folin's method.

The results showed creatine to be absent in urine both during normal and exercise days. Twenty-four hour excretion of creatinine was greater during the exercise days as compared with the normal days in all subjects with the exception of one subject in series I and one subject in series III. Statistical analysis showed that increase in the excretion during the exercise days in series III and IV was significant. There was an increase in the mean value of creatinine excretion during exercise days in series I and II as well but this was not statistically significant. In series III and IV, the average 24 hour excretion of creatinine was higher in days of higher work load, but the difference was not statistically significant. Average hourly excretion fell during the recovery periods as compared to the exercise period in all the four series. The fall in series II and IV were statistically significant.

These researchers state that the increased creatinine excretion during exercise may be due to the fact that during muscular contraction there is conversion of creatine phosphate into creatine and inorganic phosphate, both of which are diffusible. It is reasonable to suppose, the researchers further state, that some of the creatine escapes into general circulation, gets coverted into creatinine and is excreted in urine (Srivastava et al., 1967).

Refsum and Stromme (1974) studied urea and creatinine

production and excretion in urine during and after long distance cross country skiing in well trained men. In the main investigation in 1972, 22 well trained men, aged 21-58 years, were studied. Sixteen participated in both a 90 kilometer (km) ski race and a 70 km ski race one week apart; six men took part in the 79 km ski race only. In a supplementary investigation in 1973, another 22 men, aged 21-45 years, were studied in connection with a 70 km ski race. For both races during the 1972 study, venous blood was collected on the day before the race and on the first, second and fourth days afterwards. Morning urine was collected on the race days and on the subsequent days of blood sampling. During the 1973 study, all urine was collected for three succeeding 24 hour periods from 7a.m. on the day before the race to 7a.m. on the second day after the race. During these three days, according to the researchers, the subjects maintained essentially the same daily intake of food, placing special emphasis on keeping the variations in protein intake as small as possible.

The serum creatinine and urea concentrations increased markedly both during the 90 and 70 km ski races in 1972. Serum creatinine concentration fell rapidly after the race but urea concentration returned slowly to the pre-race level. The serum creatinine and urea concentrations peaked at 65 and 75 percent higher, respectively, than resting

levels. In the 1973 study, total creatinine and urea excretion was determined, during and after a 70 km race. Both the creatinine and urea excretion was higher during the day of the race and the following day than the day before, the highest excretion being observed during the race day for creatinine and during the following day for urea.

Refsum et al.(1974) concluded that prolonged heavy exercise leads to marked increases in serum urea and creatinine concentrations as well as increased urinary urea and creatinine excretion. The researchers stated that the data indicates an increased protein catabolism with heavy muscular exercise. Their results seem to concur with those of Srivastava et al.(1967) that heavy exercise increases protein catabolism as indicated by nitrogen balance studies and creatinine excretion.

Conzolazio et al.(1975) conducted a study on protein metabolism in two groups of young men undergoing intensive physical training. The results in their study differed from the previous two studies. The two groups of men consumed two levels of protein, 1.4 and 2.8g per kg body weight, during a 40 day experimental period. These two levels of protein were used to determine if the higher levels enhanced physiological work. The physical activity included treadmill walking, riding a bicycle ergometer, calesthenics, isometric exercises and sporting activities. Urine and

fecal collections were made continuously during the entire study and were used to compute the nitrogen balances. Sweat samples were collected and analyzed for nitrogen. Blood samples were drawn in a fasting state for analysis of protein. Results showed that nitrogen balances exclusive of sweat losses were positive during the entire study for both groups. Those researchers concluded that muscular work does not result in an increased destruction of cellular protein so an increase in dietary protein for exercise is probably not necessary.

Tandon, Pant, Negi and Mehrotra (1978) studied the changes occurring in the total protein and non-protein nitrogen (NPN) content in young male and female medical students immediately after exhaustive exercise. Exhaustive level of exercise was defined as that level beyond which the individuals felt complete inability to ply the ergometer pedal any more. The female subjects became fatigued earlier than the males in the majority of the cases. The individuals were fasted for 12 hours before starting exercise and were given only water ad libitum before and during exercise (for reasons unexplained). Blood samples were collected before starting and immediately after finishing the exercise. Serum proteins and total serum NPN content and blood urea were determined.

At the end of exercise the total serum protein levels

in both groups significantly declined and the NPN, including blood urea, was significantly increased. The magnitude of increase and decrease in these levels did show sex differences. In females, the percentage fall in the total serum protein level (19.3 percent) was more than in the males (9.6 percent). Also, the total NPN content rise was more in the females than in the males. The rise in urea content, when estimated separately, was found to be greater in the males (31.0 percent) than in the females (25.9 percent). All the observed differences were found to be statistically significant.

These researchers concluded that the fall occurring in the plasma protein level as a result of exhaustive exercise might minimally be the result of (a) significant proteinuria and (b) drainage of the plasma proteins towards the tissues. The reason the researchers give for the rise in NPN is the extensive breakdown of the tissue proteins during exhaustive exercise. The researchers state that they do not have an explanation for the observed differences in the magnitude of chemical changes during exercise between the males and the females. The results of this study are difficult to interpret because the length of the study was short (the subjects only exercised one time).

Marable et al.(1979) found that with muscle building exercise, urinary nitrogen is significantly decreased,

contradicting the results of the previous studies discussed. Their study included four groups of college aged men who consumed two levels of protein (approximately 0.8 or 2.4g per kg body weight) for 28 days while participating in a progressive resistance exercise program. The objective of the study was to observe the effects of feeding protein at two levels and a progressive resistance exercise regime on urinary nitrogen and creatinine excretion. The subjects, 12 healthy males divided into four groups, consumed either approximately 0.8g protein per kg body weight per day or approximately 2.4g protein per kg body weight per day. These two protein levels represent the Recommended Dietary Allowance (RDA) and three times the RDA, respectively, for protein. Caloric intakes were initially set at approximately 41 kcal per kg body weight per day for the non-exercise control groups and approximately 62 kcal per kg body weight per day for the exercise groups. The exercise groups participated in a weight training regimen six days per week. All subjects made timed 24 hour urine collections and urinary nitrogen and creatinine were assayed daily. Food intake nitrogen was also determined.

The results showed that, at both protein intake levels, the exercising subjects excreted significantly less urinary nitrogen than did the non-exercising controls. The exercising subjects were in positive nitrogen balance. Mean

creatinine excretions for the last 14 days of the study were not significantly different for any group. Several explanations were given by the researchers for the decreased urinary nitrogen in the exercise groups, including the higher caloric intake, the type and duration of exercise, greater weight gain by exercising subjects and/or possible increased sweat nitrogen losses. The exercise, which was weight training, is different from the exhaustive exercise in the Tandon et al.(1978) study and the Refsum et al.(1974) study and could provide a possible explanation for the differing results between studies.

Dohm, Puente, Smith and Edge (1978) studied the effects of an acute endurance exercise bout on tissue protein levels and urea excretion. These researchers used male Holtzman rats in their study. The rats were divided into two groups: untrained, which remained sedentary in their cages, and trained, which were subjected to daily treadmill running one hour per day, six days per week for six weeks. During the last week of training a 24 hour urine collection was taken on 16 trained rats that had been rested 24 hours before collection. A 24 hour urine collection was taken for a second group of seven trained rats that were exhaustively exercised immediately before the urine collection was taken. During the seventh week of the experiment, untrained, trained rested and trained exhausted rats were sacrificed

and the gastrocnemius muscles (the large muscle of the posterior portion of the lower leg; it is the most superficial of the calf muscles, (Thomas, 1977)) were extracted and protein concentration was assayed. Results showed that concentrations of soluble and myofibrillar protein were decreased by training. Also, an acute exhaustive bout of exercise also caused a lowering of muscle protein concentration. Urea excretion was not significantly increased as a result of exhaustive exercise, concurring with the results of Marable et al.(1979). This experiment was repeated with the same results. Three possible explanations were given for the loss of muscle protein; a decrease in protein synthesis, an increase in protein degradation and "leakage" of protein from the muscle.

These studies and other studies looking at protein metabolism with exercise indicate that more research needs to be done on this subject. The conflicting results of the studies discussed, the continuing beliefs by many athletes that protein supplementation beyond the RDA is necessary for improved performance, and the fact that very little research on females has been conducted in this area suggest the need for further research.

## HYPOTHESIS

The null hypothesis tested in this study was: There is no significant loss of body protein as measured by urinary creatinine and urinary urea in untrained females beginning a training program. The independent variable in this study was an exercise training program. The dependent variables were urinary creatinine and urea excretion which were used to determine protein loss.

# METHODS

## Selection

The population selected for this study included seven non-smoking healthy females, aged 21-35 years, who had no previous exercise training. Training was determined by a treadmill test. The test consisted of walking at a constant 4.0 mph rate on a Quinton model 1444-B treadmill. The treadmill elevation was increased 5.0 percent grade every three minutes beginning at zero grade until the final 20.0 percent grade was reached. Prior to completing 15 minutes on the treadmill, the women were expected to reach 85 percent of predicted maximum heart rate (Zohman, 1973).

All subjects were examined by a physician to determine if there were any physical problems which would preclude participation in this study.

## Diet

Prior to the beginning of the study, subjects were requested to keep a diet history for five days beginning on Sunday and ending on Thursday. These diets were analyzed for protein content using the Ohio State Nutrient Data Base and Nutrient Analysis program on the Texas Woman's University Dec 20 computer. These diets were then modified individually when necessary to provide at least the RDA for

protein each day. The diet was adjusted to provide a daily variance of no more than 10.0g of protein and a total protein level not exceeding the RDA by more than 20 percent. These modified diets were then consumed by the subjects for the eight weeks of the study, Sunday through Thursday. Friday and Saturday the subjects were allowed to eat ad libitum to aid compliance. If the subjects deviated from the baseline diet they were requested to record these deviations. These changes in the diet were then analyzed to determine actual protein consumption. The subjects consumed no additional protein supplements.

Urea and Creatinine Samples

On Wednesday and Thursday the week prior to the beginning of the study, the subjects collected 24 hour urine samples. The samples were collected in two liter plastic bottles which contained 5.0 milliliters (ml) of 3N hydrochloric acid (Hcl). Each week for eight weeks the subjects collected 24 hour urine samples on Wednesday and Thursday to be used for determining any significant change in urea and creatinine excretion. After collection the sample volumes were measured and aliquots were placed in small glass vials and frozen.

Body Density

The week before the study began, the subjects' heights were measured. Weights were determined on a balance beam scale prior to the study and once each week thereafter. Anthropometric measurements were determined prior to the study and once each week thereafter. Triceps skinfold was measured on the left arm. Skinfolds were also measured on the left side above the iliac crest and the mid-front thigh. Body density was determined by using the sum of the three skinfold measurements in the following equation (Jackson, 1978), (unpublished data validated by Dr. Harley Hartung, 1980):

$$D = 1.105339 - 0.0011964(\text{sum } 3) + 0.00000038(\text{sum } 3) - 0.0001069(\text{age})$$

Percent fat was calculated using the following equation (Siri, 1961):

$$\text{Percent fat} = (4.95/D) - 4.5 \times 100$$

Training

Exercise consisted of walking and/or jogging 30 minutes four times per week for eight weeks on the treadmill. The subjects measured and recorded their pulse every 10 minutes to insure they were not exceeding their 85 percent maximum heart rate. Treadmill tests were repeated at four weeks and eight weeks to determine if a training effect had occurred.

Urea Determination

To determine urinary urea nitrogen, the Evans method was used (Evans, 1968). The principle of the method is that urea reacts with acidic diacetyl monoxime to produce a pink color. Thiosemicarbazide intensifies the color and decreases sensitivity of the product to light. The reagents used were a 2.5 percent diacetylmonoxime solution, a 0.25 percent thiosemicarbazide solution, 5.0 percent tricarboxylic acid solution and an acid reagent which consisted of one liter of water, 80.0 ml of concentrated sulfuric acid, 10.0 ml of 85 percent phosphoric acid and 0.5g ferric nitrate. The standard was obtained from a blood urea nitrogen reagent set developed by Harleco, a division of the American Hospital Supply Corporation. The standard contained one milligram (mg) nitrogen per one ml solution.

The urine sample was diluted 1:150 with distilled water. Twenty-four ml of stock diacetylmonoxime and 10.0 ml of stock thiosemicarbazide were mixed and diluted to 100.0 ml with distilled water. Five parts of the acid reagent was then added to one part of the diacetylmonoxime-thiosemicarbazide to form a color reagent. Five ml of this color reagent was then added to each test tube containing 0.5 ml of the diluted urine. These tubes were heated in boiling water for exactly eight minutes. The tubes were cooled to room temperature in a tap water bath. The optical

density of the samples were then read in a Bausch and Lomb spectrophotometer at 520 nanometers against a blank which contained 5.0 percent tricarboxylic acid.

Creatinine Determination

Urinary creatinine was determined using a method very similiar to the Folin (1914) procedure. The principle of the method is a color reaction which occurs with creatinine upon exposure to picric acid along with sodium hydroxide. The reagents used were 1.0 percent picric acid and 15.0 percent 3.75N sodium hydroxide.

The urine sample was diluted 1:100. Added to the test tube was 1.5 ml of diluted urine, 1.0 ml of distilled water, 1.0 ml picric acid and 0.2 ml sodium hydroxide. This solution was allowed to react for 15 minutes and results were read in a Bausch and Lomb spectrophotometer at 520 nanometers. The results of the study were analyzed statistically using analysis of variance at the $p < 0.05$ level of probability.

## RESULTS AND DISCUSSION

All subjects initially selected for the study completed the study. The exercise training, as discussed previously, consisted of walking/jogging on a treadmill. To maintain the training effect in the subjects, the speed and/or elevation of the treadmill was increased gradually over the eight week period according to individual subject tolerance. A training effect was measured by the ability to sustain heart rate at 70 to 85 percent of predicted maximum heart rate with the continuing increase in speed and/or elevation of the treadmill. All subjects maintained a training effect by increased speed and elevation of the treadmill.

There was a skewed age distribution in the group, with a single subject who was 34 years old. The other subjects ranged in age from 21 to 24 years. The body density of the older subject (subject six) was consistently less dense than the other subjects, so data was analyzed both with and without the data of subject six to determine any variations due to age and density.

During the eight week study, the subjects consumed a protein controlled diet for five days each week. Nitrogen intake was calculated by dividing protein intake by 6.25, since nitrogen content in protein is generally considered to

be 16 percent (Krause and Mahan, 1979). For all subjects, the range of nitrogen intake over the eight week period was 6.38 to 16.56g. No individual nitrogen intake varied more than 3.0g; the wide discrepancy above was due to inter-subject not intra-subject variability in nitrogen intake. (See Table 1.)

TABLE 1

MEAN NITROGEN INTAKE IN GRAMS FOR FIVE DAY DIET PERIOD INCLUDING DAILY RANGE

| | Mean | High | Low | Std Dev'n |
|---|---|---|---|---|
| Day 1 | 11.59 | 16.96 | 08.71 | 01.96 |
| Day 2 | 11.25 | 13.91 | 06.36 | 01.62 |
| Day 3 | 11.26 | 14.12 | 06.91 | 01.42 |
| Day 4 | 11.81 | 16.36 | 08.16 | 01.66 |
| Day 5 | 11.11 | 13.22 | 07.86 | 01.63 |

Weight for each subject did not change significantly during the eight week study. Mean weight varied, for the eight weeks, from 131.87 pounds to 132.83 pounds.

Anthropometric measurements, including triceps skinfold, suprailiac, and thigh were not significantly different during the experiment for the subjects as measured by one way analysis of variance. Triceps skinfold measurements, although they did not significantly change, did show a decrease for all subjects over the eight week

period. From week one to week eight the average decrease was one to ten millimeters in skinfold thickness among all subjects (see Appendix A). The data on subject six showed the greatest decrease in skinfold measurement. Subject six also lost a greater amount of weight, five pounds, than any of the other subjects. Thigh and suprailiac showed little overall difference in measurement for all subjects over the eight weeks (see Appendix B and C).

Body density and percent fat were calculated from the anthropometric measurements (see Methods). Body density ranged from 0.98 to 1.06 with subject six being consistently less dense (0.98-1.01) than the other subjects. Mean density for all subjects was 1.04.

When data for all subjects was considered, body density varied significantly with age of the subject. However, when the data of subject six was removed there was no significant effect of age on body density. The data suggests that at least part of the variability is due to age specific changes. With the data of subject six excluded from the analysis, there was a significant change in body density during the eight week experimental period among a more age homogeneous subgroup (see Table 2).

TABLE 2

EFFECTS OF AGE AND TIME IN STUDY ON BODY DENSITY

| Variable | All subjects | | | Without subject six | | |
|---|---|---|---|---|---|---|
| | F ratio | df | F signif | F ratio | df | F signif |
| Time of study | 01.15 | 7 | NS* | 02.55 | 7 | 00.03 |
| Age of subject | 68.80 | 1 | 00.00 | 00.34 | 1 | NS |
| Time with age | 02.78 | 7 | 00.02 | 02.51 | 7 | 00.03 |

*Not significant

A Student-Newman-Keuls post-hoc analysis showed that week six differed significantly from weeks one and two when the data of subject six was removed. See Figure 3.

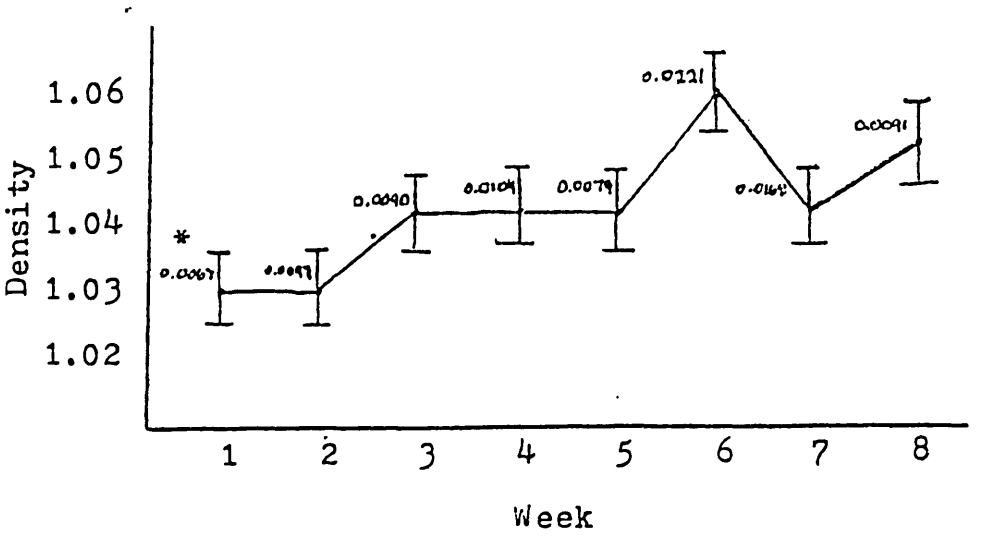


Fig. 3. Mean density and standard deviation by week (data of subject six excluded).
*Standard deviation

Body density during this exercise program was expected to increase, as prior exercise studies also found changes in

body density over time (Girandola, 1976 and Moody et al., 1972). Moody et al.(1972) did a study on high school girls. The girls participated in a walking/jogging program for 15-19 weeks. The researchers found a significant increase in body density but total body weight decreased only slightly. The results of Moody et al.(1972) correspond with the results of this study. In this study body density did increase over time. The walking/jogging exercise program for eight weeks is the probable explanation for the increase in body density.

Since there was a change in body density, percent body fat and lean body mass were analyzed to determine what changes, if any, occurred in their composition. There was no significant change in percent body fat during the study as measured by one way analysis of variance.

Lean body mass was calculated using weight and percent body fat (Brozek, Grande, Anderson and Keys, 1963):

Weight - (Weight x (Percent fat/100))

Lean body mass also showed no significant change over the eight week period by analysis of variance. Because weight and percent fat showed no significant change during the study it was expected that lean body mass would also show no significant change. The minimal changes in percent body fat and lean body mass could indicate that body density changes might have been due to improvement in skinfold measurement

techniques rather than to actual body density changes. Normally, a greater change in percent body fat and lean body mass might be expected with change in body density.

Creatinine results were inconclusive because of difficulties with the assay techniques. Preparation of standard samples and an accurate standard curve (Pearson R = 0.958, $p < 0.0017$) showed that the lab techniques were performed correctly. The assays performed, however, did not provide consistent results although the tests were duplicated. There were not sufficient samples left to repeat the test.

One possibility for the poor creatinine results could be that the acid used for the preservation of the samples affected the creatinine. Also, Folin (1914) states that in analyzing creatinine in urine it is advantageous to work with reasonably fresh and well preserved urine. The samples were stored for two to ten weeks and were thawed and refrozen, so breakdown of creatinine could have occurred, creating the false results. No current research was found to substantiate Folin's assertion.

The variation in urea nitrogen excretion by week, as determined by analysis of variance, did show a significant change ($p < 0.05$). Mean nitrogen excretion for all subjects ranged from 1.08 to 4.65g. A Student-Newman-Keuls post-hoc analysis showed nitrogen excretion during week one was

significantly higher than all other weeks; and week three excretion was significantly lower than weeks one, four, five, seven and eight (see Figure 4).

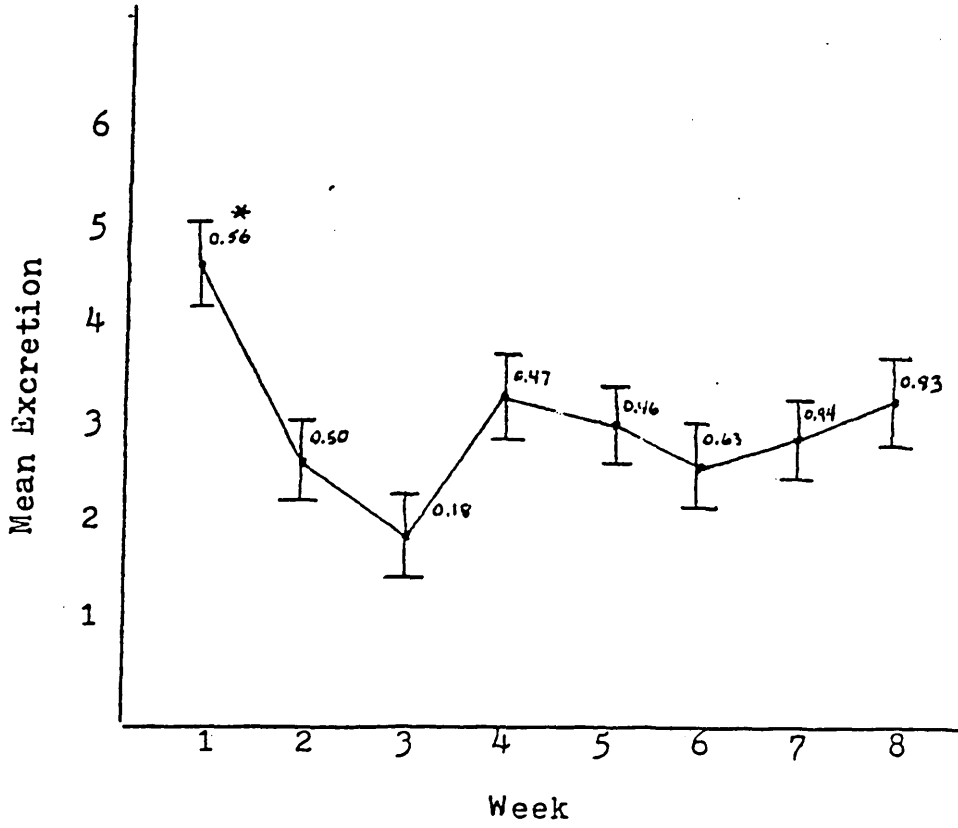


Fig. 4. Mean nitrogen excretion in grams with standard deviation for eight weeks (including data of all subjects).
*Standard deviation

Regression analysis did not indicate a significant correlation between nitrogen intake and urea nitrogen excretion. Age, and body density of the subjects, also had no correlation with urea nitrogen output.

Urea nitrogen excretion in a normal adult usually averages approximately 7.0 to 16.0g (Hawk, Oser and

Summerson, 1954). In this study the nitrogen excretion was lower than the figures above indicate, ranging from 1.08 to 4.65g. According to the literature, during an exercise program nitrogen excretion should not differ by grams but by milligrams (Haralambie et al., 1976, Gontzea et al., 1974) from nitrogen intake. The differences in nitrogen intake to nitrogen excretion in this study was 6.75 to 10.32g.

The lab work to obtain these results was done as properly as time and equipment permitted. Pre-testing of samples was conducted to ensure proper technique. The standard curve, measured prior to testing, had a Pearson R of 0.998 with $p < 0.001$.

Although the excretion of nitrogen was low there was an obvious trend in this nitrogen excretion (see Figure 4). There was a sharp decrease in nitrogen excretion in the first weeks of training followed by a gradual increase over the eight week period. If an exercise program was to affect the protein metabolism in previously untrained individuals it seems likely that it would occur as indicated in this study; that is, as exercise begins, the body adjusts to the change then gradually adapts to the increased demands as the body becomes trained. Gontzea et al.(1975) state that body reactions to the same stimulation repeatedly applied become less and less ample. Marable et al.(1979) state that urinary nitrogen excretion does decrease with a muscle

building exercise program and further state that this could lead to an increased need for dietary protein due to increased use of protein by the body during exercise. Also, Dohm et al.(1980) state that amino acid utilization is increased during exercise, affecting a change in protein metabolism and therefore urea metabolism. So, although the excretion of urea nitrogen in this study was low, the observed changes are consistent in direction, if not degree, with previous studies.

One explanation for the trend in urea nitrogen excretion could be that the excretion was significantly diminished. Haralambie et al.(1976) state that the urinary urea elimination rate is somewhat impaired by exercise. These researchers state that urea clearance has been reported to drop by 56 percent during a 70 km ski race. The researchers also report that exercise on a bicycle ergometer caused a mean decrease in urea elimination of 47.6 percent.

Another explanation for the low excretion of urea could be that there was a significant nitrogen excretion from other routes not studied, for instance, fecal excretion or sweat. Marable et al.(1979) and Haralambie et al.(1976) both state that decreased urinary urea output may be caused by increased sweat losses. These routes for nitrogen excretion were not considered in this study for research. Gontzea et al.(1974) emphasize that it is important to

consider all excretion routes, including feces and sweat as well as urine.

Another possibility for the low excretion could be deterioration of the urea in the stored samples prior to testing. The samples were preserved with 5.0 ml 3N Hcl and were then frozen for later analysis. According to past researchers, urea will decompose if held for a long period of time. Both Laidler and Hoare (1949) and Van Slyke and Cullen (1914) state that urea determinations should be carried out on fresh or recently collected and preserved samples of urine because on long standing, even with preservatives, significant amounts of urea may undergo hydrolysis to form ammonia. Kennedy (1975) states that urea slowly decomposes to cyanate and ammonium ion in aqueous solution at high concentration. If it is assumed that all samples decomposed in a similar manner and to a similar degree, there might not have been enough urea left to indicate the true amount excreted but there may have been enough to indicate the trend that does appear in the result.

Another aspect which may have affected the low levels of urea nitrogen is the handling of the samples. The samples were thawed and refrozen (for analysis of another compound) adding to the possibility of deterioration.

The most important aspect to consider is that, even though urea excretion was low, there is a trend presented in

the urea excretion that could indicate that urea nitrogen metabolism does change with the introduction of an exercise program in untrained individuals. Urea metabolism did show a change in this study and so the protein metabolism in the exercising females could have changed, implying that there could be a need for supplementary protein, in excess of the RDA, with a regular exercise program, at least in the early weeks of the training program.

## CONCLUSION AND IMPLICATIONS FOR FUTURE RESEARCH

In this study, a group of seven untrained women, ages 21-35 years, participated in a walking/jogging program for eight weeks. Dietary protein intake was controlled for five days each week.

The question asked in this study was: Does an exercise training program affect protein metabolism in previously untrained healthy women? In an attempt to answer this question, urinary urea and creatinine were analyzed during the eight week period. The women exercised by walking/jogging on a treadmill four days a week for eight weeks. Twenty-four hour urine samples were collected twice a week for eight weeks. Urea and creatinine assays were performed using these samples. Anthropometrics and weight were measured once a week.

Body density, determined by anthropometrics, did show a significant change during the study. Density steadily increased during the study as exercise progressed (refer to Figure 3). Previous research supports this increase in density with exercise. There was a drop in density in the last two weeks of training, which could have been caused by a change in body density measurements or change in

diet and exercise compliance by subjects towards the end of the study. The drop in density was not significant. Body density changes did not show a significant correlation with urea nitrogen excretion.

The null hypothesis of this study was: There is no significant loss of body protein as measured by urinary creatinine and urea in untrained individuals. Urinary creatinine results proved inconclusive and could not be used to test the hypothesis. Urinary urea excretion showed a decrease during the first weeks of training with a gradual increase in excretion in the later weeks, almost back to beginning excretion levels. This decrease in urea excretion could mean that protein is being used by the body to a greater extent than normal during exercise training. In theory, the null hypothesis could be accepted, because there is no significant loss of protein with exercise training as could be ascertained by this study, but there is a definite sign of some change in protein metabolism (as seen by decreased urea nitrogen excretion) in concurrence with the exercise training program. This could mean that a moderate increase in protein consumption, beyond normal requirements, could be useful in the beginning weeks of an exercise program.

Future research studies on protein metabolism with exercise should allow two weeks prior to beginning the study

to establish baseline diet and baseline urinary urea and creatinine excretion. Also, urine samples should be analyzed as quickly as possible to prevent deterioration in urea and creatinine. More subjects should be used in the study to provide better statistical accuracy, and age and possibly body density should be controlled to provide more uniformity in results. Using blood samples to test free amino acid levels might also prove helpful in testing protein metabolism with exercise.

## APPENDIX A

MEAN TRICEPS SKINFOLD MEASUREMENTS IN MILLIMETERS WITH RANGE AND STANDARD DEVIATION

| | Mean | High | Low | Std Dev'n |
|---|---|---|---|---|
| Week 1 | 18.57 | 31.00 | 12.00 | 06.85 |
| Week 2 | 18.71 | 27.00 | 12.00 | 04.82 |
| Week 3 | 19.43 | 26.00 | 11.00 | 04.83 |
| Week 4 | 18.57 | 26.00 | 10.00 | 05.71 |
| Week 5 | 17.29 | 29.00 | 12.00 | 05.71 |
| Week 6 | 16.71 | 26.00 | 10.00 | 05.50 |
| Week 7 | 16.43 | 22.00 | 10.00 | 04.28 |
| Week 8 | 14.57 | 21.00 | 10.00 | 03.51 |

## APPENDIX B

MEAN THIGH SKINFOLD MEASUREMEMTS IN MILLIMETERS WITH RANGE AND STANDARD DEVIATION

| | Mean | High | Low | Std Dev'n |
|---|---|---|---|---|
| Week 1 | 30.57 | 46.00 | 24.00 | 07.66 |
| Week 2 | 31.43 | 42.00 | 22.00 | 06.35 |
| Week 3 | 27.57 | 34.00 | 22.00 | 04.76 |
| Week 4 | 25.43 | 32.00 | 18.00 | 05.50 |
| Week 5 | 24.71 | 37.00 | 19.00 | 06.05 |
| Week 6 | 25.86 | 38.00 | 18.00 | 08.13 |
| Week 7 | 28.00 | 41.00 | 19.00 | 08.60 |
| Week 8 | 27.43 | 36.00 | 18.00 | 05.60 |

## APPENDIX C

MEAN SUPRAILIAC SKINFOLD MEASUREMENTS IN MILLIMETERS WITH RANGE AND STANDARD DEVIATION

| | Mean | High | Low | Std Dev'n |
|---|---|---|---|---|
| Week 1 | 14.00 | 26.00 | 09.00 | 05.74 |
| Week 2 | 13.43 | 25.00 | 09.00 | 05.47 |
| Week 3 | 13.43 | 24.00 | 09.00 | 05.16 |
| Week 4 | 13.57 | 24.00 | 08.00 | 05.29 |
| Week 5 | 12.00 | 18.00 | 08.00 | 03.42 |
| Week 6 | 12.71 | 20.00 | 08.00 | 04.39 |
| Week 7 | 13.29 | 21.00 | 08.00 | 05.12 |
| Week 8 | 12.29 | 21.00 | 07.00 | 04.50 |

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