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The Effects of Supplemental Protein on Urea Nitrogen Excretion in Women 40-70 Years Old

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THE EFFECTS OF SUPPLEMENTAL PROTEIN ON UREA NITROGEN EXCRETION IN WOMEN 40–70 YEARS OLD

by

Tammy D. Moran

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Health, Physical Education, and Recreation

Western Michigan University
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1999
ACKNOWLEDGMENTS

The completion of this thesis is met with much jubilation and cheer. I have to admit, there were times when I thought it would never end! Now that the labor is complete, I must thank everyone who made it possible for this original proposal to become research reality. First, a sincere thank you to the members of my committee, Dr. Moss, Dr. Bolin, Dr. Dawson, and Dr. Zabik. I am indebted to you for your time and wisdom. I am especially grateful to Dr. Zabik for his patience and humor (especially in the hot chemistry lab)! A special thank you to Dr. Bolin, who sacrificed his free days to make sure the assay was working properly. Also, thank you to Dr. Dawson for always helping us out when called upon. In addition, thanks to the chemistry lab for always allowing us entry and use of the equipment.

Thank you to my family members who felt obligated to serve as participants. Also, to my Kalamazoo and Elsie friends who diligently collected the much needed specimen! Without you, this wouldn’t have happened. A special thank you to my mom for keeping me company while on the collection route and for helping me see the light at the end of the tunnel.

Finally, last but not least, a loving thank you to my husband for allowing me the privilege of being a full-time student. I appreciate the countless words of encouragement, as well as the silence you offered when I needed a sounding board. I dedicate this thesis to everyone who never bored of hearing me talk about it!

Tammy D. Moran
The problem of this study was to determine the effect of supplemental liquid protein in women 40–70 years of age over a 4-week period. Weeks 1 and 3 served as the control period. During this time, no particular diet regime was required, but protein drinks were prohibited. The treatment condition occurred during Weeks 2 and 4. During this time, subjects consumed two protein drinks each day for a period of 7 days. Twenty-four hour urine collections occurred at the end of each week. Urine was analyzed for the presence of urea nitrogen. The dependent variables were urinary urea nitrogen, self-perceived wellness levels, and body weight. The results indicated that supplemental liquid protein of 30 g per day does have an effect upon urea nitrogen excretion in women 40–70 years of age. The supplemental protein did not affect self-perceived sleep, stress, food intake, exercise, or body weight levels. Energy levels were, however, higher during the supplement weeks than during the control weeks.
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CHAPTER I

INTRODUCTION

A well-balanced diet is a vital key to achieving optimal health. Unfortunately, this fast-paced world we live in does not always allow the time necessary for the preparation of nutritious meals. As a result, supplemental nutrient drinks have become big business. Many supplemental drink manufacturers specifically target the 50-something population. Television advertisements for some drinks promise revived, youthful energy and vigor upon consumption. The drinks vary, not only in flavor, but also in regular and high-protein formulas.

With such a wide variety of drinks on the supermarket shelves, consumers should have the knowledge that will enable them to make educated, economical decisions with regard to the nutritional value of additional protein. Very little research existed that supported the need for supplemental protein in an average person’s diet.

Statement of the Problem

The problem was to determine the effect of supplemental dietary protein on urea nitrogen excretion, self-perceived wellness levels, and body weight in 40-70 year old females. Urinalysis for urea nitrogen, once a week, determined protein excretion levels. Protein diets were studied for 4 weeks. No specific activities were required of the subjects.
Purpose of the Study

The purpose of the study was to provide more information about the usefulness of additional protein contained in nutrient drinks to apparently healthy consumers. This study will determine if there is a need for supplemental protein in the diet, if the recommended daily allowance of protein is met. Analyzing the need for extra protein may help consumers make informed decisions when considering the purchase of high-protein drinks.

Delimitations

The following delimitations were established for this study:

1. The female volunteer subjects \((N = 10)\) were recruited from known acquaintances (see Recruitment Form, Appendix A).

2. Participants ranged in age from 40–70 years.

3. Participants were placed into the control regimen for 7 days and then into the treatment regimen for 7 days. This protocol was repeated once.

4. Two conditions were studied: (1) participant’s normal diet, the control; and (2) participant’s normal diet plus a high-protein formula drink, the treatment. Participants consumed their normal diet for 1 week. This condition was followed by consumption of their normal diet plus one 8-oz serving of a high-protein formula at approximately mid-morning and another at approximately mid-afternoon each day for 7 days.

5. Throughout the duration of the study, no specific activities were required of the subjects. However, during the control weeks protein drinks were prohibited.
6. Urinalysis for urea nitrogen, following each weekly dietary regimen, determined protein excretion levels. A total of four 24-hr collections were independently analyzed using a spectrophotometer.

7. Self-perceived wellness levels were analyzed each evening.

8. Body weight was recorded and urine samples were collected every 8th day.

9. The study was conducted for a period of 4 weeks from January 24 through February 22, 1999.

Limitations

The study was limited by the following facts:

1. The sample size of the study was small \(N = 10\). Extrapolation of the data to a larger population should be made with caution.

2. The activity levels of the subjects were not monitored during the course of the study. Varying activity levels may affect protein metabolism.

3. The use of only one brand of supplemental high-protein drink was studied; other drinks may produce different results.

4. The duration of the study was short. Observation of subjects, over an extended period of time, may have produced different results.

Assumptions

The basic assumptions of the research were as follows:

1. All subjects completed the pretest questionnaire accurately and honestly.

2. The test for urea nitrogen produced accurate results.

3. The subjects were able to adequately collect and appropriately store their urine output for each 24-hr collection.
4. Subjects were able to record levels of self-perceived wellness accurately and honestly.

5. The 1-week control periods allowed for complete protein turn-over in all subjects.

6. Increased urinary urea nitrogen levels were the result of increased protein in the diet and increased protein catabolism.

7. Ammonia in any of the reagents or in the atmosphere of the room in which the procedure was carried out did not result in falsely high values.

Research Hypotheses

The study was conducted to test the following hypotheses:

1. Participants’ excretion of urea nitrogen during the 24-hr collection period will be greater for the supplementation weeks than for the control weeks.

2. Participants’ excretion of urea nitrogen will be consistent across the two 24-hr collection periods for the control weeks.

3. Participants’ excretion of urea nitrogen will be consistent across the two 24-hr collection periods for the two supplementation weeks.

4. Participants’ perceived wellness levels will be the same for the supplementation weeks as for the control weeks.

5. Participants’ body weight will increase during their 4-week participation in the study.

Definition of Terms

The following definitions and terms are important to the understanding of this study:
1. **Urea nitrogen**: A compound that is identifiable in 60–90% of all nitrogen-containing material in urine. It is derived from ammonia that is produced by deamination of amino acids, which combines with carbon dioxide to form urea (Tortora & Grabowski, 1996).

2. **Nitrogen balance**: A condition in which bodily protein is being degraded and synthesized at equal rates. It is a reflection of overall balance between protein synthesis and breakdown rates.

3. **Spectrophotometer**: A device which allows color reagents to be analyzed in order to determine nitrogen urea concentrations (Chaney & Marbach, 1962).

4. **High-protein formula**: A drink containing 15 g of protein, 6 g of fat, and 240 calories.

5. **Supplemental protein**: Protein consumed above the recommended daily allowance.

6. **Protein turnover**: The complete breakdown and synthesis of bodily protein.
CHAPTER II

REVIEW OF LITERATURE

Introduction

As a supplement, protein has become very marketable. Some products are advertised as providing the protein necessary for producing greater muscle mass (Rand, 1997) and supplying youthful energy to those who consume it. These claims attract many people who are searching for a quick solution to their perceived low energy levels. Unfortunately, older adults are specifically targeted by manufacturers of these supplements.

Due to the increased promotion of supplemental protein products, it is vital that older individuals be aware of their protein needs and how these supplements may affect protein retention. Also, an understanding of the fate of excess protein in the diet, physiologically, may aid consumers in making more informed choices concerning the nutritional value of foods that they eat.

Protein Intake

Protein is necessary for development and growth of the human body. Estimating the amount of protein required varies depending upon age and activity level. Unfortunately, studies recommend protein-intake based upon requirements for a healthy, 18-year-old male.
Protein is often obtained through the use of protein drinks. These drinks are a quick and effective way to meet daily protein requirements. However, not only are they costly, but they may also be high in calories and fat.

The need for supplementing protein in the diet has long been debated. Some research purports specific health risks associated with additional protein, while others find no negative health effects related to high protein consumption.

RDA of Protein

At the present time, the Recommended Dietary Allowance (RDA) is 0.8 g of protein per kilogram of body weight per day. This standard was set by the Food and Nutrition Board of the U.S. National Research Council (Campbell, Crim, Dallal, Young, & Evans, 1994). Protein requirements are estimated from nitrogen balance data that compare nitrogen intake with nitrogen output (deGroot & vanStaveren, 1996). The nitrogen-balance technique has been criticized by Millward, Jackson, Price, and Rivers (1989); however, it continues to be accepted on a national and international level as a means for estimating dietary protein requirements (Campbell et al., 1994).

The current RDA for protein is based upon data collected using sedentary male subjects, over 18 years of age (Ahmed, 1992; Meredith, Zackin, Frontera, & Evans, 1989). Unfortunately, adequate protein-intake information is scarce for adult women over the age of 65 years. The World Health Organization (WHO) recommends 0.75 g per kilogram of body weight per day for people 65 years of age and older. This suggestion set forth by WHO, however, is based upon extrapolations of data from nitrogen-balance studies performed on healthy young men (Pannemans, Halliday, & Westerterp, 1995). These studies failed to take into account physical
activity levels, caloric consumption, and health problems in many older individuals. These factors may contribute to different protein requirements for this age group (Campbell et al., 1994). Other researchers (Bunker, Lawson, Stanfield, & Clayton, 1987) reported that the protein needs of older individuals may be met by 1 g of protein per kilogram of body weight per day. A higher protein requirement suggests a lower efficiency of dietary protein utilization in older adults, despite decreased muscle mass (Campbell et al., 1994). Also, it is still uncertain whether older women require more or less protein than older men. Pannemans et al. (1995) found protein-turnover (PT) rates to be notably higher in men than in women. The differing PT rates may be due, in part, to hormonal differences between the sexes.

**Protein Drinks**

Some high protein formulas contain as much as 15 g of protein in an 8-oz can. Unfortunately, a recommended dosage is generally not specified by the manufacturer. Other drinks range from 10 to 13.5 g of protein per 8-oz serving. Along with the additional protein, consumers receive 6 g of fat and a total of 240 calories. The fiber in most high-protein drinks is minimal, if not absent, and calcium content varies from 20% to 50% of the Food and Drug Administration (FDA) daily value (based on a daily 2,000 calorie diet). In addition, some current protein drink products are lacking in omega 3 fatty acids and phytochemicals that have been shown to prevent cancer and heart disease (Sugarman, 1996).

The main ingredients in most liquid supplement drinks are water, sugar, oils, milk or soy proteins, vitamins, minerals, and artificial flavors. It is believed that these ingredients may actually cause weight gain due to the high fat and caloric content (Sugarman, 1996). Dahilig (1997) also pointed to the dangers of some protein drinks
that contain ephedrine, which is a stimulant that raises blood pressure and increases heart rate.

High-Protein Diets

The effects of high-protein diets have been greatly debated. Brenner, Meyer, and Hostetter (1982) found a possible link between high-protein diets and an increased risk of renal disease. It is believed that an additional workload is placed on the kidneys due to processing of nitrogen wastes generated by protein catabolism. However, other studies on rats that received an 80% protein diet for more than half of their life span did not reveal any damaging side-effects (Lemon, 1996).

In addition, researchers have also suggested that high-protein diets lead to an increased loss of urinary calcium, which may have a negative impact on the health of bone (Allen, Oddoye, & Margen, 1979). Also of concern is the risk of dehydration, due to the catabolism of amino acids into urea. The body requires a sizeable quantity of water in order to excrete urea in the urine, and, as a result, greater water consumption would be essential if a high-protein diet was consumed (Christian & Greger, 1994).

Perhaps the greatest concern related to high-protein diets is the intake of large amounts of individual amino acids. Complications include absorption difficulties, altered neurotransmitter activity, metabolic imbalances, and toxicity (Lemon, 1996). Also of interest, especially to the older population, is research that indicates raising the daily protein intake to twice the RDA levels results in an increase in protein breakdown and protein synthesis (deGroot & vanStaveren, 1996).

In contrast, some researchers believe there may be health concerns associated with decreasing the RDA for protein. For example, inadequate protein may cause the
development of amenorrhea in women. With regards to older adults, low protein intake results in decreased muscle function and an impairment of cellular immune responses (deGroot & vanStaveren, 1996).

Metabolism of Protein

Protein has a variety of physiological functions. The role of protein is generally considered limited for most types of muscular activity. However, it is now evident that protein catabolism is increased by endurance activities lasting longer than 60 min.

Sedentary Individuals

During digestion, protein is broken down into amino acids (Tortora & Grabowski, 1996). These amino acids can enter the body's free amino acid pool from either the protein foods ingested, from the breakdown of body protein, or as nonessential amino acids synthesized from carbohydrates or fats. Ideally, these processes are in equilibrium so that protein synthesis using amino acids from the free pool replaces the breakdown of body protein (Lemon, 1996). Therefore, a continuous input of new amino acids into the free pool is necessary to compensate for the loss and constant reordering of material within the body (Brooks, Fahey, & White, 1996). When protein ingestion is insufficient, there are inadequate amino acids entering the free pool to replace those lost from the degradation of protein. Ultimately, this situation can lead to loss of muscle mass and strength which may have an adverse effect upon performance and health. In contrast, if protein intake is extreme, the additional amino acid carbons are oxidized or converted to fat or
carbohydrates and stored. The surplus nitrogen is excreted primarily as urea in the urine (Lemon, 1996).

**Active Individual**

Physical activity has drastic effects on protein metabolism. It has been determined that protein is utilized to a greater extent during exercise than was once assumed (Lemon & Mullin, 1980). Endurance exercise causes an increase in mitochondrial protein synthesis, and amino acid oxidation is also enhanced significantly (Friedman & Lemon, 1989). Furthermore, exercise causes a net efflux of most amino acids from the liver. Additional research indicated a substantial decrease in the rate of protein synthesis during exercise. As a result, the increased level of liver amino acids, circulating in the blood, is made available to muscle as an energy source without causing an increase in muscle protein catabolism (Evans, Fisher, Hoerr, & Young, 1983).

**Measurement of Protein Excretion**

Protein turnover can be analyzed in several ways. Tests measuring various waste products of protein catabolism, in the blood or urine, are most frequently used. Although some techniques are more popular than others, Waterlow, Garlick, and Millward (1978) determined that comparative methods of measuring total protein turnover are likely to produce similar results.

**Blood Urea Nitrogen**

One end-product of protein metabolism is urea. Urea is synthesized from ammonia in the liver through the metabolic process known as the urea cycle. This
cycle removes ammonia, which is a toxic by-product of amino acid metabolism. Urea has no metabolic function after it is synthesized. Therefore, it is removed from the blood by the kidneys and excreted in the urine. Urea can be measured in the blood using the diagnostic test known as BUN (Blood Urea Nitrogen). An increase in plasma urea concentrations can be attributed to several factors, including a high protein diet and increased protein catabolism. Typically, blood urea levels range from 2.5–6.5 mmol/L (Higgins, 1994).

**Urinary Urea Nitrogen**

Urea may also be measured in the urine using a variety of assays. Urinary urea nitrogen is often used to determine nitrogen balance in subjects receiving additional nutritional support (Traub, 1996). This study, in particular, utilized a combination of reagents to initiate the catalyzed endophenol reaction for the determination of ammonia and urea levels. Ammonia and urea were measured after being hydrolyzed with urease (Chaney & Marbach, 1962). Typically, urinary urea nitrogen levels range from 7 to 16 g per 24-hr period. An increase in these numbers can be attributed to several factors, including increased protein in the diet and an increase in protein catabolism (McBride, 1998). Typical urine output for adults as noted by Walters, Estridge, and Reynolds (1996) is 600–2000 ml per 24-hr period.

Several relatively new ways exist to detect trace amounts of protein in the urine. Some of these newer techniques include radioimmunoassay, and immunonephelometric procedures. These methods, however, are not considered simple screening devices. Easier methods of measuring protein include the Chemstrip Micral Urine Test Strip (Boehringer-Mannheim), and the Micro-Bumintest (Bayer). The Micro-Bumintest, for example, is designed to measure minute amounts of
albumin in the urine. This test requires a drop of urine be placed on a tablet containing salicylic acid and bromophenol blue indicator. Any ring or bluish spot on the tablet indicates a positive reaction (McBride, 1998).

Anthropometric Measurements

Anthropometric measurement is the measurement of size, weight, and proportions of the human body. The most common measurements taken include weight, circumference of various body parts, skinfold thickness, and hydrostatic weighing. Measurements are compared with standardized reference tables for the individual being assessed. Results that deviate from the standards may be indicative of malnutrition or overconsumption of nutrients (Malasanos, Barkauskas, & Stoltenberg-Allen, 1990).

Scales

Many types of instruments are regularly used for measuring weight, but the preferred instrument usually is a balance beam scale with nondetachable weights. Digital electronic platform scales are increasing in popularity and can be modified to weigh individuals in a compromised position. When serial weights are recorded, it is important for the person to be weighed on the same scale, at approximately the same time of day, with approximately the same amount of clothing (Malasanos et al., 1990). These factors will allow for an accurate comparison among recordings.
Circumference

The head, chest, and mid-arm are common circumference measurements. Mid-arm circumferences are useful when evaluating somatic protein stores and are most commonly used when assessing malnutrition (Malasanos et al., 1990).

Skinfolds

Body composition can be determined by measuring the thickness of skinfolds at predetermined locations. Measuring the thickness of a skinfold requires pulling a fold of skin and fat away from the underlying muscle. The sum of the skinfolds can be used to determine changes in body fat following diet or exercise (Howley & Franks, 1992).

Hydrostatic Weighing

This relatively noninvasive technique measures body density from the amount of water that is displaced around the individual’s submerged body. The measurement of body density is translated to provide an estimation of body fat percentage. The disadvantages to this procedure are the time necessary to gather data and the inability of many participants to perform the test properly (Howley & Franks, 1992).

Research Designs for Diet Studies

Many studies have been conducted that attempt to determine PT in the human body. These studies range from analyzing PT in older adults to determining the effect of endurance exercise upon protein retention. The structure of various macro nutrient studies are useful when choosing a protocol design.
Protein

In order to determine PT in elderly men and women, Pannemans et al. (1995) used 28 subjects. All subjects were given two different diets of either 12% or 21% protein for a period of 3 weeks. All meals were provided daily at the home of each subject. Subjects were not allowed to drink or eat anything else except water, coffee, and tea. All subjects served as their own control. Nitrogen balance was determined from 24-hr urine samples collected during the last 2 days of the week for each diet. Urine collection started with the second void of the morning and included the first void of the next day. Urinary ammonia and urea concentrations were analyzed with a spectrophotometer. Nitrogen content was measured with a Heraeus analyzer. Corrections were made for other obligatory losses with the formula of 8 mg N/kg body weight. It was found that urinary nitrogen was significantly higher with the 21% protein diet.

In a related study, Friedman and Lemon (1989) analyzed the effect of chronic endurance exercise on retention of dietary protein. For this study, two dietary protocols that were lacto-ovo-vegetarian in nature were used. Each diet provided either 0.8 g protein per kg of body weight per day or 1.6 g protein per kg of body weight per day. Subjects were familiarized with a food exchange list and given detailed instructions regarding how many servings should be consumed from each food group. Each diet treatment lasted 6 days, with 2 weeks separating consecutive treatments. Under both dietary treatments, subjects completed the same 6-day exercise program. The exercise program consisted of their regular workout and a treadmill run. Urine collections were made during days 4, 5, and 6 of the diet
treatment. All subjects recorded their food and drink intake throughout each dietary trial.

It was found that urinary urea nitrogen excretion tended to be higher while on the lower protein diet. However, the results were not statistically significant. It is believed that more body protein was broken down during the lower protein diet in order to meet overall protein needs.

**Carbohydrate**

Herron (1994) tested the effect of diet on mood in the elderly. Subjects consisted of 3 males and 25 females ranging in age from 63–83 years. All subjects were fed either a high protein, high carbohydrate or control snack. In the first part of the study, subjects kept 3-day food-intake preprandial and postprandial mood records. In the experimental portion, subjects served as their own controls and ate either a protein, carbohydrate, or control snack, in random order. Mood was determined immediately before and 1 hr after the snacks were eaten. It was found that protein did not improve vigor, but the carbohydrate snack significantly improved depression and mood scores.

**Summary**

Daily protein intake amounts have been determined for the sedentary, adult male. Unfortunately, controversial information exists with regards to the protein needs of women and older individuals.

Supplemental protein drinks come in many varieties and formulas. New brands are continually being placed on supermarket shelves that contain different
levels of protein, fat, and carbohydrates. Most of the drinks contain many calories with little or no fiber content.

Additional protein intake, above the recommended allowance, has been debated by many researchers. Some studies reveal no hazards involved with extra protein intake, but others have shown significant health risks. Others have even suggested that increased protein may actually be beneficial to the overall health of an individual.

Protein appears to be metabolized differently depending upon the activity level of the individual. It also is believed that activity level affects bodily protein requirements. Several techniques exist to measure protein excretion levels. The most common involve analysis of urine or blood. Both procedures are fairly noninvasive, but require relatively complex analyzing systems.
CHAPTER III

METHODS AND PROCEDURES

Supplement protein drinks have become a staple in the diets of many people. The older population, in particular, has been targeted by food supplement manufacturers to consume these expensive, high-protein drinks. In this study, a selected urinary urea nitrogen test, body weight measurements, and self-surveys were used to determine the effects of supplementing the diet with a high-protein formula drink. The following topics are covered in this chapter: (a) subjects, (b) research design, (c) equipment, (d) data collection procedures, (e) measurement and collection procedures, and (f) statistical analysis.

Subjects

The subjects for this study were 10 randomly selected female volunteers recruited from known acquaintances. The ages of the subjects were 40–70 years. Each subject signed a consent form. Approval to conduct this study was given by Western Michigan University’s Human Subjects Institutional Review Board (see Consent Form, Appendix B and Approval Letter, Appendix C). All subjects chosen were apparently healthy, as defined by the American College of Sports Medicine. In addition, all participants had no history of renal problems and had maintained a stable weight of plus or minus 5 lb for at least 6 months prior to participating in the study. Participants were screened to determine eligibility for participation (see Screening Form, Appendix D).
Research Design

This study measured three dependent variables: (1) urinary urea nitrogen, (2) self-perceived wellness levels, and (3) body weight. The independent variables consisted of a control and a treatment condition. The research design was repeated measures. Participants were, on a weekly basis, alternately exposed to the control and treatment conditions, twice in succession.

Equipment

This study utilized the following equipment:

1. Urinary urea nitrogen concentrations were measured spectrophotometrically by standard enzymatic methods on a Hitachi spectrophotometer, Model 100-10 (Hitachi, Ltd. Tokyo, Japan). Urea nitrogen values were corrected for ammonia content in the urine.

2. Body weight was determined on the Memorie Bath Scale (Hanson, Japan).

3. Self-perceived sleep, food intake, exercise, stress, and energy levels were evaluated by each participant, on a numerical scale (see Journal Form, Appendix E).

Data Collection Procedures

The 10 participants were placed into the control regimen for a period of 7 days. No specific dietary program was required; however, protein drinks were prohibited. On the 7th day, 24-hr urine collections began with the second void of the morning and ended with the first void of the 8th day. For the next 7 days the participants consumed one 8-oz high-protein formula drink at approximately mid-morning and another at approximately mid-afternoon each day, for 7 days. On the 7th
day, 24-hr urine collections began with the second void of the morning and ended with the first void of the 8th day. The control and treatment sequence was again repeated. Subjects participated for a total of 4 weeks.

Measurement and Collection Procedures

Urine Collection Procedures

Urine collection procedures were as follows:

1. Collection began with the second void of the 7th day and ended with the first void of the 8th day.

2. During the 24-hr collection period, subjects stored each void in a brown container that was kept in the refrigerator.

3. After the final void, subjects marked the volume and separated the sample into four individual containers and discarded the remaining specimen. The four containers were then frozen until urinalysis in the laboratory occurred.

Urea Nitrogen Measurement Procedures

After thawing the four containers, the following assay procedures sequentially occurred:

1. After agitating each container, a 1 ml sample of urine was pipetted and placed in a separate sterile container.

2. Using a graduated cylinder, 10 ml of distilled water was added to each sterile container. Each container was then agitated to ensure complete dilution.

3. Four 50 µl samples of urine were pipetted from each of the four diluted samples for each subject and placed in separate test tubes. The 16 test tubes were
appropriately labeled. Of the 4 test tubes per participant sample, 2 test tubes received 150 µl of distilled water and 2 received 350 µl.

4. For each of the four samples, the first two test tubes received 200 µl of urease added under the surface of the diluted urine using a pipette. Test tubes were pipetted every 15 s and each was immediately transferred to a circulating water bath (34°C) for a 20 min incubation period.

5. The second two test tubes, for each of the four samples, were also placed in the water bath but did not receive the urease. This procedure allowed the investigators to account for the ammonia content in the urine.

6. To generate a standard curve, 0 µl, 25 µl, 50 µl, 75 µl, and 100 µl of urea nitrogen standard was pipetted in duplicate into 10 test tubes. A proportional amount of distilled water was pipetted into each test tube to create a 200 µl sample in each tube. Each test tube was also incubated with 200 µl of urease for 20 min.

7. After the 20 min incubation, 5 ml of Reagent A, phenol and sodium nitroprusside, was systematically pipetted into all test tubes at 15 s intervals.

8. Immediately following, 5 ml of Reagent B, sodium hydroxide and sodium hypochlorite, was added to all the test tubes.

9. After adding Reagent A and B, the test tubes remained in the water bath for 40 min. All test tube samples were poured into cuvettes and analyzed spectrophotometrically at a wave length of 560 mm.

10. Urea nitrogen levels, ammonia excretion and standard curve data were recorded and filed (see Data Collection Form, Appendix F).
Self-Perceived Wellness Levels

Self-perceived sleep, food intake, exercise, stress and energy levels were evaluated by each subject, before bedtime, on a numerical scale. Participants were given the opportunity to provide a brief written description to clarify the numerical value chosen. Journal forms were returned to the researcher on the final day of the study. All seven days of each week were analyzed to evaluate the effectiveness of the protein supplement as it related to self-perceived wellness levels.

Weighing Procedures

Participants were weighed every 8th day. The participants wore long pants, a long sleeved shirt, and no shoes for each of three weight measurement trials. Measures were recorded to the nearest 0.25 lb. The mean of the three trials served as the dependent variable for each of the four weeks.

Statistical Analysis

Data were analyzed by inferential and descriptive statistics. An initial analysis was performed to determine the internal consistency of the assay procedures associated with the 24-hr urea nitrogen collection and analysis. The data from the initial analysis proved consistent; therefore, the dependent variable for the second analysis was the mean of the four 24-hr urea nitrogen samples. A randomized block factorial ANOVA with two research variables, treatment (two levels, high-protein drink and no drink) and trials (2 weeks) was used to analyze the urea nitrogen levels. Descriptive statistics, including means and standard deviations, were used to analyze self-perceived wellness levels and body weight.
CHAPTER IV

RESULTS AND DISCUSSION

Introduction

The problem of this study was to determine the effect of supplemental dietary protein in women 40–70 years of age. Specifically, this study investigated the effects of a 2-week high-protein drink supplement on urea nitrogen excretion, self-perceived wellness levels, and body weight. The results will address: (a) independent variables, (b) subject demographics, (c) reliability of the 24-hr urinary urea nitrogen excretion, (d) 24-hr urinary urea nitrogen excretion, (e) self-perceived wellness levels, and (f) body weight. A discussion follows the results section. A randomized block factorial ANOVA design was used to analyze the dependent variables of urinary urea nitrogen excretion, wellness levels, and body weight. The independent variables in the design consisted of a control and a treatment condition. The .05 level of confidence was used to determine statistical significance.

Results

Independent Variables

During Weeks 1 and 3, subjects participated in the control portion of the study. No particular dietary regime was dictated during the control weeks; however, use of protein drinks was prohibited.
During Weeks 2 and 4, subjects participated in the treatment segment of the study. No particular dietary regime was mandated; however, consumption of one 8-oz high-protein drink at approximately mid-morning and another at mid-afternoon occurred on each day.

Subject Demographics

The average age for the subjects was 50.8 years, with a standard deviation of 3.97. Although the study began with 11 subjects, only 10 completed data collection. The mean urea nitrogen level for the subjects during Week 1 was 8.89 g per 24 hr. The initial mean body weight was 80.4 kg with a standard deviation of 20.38 kg.

Reliability of the 24-Hour Urinary Urea Nitrogen Excretion Measurements

The means and standard deviations for urinary urea nitrogen excretion are included in Appendix G. The randomized block ANOVA summary for the 24-hr urinary urea nitrogen excretion measurements is presented in Table 1. This initial analysis consisted of two factors: weeks (4) and samples (4). The analysis indicated the following:

1. A significant difference in 24-hr urea nitrogen excretion was found among subjects, $F(9, 135) = 104.17, p < .05$.

2. A significant difference existed for the main effect, weeks, $F(3, 135) = 18.76, p < .05$.

3. No significant difference existed for the main effect, samples, $F(3, 135) = 0.34, p > .05$. The means for samples 1, 2, 3, and 4 were 8.32 g, 8.61 g, 8.43 g, and 8.14 g, respectively.
4. No significant interaction effect, weeks by samples, was found, $F(9, 135) = 0.46, p > .05$.

The results of the samples indicated that pipetting and diluting procedures were consistent.

**Table 1**

**ANOVA Summary for 24-Hour Urea Nitrogen Excretion**

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>$F$</th>
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<tbody>
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<td>9</td>
<td>107.79</td>
<td>104.17*</td>
</tr>
<tr>
<td>Weeks (W)</td>
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<td>3</td>
<td>82.48</td>
<td>18.76*</td>
</tr>
<tr>
<td>Samples (S)</td>
<td>4.52</td>
<td>3</td>
<td>1.51</td>
<td>0.34</td>
</tr>
<tr>
<td>W x S</td>
<td>18.31</td>
<td>9</td>
<td>2.04</td>
<td>0.46</td>
</tr>
<tr>
<td>Residual</td>
<td>593.39</td>
<td>135</td>
<td>4.40</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.

**24-Hour Urinary Urea Nitrogen Excretion**

Since no significant difference was found among the samples, the mean of the four samples was used as the dependent variable in analyzing the research hypotheses in this investigation. A randomized block factorial design using two factors, conditions (2) and weeks (2), was utilized. The results presented in Table 2 revealed the following:

1. A significant difference in 24-hr urea nitrogen excretion was found among subjects, $F(9, 27) = 7.07, p < .05$. 
2. No significant difference, in 24-hr urea nitrogen excretion, was found for the main effect, conditions, $F(1, 27) = 0.02, p > .05$. The means for the control and supplement conditions were 8.33 g and 8.42 g, respectively.

3. A significant difference was found in 24-hr urea nitrogen excretion, for the main effect, weeks, $F(1, 27) = 5.57, p < .05$. The means for Weeks 1 and 2 were 7.65 g and 9.11 g, respectively.

4. A significant interaction effect, conditions by weeks, was found, $F(1, 27) = 10.63, p < .05$.

Table 2

ANOVA and Simple Main Effect Summaries for 24-Hour Urea Nitrogen Excretion Collapsed for Mean Sample Values

<table>
<thead>
<tr>
<th>Source</th>
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</thead>
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<td>9</td>
<td>26.95</td>
<td>7.07*</td>
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<tr>
<td>Conditions (C)</td>
<td>0.08</td>
<td>1</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>21.24</td>
<td>1</td>
<td>21.24</td>
<td>5.57*</td>
</tr>
<tr>
<td>$C \times W$</td>
<td>40.53</td>
<td>1</td>
<td>40.53</td>
<td>10.63*</td>
</tr>
<tr>
<td>$C$ at $W1$</td>
<td>18.46</td>
<td>1</td>
<td>18.46</td>
<td>4.84*</td>
</tr>
<tr>
<td>$C$ at $W2$</td>
<td>22.16</td>
<td>1</td>
<td>22.16</td>
<td>5.81*</td>
</tr>
<tr>
<td>$W$ at control</td>
<td>1.54</td>
<td>1</td>
<td>1.54</td>
<td>0.40</td>
</tr>
<tr>
<td>$W$ at supplement</td>
<td>60.23</td>
<td>1</td>
<td>60.23</td>
<td>15.80*</td>
</tr>
<tr>
<td>Residual</td>
<td>102.94</td>
<td>27</td>
<td>3.81</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.
Since the interaction effect for conditions by weeks was significant, a simple main effects test was calculated. The results were:

1. The control condition was significantly different from the supplement condition at Week 1, $F(1, 27) = 4.84, p < .05$. The means for control and supplement conditions at Week 1 were 8.61 g and 6.69 g, respectively.

2. The control condition was significantly different from the supplement condition at Week 2, $F(1, 27) = 5.81, p < .05$. The means for control and supplement condition at Week 2 were 8.06 g and 10.16 g, respectively.

3. Weeks 1 and 2 for the control condition were not significantly different, $F(1, 27) = 0.40, p > 0.05$. The means for Control Weeks 1 and 2 were 8.61 g and 8.06 g, respectively.

4. Weeks 1 and 2 for the supplement condition were significantly different, $F(1, 27) = 15.80, p < .05$. The means for Supplement Weeks 1 and 2 were 6.69 g and 10.16 g, respectively.

**Self-Perceived Sleep Levels**

The means and standard deviations for self-perceived sleep, food intake, exercise, stress, and energy levels are included in Appendix H. The ANOVA summary for self-perceived sleep levels is presented in Table 3. The ANOVA for sleep levels indicated the following results:

1. A significant difference was found among subjects, $F(9, 243) = 5.34, p < .05$.

2. No significant difference existed for the main effect, conditions, $F(1, 243) = 0.08, p > .05$. 
3. No significant difference existed for the main effect, weeks, $F(1, 243) = 1.76, p > .05$.

4. A significant difference existed for the main effect, days, $F(6, 243) = 1.89, p < .05$.

5. No significant difference existed for the first- or second-order interaction effects.

Table 3
ANOVA Summary for Self-Perceived Sleep Levels

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
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<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<td>Subjects</td>
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<td>9</td>
<td>2.78</td>
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</tr>
<tr>
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<td>1</td>
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<td>0.08</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>0.91</td>
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<td>0.91</td>
<td>1.76</td>
</tr>
<tr>
<td>Days (D)</td>
<td>5.85</td>
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<td>0.98</td>
<td>1.89*</td>
</tr>
<tr>
<td>C × W</td>
<td>0.91</td>
<td>1</td>
<td>0.91</td>
<td>1.76</td>
</tr>
<tr>
<td>C × D</td>
<td>3.14</td>
<td>6</td>
<td>0.52</td>
<td>1.00</td>
</tr>
<tr>
<td>W × D</td>
<td>2.44</td>
<td>6</td>
<td>0.41</td>
<td>0.79</td>
</tr>
<tr>
<td>C × W × D</td>
<td>1.64</td>
<td>6</td>
<td>0.27</td>
<td>0.52</td>
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<tr>
<td>Residual</td>
<td>125.81</td>
<td>243</td>
<td>0.52</td>
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</tr>
</tbody>
</table>

*Significant at .05 level.

Self-Perceived Food-Intake Levels

The ANOVA summary for self-perceived food intake is presented in Table 4.

The ANOVA for food intake indicated the following results:
1. No significant difference existed among subjects, $F(9, 243) = 1.55, p > .05$.

2. No significant difference existed for the main effect, conditions, $F(1, 243) = 3.75, p > .05$.

3. No significant difference existed for the main effect, weeks, $F(1, 243) = 0.43, p > .05$.

4. No significant difference existed for the main effect, days, $F(6, 243) = 0.47, p > .05$.

5. No significant difference existed for the first- or second-order interaction effects.

Table 4
ANOVA Summary for Self-Perceived Food-Intake Levels

<table>
<thead>
<tr>
<th>Source</th>
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<td>Subjects</td>
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<td>9</td>
<td>0.65</td>
<td>1.55</td>
</tr>
<tr>
<td>Condition (C)</td>
<td>1.58</td>
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<td>1.58</td>
<td>3.75</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>0.18</td>
<td>1</td>
<td>0.18</td>
<td>0.43</td>
</tr>
<tr>
<td>Days (D)</td>
<td>1.17</td>
<td>6</td>
<td>0.20</td>
<td>0.47</td>
</tr>
<tr>
<td>$C \times W$</td>
<td>0.29</td>
<td>1</td>
<td>0.29</td>
<td>0.69</td>
</tr>
<tr>
<td>$C \times D$</td>
<td>2.40</td>
<td>6</td>
<td>0.40</td>
<td>0.95</td>
</tr>
<tr>
<td>$W \times D$</td>
<td>1.80</td>
<td>6</td>
<td>0.30</td>
<td>0.71</td>
</tr>
<tr>
<td>$C \times W \times D$</td>
<td>2.69</td>
<td>6</td>
<td>0.45</td>
<td>1.07</td>
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<tr>
<td>Residual</td>
<td>102.44</td>
<td>243</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.
Self-Perceived Exercise Levels

The ANOVA summary for self-perceived exercise levels is presented in Table 5. The ANOVA for exercise levels indicated the following results:

1. A significant difference was found among subjects, $F(9, 243) = 6.25, p < .05$.

2. No significant difference existed for the main effect, conditions, $F(1, 243) = 0.86, p > .05$.

3. No significant difference existed for the main effect, weeks $F(1, 243) = 1.15, p > .05$.

Table 5
ANOVA Summary for Self-Perceived Exercise Levels

<table>
<thead>
<tr>
<th>Source</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>34.29</td>
<td>9</td>
<td>3.81</td>
<td>6.25*</td>
</tr>
<tr>
<td>Condition (C)</td>
<td>0.52</td>
<td>1</td>
<td>0.52</td>
<td>0.86</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>0.70</td>
<td>1</td>
<td>0.70</td>
<td>1.15</td>
</tr>
<tr>
<td>Days (D)</td>
<td>9.74</td>
<td>6</td>
<td>1.62</td>
<td>2.67*</td>
</tr>
<tr>
<td>C × W</td>
<td>0.06</td>
<td>1</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>C × D</td>
<td>5.24</td>
<td>6</td>
<td>0.87</td>
<td>1.43</td>
</tr>
<tr>
<td>W × D</td>
<td>0.75</td>
<td>6</td>
<td>0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>C × W × D</td>
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<td>6</td>
<td>0.63</td>
<td>1.04</td>
</tr>
<tr>
<td>Residual</td>
<td>147.71</td>
<td>243</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.
4. A significant difference existed for the main effect, days, $F(6, 243) = 2.67$, $p < .05$.

5. No significant difference existed for the first- or second-order interaction effects.

Self-Perceived Stress Levels

The ANOVA summary for self-perceived stress levels is presented in Table 6.

<table>
<thead>
<tr>
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</tr>
<tr>
<td>Condition (C)</td>
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<td>1.03</td>
<td>1.72</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>1.58</td>
<td>1</td>
<td>1.58</td>
<td>2.62</td>
</tr>
<tr>
<td>Days (D)</td>
<td>10.77</td>
<td>6</td>
<td>1.80</td>
<td>3.01*</td>
</tr>
<tr>
<td>$C \times W$</td>
<td>1.29</td>
<td>1</td>
<td>1.29</td>
<td>2.16</td>
</tr>
<tr>
<td>$C \times D$</td>
<td>4.14</td>
<td>6</td>
<td>0.69</td>
<td>1.15</td>
</tr>
<tr>
<td>$W \times D$</td>
<td>1.00</td>
<td>6</td>
<td>0.17</td>
<td>0.29</td>
</tr>
<tr>
<td>$C \times W \times D$</td>
<td>3.49</td>
<td>6</td>
<td>0.58</td>
<td>0.97</td>
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<td>Residual</td>
<td>144.88</td>
<td>243</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.

The ANOVA for stress levels indicated the following results:

1. A significant difference existed among subjects, $F(9, 243) = 4.86$, $p < .05$. 
2. No significant difference existed for the main effect, conditions, $F(1, 243) = 1.72, p > .05$.

3. No significant difference existed for the main effect, weeks, $F(1, 243) = 2.62, p > .05$.

4. A significant difference existed for the main effect, days, $F(6, 243) = 3.01, p < .05$.

5. No significant difference existed for the first- or second-order interaction effects.

**Self-Perceived Energy Levels**

The ANOVA summary table for the self-perceived energy levels is presented in Table 7. The ANOVA for energy level indicated the following results:

1. A significant difference existed among subjects, $F(9, 243) = 5.49, p < .05$.

2. A significant difference existed for the main effect, conditions, $F(1, 243) = 7.09, p < .05$.

3. No significant difference existed for the main effect, weeks, $F(1, 243) = 0.15, p > .05$.

4. No significant difference existed for the main effect, days, $F(6, 243) = 0.91, p > .05$.

5. No significant difference existed for the first- or second-order interaction effects.
Table 7
ANOVA Summary for Self-Perceived Energy Levels

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
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</thead>
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<td>2.20</td>
<td>5.49*</td>
</tr>
<tr>
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<td>2.80</td>
<td>7.09*</td>
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<tr>
<td>Weeks (W)</td>
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<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>Days (D)</td>
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<td>6</td>
<td>0.36</td>
<td>0.91</td>
</tr>
<tr>
<td>C × W</td>
<td>0.23</td>
<td>1</td>
<td>0.23</td>
<td>0.58</td>
</tr>
<tr>
<td>C × D</td>
<td>3.25</td>
<td>6</td>
<td>0.54</td>
<td>1.36</td>
</tr>
<tr>
<td>W × D</td>
<td>4.79</td>
<td>6</td>
<td>0.80</td>
<td>2.03</td>
</tr>
<tr>
<td>C × W × D</td>
<td>2.42</td>
<td>6</td>
<td>0.40</td>
<td>1.01</td>
</tr>
<tr>
<td>Residual</td>
<td>96.03</td>
<td>243</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.

Weight

The means and standard deviations for weight over the 4-week investigation are included in Appendix I. The ANOVA summary table for weight is presented in Table 8. The ANOVA for weight indicated the following:

1. No significant difference existed among subjects, $F(9, 27) = 1.42, p < .05$.
2. No significant difference existed for the main effect, conditions, $F(1, 243) = 0.03, p > .05$.
3. No significant difference existed for the main effect, weeks, $F(1, 243) = 0.03, p > .05$. 

4. No significant difference existed for the first-order interaction effect, condition by weeks.

Table 8
ANOVA Summary for Body Weight

<table>
<thead>
<tr>
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<th>F</th>
</tr>
</thead>
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<td>1.42</td>
</tr>
<tr>
<td>Condition (C)</td>
<td>0.00</td>
<td>1</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Weeks (W)</td>
<td>0.03</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>C × W</td>
<td>0.42</td>
<td>1</td>
<td>0.42</td>
<td>0.48</td>
</tr>
<tr>
<td>Residual</td>
<td>23.81</td>
<td>27</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level.

Discussion

Based upon the results of Table 1, it would appear that measurement errors due to sample dilution and pipetting procedures were controlled. It was found that there was a significant difference for the main effect, weeks, but there were no significant differences among the 24-hr urea nitrogen samples. Since significant differences were not found among the samples, the data presented in Table 2 represent the mean of the four samples. Analysis of the 24-hr urea nitrogen samples indicated that a significant difference existed among the subjects, as well as among the weeks. No significant difference was found for the main effect, conditions. However, there was a significant interaction effect between conditions by weeks; therefore, simple main effects were calculated. Calculation of simple main effects
revealed a significant difference between Control Week 1 and Supplement Week 1. It was hypothesized that the supplementation condition would produce higher urea nitrogen excretion levels. However, the means for urea nitrogen excretion indicated levels were lower for Supplement Week 1 than Control Week 1.

No information was found in the literature pertaining to urea nitrogen excretion in older women over a 4-week period. Therefore, it is difficult to ascertain the reason for decreased urea nitrogen excretion for Supplement Week 1, as compared to Control Week 1. Although independent laboratory tests supported the findings, perhaps the investigator made errors in the urea nitrogen analysis. It is also possible that the subjects failed to store the specimen properly for Supplement Week 1. It may also be hypothesized that the subjects drastically increased their exercise regime from Supplement Week 1 to Supplement Week 2. This may have resulted in an increase in protein catabolism which would be reflected in the higher urea nitrogen excretion levels. However, the exercise journal reflects no significant changes in exercise throughout the course of the study. It may also be possible that during the initial supplement period the subjects were slightly dehydrated, which may have impeded the catabolism of amino acids into urea. However, the urine volumes expressed in Appendix J do not reflect a significant change throughout the study.

For Week 2 of the control period, subjects returned to values similar to Control Week 1. Supplement Week 2 produced an elevation in urea nitrogen excretion above Control Weeks 1 and 2. Supplement Week 2 was significantly higher than Supplement Week 1. It was theorized that the subjects became familiar with the supplement protocol for Week 1. Therefore, for Supplement Week 2 they consumed the drink in addition to their normal diet.
No significant differences were found in the amount of urea nitrogen excretion between Control Week 1 and Control Week 2. Although urea nitrogen excretion levels during the control weeks were relatively stable, the supplement weeks fluctuated from lower than control levels to ultimately higher than control levels.

It was also noted that urea nitrogen excretion for Supplement Week 1 was significantly lower than Supplement Week 2. The low amount of urea nitrogen excretion for Week 1 may be attributed to a low protein intake, despite supplementation. Although the food-intake journal reflects no significant difference between the weeks, it is possible that the subjects may have used the supplement drink as a meal replacement, rather than as a dietary supplement. This may have reduced protein consumption to a lower level than each subject’s normal intake, and thus may have put the subjects into slight negative nitrogen balance. This theory, however, is in direct conflict with the findings of Friedman and Lemon (1989). Their research indicated that urinary urea nitrogen excretion tends to be higher when individuals consume a low protein diet. It should be noted that the investigation of Friedman and Lemon analyzed the effect of chronic exercise on protein retention in endurance athletes. The average age of the athletes was 27 years.

The purpose of the self-perceived wellness journal was to support urea nitrogen excretion results, as well as to determine the psychosomatic effect of the drink upon the subjects. It was found that sleep levels remained unchanged during the course of the investigation. However, significantly more sleep was obtained during the weekend than during weekdays. The ANOVA for food intake revealed no significant difference in food consumption for the duration of the study. In addition, self-perceived exercise levels were similar except during the weekend. The results
indicated lower amounts of exercise during the weekend than during the week. Similar results were found pertaining to stress. The subjects reported stress levels significantly lower during the weekend than during the week. Ironically, energy levels were reported to be significantly higher during supplement weeks than during control weeks. However, a significant difference did not exist among weeks or days for self-perceived energy levels. This contradicts the findings of Herron (1994), who found protein snacks did not have an effect upon energy levels.

The anthropometric measure of body weight was utilized to determine if the additional protein or fat would be stored by the body or excreted in the urine. If the protein was stored, body weight should have increased. According to the results presented in Table 8, it appeared that weight was unchanged over the course of the 4-week investigation. This may be attributed to complete protein utilization; however, many extraneous factors may also be responsible.
CHAPTER V

SUMMARY, FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Introduction

The purpose of this study was to determine the effect of 2 weeks of intermittent high-protein drink supplementation upon urea nitrogen excretion. The research is discussed under the following headings: (a) Summary, (b) Major Findings, (c) Conclusions, and (d) Recommendations.

Summary

This study investigated the effects of supplemental protein on older women (ages 40–70 years), over a 4-week period. Supplementation was delivered in the form of a liquid drink and was consumed in addition to their normal diet. Urea nitrogen excretion was measured to determine whether the supplemental protein would be utilized by the body or excreted in the form of urea nitrogen in the urine. A self-perceived journal was also documented by each subject to rate the amount of sleep, food intake, exercise, stress, and energy obtained over the 4-week period.

The 10 female subjects initially experienced a 7-day control period. During this time, normal diet was consumed and protein drinks were prohibited. For 7 days following the control period, subjects experienced the treatment period. During this time, subjects consumed their normal diet in addition to drinking one 8-oz high-protein drink at approximately mid-morning and another at mid-afternoon each day.
This control and treatment protocol was repeated once. The subjects were instructed to begin the 24-hr urine collection with the second void of the morning of the 7th day. The collection ended with the first void of the 8th day. Urinary urea nitrogen excretion was measured using a combination of reagents to initiate a catalyzed reaction. Urea nitrogen concentration levels were determined with a Hitachi spectrophotometer, Model 100-10. Samples were read at a wavelength of 560 mm.

Self-perceived sleep, food intake, exercise, stress, and energy levels were evaluated every evening by each subject on a numerical scale. Body weight was recorded every 8th day using a Memorie Computer Bath Scale (Hanson, Japan). The mean of three trials represented the subject’s weight for each week.

Seven randomized block factorial ANOVAs were calculated using the following dependent variables: (a) urea nitrogen excretion, (b) sleep level, (c) food-intake level, (d) exercise level, (e) stress level, (f) energy level, and (g) weight. The independent variables in the design were the repeated measures of the control and treatment conditions. All hypotheses were tested statistically at the .05 level of significance.

Major Findings

1. The initial analysis of variance revealed there was not a significant difference found among samples, $F(3,135) = 0.34, p > .05$. This indicated the pipetting procedure used was consistent and not a source of error.

2. The second analysis of variance utilized the mean of the four 24-hr urea nitrogen samples as the dependent variable. It was found that there were significant simple main effects. For instance, there was a significant difference between the control and supplement condition at Week 1, $F(1, 27) = 4.84, p < .05$. It was
expected that the supplement week would produce higher urea nitrogen excretion levels; however, Week 1 revealed just the opposite. It was found that the control condition produced higher urea nitrogen excretion levels than the treatment condition. The means for the control and supplement condition at Week 1 were 8.61 g and 6.69 g, respectively. It was believed that the subjects used the supplement drink as a meal replacement, thus lowering their normal protein consumption. This may have put the subjects into a slight negative nitrogen balance state.

3. There was a significant difference found between the control and supplement condition at Week 2, $F(1, 27) = 5.81, p < .05$. During Week 2, the treatment condition produced higher urea nitrogen excretion levels than the control condition. This was to be expected due to the increased amount of protein introduced into the diet. The means for the treatment and control condition at Week 2 were 10.16 g and 8.05 g, respectively.

4. There was no significant difference between Control Weeks 1 and 2, $F(1, 27) = 0.40, p > .05$. The means for Control Weeks 1 and 2 were 8.61 g and 8.05 g, respectively.

5. There was a significant difference between Supplement Weeks 1 and 2, $F(1, 27) = 15.80, p < .05$. Supplement Week 1 produced lower urea nitrogen excretion levels than Supplement Week 2. The means for Supplement Weeks 1 and 2 were 6.69 g and 10.16 g, respectively. It was theorized that once the subjects became familiar with the supplement regime, they were able to consume their normal diet in addition to the protein drink.

6. The analysis of variance for sleep revealed a significant difference for the main effect days, $F(6, 243) = 1.89, p < .05$. It was found that more sleep was obtained on the weekend than on the weekdays.
7. The analysis of variance for exercise revealed a significant difference for the main effect, days, $F(6, 243) = 2.67, p < .05$. It was found that more exercise was obtained on the weekend than on the weekdays.

8. The analysis of variance for stress revealed a significant difference for the main effect, days, $F(6, 243) = 3.01, p < .05$. It was found that stress was less likely during the weekend than during the weekday.

9. The analysis of variance for energy revealed a significant difference for the main effect, conditions, $F(1, 243) = 7.09, p < .05$. It was found that more energy was perceived during supplement weeks than during control weeks.

10. The analysis of variance for body weight revealed no significant difference for the main effect, conditions, $F(1, 27) = .03, p > .05$. Weight did not change over the course of the 4-week investigation, despite the additional protein.

Conclusions

Based on the results of this study, it appeared that 4 weeks of dietary supplementation of 30 grams of protein, per day, by women aged 40–70 years, does have a significant effect upon urinary urea nitrogen excretion levels and self-perceived energy levels. However, sleep, food intake, exercise, stress, and body weight were unaffected by protein supplementation.

Recommendations

Further research is need to confirm the present findings and to examine the effects of high-protein drinks over a longer supplementation period. Several suggestions to improve the testing of urea nitrogen include: (a) immediate analysis following the 24-hr specimen collection, (b) preservation of urine integrity before
freezing with a bacterial inhibitor, (c) requiring subjects to journal specifically all food consumption, and (d) requiring subjects to journal specifically all exercise.
Appendix A

Recruitment Script
Tammy Moran, an HPER graduate student, is looking for volunteers to participate in her master’s thesis research. The study is titled, “The effects of supplemental protein on urea excretion in older women.” The study requires 10 female volunteers, between the ages of 40 and 70 years, who are free of renal disease and have maintained a stable weight of plus or minus 5 pounds over the last 6 months.

Volunteers will be asked to complete a health history questionnaire to determine if they qualify to participate. Coronary artery disease, renal disease, diabetes, pregnancy or other health conditions that may put a participant at risk will result in exclusion from the study. If accepted, the subject must read and sign a consent form prior to participating.

Participants will be asked to commit to a 4-week dietary regimen. Location of supplementation and urine collection will be determined by the subject. Subjects will be required to consume an 8-oz high-protein drink twice a day for a period of 6 days during Week 2 and Week 4. In addition, four 24-hour urine collection samples will be required over the 4-week period. Body weight will also be determined following each dietary protocol as well as recordings of self-perceived energy levels.

At any time, voluntary termination of involvement in the study is permitted for any reason. All test information is strictly confidential, although copies of your individual test results will be provided to you upon your request. If you are interested in getting more information or volunteering for the study, please fill out the information below or contact Tammy Moran by phone at (616) 692-2850. Thank you.

Name

Phone number
Appendix B
Consent Form
Title: The effects of supplemental protein on urea excretion in women 40 to 70 years old

Principal Investigator: Dr. Roger Zabik

Research Associate: Tammy D. Moran

I have been invited to participate in a research project entitled “The effects of supplemental protein on urea excretion in women 40 to 70 years old”. I know that this research is intended to determine what effects supplemental protein drinks have on urea nitrogen excretion and self-perceived energy levels. This project is a master’s thesis for Tammy Moran in the Department of Health, Physical Education, and Recreation at Western Michigan University.

By consenting to participate in this study, I am indicating that I will participate in a 4-week regimen. Participation will involve four 24-hour urine collections. Instructions for collecting each 24-hour urine specimen are as follows: 1) When subjects first arise on Saturday morning, they will empty their bladder and will not save this urine specimen 2) From this time on, subjects will save, in the urine collection container provided, all of the urine that is passed for the next 24 hours up to and including the final specimen that is first passed on Sunday morning 3) Subjects will keep the urine specimen refrigerated 4) Subjects will either telephone for urine collection pickup, or they will deliver the chilled specimen to the Exercise Physiology Laboratory, located in the Student Recreation Center at Western Michigan University by 6pm on Sunday. During supplemental protein weeks, I will be required to consume one 8oz can of a liquid protein drink at approximately mid-morning and mid-afternoon of each day. I will either telephone for urine collection pickup or I will deliver the chilled specimen to the Exercise Physiology Laboratory, located in the Student Recreation Center at Western Michigan University by Sunday. At the time of pickup or delivery, I will be asked to stand on a scale in order to record body weight. In addition, I will be asked to evaluate and record my energy, sleep, exercise, stress and food intake levels on a daily basis, before bedtime, for the duration of the four weeks on supplied journal forms. I will be asked to evaluate the quantity of my sleep, how much food I ate, how much exercise I obtained, how much stress I encountered and my perceived energy level on a numerical scale. I may also provide comments if I see fit that may help qualify my numerical choice.

I may benefit from participation by developing a better understanding of the importance of protein in the diet as well as the need to perhaps supplement the diet with protein drinks. I may also benefit from receiving my own results and having them explained thoroughly to me. I may also learn more about my energy level from my journal entries.

As in all research, there may be unforeseen risks to the participant. If an accidental injury occurs, appropriate emergency measures will be taken, however, no compensation or treatment will be made available to me except as otherwise stated in this consent form. There are potential
risks involved, such as dehydration or renal complications, and all appropriate measures will be taken to minimize these risks. The investigators and assistants involved in the study are CPR and First Aid trained and emergency procedures are posted in the Exercise Physiology Laboratory. I may terminate my involvement with this research at any time without prejudice or penalty. All information is confidential and my name will not appear in any reports or presentations other than on a list of identification codes and on this form, which will be handled only by those involved in administering the exercise tests. All data will be retained for a period of 3 years in a locked file controlled by the principal investigator. At the conclusion of the study, a copy of the results will be made available upon my request.

If any questions or concerns arise during the course of this study, I may contact Tammy Moran at (616) 692-2851 or Dr. Zabik at 387-2720. I may also contact the Chair, Human Subjects Institutional Review Board at 387-8293 or the Vice-President for Research at 387-8298 if questions or problems arise during the course of the study. My signature below indicates that I have read the purpose and requirements of this study and I agree to participate.

This consent document has been approved for use for one year by the Human Subjects Institutional Review Board (HSIRB) as indicated by the stamped date and signature of the board chair in the upper right corner. Subjects should not sign this document if the corner does not show a stamped date and signature.

Participant Signature
Date

Research Associate Signature
Date
Appendix C

Human Subjects Institutional Review Board Approval Letter
Date: 23 November 1998

To: Roger Zabik, Principal Investigator
    Tammy Moran, Student Investigator for thesis

From: Sylvia Culp, Chair

Re: HSIRB Project Number 98-10-05

This letter will serve as confirmation that your research project entitled "The Effects of Supplemental Protein on Urea Nitrogen Excretion in Women 40-70 Years Old" has been approved under the full category of review by the Human Subjects Institutional Review Board. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

Please note that you may only conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

Please remember to include the HSIRB project number on all future correspondence in order to speed processing.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: 23 November 1999
Appendix D

Subject Screening Form
SUBJECT SCREENING FORM

Code number: __________________________

Please answer "yes" or "no" to the following questions regarding your health status.

_____ 1. Have you been diagnosed with cardiac disease (angina, heart attack, congestive heart failure, coronary artery disease, or vascular disease), diabetes, renal disease, or pulmonary disease (chronic obstructive pulmonary disease, emphysema)?

_____ 2. Do you smoke?

_____ 3. Have you been diagnosed with high blood pressure, or do you take medication for high blood pressure?

_____ 4. Do you exercise regularly (30 consecutive or intermittent minutes, at least 3 times per week)?

_____ 5. Have you maintained a constant body weight of plus or minus 5 pounds within the past 6 months?

_____ 6. Do you take any medication, prescribed or over the counter? If so, what are you taking?

_____ 7. Do you use illicit drugs? (marijuana, cocaine, amphetamines)?

_____ 8. Are you capable of completing 24-hour urine collections if given detailed instruction?

_____ 9. Do you have any food allergies? If so, please indicate__________________________

Failure to answer any of the above questions will result in elimination from the study. Only those subjects who qualify as "apparently healthy" or at "increased risk" according to the American College of Sports Medicine (ACSM, 1995) will be allowed to participate in the study. Individuals who answered "yes" to more than one item for cardiovascular risk factors 1-3, will not qualify as "apparently healthy" or "increased risk" and will not be allowed to participate in the study. An individual judgment will be made concerning the responses to items 4-9 based on the impact of protein supplementation to the particular individual.
Appendix E
Journal Form
Please respond to the questions below by choosing the appropriate numerical value from the scale that is listed above the questions. Place the numerical value in the space before each question. Use the lines below each question to provide any comments that might clarify your numerical response.

1. much less than usual amount
2. less than usual amount
3. usual amount
4. more than usual amount
5. much more than usual amount

_______ How much sleep did you obtain last night?

_______ How much food did you eat today?

_______ How much exercise did you get today?

_______ How would you rate your stress level today?

_______ How would you rate your energy level today?
Appendix F

Data Collection Form
Data Collection Form

Subject Code __________ Age __________

**Body Weight (kg)**

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**Journal Responses**

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- **Sleep**

- **Food**

- **Exercise**

- **Stress**

- **Energy**

**Urea Nitrogen**

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Appendix G

Urinary Urea Nitrogen Excretion
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Urea Nitrogen Excretion Means in Grams
Appendix H

Self-Perceived Wellness Levels Means and Standard Deviations
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Appendix I

Weight Means and Standard Deviations
Weight Means in Kilograms

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Appendix J

Urine Volume Means and Standard Deviations
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