Dietary Protein and Weight in Midlife Adults

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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April 2013

Acknowledgements

Thank you to my advisors Dr. Susan Raatz, and Dr. Marla Reicks for their hours of input and guidance through the rigorous challenge of doctoral development.

Thank you to my doctoral committee, Dr. Craig Hassel, Dr. Shalamar Sibley, Dr. Len Marquart, Dr. Zata Vickers, Dr. Susan Raatz, and Dr. Marla Reicks for the depth and breadth of knowledge that made this project an especially interesting one.

Thank you to my family for their patience, prayers, and continued support through the years of waiting and growing.

Dedication

Oh, the depth of the riches and wisdom and knowledge of God! How unsearchable are his judgments and how inscrutable his ways! "For who has known the mind of the Lord, or who has been his counselor?" "Or who has given a gift to him that he might be repaid?" For from him and through him and to him are all things. To him be glory forever. Amen. Romans 11:33 – 36 ESV.

If anyone imagines that he knows something, he does not yet know as he ought to know. I Corinthians 8:2 ESV.

Abstract

Current dietary recommendations include 0.8 g/kg/day of protein to meet metabolic requirements of nitrogen and amino acids; however, a growing body of data has identified positive changes in weight, body composition, blood pressure, and metabolic markers with increased intake of protein. Related to increased protein intake, research has been directed toward various sources of protein and composition responsible for specific metabolic responses. Therefore, more research is needed to evaluate the effective use of protein to improve body weight and composition, and perceptions consumers have regarding the role of dietary protein in weight control/maintenance.

The focus of this dissertation was two-fold, 1) to evaluate the effectiveness of increased protein intake for promoting weight loss and improving body composition in a controlled weight loss study, and 2) to evaluate the use of the practice of "eating more protein" to prevent weight gain among midlife women. Additionally, the activity of the Renin-Angiotensin system (RAS) was evaluated to assess related metabolic effects with protein intake.

The first study described the effect of three weight loss diets on body composition, blood pressure, and RAS metabolites. Midlife participants were randomly assigned a control diet (15% protein), a mixed protein diet (30% protein), and a whey protein diet (15% mixed, 15% whey) condition for a 5 month period. Total body weight and fat loss between groups was not significantly different, but a trend toward greater body weight and fat loss was observed with the whey protein diet. No differences in RAS metabolites were observed between diets, but a statistically significant decrease in

systolic blood pressure was observed with the whey protein diet. These results confirm that reduction of energy intake is the primary effective step in weight loss, but secondary effects of regional fat loss and decreased blood pressure may be achieved with a high protein diet containing whey protein.

The second study described survey results of a national panel of midlife women regarding weight maintenance practices and weight self-efficacy. In this cross-sectional survey, "eating more protein" was identified as the fourth most common practice used to prevent weight gain. Self-reported weight loss over 2 years was associated with reporting the use of the eating more protein practice. Although those who gained and lost weight reported similar weight maintenance practices, those who lost weight had significantly higher Weight Efficacy Lifestyle scores than those who gained weight. Educating individuals on the best use of protein to encourage successful weight maintenance may enhance the results.

In conclusion, while many metabolic effects have been identified with increased protein intake, the best use practices for weight maintenance and weight loss continue to be a significant research topic. Increased protein intake has been associated with increased satiety and insulin sensitivity, and decreased blood pressure. Whey protein intake in a high protein weight loss plan may further result in regional fat loss and decreased blood pressure, but the specific mechanisms have not been determined. Among a national sample, midlife women reported eating more protein to maintain weight, and high self-efficacy was associated with successful weight maintenance.

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Chapter One: Introduction

Obesity is a global epidemic associated with an increasing number of deaths each year. The World Health Organization has reported that annual deaths related to being overweight claimed 2.8 million lives worldwide (World Health Organization, 2011). Within the United States (US), a higher prevalence is evident among midlife adults (40 – 60 years) compared to younger adults (20 – 39 years)(Flegal, Carroll, Ogden, & Lester, 2010). On a personal level, the medical costs within the US for an obese individual have been estimated to be $1,400 greater annually (about a 42% increase) compared to a normal weight individual (Finkelstein, Trogdon, Cohen, & Dietz, 2009). Consequently, obesity is one of the greatest contributors to rising health costs in the US.

To address the current obesity epidemic, high protein (HP) reduced energy diets have been suggested as a weight loss strategy. While some positive results from the use of high protein diets have been reported, there are insufficient data to suggest a high protein diet is superior to other reduced-energy strategies. Some practitioners have been hesitant to promote a high protein diet based on concerns of possible promotion of bone loss, or kidney stress during such a diet. In addition, a successful weight loss strategy should not be based upon weight loss alone, but should include improvements in body composition, metabolic markers such as insulin and glucose, and measures of weight loss self-efficacy.

This dissertation addresses four general questions related to weight management and protein intake. First, how does the macronutrient composition of the diet and the protein quality affect weight loss? Second, how does macronutrient composition and protein quality affect body composition? Third, how does macronutrient composition

and protein quality affect biomarkers related to weight loss, including insulin, glucose, and metabolites related to the renin angiotensin system (RAS)? Fourth, how does eating more protein compare as a weight maintenance practice to other known practices chosen by midlife adults for weight maintenance? Answers to these four questions will provide a more complete analysis of the HP diet and its application.

This work included a clinical study to evaluate HP intake as a weight loss strategy in midlife adults and a cross-sectional study of the relationship between protein knowledge, protein intake, weight maintenance and self-efficacy in midlife women based on results from a national survey. The first study was a weight loss study of midlife adults conducted at the General Clinical Research Center of the University of Minnesota. The aims of this study were: 1) to evaluate the effect of HP diets on body composition, 2) to evaluate the effect of whey protein in a HP weight loss diet, and 3) to measure the effects of diet composition on the RAS. The second study was a national survey of midlife women and their reported practices regarding weight maintenance. The aims of this study were: 1) to evaluate the frequency of use of various weight maintenance practices by midlife women from the nine regional areas of the United States and 2) to test for associations between protein intake, weight maintenance over two years, and reported frequency of eating more protein to maintain weight among midlife women. The results of the weight loss study, and information about the relationships between protein intake, self-efficacy, and weight maintenance from the national survey, may provide evidence for a greater understanding of the role of protein to assist individuals in the battle against obesity.

Chapter Two: Literature Review

Obesity

Definition

Obesity is quantified as a statistical relationship between weight and height. The formula kg/m^2 identified today as the Body Mass Index, or BMI, is based on the work of Lambert Adolph Quetelet, a statistician of the 1800's, who was interested in describing the "average man" (Eknoyan, 2008). The National Institutes of Health established a classification for adults based on this BMI calculation to identify the general status of the population. Overweight has been classified as having a BMI $\geq$ 25 kg/m^2 and < 30 kg/m^2, and obesity has been classified as having a BMI $\geq$ 30 kg/m^2, with extreme obesity identified as having a BMI $\geq$ 40 kg/m^2 (National Institutes of Health, 1998). BMI is a helpful tool to evaluate population trends and provide a starting point for definitions and evaluations, but additional measures are needed for individual diagnoses and counseling.

The absolute nature of a single number to describe an individual's weight status has not been satisfactory for addressing individual health protocols. For example, professional athletes may have a high BMI; however, the weight may be primarily from lean tissue and not excess fat. Also, a BMI calculation provides no differentiation between subcutaneous fat vs. visceral fat, or descriptions of regional fat deposition. However, monitoring BMI changes in the population has been effective for bringing the obesity epidemic to the public's attention. As individuals take personal responsibility and seek solutions, additional measures such as anthropometric measurements (circumference and skin folds) and total body composition measures, such as

densitometry, are used to provide a more comprehensive picture of an individual's current physical condition and give more variables for monitoring change.

1. Prevalence

At present, the US is ranked as one of the most obese nations in the world. Comparing estimated BMIs from 199 countries, the highest estimated BMI average among high-income countries was found in the US (Finucane et al., 2011). Finucane et al. (2011) found the prevalence of obesity among men was higher in North America (29.2%) than in any other region of the world, while North American women came in fourth place globally with over 30% prevalence of obesity. There are few places in the world more affected by obesity than the US, but the US is one of the few places with the resources to address this issue that is advancing throughout the world.

The US has been aware of the obesity problem for many years, and is constantly monitoring the current weight status of the population. The National Health and Nutrition Examination Survey (NHANES) has been a consistent tool used since the 1970's to evaluate health status including obesity. According to the measuring cycle that concluded in 2008, the categories of overweight and obese, collectively, have reached a plateau among adult men at about 72%, and among adult women at about 64% of the population (Flegal et al., 2010). While just twenty years ago, no state reported an obesity rate in the population greater than 15%, now twelve states have reported an obesity rate of over 30% and no state has reported an obesity rate less than 15% (Trust for America's Health, 2011). Though some surveys have indicated slower increases or plateaus, there

have not been any significant solutions provided that demonstrate a halt or reversing of the obesity trend.

2. Trends

For more than three decades, an increasing trend in body weight has been observed among all age groups in the United States according to NHANES data. Comparing data from NHANES I (1970-1974) to NHANES III (1990-1994), the prevalence of obesity increased among adults age 20 – 74 years old from 9.5% among men and 10.5% among women to 14.6% among men and 14.2% among women, respectively (Flegal, Carroll, Kuczmarski, & Johnson, 1998). Continued data collection from NHANES (1999-2000) and NHANES (2007-2008) showed increased obesity among adults $\geq$ 20 years old from 27.5% among men and 33.4% among women to 32.3% among men and 35.5% among women, respectively (Flegal et al., 2010). A greater than three-fold increase in obesity has been observed between the first NHANES measures to the most recent, resulting in the current statistical equivalent of 1 in 3 adults within the US being obese.

In the US, regional trends have been observed in the measures of obesity. The US Centers for Disease Control and Prevention (CDCP) (http://www.cdc.gov/) has monitored the progression of obesity across the country with the Behavioral Risk Factor Surveillance System (http://www.cdc.gov/brfss/index.htm), which uses a telephone survey on an on-going schedule to monitor changes on a variety of health behavior issues. All data collected are self-reported, therefore there are some differences compared to objective measures completed by NHANES. According to CDCP statistics,

Mississippi was the first state to report more than 25% of the population as obese in 2001. By 2005, Mississippi, Alabama, and West Virginia reported obesity prevalence ≥ 30%. By 2010, all of the southern states reported obesity rates greater than 25% with the majority of southern states exceeding 30% (Centers for Disease Control and Prevention, 2011).While all states have reported obesity prevalence at about 20%, the southern states have shown the greatest prevalence, which may be related to particular dietary habits, genetic traits, and demographic characteristics unique to the region.

Among age groups, midlife adults (40 – 60 years) have been observed to have a greater prevalence of obesity than any other age group. This relationship has been observed from the first NHANES to the present. In NHANES I, those men reported to be obese with BMI between 30 and 34.9 included 5.9% of young men (20-29 years) and 12.0% of midlife men (50 – 59 years) (Flegal et al., 1998). Thirty-four years later, the national prevalence of obesity included 27.5% of young men (20-39 years) and 34.3% of midlife men (40 -59 years) (NHANES 2007-2008) (Flegal et al., 2010). In NHANES I, those women reported obese with BMI between 30 and 34.9 included 4.9% of young women (20-29 years) and 13.9% of midlife women (50 – 59 years) (Flegal et al., 1998). From the most recent records, the increase in obesity prevalence resulted in 34.0% of young women (20-39 years) and 38.2% of midlife women (40 -59 years) with a BMI between 30 and 34.9 (NHANES 2007-2008) (Flegal et al., 2010). Greater increases in obesity have been observed in the younger age groups, but the midlife group maintains the largest prevalence of obesity at this time.

In addition to age differences, racial differences in obesity trends have been observed in the US. NHANES II (1976 – 1980) data showed that 12% of non-Hispanic black men and 12.4% of Mexican-American men had a BMI between 30 and 34.9 compared to 9.7% of non-Hispanic white men (Flegal et al., 1998). For the same survey, 18.1% of non-Hispanic black women and 15.5% of Mexican-American women were in the Class I obesity category compared to 9.1% of non-Hispanic white women. From NHANES data (2007-2008), obesity was observed in 37.3% of non-Hispanic black men and 35.9% Mexican American men compared to 31.9% of non-Hispanic white men (Flegal et al., 2010). The women participating in this survey demonstrated similar increases with 49.6% of non-Hispanic black women and 45.1% of Mexican-American women being obese compared to 33.0% of non-Hispanic white women. Consequently, the demographic group with the greatest prevalence of obesity in the US is the midlife, black woman, living in the southern states.

Factors

The most basic concept related to the obesity epidemic is the concept of energy imbalance. This concept is based on the thermodynamic principle that energy cannot simply appear or disappear; there is always an accounting for every calorie. Therefore, energy balance is described with the equation: energy in = energy out. In more detail, Caloric intake = Metabolism + Physical Activity + Energy Storage, which represents the distribution of energy that is taken in. Positive energy imbalance is the state where more calories have been taken in than metabolism or physical activity can use resulting in the

remaining energy being stored in the body. The stored energy is typically stored as adipose tissue.

1. Excessive Energy Intake

Much of the increased energy intake observed in the past thirty years has come from increased carbohydrate consumption. Based on NHANES data collected from 1971 - 2000, men and women increased their calorie intake by 168 and 335 kcal/day, respectively, while the composition of carbohydrate in the diet increased by more than 6% for men and women (Wright, Kennedy-Stephenson, Wang, McDowell, & Johnson, 2004). Comparison of NHANES 1 (1971) to NHANES 2006 data showed trends in energy from macronutrient intakes including increases for carbohydrates (44% to 48.7%), and decreases for fats (36.6% to 33.7%) and proteins (16.5% to 15.7%) (Austin, Ogden, & Hill, 2011). Based on food group trends, Americans have consumed recommended levels of protein and grain products, but the average American has been consuming 30 teaspoons of added sugars and sweeteners daily (450 kcal), when the recommendations are to limit added sugars to 8 teaspoons daily (120 kcal) (Wells & Buzby, 2008). Sweetened beverages have been a major contributor to weight gain over the past two decades (Duffey & Popkin, 2011; Mozaffarian, Hao, Rimm, Willett, & Hu, 2011). This increased carbohydrate consumption alone can account for a significant proportion of the weight increase observed in the US.

Total energy intake has been evaluated by the components of portion size, eating occasions, and energy density. When these factors were analyzed statistically, the primary change in the adult American lifestyle was an increase in the number of eating

occasions which contribute to additional calories (Duffey & Popkin, 2011; McCrory, Howarth, Roberts, & Huang, 2011). However, a direct relationship between an increased number of eating occasions and increased adiposity has not been established (McCrory et al., 2011). Mills et al. (2011) found that as the number of eating occasions increased across all weight status categories among midlife women, energy intake also increased. Therefore, eating frequency was associated with energy intake, but not weight status. American culture has found more numerous applications for celebrating with food, but some individuals seem to adjust their total caloric intake better than others.

From the perspective of portion sizes, the American population has received significantly larger portion sizes at restaurants than what is recommended and these portions are larger than portions served in other countries by the same food service corporations (Young & Nestle, 2002). Even with a growing number of health organizations calling for restraint on portion control, the leading fast food restaurants have continued to offer large portions (Young & Nestle, 2007). Creative names such as "Biggie," "Big Gulp," and "Super Size" clearly indicates large portions, so while the food industry may be blamed for larger portion sizes, they are not guilty of false advertising.

2. Reduction in Energy Expenditure

In addition to an increase in energy intake, Americans have decreased their physical activity levels as technology increasingly completes the work that previously required physical labor. This decrease in physical activity results in less calories being used in daily activity resulting in an energy imbalance or a net positive energy balance as more calories are eaten than are used for activity. The decrease in physical activity can

be related to changes in the work place and changes in the way leisure time is filled. Reviewing classification of physical activity records from the Bureau of Labor Statistics between 1960 and 2006, the typical energy expenditure related to work in America has decreased an equivalent of 100 calories/day for both men and women (Church et al., 2011). Additionally, the US government has provided physical activity recommendations for how Americans can increase physical activity during recreation hours (USDHHS, 2008), but evaluations have indicated minimal improvements among US adults (Carlson, Fulton, Schoenborn, & Loustalot, 2010; Tucker, Welk, & Beyler, 2011). Consequently, decreased physical activity results in greater energy imbalance and greater risk of obesity and related diseases.

Along with decreased physical activity, a measurable decrease in metabolic activity or Basal Metabolic Rate (BMR) occurs with age. BMR represents the lowest rate of metabolic activity required to sustain life in an organism and is calculated by measuring the production of carbon dioxide while the organism is at that lowest level of activity (Henry, 2005). Comparison of BMR with progression of age has shown that metabolic activity decreases according to indirect calorimetry methods (Cole & Henry, 2005) and by doubly labeled water methods (Roberts & Dallal, 2005). A decrease in metabolic activity could theoretically increase the energy surplus resulting in weight gain if there is no corresponding reduction in energy intake. However, a study of obese postmenopausal women matched with non-obese postmenopausal women compared resting energy expenditure (REE) at baseline, after weight loss, and four years later, and found that REE did not significantly change between weight loss and four years

(Weinsier et al., 1995). The obese women lost a significant amount of weight to reach normal BMI and equivalent REE to the control group of non-obese women. Four years later, the women who had lost weight had gained a significant portion of the weight again but had maintained a REE similar to the non-obese women who maintained normal BMI. The conclusion in this study was that differences in physical activity and energy intake, and not changes in REE, were the primary reasons for weight regain in the postmenopausal years.

The menopause transition for women can produce changes in body composition due to the change in the production of estrogen and progesterone. A cross-sectional study of midlife Caucasian and Chinese women showed a decrease in lean mass among all women as age increased, and an increase in fat mass related to menopause state among Chinese women, but not among Caucasian women (Sternfeld, Bhat, Wang, Sharp, & Quesenberry, 2005). Sternfeld et al (2005) found that body fat (%) and waist circumference were inversely related to vigorous physical activity regardless of age or menopause status, and total physical activity was not related to lean mass. These findings were supported by a cross-sectional analysis of magnetic resonance imaging measures among European American and African American women between 18 and 80 years of age (Demerath et al., 2011). Demerath et al. (2011) observed increased visceral fat deposition and decreased subcutaneous fat mass with age among all women, but increased physical activity was related to lower visceral fat mass. In a longitudinal study of Caucasian women who had transitioned from pre- to post-menopause, lean mass and fat mass did not change significantly, but the abdominal fat depot was increased 35% at

post- compared to pre-menopause (Franklin, Ploutz-Snyder, & Kanaley, 2009). These women had maintained the same weight and reported the same physical activity at the follow-up eight years later. From these studies, measured increases in fat are related to changes in deposition or reduction in lean mass as a result of the hormonal changes caused by menopause.

Attempts to attenuate the physiological effects brought on by menopause using hormone replacement therapy (HRT) demonstrate the effect that hormone changes have on fat deposition. In a parallel study of three different HRT protocols, all fifty nine overweight postmenopausal subjects had measured decreases in waist circumference and subcutaneous fat, but insignificant changes in body weight and BMI after 6 months of treatment (Yuksel et al., 2007). The HRT treatment seemed to reverse the trend of abdominal fat deposition. However, other HRT protocols have not observed this change in fat deposition. In comparison, a two year study of a HRT treatment with seventy six women found no significant changes in weight or any fat measures at follow-up (Sites et al., 2005). The postmenopausal women in the two year study were within normal BMI at the beginning of the study, so lifestyle management skills or the difference in HRT protocol probably influenced the results.

Although hormonal changes may affect alterations in body composition, these changes do not seem to affect the energy balance equation as significantly as energy intake and physical activity. Even in postmenopause the most significant factor related to body fat was physical activity (Sternfeld et al., 2005). The biochemical processes that result in decreased BMR or lean mass with age may be important pathways to consider

regarding dietary composition, but overall energy intake will still be a key factor to reaching energy balance.

Consequences among Midlife Women

As noted earlier, midlife women in the US have the greatest prevalence of obesity compared to other age groups. Among midlife women, age 40 to 60 years of age, 38% of the population was obese. With respect to racial distinctions, a greater proportion of Black women (52%) in the midlife group were obese, followed by Hispanic women (49%), and Caucasian women (36%) (Flegal et al., 2010). Some investigators have suggested the trend among all women may have reached a plateau in 2004 with 33.2% obese, but no decrease in obesity has been observed at the national level for any demographic group.

1. Increased Risk of Chronic Diseases

With an increase in body weight, the risk of mortality increases. Based on a Nurses' Health Study cohort, a 24 year follow-up study demonstrated that obese women had two times the relative risk of mortality compared to lean women (Hu et al., 2004). Based on data from death certificates, obese subjects had a 3-fold increase in heart disease and an almost 66% increase in cancer compared to lean subjects. Another research team analyzed data from the Nurses' Health Study and found that BMI was a significant predictor of relative risk of type 2 diabetes (Colditz, Willet, Rotnitzky, & Manson, 1995). In a model developed based on data from the Framingham Heart Study and other sources, as BMI in midlife women increased from 22 to 37, the estimated risk of type 2 diabetes increased from 3.2% to 11.2%, respectively (Thompson, Edelsberg,

Colditz, Bird, & Oster, 1999). These data demonstrate the strong correlation between obesity, heart disease, and type 2 diabetes.

In addition, an association has been observed between obesity and various types of cancer. In a recent review, higher risk of endometrial, cervical, and breast cancer were associated with obesity and may be related to increased hormone concentration derived from the increased fat tissue (Kulie et al., 2011) as well as increased systemic inflammation (Dossus et al., 2010). A cohort study in England followed 1.2 million midlife women for five years to observe incidence of cancer and cancer mortality and found a 2.73 relative risk of endometrial cancer incidence, and 2.54 relative risk of esophageal cancer incidence in obese women, relative to normal weight women (Reeves et al., 2007). Reeves et al (2007) estimated that 51% of endometrial cancer was related to being overweight. Significant increases in endometrial and breast cancer have been observed in overweight women after menopause (World Cancer Research Fund / American Institute of Cancer Research, 2007). Indeed, while increased body fat may be protective against breast cancer in pre-menopause, no such protection is provided after menopause (Reeves et al., 2007). Therefore, a motivational factor to lose weight or prevent weight gain may be the desire to reduce the risk of cancer, heart disease, and diabetes.

2. Decreased Life Expectancy

An additional motivational factor for avoiding obesity or for losing weight may be the desire to live a long and healthy life. The Prospective Studies Collaboration combined 57 prospective studies and observed that the average obese individual had a

reduction of 2 – 4 years in life expectancy, and a morbidly obese individual had a reduction of 8 – 10 years in life expectancy (Prospective Studies Collaboration, 2009). The effect of weight reduction on cancer mortality was evaluated in a retrospective cohort study of gastric bypass subjects compared to controls (Adams et al., 2009). Based on the cohort of midlife adults, cancer mortality was reduced by 46% in the bypass group compared to the control group. Indeed, rates of coronary artery disease decreased (56%) and diabetes decreased (92%) in the same cohort comparison (Adams et al., 2007). Gastric bypass surgery is not an ideal solution; however, the positive effect of weight loss on these risk factors should motivate individuals and health organizations to pursue the benefits of healthy weight maintenance.

3. Economic Issues

Additional motivation for pursuing healthy weight maintenance may be based on the economic impact of obesity. Between 1987 and 2003, the top ten medical conditions presenting the greatest rise in prevalence included five conditions often associated with obesity (Thorpe, 2006). These five conditions (hypertension, hyperlipidemia, arthritis, pulmonary disorders, and heart disease) were related to a 19% increase in medical spending during the same time period (Thorpe, 2006). The increased cost translates to an additional annual expense of $266 and $1723, per overweight and obese individual, respectively (Tsai et al., 2011; Tsai, Williamson, & Glick, 2011). Tsai et al. (2011) estimated that these numbers represented about $114 billion in national health care costs in 2008 related to obesity. Therefore, it is in the economic interest of the nation for weight maintenance to be promoted more effectively.

For the individual, increased health expenses may drain personal financial resources, and this stress may be realized more by obese women compared to obese men. Using data from the Medical Expenditure Panel Survey, estimates comparing differences in medical expenditures for individuals with increasing BMI classifications showed increasing expenditures with increasing BMI across all race and gender comparisons (Finkelstein et al., 2008). In this analysis, white women reported the greatest increases and sustained expenditures compared to white men, and Black Americans. In a comparison of NHANES I Epidemiologic Follow-up Study data and Medicare files, a simulation estimated lifetime Medicare spending for the average 45-year-old until time to death (Cai, Lubitz, Flegal, & Pamuk, 2010). In all categories of BMI, women would incur greater Medicare spending. Perhaps the greatest impact on personal and national finances may be realized by addressing the obesity prevalence in the nation.

Approaches to Treatment

Numerous organizations have developed recommendations to address the obesity epidemic in the US (American Dietetic Association, 2009; Blue Cross and Blue Shield, 2011; CDCP, 2011; National Heart, Lung, and Blood Institute, 2011; National Instituted of Health, 2009). The National Institutes of Health (NIH) direct major funding to numerous obesity studies each year to add to the knowledge base to effectively fight obesity. Health insurance agencies have initiated policies to encourage healthy lifestyle decisions, such as Blue Cross Blue Shields "Do" campaign (Blue Cross and Blue Shield, 2011), as well as fee reductions for maintaining a healthy BMI. However, a significant

motivation and action among large groups of individuals necessary to change the trends of obesity in the US are lacking.

1. The Position of the Academy of Nutrition and Dietetics

The Academy of Nutrition and Dietetics (AND) provides nutrition and dietary recommendations to the general public through its membership of Registered Dietitians (RDs). The official position of the AND on weight management recommends 1) assessment of BMI and BMR to establish goals and outcomes for changing weight, 2) a reduced-calorie diet to produce a 500 to 1,000 kcal deficit per day preferably by reducing fat and carbohydrate content, 3) reducing portion sizes to help generate the deficit in calories, 4) distributing carbohydrate intake evenly throughout the day, and 5) a complete program including a dietary plan, physical activity plan, problem solving and management resources (American Dietetic Association, 2009). Within these guidelines, a reduced-energy diet by means of reducing fat content is identified as "the best studied" and "most frequently recommended" diet strategy (American Dietetic Association, 2009). These guidelines provide the framework of general principles followed by RDs throughout the US as they counsel clients regarding weight loss.

2. Dietary Guidelines for Americans

Additional guidelines on healthy dietary practices are available to the public in the Dietary Guidelines for Americans 2010 document presented by the US Department of Health and Human Services (USDHHS) and the US Department of Agriculture (USDA). Within this document, energy balance is identified as the model for promoting weight management (USDA and USDHHS, 2010). Energy balance can be achieved through

changes in physical activity and changes in macronutrient intake adaptable to the individual lifestyle. Macronutrient intake is to be maintained within the levels of Acceptable Macronutrient Distribution Ranges (AMDRs), which recommend total calorie intake to be distributed within the range of 10 – 35% from protein, 45 -55% from carbohydrates, and 20 – 35% from fat (Otten, Hellwig, & Meyers, 2006). Specific recommendations within the Dietary Guidelines include reducing or eliminating sweetened beverages and reducing saturated fat intake, while increasing vegetable and lean protein servings (USDA and USDHHS, 2010). Within the most recent policy document(USDA and USDHHS, 2010), slightly more attention is given to the role protein may play in providing increased satiety to weight maintenance goals because of the increased public attention to HP diets for weight loss compared to previous Dietary Guidelines (USDA and USDHHS, 2005).

High Protein Diets

Definition

The description of a HP diet is based in part on the Recommended Daily Allowance (RDA) established by the Institute of Medicine, and is on the upper range of the AMDR. The RDA for protein intake is established at 0.80 g/kg/day for both men and women (Otten et al., 2006). According to data collected by the USDA in the "What We Eat in America," NHANES 2007-2008 study, the average consumption of protein by male and female adults was about16.0% of energy intake (USDA, 2010). With the rate of protein consumption at 16% of energy intake among the adult population, a diet with

increased protein ≥ 25% of energy intake from any source of protein is described as a HP diet in the literature.

Animal Studies

Effects of changes in diet composition have been easily observed in numerous rat studies and provide indications of similar measures to observe in human studies. In a study stimulating weight gain, followed by weight loss, and then followed with different diet compositions, Sprague-Dawley rats given a HP (60% of energy as protein) diet after weight loss maintained similar fat mass and fat cell size to the control group (25% of energy as protein), which had never gained the excess weight (Jen, 1988). In comparison, rats placed on the control diet after losing weight tended to eat more and increase their adipose stores again. At the end of the study, those rats on the HP diet had restored lean body mass to similar levels as the control group and had significantly less percent body fat compared to the high fat and high carbohydrate diet groups. Although a diet with 60% of energy as protein would not be reasonable for human consumption, an increase in protein content in daily practice may provide similar benefits for weight control.

Dietary protein content may be an important factor to which the body responds by prompting increased consumption until the protein needs are met. Sprague-Dawley rats were randomized to five different isocaloric diet groups containing increasing amounts of protein (5, 10, 20, 35, 60% energy as protein) and decreasing amounts of carbohydrate (70, 65, 55, 40, 14% energy as carbohydrate) for eight days, followed by the choice to

consume a diet with 5% protein or 35% energy as protein (White, Porter, & Martin, 2000). While no difference in daily food intake was observed between groups, increased selection of the HP diet was observed in those rats that had the least protein in their first randomized diet. White et al. (2000) went on to test if increased methionine would result in reduced food intake in a diet that included protein at 10% of energy and found that increased methionine alone was not sufficient to reduce the daily food intake among the rats. In both of these studies, decreased protein content in the diet resulted in increased body fat in those rats that were fed a low protein diet, but HP diets resulted in greater lean body mass.

Protein content of the diet can be used to regulate food intake and body fat mass in rats. In a set of studies looking at the protein to lipid ratio, Wistar and Sprague-Dawley rats were randomized to different diet compositions that had increasing protein and lipid content with some diets being carbohydrate free to test the hypothesis that the combination of fat and carbohydrate leads to energy imbalance (Marsset-Baglieri et al., 2004). Indeed, the rats with the greatest increase in weight were those on a high fat and carbohydrate diet. When the carbohydrate was replaced with protein, although the diet could be described as isocaloric, the daily food intake decreased significantly. Even though the rats were on high fat diets, the increased protein preserved lean body mass and did not promote increased fat mass.

The carbohydrate to lipid ratio becomes a key combination to overall energy intake and fat mass changes. In a study looking at changes in the carbohydrate:lipid ratio in HP diets, Wistar rats were randomized after an adaption period and fed for forty-two

days (Pichon, Huneau, Fromentin, & Tome, 2006). As the carbohydrate:lipid ratio decreased, weight gain decreased. Rats on the carbohydrate-free diet were slightly smaller, but this decrease in size was accounted for by the decreased fat stores because lean body mass was greater. Pichon et al (2006) found that the higher carbohydrate:lipid ratio was related to higher liver fatty acid synthase enzyme activity. Consequently, the carbohydrate content in the diet was the chief factor regulating how the lipids were metabolized in these rats. The higher the carbohydrate content, the greater quantity of fat was stored, while absence of carbohydrate forced the body to metabolize more of the fat for immediate energy use.

Human Studies

Studies among human subjects also suggest benefits from a HP diet. Recent studies have documented beneficial effects with respect to an increase in satiety, improved insulin sensitivity, increased thermogenesis, and decreased blood pressure with increased protein intake.

1. Satiety

In crossover designed studies, subjects reported greater satiety after consuming HP meals compared to low protein (LP) meals (Lejeune, Westerterp, Adam, Luscombe-Marsh, & Westerterp-Plantenga, 2006; Smeets, Soenen, Luscombe-Marsh, Ueland, & Westerterp-Plantenga, 2008). Plasma concentrations of glucagon-like peptide 1 (GLP-1) and ghrelin were examined to observe associations with reported satiety. After consuming a HP meal, the plasma concentration of GLP-1 was briefly lower compared to consumption of the LP meal (Smeets et al., 2008); however, a significant difference was

not observed in plasma concentrations of GLP-1 and ghrelin during a 24-hour study of HP meals compared to LP meals (Lejeune et al., 2006). In a 16-week study, subjects placed on a HP ad libitum diet demonstrated satiety by decreasing their calorie intake by 441± 63 kcal/d from the isocaloric levels at the start of the study (Weigle et al., 2005). The decrease in calorie intake per day resulted in about a 5 kg loss in weight over the 16 weeks of the study, a decrease in leptin concentrations, and an increase in ghrelin area under the curve (AUC) values compared to baseline. The increased plasma concentration of ghrelin did not produce an increase in ad libitum consumption, which suggested the dietary protein had a greater satiating effect. Similar decreases in calorie intake (-483 kcal/day in overweight individuals) as a result of increasing protein consumption to 25% of energy intake was predicted from NHANES 2005-2006 data (Austin et al., 2011). This decrease in calorie intake due to the satiety effect of protein was observed in a three-week trial of isocaloric diets, where men were randomized to HP (1.00g/kg/day), moderate protein (0.75 g/kg/day), and LP (0.50 g/kg/day) assignments (Apolzan, Carnell, Mattes, & Campbell, 2007). The desire to eat rating increased as protein intake decreased. Those consuming the HP diet demonstrated a significantly lower hunger rating. These studies evaluated satiety based on subjects self-report or eating activity, since a clear biochemical marker associated with satiety has not been identified.

2. Insulin Sensitivity

HP diets have demonstrated a significant effect on increasing insulin sensitivity, maintaining glucose homeostasis, and decreasing lipid concentrations of the blood. Both in weight maintenance and in weight loss diets, the effect of HP diets was a lowering of

blood glucose and an increased sensitivity to insulin (Gannon, Nuttall, Saeed, Jordan, & Hoover, 2003; Layman et al., 2003; Layman, Shiue, Sather, Erickson, & Baum, 2003). Gannon et al. (2003) reported a decrease in glycated hemoglobin, an increase in plasma glucagon, and a decrease in triacylglycerol when untreated diabetic subjects crossed over to a 30% protein weight maintenance diet. Layman et al. (2003) found similar improvements with healthy overweight midlife women on a HP weight loss diet and attributed this to increased consumption of branch chain amino acids (BCAA) which interact with the gluconeogenesis pathway (Layman et al., 2003; Layman, Shiue et al., 2003). Nuttall et al. (2003) observed an increased amount of amino acids retained *in vivo* in a group of untreated diabetic subjects consuming a HP diet (30% protein) as increased consumption of protein did not result in a linear increase in urea production (Nuttall, Gannon, Saeed, Jordan, & Hoover, 2003). The increased protein intake resulted in an improved blood glucose response and additional protein synthesis may account for some of the missing nitrogen.

3. Thermic Effect

Increased protein intake results in an increased thermic effect due to protein metabolism. The increased energy expenditure is associated with gluconeogenesis, ureagenesis, and protein synthesis from peptide bond formation, all of which release energy during chemical processing. The rate of gluconeogenesis, the production of *de novo* glucose, has been described as a constant rate with variation between individuals (Nuttall, Ngo, & Gannon, 2008). However, as amino acids become the substrate for glucose production, deamination of amino acids is required with subsequent removal of

the ammonia product through the urea cycle, an activity of the liver. A comparison between a 70% carbohydrate diet and a 70% protein diet in a twelve-hour period showed a significant increased thermic effect with the HP diet compared to the high carbohydrate (HC) diet (Robinson et al., 1990). The increased thermic effect was related to increased protein synthesis and increased ureagenesis while consuming the HP diet. Therefore, the significant portion of thermic effect produced from increased protein intake was associated with ureagenesis and protein synthesis as gluconeogenesis remains relatively constant.

The increased thermic effect of a HP diet has been observed in a number of settings. Through an infusion of an amino acid solution, Tappy et al. demonstrated that both obese and lean subjects had equivalent, measurable increases in thermic expenditure (Tappy, Jequier, & Acheson, 1993). All subjects showed an increase in energy expenditure equivalent to 22% of the energy value of the amino acids infused. Another crossover-designed study demonstrated increased thermogenesis from HP diets through increased body temperature and increased resting energy expenditure (REE) following HP meals (Johnston, Da, & Swan, 2002). In a four-week hypocaloric diet study, Baba et al. (1999) provided all the food to obese hyperinsulinemic subjects and found the HP diet did not cause as great a decrease in REE as the HC diet (Baba et al., 1999). The investigators noted a higher expenditure of energy and a significant decrease in total body water in the HP subjects as measured by bioelectrical impedance. An eight-week restricted-calorie HP diet did not result in a difference in REE in type 2 diabetics compared to a LP diet, but an increased thermic effect of food was observed for women

on the HP diet (Luscombe, Clifton, Noakes, Parker, & Wittert, 2002). However, this study measured the change in REE following four weeks of energy balanced meals, so the decreased REE observed in the study by Baba et al. (1999) may have been associated with negative energy balance and not diet composition. With HP diets, both men and women had increased thermogenesis, but men demonstrated greater responses (Luscombe et al., 2002). The observed thermogenesis following protein consumption has been suggested as a partial explanation for increased weight loss observed with HP diets.

4. Blood Pressure

Increased protein intake has been associated with decreased blood pressure in a number of cross-sectional studies. From a study of a Japanese population, measured increments of total protein intake were associated with decreases in systolic and diastolic blood pressure (Umesawa et al., 2009). A recent study involving elderly subjects in Brazil showed that increased hypertension was correlated with insufficient protein intake (Lopes, dos Santos, Lima-Costa, & Caiaffa, 2011). In contrast, a survey of older residents in the Netherlands found no relationship between risk of hypertension and intake of total protein in subjects less than 70 years old, but an increased risk of hypertension when more animal protein was consumed by those older than 70 years (Altorf-van der Kuil et al., 2010). When animal protein intake was specifically measured by urinary 3-methylhistidine excretion, a significant relationship between increased protein intake and decreased blood pressure was noted in the Chinese population (Liu, Ikeda, & Yamori, 2002). In the INTERMAP Study with enrolled subjects from the US, China, Japan, and the United Kingdom, regression analysis identified vegetable protein as

the significant nutrient with an inverse relationship to blood pressure (Elliot et al., 2006). In a meta-analysis of previous studies, Liu et al. (2002) found that nine studies with a total of 20,904 men and women provided strong evidence for a decrease in blood pressure associated with dietary protein intake (Liu, Ikeda , Sullivan , Ling, & Yamori, 2002). In a variety of populations, and with different measures, a significant relationship between protein intake and blood pressure has been found through cross-sectional studies. However, the question that remains unanswered is whether the additional protein intake or the decrease in carbohydrate intake is causing the decrease in blood pressure, since the two macronutrients are typically substituted for one another.

Increased protein intake and its effect on blood pressure have been more closely evaluated through a number of human clinical trials. In a 5-month weight loss study of 100 subjects, the subjects in the HP group lost a similar amount of weight as the HC group; however, the systolic blood pressure of the HP group was significantly lower (Muzio, Mondazzi, Harris, Sommariva, & Branchi, 2007). Similar results were observed with 82 subjects in a weight maintenance study. Following a recent weight loss, both the HP and HC subjects maintained weight loss for twelve months, but the HP subjects measured a 14.3 mmHg decrease from baseline in systolic blood pressure compared to a 7.7 mmHg decrease for the HC subjects (Delbridge, Prendergast, Pritchard, & Proietto, 2009). Both studies involved free-living subjects participating in weight loss and weight maintenance protocols, so some of the changes can also be attributed to the change in weight.

Two recent studies evaluated the replacement of carbohydrate with protein to clarify if increased protein or decreased carbohydrate affected blood pressure. When animal protein (36-50 g/day) was substituted for carbohydrate with 30 hypertensive individuals for eight weeks, systolic blood pressure decreased significantly compared to the control group, while body weight remained the same (Hodgson, Burke, Beilin, & Puddey, 2006). The Omniheart randomized trial reported the results of a cross-over study in which carbohydrate was replaced with protein or with unsaturated fat for six-week periods (Appel et al., 2005). Protein and unsaturated fat both resulted in a lowering of blood pressure when the subjects switched to these diets from the higher carbohydrate diet, but only the higher protein diet lowered blood pressure to a statistically significant level for the pre-hypertensive group. In addition, the hypertensive subjects observed a greater decrease in blood pressure than the pre-hypertensive subjects. The increased protein consumed in the Omniheart study came from a variety of animal and plant sources. A mixed source of protein was also used in the PREMIER clinical trial in which about 66% animal protein and 34% plant protein comprised the daily protein intakes (Lin et al., 2010). In this trial, an increased intake of the plant protein (0.4% total kcal) was significantly associated with decreased blood pressure at six months, but not at the end of the 18-month trial (Wang et al., 2008). While this study among hypertensive subjects demonstrated a small initial effect from plant protein, the change in blood pressure was not sustained, which may be attributed to an insignificant change in diet.

Plant protein intake has been identified in a prospective study as a significant factor for reducing hypertension in younger subjects. In a prospective study of a Spanish

cohort of university students (n=5,880), follow up analysis showed that increased vegetable protein was significantly correlated with decreased risk of hypertension (Alonso, Beunza, Bes-Rastrollo, Pajares, & Martinez-Gonzalez, 2006). In a similar population, the CARDIA study evaluated dietary intake with nutrient measures from blood samples taken from 18 to 30 year olds across the US (Steffen et al., 2005). Results from this fifteen-year study showed that plant food intake was inversely related to blood pressure after adjusting for confounding factors. The general description of plant food intake seems more appropriate than vegetable protein because of the unknown influences of phytonutrients associated with vegetable intake. The influence of vegetable protein on blood pressure among hypertensive subjects in the PREMIER study seems to be small. The effect of plant food on younger subjects is positive in these studies, but Steffen et al. (2005) pointed out that increased plant food is also related to healthier behaviors such as exercise, use of supplements, lower caloric intake, and less smoking. Consequently, the influence of vegetable protein on blood pressure has only been inferred through statistical analysis and not by clinical trial. While the increased intake of vegetable matter may result in a decrease in blood pressure, the effect may be more likely to be related to increased fiber and phytonutrients than to the protein content of the vegetables.

Conclusion

A variety of animal and human clinical studies with both men and women have demonstrated positive effects of increased protein intake on weight loss, insulin sensitivity, satiety, and decreased blood pressure. Increased satiety and thermogenesis may be key factors contributing to the observed weight loss and may offer additional

control to the individual who desires to change dietary habits that have contributed to weight gain. Increased insulin sensitivity and decreased blood pressure provide further incentive that a healthy weight goal can be reached by changing the dietary composition.

Protein Metabolism

The metabolism of protein is an active process taking place in every cell of the body. The process has also been called protein turnover in an attempt to describe the input and output of proteins, or the catabolism and anabolism of amino acids and the biochemical cycles involved. The complete description of cellular protein metabolism is beyond the work of this dissertation, but a brief review of some of the primary metabolic pathways within the liver and within the cell in regulating the amino acid pool will be presented.

Primary pathways in the Liver

The liver is a central participant in the regulation of amino acids in the amino acid pool. After a meal, the concentration of amino acids first increases in the blood of the portal vein and the hepatocytes begin to selectively absorb the amino acids depending upon the current physiological needs. The liver will take up about 50 – 60% of the amino acids from a meal (Gropper, Smith, & Groff, 2009); however, branch chain amino acids are not significantly absorbed in the liver, but are taken up primarily by the muscle tissue for metabolism (Stipanuk, 2000a).

Once the amino acid is within the cytosol, a number of reactions are possible. If the amino acid is not used to build a new protein, the amino acid can be transaminated

into another amino acid, or deaminated to a carbon skeleton to be transformed into glucose or another product and the remaining ammonia group used to make urea or added to another carbon skeleton. Since there is no specific storage for "extra" protein in the body, a specific biochemical process must be initiated to determine the fate of the amino acid.

1. Transamination

Transamination involves the transfer of the amino group from the carbon skeleton of the original amino acid to a new carbon skeleton. For each amino acid, a specific aminotransferase will catalyze the transfer of the amino group in the presence of pyridoxal 5`-phosphate (PLP). Among the most active in the aminotransferase family are the alanine aminotransferase (ALT) and the aspartate aminotransferase (AST), which transfer amino groups from alanine, glutamate, and aspartate (Gropper et al., 2009).

In the presence of the enzyme and PLP, the amino group is removed from the amino acid leaving a carbon skeleton, or keto acid as shown in Figure 2 – 1. The ketone

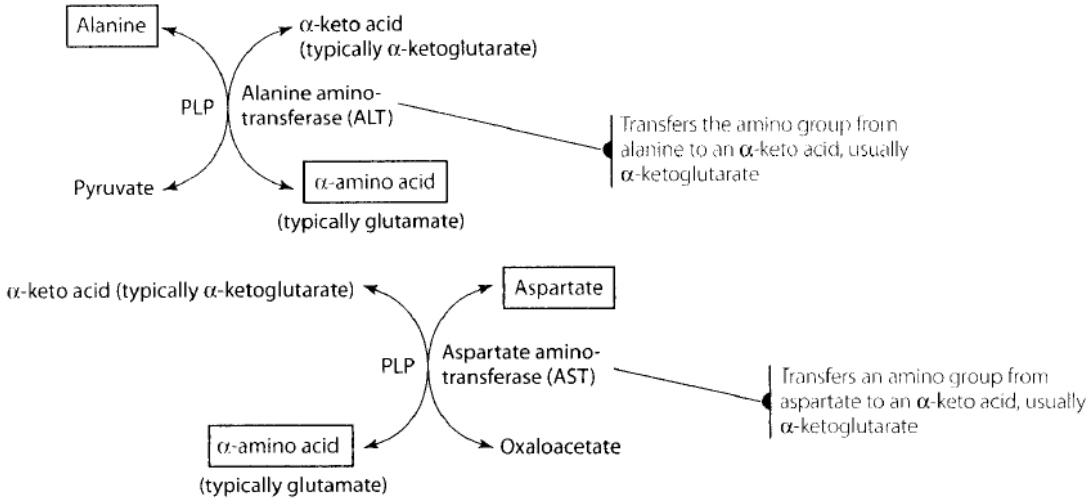

Figure 2 – 1. General transamination pathway (Gropper et al., 2009)

acid is available for transamination or processed further to acetyl CoA. Each of the amino acids has a pathway to enter the citric acid cycle to generate ATP as shown in Figure 2 – 2. Therefore, if the body does not use the amino acid in protein synthesis, the biochemical pathways will transform the molecule for energy production or other non-protein reactions.

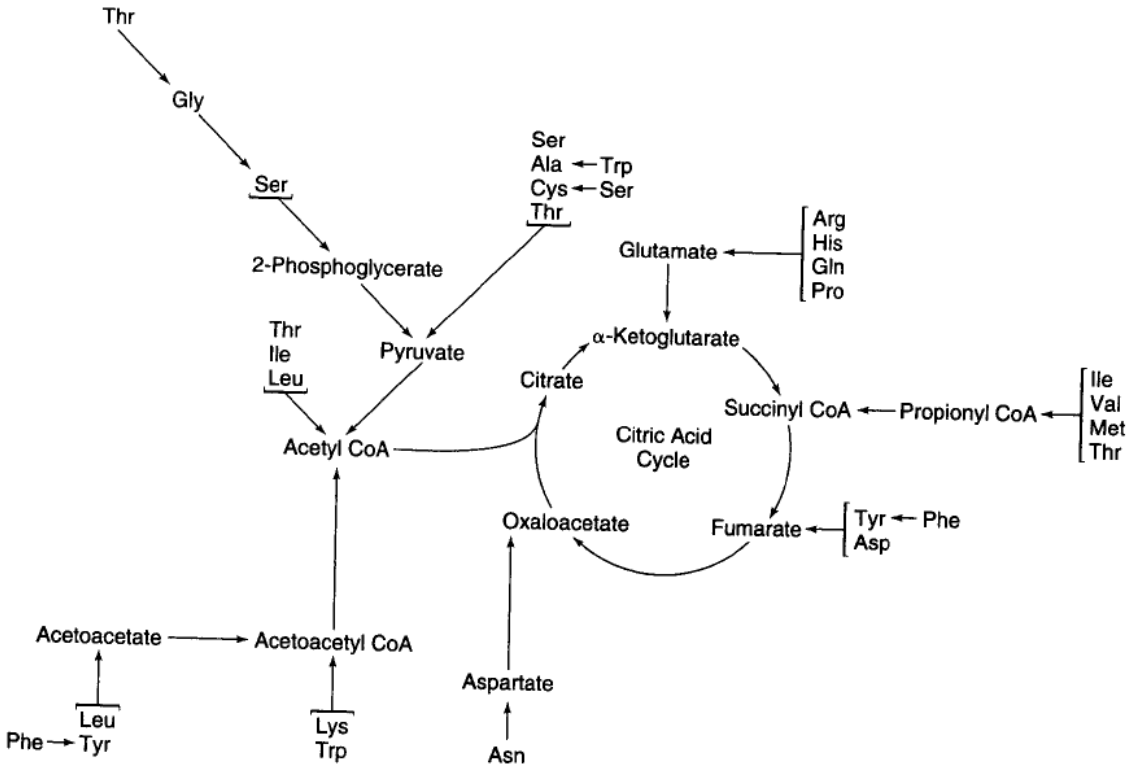

Figure 2 – 2. Biochemical pathways of AA to the Citric Acid Cycle (Stipanuk, 2000a)

Transamination provides adaptability in the amino acid pool depending on the biochemical needs of the body at the moment. Transamination will change the identity of the amino acid in preparation for protein synthesis, or gluconeogenesis and ureagenesis.

2. Deamination

Deamination is the biochemical process of removing the amino group when the carbon skeleton will be used for a new chemical structure, and not to build a new amino acid as in transamination. This process can be catalyzed by lyases, dehydratases, or dehydrogenases primarily in the periportal hepatocytes (Gropper et al., 2009). Many of the transaminases catalyze the formation of glutamate, which is then deaminated by glutamate dehydrogenase, which yields α-ketoglutarate and ammonia (Stipanuk, 2000a). The α-ketoglutarate can be shuttled to the citric acid cycle (Figure 2 - 2) or used to generate more glutamate and the ammonia becomes a reactant for ureagenesis.

3. Gluconeogenesis

Once the carbon skeleton, or α keto acid, is transformed into a reactant of the citric acid cycle within the mitochondria, then oxaloacetate can be shuttled toward production of phosphoenol pyruvate (PEP) to promote production of glucose through gluconeogenesis. Gluconeogenesis is an active biochemical reaction occurring primarily in the liver and as needed in the kidneys (Stipanuk, 2000b). The process of producing glucose from oxaloacetate requires the use of energy to transform oxaloacetate into malate, aspartate, or PEP as shown in Figure 2 – 3. Each of these products can leave the mitochondria to produce PEP in the first step toward reverse glycolysis.

Amino acids whose carbon skeletons can be transformed into intermediates of the citric acid cycle are described as glucogenic, while amino acids whose carbon skeletons are typically transformed into keto acids are described as ketogenic. Only leucine and lysine are totally ketogenic as their carbon skeleton generates acetyl CoA, which can be

transformed into ketone bodies. All other amino acids are described as glucogenic, or partially glucogenic and ketogenic (Gropper et al., 2009).

Gluconeogenesis is dependent upon the availability of glucogenic reactants to complete the reaction. Because the primary fuel for the liver is derived from fatty acids, the amino acids, lactate, pyruvate, and fructose are always available above and beyond the fuel need of the liver (Nuttall et al., 2008). A review of the literature

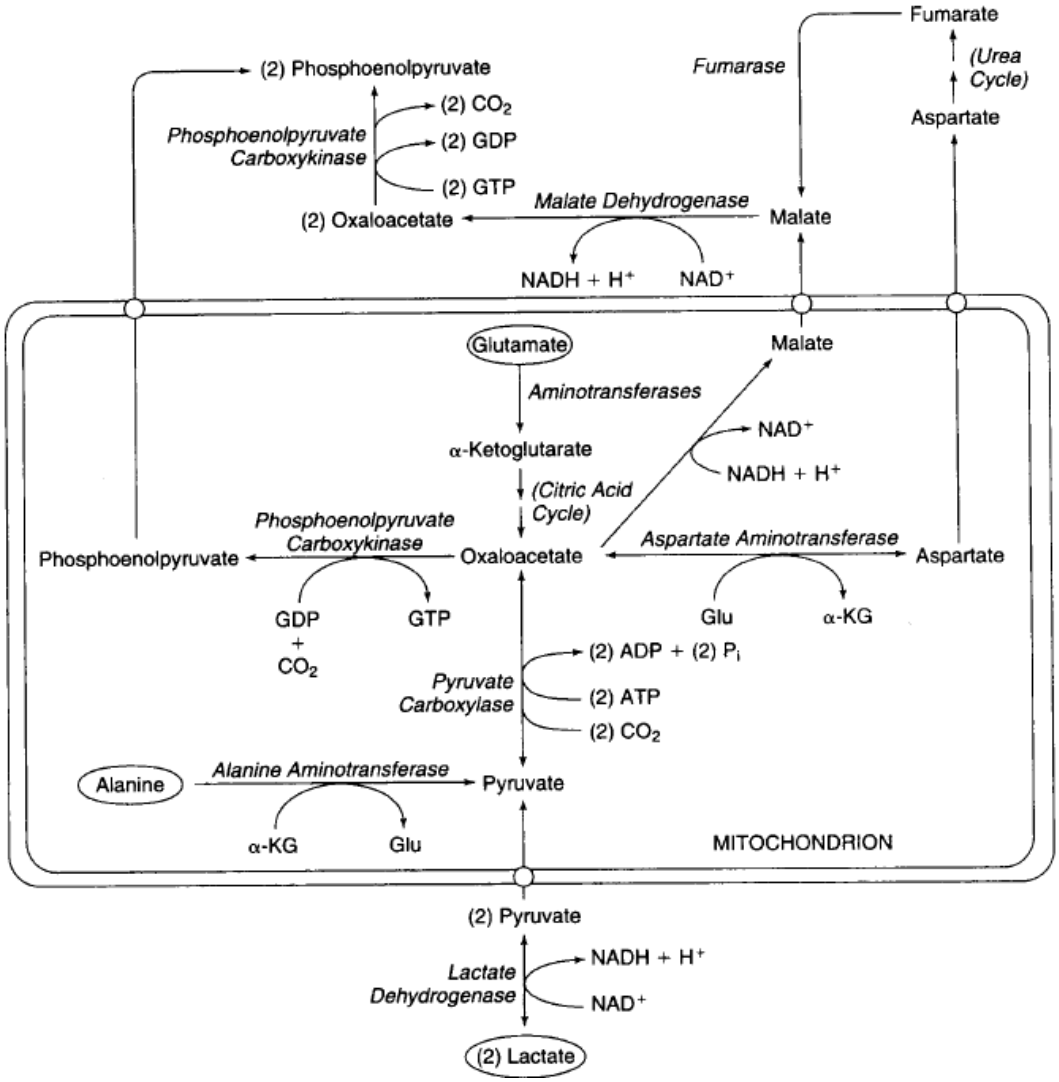

Figure 2 – 3. Gluconeogenic pathways from the mitochondria (Stipanuk, 2000b)

by Nuttall et al. (2008) found the rate of gluconeogenesis to be constant with very little change due to fasting or recent food intake. In healthy lean and obese individuals, changes in endogenous glucose output have been observed, but this change in output is due to the change in glycogenolysis rate (Chevalier et al., 2006; Nuttall et al., 2008). Changes in gluconeogenesis have been observed in catabolic conditions such as type 2 diabetes (Basu, Schwenk, & Rizza, 2004; Boden, Chen, & Stein, 2001) and lung cancer (Leij-Halfwerk et al., 2000). The normal state of gluconeogenesis is a well-controlled, constant turnover of carbon skeletons into PEP and eventually glucose.

4. Ureagenesis

With the uptake of the carbon skeleton of the amino acid into the gluconeogenesis pathway, the ammonia group must be processed immediately to reduce toxicity. The liver is the primary organ that contains all the enzymes required for processing the ammonia into urea, though some urea production has been observed in the kidneys (Haines, Pendleton, & Eichler, 2011). The rate of ureagenesis under normal conditions has the capacity to process the quantity of ammonia produced in catabolic processes (Morris, 2002). The urea produced is released into the blood stream to be taken up by the kidney for excretion.

The urea cycle is a tightly governed process involving enzymes inside and outside the mitochondria (Haines et al., 2011). Ammonia reacts with carbon dioxide in the presence of carbamoyl phosphate synthetase in the mitochondria to form carbamoyl phosphate. Carbamoyl phosphate bonds to the ammonia group of ornithine in the

presence of ornithine transcarbamoylase to form citrulline, which is able to leave the mitochondria. The citrulline reacts with aspartate to bond with a third ammonia group forming argininosuccinate. Argininosuccinase removes the aspartate carbon skeleton to form fumurate, which can be incorporated into the Krebs cycle, or arginine. In the presence of arginase, the arginine molecule is split into two molecules, urea and ornithine, and ornithine returns to the mitochondria to repeat the cycle, as shown in Figure 2 – 4.

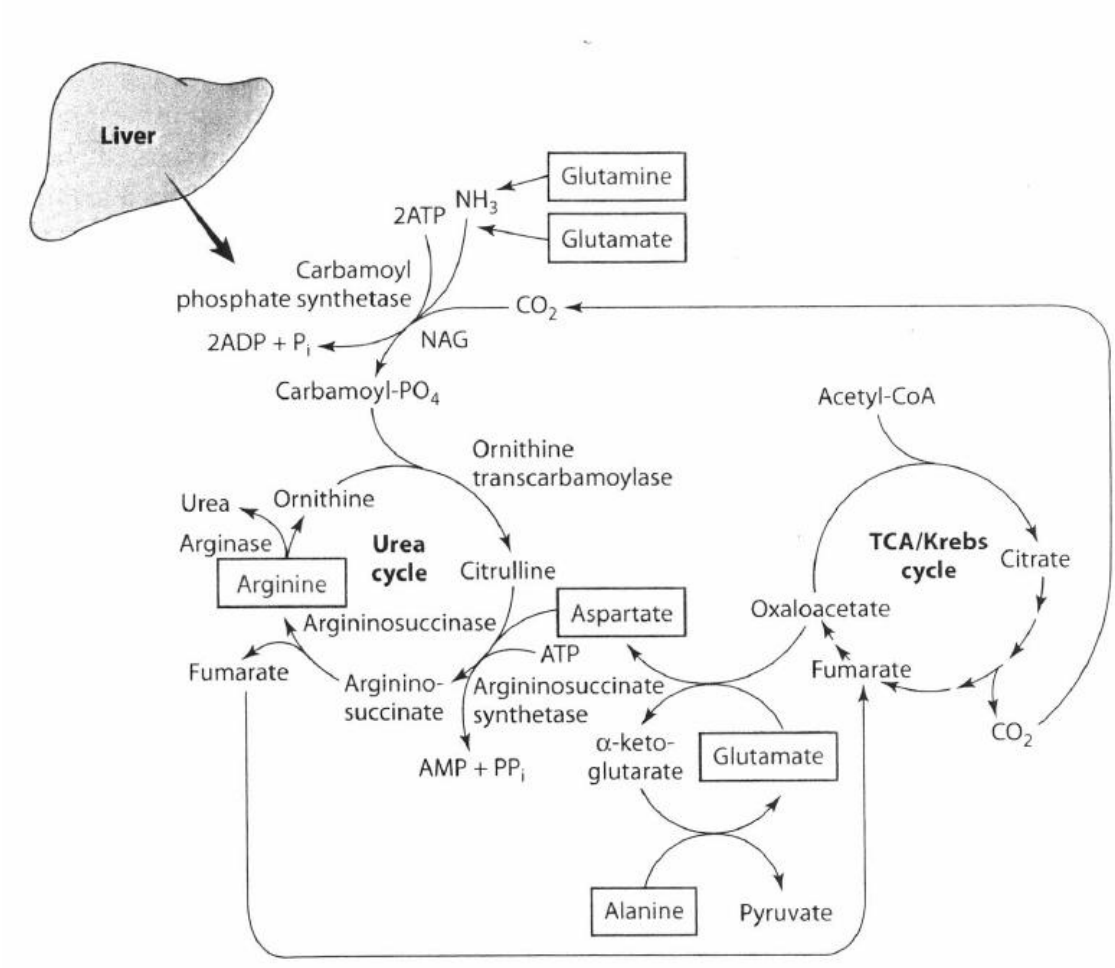

Figure 2 – 4. Pathways associated with urea cycle (Gropper et al., 2009)

General Protein Turnover

1. Protein anabolism

As described above, amino acids can be processed as an energy source as needed. However, the primary use of amino acids in the body is to build the unique proteins necessary for daily cellular activity. This anabolic process has become more clearly defined in recent years providing identification of some of the key mechanisms that moderate protein synthesis in the cells.

Increased cytosolic concentrations of amino acids, either from intracellular catabolism or plasma membrane import, will signal multiple responses. One of the key mechanisms regulating these multiple responses is the mammalian target of rampamycin (mTOR). Pathways signaling mTOR include protein kinase B (PKB), (Pallafacchina, Calabria, Serrano, Kalhovde, & Schiaffino, 2002; Sandri, 2008) insulin-like growth factor 1 (IGF-1), (Song et al., 2005) and the concentration of amino acids in the cytosol (Sancak et al., 2010). The activated mTOR complex signals the beginning of the anabolic process by phosphorylating the inhibitor molecule, eukaryotic initiation factor 4E binding protein 1 (eIF4E-BP1), (Proud, 2002; Sandri, 2008; Yang et al., 2008) and the synthesis molecule ribosomal protein S6 kinase 1 (S6K1) (Yang et al., 2008). The phosphorylation of eIF4E-BP1 results in the release of the eukaryotic initiation factor 4E (eIF4E) and its relocation and binding to an initiation factor complex that initiates the translation process (Proud, 2002). The regulation of mTOR continues to be investigated, including the differential effects of mTOR1 and mTOR2 (Yang et al., 2008), but its role in responding to cytosolic amino acid levels is firmly established.

While protein synthesis is initiated by mTOR signaling, there is a physiological limit to the capacity for protein synthesis. In an 8.5-hour observation of human subjects, protein synthesis increased with increased PKB and S6K1 signaling after the initial bolus of protein, but declined after 90 minutes despite continued amino acid concentrations maintained by intravenous feeding (Atherton et al., 2010). Atherton et al. (2010) reported that any continued synthesis may have been immediately degraded due to rising stress responsive factors. In a 4-hour observation of six young men following an exercise session, protein synthesis increased in a dose-dependent manner up to 20 g of protein with no change in signaling of S6K1 or eIF2B. Additional protein intake did not increase muscle protein synthesis or albumin protein synthesis (Moore et al., 2009). In another study, a 10 g intake of essential amino acids containing 1.8 g leucine produced the maximal effect of protein synthesis in young men and women (Glynn et al., 2010). Glynn et al. (2010) suggested a limitation in transport across the cell membrane may be the limiting factor to explain why increased protein synthesis was not observed with higher leucine intake, but increased catabolic signaling could also limit protein synthesis.

Protein synthesis rate can be affected by protein type, age of the individual, and level of physical activity. In a study of 6 young men, a comparison of whey, soy, and casein proteins was made to evaluate the effect on muscle protein synthesis (MPS) at rest and after exercise. The results showed that whey protein promoted MPS to a significantly greater extent than soy or casein in both rest and after exercise (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). The authors suggested the increased quantity of leucine in whey protein as a potential trigger signaling MPS. In a larger study

of 24 young men, smaller quantities of protein were supplemented with leucine or all other essential amino acids without leucine. The supplemented intake was able to stimulate MPS as effectively as 25 g of whey protein, however, only the 25 g whey protein intake level sustained MPS (Churchward-Venne et al., 2012). Comparing digestion and absorption of protein between young and old men, the MPS rate was not significantly different after a single bolus of 35 g casein protein (Koopman et al., 2009). However, when comparing whey protein with casein protein, elderly men had a higher postprandial leucine balance after consuming whey protein compared to young men although the rate of protein synthesis was similar between age groups (Dangin et al., 2003). In a crossover study comparing a HP diet to a typical protein diet, ten days on the HP diet did not show significant increases in protein synthesis or mitochondrial activity in the young or older participants (Walrand et al., 2008). In order to increase protein synthesis, aerobic exercise was effective as the increased vasodilation provides increased nutrient exposure to muscle cells of the body in both young and elderly individuals (Timmerman et al., 2010; Timmerman et al., 2012). Consequently, quality of the protein and protein accessibility to the cell are more significant factors affecting protein synthesis than age alone. However, age may affect which protein is more efficiently digested and the level of activity that can stimulate additional protein synthesis.

2. Protein catabolism

Protein catabolism is the reverse process of anabolism and provides the final link to complete protein turnover. The whole process of protein turnover has been calculated to synthesize and degrade more than 280 grams per day in a typical 70 kg male (Gropper

et al., 2009; Mitch & Goldberg, 1996). The catabolic process is essential for identifying and removing malfunctioning molecules, sequestering and silencing receptor signals, removing and communicating foreign infectious bodies, and recycling intracellular molecules. Two primary catabolic pathways of the cell are the lysosomal pathway and the ubiquitin-proteasome pathway. These two pathways along with numerous cytosolic peptidases identify and breakdown marked molecules into reusable amino acids for the cell, which is the primary purpose of catabolism.

The complex system of molecules that circulate in cytosol and monitor protein quality is not completely understood, but some key participants have been identified. Among these participants are heat shock proteins (Hsp70s), ubiquitin-proteosome enzymes (E1, E2, and E3), and chaperone molecules, BAG-1 and BAG-3 (Gamerdinger, Carra, & Behl, 2011; Kriegenburg, Ellgaard, & Harmann-Petersen, 2012). The Hsp70 family of proteins scouts the cytosol and identifies misfolded proteins. The marked protein that cannot be correctly folded then forms a complex with a BAG molecule and is directed toward an E3 molecule to be tagged with ubiquitin. The E1 and E2 proteins bring ubiquitin to the E3 molecule for transfer to the misfolded protein (Ciechanover, 2012; Mitch & Goldberg, 1996). Once the misfolded protein is marked with a chain of ubiquitin, it is transferred to the appropriate disposal site. When a BAG-3 marker is bonded to the protein, the complex will move toward macroautophagy in the lysosome, and when a BAG-1 marker is bonded to the protein, the ubiquinated molecule will be processed by the proteosome (Gamerdinger et al., 2011).

Lysosomal catabolism facilitates a variety of processes that isolate a target molecule or organelle and then breaks it down. The targeted item may be received through pinocytosis, phagocytosis, or mediated by receptors on the cell membrane. The targeted item may also be sequestered through macroautophagy, such as isolating mitochondria, or through microautography, such as isolating a globular protein (Ciechanover, 2012). These sequestered items are contained in a vacuole that is transported to the lysosome, which fuses with the vacuole and introduces numerous acid hydrolases, including proteases and cathepsins, and increased acidity to break down molecular and organelle structures to simple amino acids, dipeptides, and sugar molecules the cell can use (Muller, Dennemarker, & Reinheckel, 2012). In addition to recycling, the lysosome provides critical communication assistance to the cell. The vacuoles may contain recently activated cell membrane receptors requiring de-activation, such as an activated low density lipoprotein (LDL) receptor bonded to a LDL molecule. In this case, the lysosome processes the cholesterol into the cell and returns the LDL receptor back to the cell membrane (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002b). In another example, antigenic material from foreign bacteria can be processed in the lysosome and then bonded to a major histocompatibility complex (MHC) class II molecule that will present the material on the cell surface for activating the immune system (Muller et al., 2012). The lysosome is an active site not only for recycling of building materials, but also for the processing and conservation of receptors, and communication with the immune system.

Passage through the lysosome membrane is controlled by a series of transporter proteins which regulate the items being processed for digestion, the acidity of the lumen, and the digested products released into the cytosol. The lysosomal-associated membrane protein 2 (LAMP2) receptor recognizes misfolded proteins chaperoned to it by the Hsp70 and BAG-3 complex and shuttles the complex into the lysosome (Gamerdinger et al., 2011). The acidity of the lysosome is maintained at a pH of 5.0 by H^+ - ATPase pumps in the membrane (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002b). When the digestion is completed, the products are transported into the cytosol by a variety of transporters. The lysosomal amino acid transporter (LYAAT -1) has been identified as an active transporter of neutral amino acids (Boll, Daniel, & Gasnier, 2004; Sagne et al., 2001). The dimeric amino acid, cystine, is transported from the lysosome through a cystinosin transport protein (Ruivo et al., 2012). Transporters for monosaccharide products of the lysosome have also been identified (Winchester, 2005). These few examples highlight the complexity of this organelle that significantly contributes to the process of catabolism in the cell.

The ubiquitin-proteosome degradation pathway is the primary route of protein degradation in the cell and is a tightly controlled process. Energy–activated degradation via the proteosome may account for up to 80% of normal protein turnover in the cell (Fukasawa, Fujigaki, Hishida, & Kitagawa, 2012). Once the misfolded protein has been repeatedly ubiquinated, the complex is chaperoned to the proteosome which recognizes the ubiquitin link to the lysine amino acid of the protein and accepts the protein for digestion (Ciechanover, 2012). The proteosome is a barrel–shaped stack of four

heptametric rings containing the proteases within the central core area, and each end is protected by a cap that controls the proteins coming in (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002a). The cap allows unfolded proteins to proceed through the center of the barrel where the protein is broken into small peptides and released at the other end (Ciechanover, 2012; Mitch & Goldberg, 1996). The smaller peptides released from the proteasome are processed by additional proteases located in the cytosol, such as tripeptidyl peptidase II (TPPII) (Rockel, Kopec, Lupas, & Baumeister, 2012). The original ubiquitin is released at the entrance of the proteosome to be reused by E1 with another identified protein. With numerous proteasomes located throughout the cell, protein quality is maintained and a regular supply of amino acids is provided for the anabolic needs of the cell.

Specific Amino Acid Balances

1. Leucine

Leucine has been identified as a prominent amino acid in cellular metabolism. This prominence is related to the interaction of leucine with two significant biochemical pathways, the mTOR pathway and the pathway of the branched chain α-ketoacid dehydrogenase (BCKD) complex. The interaction of leucine with each of these pathways will be briefly described.

The mTOR mechanism is a key regulator of protein metabolism associated with inducing protein anabolism as well as inhibiting cellular autophagy, and leucine has been observed as a key nutrient that directs mTOR activity. Protein synthesis is initiated when the mTOR complex is signaled by insulin or by amino acid concentration (Sancak et al.,

2010). Compared to other amino acids, leucine provides enhanced stimulation (Garlick, 2005; Hara et al., 1998). In fact, the removal of leucine from the amino acid pool almost completely halts protein synthesis even in the presence of insulin (Hara et al., 1998). The concentration of leucine in the cell is communicated by leucyl aminoacyl-tRNA synthetase (LRS), which has been determined to have a specific binding site with part of the mTOR complex (Bonfils et al., 2012; Han et al., 2012). Current research suggests increased cellular leucine increases LRS activity resulting in increased mTOR activity initiating protein synthesis and disengaging autophagy (Dodd & Tee, 2012). Consequently, leucine has been identified as a key nutrient for signaling protein metabolism.

Leucine is one of the three BCAA, which are primarily catabolized in a two–step process of transamination by branched-chain aminotransferases (BCATs) to branch chain α-ketoacids (BCKAs) and then reduced to acyl-CoAs in a rate limiting step by the BCKD complex. BCAA homeostasis is maintained by a competition between protein phosphatase, PP2Cm, which activates BCKD by phosphorylation, and branch-chain dehydrogenase kinase (BDK), which turns off the complex by dephosphorylation (Harris, Joshi, & Jeoung, 2004; Zhou, Lu, Gao, Wang, & Sun, 2012). When higher levels of BCAAs are presented, increased levels of the BCKAs are generated, which inhibit BDK from turning off the BCKD complex (Hutson, Sweatt, & LaNoue, 2005). In this way, leucine's effect on metabolism can be limited as a higher concentration promotes its own oxidation (Harris et al., 2004). In some genetic disorders, BCKD does not complete the oxidation resulting in excess BCAA in a condition known as maple syrup urine disease;

however, without this congenital defect, there is little evidence that toxicity could be generated from over consumption of leucine (Yudkoff et al., 2005). The BCKD complex is active in skeletal muscle, brain, adipose tissue, heart, kidney, liver, and intestine (Brosnan, 2003). In zebra fish and adult mice, a high expression of PP2Cm has been identified in heart and brain tissue (G. Lu et al., 2007). This increased expression of PP2Cm may reflect a need for faster processing of BCKAs in these tissues. Consequently, in a normal metabolic state, consumption of leucine provides a signal for cellular metabolic activity that is tightly controlled by mTOR and BCKD mechanisms in many tissues, which work to maintain homeostasis of amino acids.

Researchers are currently investigating how leucine may be best utilized to promote healthy metabolism. Recent studies by Newgard et al. (2009), comparing metabolic profiling of human subjects and follow-up feeding studies with Wistar rats, showed that increased BCAAs were related to increased insulin resistance (Newgard et al., 2009). In comparison, Macotela et al. (2011) reported that feeding leucine water to mice improved metabolic measures, including increased insulin sensitivity (Macotela et al., 2011). The animal studies compared models of weight gain over time with ad libitum diets. More consistently, humans are interested in losing weight. Considering that BCAAs make up about 20% of average protein intake (Layman & Walker, 2006), a minimum of 2.5 g leucine per meal has been suggested to trigger optimum protein synthesis during weight loss (Devkota & Layman, 2010). Devkota and Layman (2010) estimate the current daily leucine intake by the typical American would need to be almost doubled to meet the 9 g/day intake for optimum protein synthesis. Because the BCKD

pathway restricts excess BCAAs, leucine can be transformed to a fuel source in muscle tissue. The ATP generated by oxidation of leucine is greater than the ATP generated by oxidation of glucose when compared on a molar basis (Gropper et al., 2009). Therefore, a proper functioning BCKD pathway should maintain the optimum level of leucine for mTOR signaling, while converting the excess leucine into energy without increasing insulin resistance. By optimizing the leucine trigger, improved metabolic activity may be promoted, which is consistent with other benefits of HP diets suggested previously.

2. Alanine

Alanine is present in the plasma at one of the highest concentrations among the various amino acids. In healthy, resting subjects, alanine arterial concentration is superseded only by glutamine, and is significantly higher compared to the total alanine content in muscle, which is just above 9% of total amino acids in humans (Clowes, Randall, & Cha, 1980). The high plasma concentration is not due to lack of clearance as about 70% of ingested alanine was absorbed on the first pass in circulation through the splanchic organs of humans (Battezzati, Haisch, Brillon, & Matthews, 1999). Additional studies have reported that more than 40% of alanine released from muscle tissue was generated from plasma glucose (Perriello et al., 1995). Therefore, alanine may be a significant biomarker of metabolic processes in the human body.

Alanine is a primary product of a transamination reaction with pyruvate in the muscle cell. Glycolysis yields pyruvate, which may react with glutamate in the presence of alanine transaminase to produce alanine (Gropper et al., 2009; Wagenmakers, 1998). The ammonia group from glutamate may come from leucine, isoleucine, asparagine,

aspartate, valine, or glutamine, which are the six amino acids the muscle cell can oxidize (Wagenmakers, 1998). During exercise, alanine plasma concentrations increase significantly during the first half hour (Wagenmakers, 1998). In these transactions, alanine relieves the muscle cell of an ammonia group and provides a harmless way of transfer to the liver for disposal. In addition, alanine can also function as a signal to the pancreas of the amount of energy the muscle cell is releasing.

As alanine concentration increases, cellular activity in the pancreas beta cell is modulated. The oscillatory activity of in vitro mouse beta cells increased in a medium containing amino acids resulting in greater sensitivity to glucose compared to medium containing glucose alone (Bolea, Pertusa, Martin, Sanchez-Andres, & Soria, 1997). In this study, leucine, alanine, isoleucine, and arginine were identified as the key amino acids affecting the response. In the BRIN-BD11 cell line and rat islets, intake of alanine has been reported to exceed intake of glutamine by 10-fold and results in significant insulin secretion compared to medium without alanine (Dixon, Nolan, McClenaghan, Flatt, & Newsholme, 2003). In this same cell line, medium containing 10 mmol/l of alanine resulted in a more than 5-fold increase in insulin secretion, and increased the oxidation rate of glucose more than 2-fold (Brennan et al., 2002). This increased activity from alanine is supported by the 1.8 fold up-regulation of sixty-six genes in the beta cell related to metabolism, protein synthesis, apoptosis, and cellular stress response (Cunningham, McClenaghan, Flatt, & Newsholme, 2005). In human studies, the stimulation of insulin by alanine has also been documented (Gannon & Nuttall, 2010; Genuth, 1973). Therefore, increased alanine returning from the muscle cells may

sensitize the beta cells to maintain insulin secretion and increase metabolic activity to continue glucose uptake.

Alanine may also provide cellular protection based on a measured effect on transcription factors. In the BRIN-BD11 cell line, cells treated in alanine medium had a 28% increased survival rate, compared to cells not exposed to alanine (Cunningham et al., 2005). This survival rate was attributed to increased protein production of enzymes that protect against cell damage. In a human endothelial cell line, increased alanine exposure correspondingly decreased oxidative activity associated with heart disease and neurological degeneration (Grosser et al., 2004). Consequently, alanine can induce transcription of proteins that promote cell health, in addition to being a transport mechanism for ammonia, and a signaling factor for energy status.

3. Glutamine

Glutamine is the amino acid with the highest concentration in plasma and has been shown to significantly affect gluconeogenesis, cellular metabolism, and processes related to organism immunity. The concentration of glutamine has been observed to decrease in cases of sepsis and trauma (Clowes et al., 1980; Oudemans-vanStraaten, Bosman, Treskes, van der Spoel, & Zandstra, 2001), type 2 diabetes (Menge et al., 2010), and with increased physical inactivity (Agostini, Heer, Guarnieri, & Biolo, 2008). The majority of glutamine is oxidized in the splanchnic tissues in the first pass following digestion being transformed into energy for the area tissues (Haisch, Fukagawa, & Matthews, 2000). Consequently, glutamine plasma concentration is a significant biomarker related to human metabolism concerns beyond dietary intake.

Glutamine is a significant contributor of carbon to the process of gluconeogenesis, while also providing a primary pathway for the body to dispose of ammonia generated by deamination or transamination. In the liver, an ammonia group is removed from glutamine to make glutamate, which may be released back into the blood stream. Glutamate can also be generated within other cells of the body as the α-keto-glutarate of the Kreb's cycle picks up an ammonia group to form glutamate. As other amino acids like leucine or alanine are deaminated to produce energy, the ammonia group can be added to the glutamate to form glutamine. Human skeletal muscle produces up to 50 g of glutamine per day from this process (Wagenmakers, 1998). Released into the blood stream, glutamine may return to the liver to release the ammonia and be transformed back to glutamate, or the glutamine may be taken up by the kidney where the ammonia is released and the carbon chain enters gluconeogenesis. Glutamine is preferentially taken up by the kidney accounting for about 8% of total glucose production in the body (Nurjhan et al., 1995) and up to 80% of glutamine glucose production (Stumvoll et al., 1998). Through this renal pathway, glutamine contributes more new carbon to the glucose pool than alanine (Nurjhan et al., 1995; Perriello et al., 1995). In addition, glutamine gluconeogenesis has a unique pathway that generates ATP while synthesizing glucose because of the nicotinamide adenine dinucleotide (NADH) oxidized in the reaction (Stumvoll, Perriello, Meyer, & Gerich, 1999; Vinay, Mapes, & Krebs, 1978). Due to the significant plasma concentration of glutamine, and the unique gluconeogenesis process in the kidney, glutamine is a significant contributor to the glucose pool in the body.

In addition to its contribution to the glucose pool, glutamine has a significant role in a variety of cellular activities. In animal and human cell lines, increased cellular glutamine was associated with increased cellular autophagy and increased cell survival under stress by decreasing cell apoptosis (Sakiyama, Musch, Ropeleski, Tsubouchi, & Chang, 2009; van der Vos et al., 2012). A decrease in glutamine was associated with an increase in mTOR activity (Sakiyama et al., 2009), but an increase in glutamine due to cellular intake, or via cellular glutamine synthetase *de novo,* resulted in decreased mTOR activity and increased autophagy (van der Vos et al., 2012). In human neutrophils, supplemental glutamine increased phagocytosis and the ability of the neutrophils to generate reactive oxygen products (Furukawa et al., 2000). In human and rat models, increased plasma glutamine was positively associated with increased expression of heat shock protein 70 (HSP70) expression (Singleton & Wischmeyer, 2007; Ziegler et al., 2005). The increased expression of HSP70 resulted in a decreased expression of nuclear factor (NF)-κB, (Singleton, Beckey, & Wischmeyer, 2005; Singleton & Wischmeyer, 2007) which is a transcription factor for numerous inflammatory pathways. Accordingly, increased glutamine concentration supports cellular activity vital to cell health and ultimately the health of the organism.

Whey Protein

Quality

The source and quantity of protein in the diet are both important considerations. Whey protein, derived from milk, is one of the highest quality sources of protein available in food (Schaafsma, 2006). In the process of making cheese, 10L of whole milk

produce 9L of whey solution (Manso & Lopez-Fandino, 2004), composed of soluble protein remnants from aggregation of casein proteins. Typically, whey protein is dried to form a powder that is more easily stored and handled. The classifications for whey powder include: sweet whey, which contains glycomacropeptide (GMP); acid whey, which does not contain GMP; whey protein concentrate, from 34 – 80 % protein; and whey protein isolate (WPI), which contains at least 90% protein (US Dairy Export Council., 2008; Yalacin, 2006). The major whey proteins include β-lactoglobulin, α-lactoalbumin, serum albumin, immunoglobulins, and GMP. The β-lactoglobulin and α-lactoalbumin compose about 70% of the protein base (Farrell et al., 2004). All the essential amino acids have been identified in whey, along with most of the non-essential amino acids, establishing whey as a standard for comparison with other proteins. By the Protein Efficiency Ratio (PER), whey protein concentrate (WPC) is rated at 3.0, compared to casein at 2.5. By the Protein Digestibility-Corrected Amino Acid Score (PDCAAS), WPC is rated at 100% (Schaafsma, 2005). This rate of quality was questioned in a study using N-labeled WPC (Lacroix et al., 2006). After a breakfast test meal of various milk proteins, the metabolism of WPC proceeded more quickly than casein or total milk proteins and resulted in a greater percentage of deaminated amino acids and excreted nitrogen. The greater turnover of nitrogen from the WPC demonstrated the ability of the body to quickly break down the BCAA for energy instead of protein synthesis. Lacroix et al. stated that the high quality rank of WPC does not accurately reflect its contribution to anabolic processes since a greater percent of the WPC is used for energy compared to total milk protein (Lacroix et al., 2006). From this

observation, WPC provides the body with the option of a fuel source or a building material source depending upon the need at the time of ingestion.

Metabolic Response in Humans

The metabolic effects of whey protein have been observed in numerous studies. The metabolic response to whey protein when consumed alone in a prepared drink resulted in significant increases in plasma concentrations of insulin, glucose-dependent insulinotropic polypeptide (GIP), cholecystokinin (CCK), and GLP-1 (Hall, Millward, Long, & Morgan, 2003; Nilsson, Stenberg, Frid, Holst, & Bjorck, 2004). In a series of mixed meals incorporating whey protein, a significant increase in insulin was observed in both type 2 diabetic subjects and healthy subjects, but the effect on GIP was muted (Frid, Nilsson, Holst, & Bjorck, 2005; Veldhorst et al., 2009). The effect of whey protein on GLP-1 was significantly higher after lunch (Frid et al., 2005; Hall et al., 2003), which corresponds with the effect observed with HP meals discussed in the previous section. The responses elicited by whey protein suggest that additional studies are needed to evaluate the effect of incorporating whey protein into a diet.

The metabolic response observed with whey protein is associated with the high quantity of BCAA in whey protein. In a study by Layman et al., increased BCAA in the diet was suggested as one of the mechanisms involved in improving glucose homeostasis among the HP-fed subjects (Layman, Shiue et al., 2003). When amino acid mixtures were compared against whey protein in postprandial measurements, the BCAA mixtures produced similar responses for insulin, GIP, and GLP-1 (Nilsson, Holst, & Bjorck, 2007).

From this study, Nilsson et al. suggested that the BCAAs in whey are the key components producing the metabolic effects, as opposed to lactose or mineral content.

Lactotripeptides

In addition to the favorable profile of essential amino acids in whey protein, the sequence of some of the peptides found in whey protein may produce effects identical to angiotensin converting enzyme (ACE) inhibitors. When milk was fermented with lactic acid bacteria, an average decrease in systolic blood pressure of 7.0 mm Hg was observed (Hata et al., 1996; Kawase, Hashimoto, Hosoda, Morita, & Hosono, 2000; Seppo, Jauhiainen, Poussa, & Korpela, 2003). The hypotensive activity observed after consuming these dairy proteins has been suggested to be the result of the cleavage of β-lactoglobulin and α-lactoalbumin proteins into smaller peptides that interact with ACE (Metzger & Nonnemacher, 2006; Pan, Guo, Zhao, & Cao, 2011; Pihlanto-Leppala, Koskinen, Piiola, Tupasela, & Korhonen, 2000). Although larger peptides have demonstrated ACE inhibitor activity, Pihlanto-Leppala et al. (2000) proposed that tripeptides or dipeptides are transported into the blood stream intact and are able to interact with ACE. The successful passage of tripeptides into the bloodstream after consuming a protein drink has been observed (Foltz et al., 2007). A sustained long term effect from the peptides on hypertension was found in one study (Hata et al., 1996), but not in another (Jauhiainen et al., 2012). This difference between studies may be related to genetic differences between study populations (Cicero, Gerocarni, Laghi, & Borghi, 2011). The activity of dairy protein peptides provides an opportunity to consider its

application as a functional food and to investigate its interaction as a natural ACE inhibitor.

A functional food approach to hypertension and obesity has been proposed based on the active peptides containing ACE inhibitor properties generated from whey protein (FitzGerald & Meisel, 2000). Comparison of various peptide groups for ACE inhibition activity has shown the tripeptides isoleucine-proline-proline and valine-proline-proline to be among the most effective *in vitro* (Murray & FitzGerald, 2007). However, no human studies have demonstrated a significant change in the concentration of RAS components following the prolonged consumption of ACE inhibitory peptides (Aihara, Kajimoto, Hirata, Takahashi, & Nakamura, 2005; de Leeuw, van der Zander, Kroon, Rennenberg, & Koning, 2009; Jauhiainen et al., 2005; Usinger et al., 2010; van der Zander, Jakel, Bianco, & Koning, 2008). Consequently, the mechanism for decreased blood pressure after ACE inhibitory peptide consumption has not been determined.

Renin-Angiotensin System in Adipose Cells

Known mechanisms

Adipocyte cellular activity has been found to include proteins of the renin-angiotensin system (RAS), which are associated with cardiovascular activity (Engeli, Negrel, & Sharma, 2000; Gorzelniak, Engeli, Janke, Luft, & Sharma, 2002). Initially understood to regulate blood pressure, these proteins produced by the adipocytes have demonstrated unique action on the surrounding adipose tissue. Angiotensinogen (AGT) mRNA levels increase with cell maturity as measured in cell cultures (Saye, Casis,

Sturgill, Lynch, & Peach, 1989). AGT and renin, the catalyst which acts on AGT to produce angiotensin I (ATI), have also been measured in human adiopocytes with production increasing as the cells mature (Schling, Mallow, Trindl, & Loffler, 1999). The activities of these two components produce the remaining proteins of RAS within the adipose tissue.

The original understanding of RAS in the cardiovascular system identified angiotensin II (AT II) as the component responsible for vasoconstriction and increased blood pressure. AT II, converted from AT I by ACE, interacts with angiotensin receptors (AT_1 and AT_2) located in the vasculature or on adipocyte cell membranes (Goossens, Blaak, Arner, Saris, & van Baak, 2007; Janke, Engeli, Gorzelniak, Luft, & Sharma, 2002; H. Lu, Boustany-Kari, Daugherty, & Cassis, 2007). Consequently, ATII concentrations are dependent upon the precursor concentrations of AGT and renin. Obese subjects demonstrated a higher plasma concentration of AGT compared to nonobese subjects (Engeli et al., 2005; Umemura et al., 1997; Yasue et al., 2010). But with weight loss, reductions in renin, AGT, ACE, and aldosterone were demonstrated, as was a corresponding decrease in blood pressure (Engeli et al., 2005). The adipose tissue contribution of RAS components to the biological system strengthens the link between hypertension and obesity.

The ATII protein activates adipocyte lipogenesis and inhibits adipogenesis. In human cell studies, ATII promoted the increase of triglycerides within adipocytes by signaling production of fatty acid synthase and glycerol-3-phosphate dehydrogenase (Jones, Standridge, & Moustaid, 1997). ATII also restrained lipolysis activity by binding

to the AT_1 receptor in subcutaneous adipose cells (Goossens et al., 2007). Thus, adipocyte lipid storage increased and was maintained by the ATII signal. ATII promoted the storage activity of existing mature adipocytes and inhibited the formation of additional mature cells from preadipocytes (Brucher, Cifuentes, Acuna, Albala, & Rojas, 2007; Janke et al., 2002). The resulting predominance of large adipose cells has been associated with increased insulin resistance related to type 2 diabetes (Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). Therefore, a reduction in ATII signaling may reverse these effects on adipocytes.

Effects of ACE Inhibition

Production of the bioactive ATII has been reduced by ACE inhibitors resulting in decreased blood pressure and measures related to adipocyte activity. ACE inhibitors caused decreased body weight in rat and human studies (Beevers et al., 1984; Mathai, Naik, Sinclair, Weisinger, & Weisinger, 2008; McGrath et al., 1990; Santos et al., 2008). ACE inhibition has been associated with a decreased risk of type 2 diabetes in a meta-analysis of multiple human studies (Scheen, 2004). In rats treated with ACE inhibitors, blood pressure and adipocyte size decreased after a two-week treatment (Furuhashi et al., 2004). In human subjects, a two-week treatment with ACE inhibitors resulted in decreased blood pressure, increased insulin sensitivity, and increased adiponectin concentrations (Furuhashi et al., 2003). These studies provide the foundation for additional investigations into the physiological effects of ACE inhibition and the possible applications to obesity and type 2 diabetes.

Conclusions

The evidence of RAS metabolic activity in adipose cells identifies potential new biomarkers for research on the adipose cell life cycle and obesity. While pharmaceutical interventions have demonstrated alterations in adipose cell size, a specific weight loss strategy has not yet targeted the RAS mechanisms. Numerous studies of dairy protein intake have observed decreased blood pressure among subjects over a period of weeks. *In vitro* studies have demonstrated the inhibitory activity of various tripeptides and dipeptides from dairy protein. Whey protein could be easily incorporated into a diet plan, providing increased protein intake in general and possibly providing additional functional food activity through the related peptides.

What are Americans doing to control weight?

Dietary Practice Surveys

With the rise in obesity, interest in weight loss practices has increased. Based on data from the 1998 National Health Interview Survey, Kruger et al. found 38% of women and 24% of men wanted to lose weight (Kruger, Galuska, Serdula, & Jones, 2004). A 2004 HealthStyles survey of 4,345 consumers representing the U.S. population, examined the behavioral factors of those successful in maintenance of weight loss. The top strategies included: smaller quantities of food, increased fruit and vegetable intake, smaller portions, decreased fat intake, and elimination of sweetened beverages (Kruger, Blanck, & Gillespie, 2006). The survey examined behavioral patterns, such as physical activity choices, weight control strategies, and barriers to weight loss to characterize

weight loss practices for men and women, eighteen years of age and older. Although the survey evaluated multiple strategies for weight loss, the measures were restricted to reported behavior and did not include measures of attitudes or nutrition knowledge that may initiate behavior promoting weight loss. In addition to information on weight loss practices, the success accompanying a particular practice and the psychological context for choosing a particular practice may provide greater insight into what is effective.

In addition to identifying the practices chosen for weight loss, recognizing which are healthy or unhealthy may significantly influence the outcome. While many individuals can identify healthy weight-loss strategies, many may select unhealthy supplemental strategies when their goal is not reached. In one study, women who added unhealthy strategies, such as diet pills, skipping meals, or laxatives, were among those who gained the most weight in a four-year period, and reported greater negative eating attitudes (Savage & Birch, 2010). In a national study of Australian women, those women who reported use of unhealthy weight loss practices had a higher mean weight gain recorded in a two-year period (Williams, Germov, & Young, 2007). Additional measures such as food disinhibition and weight self-efficacy may determine the rationale for selection of certain practices.

National Weight Control Registry

A long term evaluation of weight loss success and maintenance has been the focus of the National Weight Control Registry (NWCR) (Wing & Hill, 2012), which was established in 1994. The NWCR has enrolled more than 10,000 individuals who have met the criteria for successful weight loss. The criteria for joining the NWCR include the

loss of thirty pounds or more, and the maintenance of weight loss of thirty pounds or more for at least one year. A study based upon participants of the registry found weight loss was related to improved self-confidence, energy, and physical health (Klem, Wing, McGuire, Seagle, & Hill, 1997). Another study found that regardless which weight loss program was used, the participants chose similar weight maintenance strategies of low calorie, low fat diets and increased physical activity (McGuire, Wing, Klem, Seagle, & Hill, 1998). Those participants who had medical reasons for losing weight were able to lose more weight and maintain the weight loss for a longer period of time (Gorin, Phelan, Hill, & Wing, 2004). Within the last ten years, a shift in eating and exercise habits has been observed with more participants successfully using low carbohydrate diets to lose weight (Phelan, Wyatt, Hill, & Wing, 2006; Phelan et al., 2007). Upon entry, participants in the NWCR are asked to complete questions from the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977; Radloff, 2000), Eating Disorders Examination (Fairburn, Cooper, & O'Connor, 2008), Symptom-Checklist-90-R (Derogatis, 2012), and Block Food Frequency questionnaire (Nutrition Quest, 2012), so results can be compared regarding weight loss and weight perceptions. Wing and Hill reported that CES-D scores showed participants as non-depressed with low frequency binge eating; however, a small percentage reported increased time spent thinking about food and weight (Wing & Hill, 2001). While the NWCR provides the most comprehensive data on weight loss and maintenance issues currently available, there is no evaluation of nutrition knowledge or self-efficacy among successful weight maintenance participants in the NWCR.

Dietary Intervention Components

Nutrition Knowledge

1. General Nutrition Knowledge Surveys

With the variety of diet practices promoted by the media and government dietary guidance, there is a concern about whether consumers have adequate nutrition knowledge to make healthy decisions, and to direct dietary behavior. While some surveys report increased nutrition knowledge with age (De Vriendt, Matthys, Verbeke, Pynaert, & De Henauw, 2009; Hendrie, Coveney, & Cox, 2008; M. C. Nelson, Lytle, & Pasch, 2009), other surveys report increased nutrition knowledge among younger individuals (Dickson-Spillman & Siegrest, 2011). Increased nutrition knowledge has a strong association with the likelihood of meeting dietary recommendations (Wardle, Parmenter, & Waller, 2000), and successful weight loss (Klohe-Lehman et al., 2006). Residents surveyed in England showed increased fruit and vegetable intake was associated with increased nutrition knowledge (Wardle et al., 2000) while a Mexican-American population did not show such an association (Sharma, Gernand, & Day, 2008). More research is needed to identify additional dietary behaviors that may be associated with increased nutrition knowledge.

2. Diet and Health Knowledge Surveys

The Diet and Health Knowledge Surveys (DHKS) were completed in 1989 – 1991 and 1994–1996 in conjunction with the Continuing Survey of Food Intakes by Individuals (CFSII) and provided a limited evaluation of national health knowledge and

attitudes among main meal providers across the US (Tippett & Cleveland, 2001). The average nutrition knowledge score increased slowly, but significantly from 1989 to 1991, with the average score higher among women than men, and positively associated with education and age (Sapp & Jensen, 1997). Sapp and Jensen (1997) reported the reliability estimate (Cronbach's alpha) of the nutrition knowledge surveys was $\leq .70$ each year, and the convergent validity was $\leq .50$ each year. From the 1995 DHKS, reliability remained unchanged and convergent validity decreased (Obayashi, Bianchi, & Song, 2003). These statistical evaluations help to describe the quality of data collected from survey tools, and values > .70 are preferred. From the 1994-1996 data, a comparison between those who said nutrition was very important and those who said nutrition was not very important showed no difference in nutrition knowledge (Bowman, 2005). The primary topics of the nutrition knowledge questions in the DHKS focused on fat, cholesterol, fiber, and calories. No questions were included concerning protein, vitamins, minerals or carbohydrates. Establishing a well-defined measure for nutrition knowledge remains a challenging goal.

While the DHKS questions attempted to measure respondents' knowledge of facts, also defined as declarative knowledge, a more recent survey attempted to measure procedural knowledge as a more accurate reflection of nutrition behavior. Procedural knowledge may be more closely related to behavior, or actions taken related to knowledge. In a Swiss population of 1043 participants, women scored higher than men, and higher education was positively associated with higher nutrition knowledge scores, but age was negatively associated with higher nutrition knowledge scores (Dickson-

Spillman & Siegrest, 2011). Dickson-Spillmann and Siegrist (2010) reported a reliability estimate (Cronbach's alpha) of 0.70 and similar correlations to previous surveys. The procedural knowledge focus may be as effective as measuring declarative knowledge, and perhaps a combination of these methods will provide a stronger model.

Self-efficacy

Within Social Cognitive Theory (Bandura, 1986), self-efficacy has increasingly been considered an important component for evaluating an individual's ability to successfully reach a weight goal (Clark, Abrams, Niaura, Eaton, & Rossi, 1991). Self-efficacy has been defined as the belief an individual has in his or her ability to successfully accomplish a behavior necessary for reaching a specific goal (Bandura, 1977). A weight management self-efficacy scale was developed and validated by Clark et al., called the Weight Efficacy Lifestyle Questionnaire (WELQ) (Clark et al., 1991; Clark & King, 2000). This scale has been used in a number of studies to evaluate changes in self-efficacy and the association with successful weight loss (Clark & King, 2000; Linde et al., 2004; Linde, Rothman, Baldwin, & Jeffery, 2006; Richman, Loughnan, Droulers, Steinbeck, & Caterson, 2001). These studies have shown that higher self-efficacy scores at pre-treatment are related to successful weight loss. However, self-efficacy was measured in the context of formal weight loss programs as a predictive tool of the subject's success, and the self-efficacy scores post-treatment did not consistently improve with weight loss. In a cross-sectional study of women who were not enrolled in a weight loss program, self-efficacy did not correspond to weight maintenance behaviors (Butler & Mellor, 2006). Therefore, participant bias may affect

the measure of self-efficacy reported in some of the weight loss studies. While higher self-efficacy scores have been associated with successful weight loss, most of these associations only describe the self-efficacy of the individuals who volunteered for a weight loss program.

The eleven studies implementing the WEL Questionnaire have primarily involved weight loss protocols. One study has used the WEL Questionnaire in a cross-sectional design, but this study involved a Malaysian population (Chang, 2007). The majority of participants in these studies have been women, so the characterization of self-efficacy in men is minimally represented. However, Linde et al. found that self-efficacy scores were predictive for women, but not for men (Linde et al., 2004). While the measure of self-efficacy has been associated with potential success in weight loss for an individual attending a weight loss program, no evaluation of weight maintenance self-efficacy in a larger study of women has been completed. A cross-sectional evaluation of weight maintenance self-efficacy among women in the US may provide a larger comparison of self-efficacy scores for future studies regarding weight maintenance and weight loss issues.

Self-efficacy is associated with some behaviors promoting weight loss. Higher self-efficacy scores have been associated with high fiber intake (Hagler et al., 2007; Schwarzer & Renner, 2000), greater fruit and vegetable intakes (Shaikh, Yaroch, Nebeling, Yeh, & Resnicow, 2008; Van Duyn et al., 2001), and decreased fat intake (K. M. Nelson, McFarland, & Reiber, 2007; Steptoe, Doherty, Kerry, Rink, & Hilton, 2000); however, each of these studies used unique measures for self-efficacy, which inhibits

generalization. In an 18 month study by Warziski et al., self-efficacy was measured with the WEL Questionnaire, and was associated with decreased fat intake and weight loss (Warziski, Sereika, Styn, Music, & Burke, 2008). In contrast, no study has investigated an association between self-efficacy and protein intake, which has been shown in some trials to increase satiety (Lejeune et al., 2006) and promote successful weight loss (Weigle et al., 2005). Therefore, an investigation of the potential association between self-efficacy and protein intake for weight maintenance would provide additional information regarding the application of a HP diet.

Conclusions

While high protein diets have been carefully researched, there has been no evaluation of how well this strategy has been received in the general population as a weight maintenance strategy. A particular selected strategy does not guarantee success in meeting the goal of losing weight. While measures of self-efficacy have been related to aspects of successful weight loss, no measure of self-efficacy has been studied in relationship to specific weight maintenance strategies. An individual's success in weight maintenance, or weight control, may have as much to do with the measure of personal self-efficacy as with the particular strategy selected.

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Chapter Three: Summary and Hypotheses

Summary

The obesity epidemic and related health issues and costs are producing an ever increasing burden upon individuals and the nation as clear solutions remain to be determined. Based on BMI measures, the US has reached a plateau with approximately two-thirds of the nation's adults overweight or obese. Among women, those who are 40 –60 years old represent the greatest percentage of obese individuals. This group may also be motivated to lose or maintain weight and may have had experience with particular strategies for losing weight. Reasons for the observed high prevalence of obesity in this age group may include dietary changes, physical activity changes, and hormonal changes. Therefore, midlife women represent an appropriate group in which to implement dietary and other weight maintenance strategies. The effectiveness and acceptability of diet strategies with this group may be especially important because midlife women serve as a role model to family members.

The high protein, reduced-energy diet has been studied in multiple controlled trials and demonstrates positive outcomes in weight loss, lean mass retention, and insulin sensitivity. High protein diet studies have reported increased satiety, improved insulin sensitivity, and increased thermic effect resulting in greater fat mass loss among subjects consuming a high protein diet. Greater satiety from protein consumption has been related to decreased energy intake. Decreasing carbohydrate intake as protein intake is increased may reduce stress on the insulin pathways resulting in greater insulin sensitivity. The biochemical processing of higher protein content in meals requires a greater percentage of energy being expended to process the increased nitrogen content of the meal resulting

in an increased thermic effect. In studies reviewed regarding the efficacy of high protein diets, no negative results have been reported that would warn against a high protein diet (up to 35% of energy) as a reasonable weight loss strategy.

Among protein sources, whey protein ranks as one of the best sources for a complete amino acid profile. Easily digested, whey proteins can be quickly utilized as amino acids or transformed through gluconeogenesis into a fuel source. In addition to the nutrition content, *in vitro* studies have shown that some whey protein molecules have ACE inhibition properties. Fermented dairy products have been prepared that induce a blood pressure lowering effect in humans and in rats. Because of the *in vitro* mechanisms observed with ACE inhibition, the *in vivo* results have been assumed to be related to the same mechanism. Whey protein may be appropriate for inclusion in a weight loss diet because of the high quality of protein and because of the potential interaction with other biological processes which may promote weight loss.

ACE inhibition is at the center of the RAS regulating blood pressure within the human body. The RAS has been observed to be an active mechanism in the regulation of adipose tissue. Some *in vivo* studies have demonstrated with pharmaceutical ACE inhibition that adipose cells will shrink in size and the subject will lose weight. ACE inhibition has not yet been measured with a functional food source *in vivo*, but decreased blood pressure has been associated with fermented dairy intake. Therefore, ACE concentration should be clinically measured as whey protein intake is increased to observe if there may be such an inhibition.

The top dietary practices reported for helping adults lose or maintain weight include exercise, smaller portions, and eating less fats and sugars. Among the various surveys that have been completed, no survey has identified increased protein intake as a weight loss strategy. Since there have been many weight loss studies that have used increased protein intake, and a few fad diets which increase protein intake, an accurate picture is needed of how increased protein intake ranks with these other common strategies.

The success of a weight maintenance strategy must include a careful consideration of the self-efficacy of the individual selecting the particular strategy. Though a number of individuals select the same strategy for losing weight, only a few will reach their goal. Various measures of self-efficacy have been associated with fruit and vegetable intake, decreased fat intake, and successful weight loss. However, no evaluation has been completed to understand the relationship of protein intake or knowledge of protein to self-efficacy of weight maintenance. One of the measures used in recent years to evaluate an individual's self-efficacy, or belief in their ability to succeed, has been the Weight Efficacy Lifestyle Questionnaire. The WELQ provides a measure for identifying whether a person may be ready to follow a particular strategy, or whether the individual may need additional support in order to be successful.

Hypotheses

Based on the literature that has been reviewed, the following conclusions have been determined and specific hypotheses have been generated:

HP diets

1. Conclusions

1) HP reduced energy diets have demonstrated increased weight loss and reduction of fat mass compared to low protein diets.

2) Adipose tissue activity is regulated by local RAS hormones.

3) Inhibition of RAS activity has resulted in decreased fat mass in rats and humans.

4) Whey proteins inhibit ACE activity *in vitro*.

5) Intake of whey proteins has resulted in decreased blood pressure in rats and humans.

Therefore, intake of whey protein may decrease fat mass by inhibiting the RAS activity regulating the adipose tissue.

2. Hypotheses

1) Reduced energy diets containing whey protein will result in greater weight loss and improvements in body composition and metabolic parameters compared to reduced energy diets of a mixed protein source.

2) Whey protein intake from a diet containing supplemental whey will be associated with decreased ACE activity compared to a reference diet.

3) Decreased ACE activity will be associated with improvements in body composition, specifically, a decrease of fat mass and an increase in lean mass.

Weight Maintenance Practices

1. Conclusions

1) No survey of current dietary practices has established how frequently individuals use the practice of eating more protein to prevent weight gain.

2) Successful use of a practice of increased protein intake may be related to the individual's knowledge of protein and nutrition requirements.

3) Successful use of a particular practice may be related to a higher self-efficacy measure such as that measured by the WEL scale.

Therefore, increased protein intake and a higher level of protein knowledge may be related to a higher WEL score.

2. Hypotheses

1) Among middle age women, reporting "eating more protein" as a practice to maintain weight will be associated with weight maintenance and increased percent energy intake from protein.

2) Among middle age women, those women who report "eating more protein" will also demonstrate a higher WEL score.

Chapter Four: Varying protein source and quantity does not significantly improve weight loss, fat loss, or satiety in reduced energy diets among midlife adults

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Nutrition Research 2011; 31:104-112

We hypothesized that a whey protein diet would result in greater weight loss and improved body composition compared to standard weight loss diets. Weight change, body composition, and renin angiotensin aldosterone system activity in midlife adults were compared between diet groups. Eighteen subjects enrolled in a 5 month study of 8 weeks controlled food intake followed by 12 weeks *ad libitum* intake. Subjects were randomized to one of three treatment groups: control diet (CD) (55% carbohydrate: 15% protein: 30% fat), mixed protein (MP) (40% carbohydrate: 30% protein: 30% fat), or whey protein (WP) (40% carbohydrate: 15% mixed protein: 15% whey protein: 30% fat). Measurements included weight, metabolic measures, body composition by dual energy x-ray absorptiometry (DXA), and resting energy expenditure. No statistically significant differences in total weight loss or total fat loss were observed between treatments; however, a trend toward greater total weight loss ($p = 0.08$) and total fat loss ($p=0.09$) was observed in the WP group compared to the CD group. Fat loss in the leg and gynoid regions was greater ($p < 0.05$) in the WP group than the CD group. No RAAS mediated response was observed, but a decrease in systolic blood pressure was significantly greater ($p <0.05$) in the WP group compared to the CD group. In summary, increased whey protein intake did not result in statistically significant differences in weight loss or in total fat loss, but significant differences in regional fat loss and in decreased blood pressure were observed in the WP group.

Introduction

In the United States, there is a greater likelihood of obesity among men and women 40 – 59 years of age compared to men and women 20 – 39 years (Flegal, Carroll, Ogden, & Lester, 2010). Obesity in midlife is associated with higher risk of metabolic disease (Colditz, Willet, Rotnitzky, & Manson, 1995; Hu et al., 2004). Energy restriction diets of various compositions have been proposed to control weight gain and promote weight loss. Multiple studies have reported successful weight loss and maintenance of lean mass using energy restricted diets of moderate to high protein content ($\geq$ 25% energy from protein) (Baba et al., 1999; Clifton, Keogh, & Noakes, 2008; Layman et al., 2003). Generally, these diets have increased protein intake from meat, eggs, dairy, and nuts and decreased the proportion of energy from carbohydrates and fats.

In a single meal study of macronutrient intake, protein decreased postprandial appetite and subsequent energy intake compared to either carbohydrate or fat (Marmonier, Chapelot, & Louise-Sylvestre, 2000). *Ad libitum* intake of high protein diets enhanced weight loss compared to diets with high carbohydrate content (Halton & Hu, 2004; Weigle et al., 2005). In a long term study, subjects following a moderate protein diet were more successful in maintaining significant weight loss and improved body composition after 12 months compared to subjects following an average protein diet plan (Layman et al., 2009).

Benefits associated with high protein reduced energy diets include enhanced satiety (Apolzan, Carnell, Mattes, & Campbell, 2007), increased thermogenesis (Halton & Hu, 2004), and improved insulin sensitivity (Gannon, Nuttall, Saeed, Jordan, &

Hoover, 2003; Layman, Shiue, Sather, Erickson, & Baum, 2003). These effects are thought to be associated with the branch chain amino acids (BCAA) found in complete proteins (Layman et al., 2003; Schaafsma, 2006). In addition, the BCAA leucine signals intracellular pathways regulating amino acid oxidation and gluconeogenesis (Layman & Baum, 2004). Whey protein (100 grams) contains about twenty-four grams of BCAA (Cribb, 2005), which have been associated with improved insulin sensitivity and satiety (Nilsson, Holst, & Bjorck, 2007). Some studies have investigated the use of whey protein in an *ad libitum* high protein weight loss diet (Frestedt, Zenk, Kuskowski, Ward, & Bastian, 2008; Treyzon et al., 2008), but to our knowledge whey protein has not been compared against other protein sources in a weight loss protocol.

Ideal weight loss results in reduction of fat mass with minimal loss of lean tissue; therefore, a better understanding of the mechanisms governing adipose activity is required. Recently, adipose metabolism has been found to be influenced by hormones previously identified in blood pressure homeostasis as the renin-angiotensin aldosterone system (RAAS) (Engeli, Negrel, & Sharma, 2000; Gorzelniak, Engeli, Janke, Luft, & Sharma, 2002). With weight loss, decreases in renin, angiotensinogen, angiotensin converting enzyme (ACE), and aldosterone have been observed (Engeli et al., 2005). In a study of the pharmaceutical ACE inhibitors enalapril and captopril, a slight loss of body weight was noted in the treatment groups (Beevers et al., 1984; McGrath et al., 1990; Santos et al., 2008), although the metabolic pathway is still unclear. Whether the decrease in RAAS activity is an effect of weight loss, or if the inhibition of the RAAS pathway also results in a loss of weight has not been specifically tested.

Recent studies have proposed that the RAAS system can be inhibited with bioactive peptides found in food sources. ACE inhibition by small peptide groups found in whey protein has been observed *in vitro* (Pihlanto-Leppala, Koskinen, Piiola, Tupasela, & Korhonen, 2000). There have been mixed results in human clinical studies with some studies reporting an average decrease in systolic blood pressure of 7.0 mmHg (Aihara, Kajimoto, Hirata, Takahashi, & Nakamura, 2005; Kawase, Hashimoto, Hosoda, Morita, & Hosono, 2000; Seppo, Jauhiainen, Poussa, & Korpela, 2003) and another study showing no significant difference in blood pressure (Lee, Skurk, Hennig, & Hauner, 2007). The specific mechanism of action by whey peptides has not been determined. However, it is believed to involve direct inhibition of ACE by binding with the zinc fingers of the molecule resulting in the inability of ACE to cleave angiotensin I into angiotensin II (Sturrock, Natesh, van Rooyen, & Acharya, 2004). It is not clear whether a decrease in blood pressure after consuming whey protein is directly related to ACE inhibition or some other mechanism.

Whey protein may be a preferred protein choice for a weight loss diet because of its high BCAA content, the potential bioactivity with the RAAS of the adipose tissue, the potential to increase satiety, and its ease of incorporation into a diet plan. We hypothesized that a whey protein diet would result in greater weight loss and improved body composition compared to standard weight loss diets. Using a randomized, parallel design of three diets, our objectives were to determine weight loss, body composition, insulin sensitivity, satiety, blood pressure, and RAAS activity in midlife adults who were following an energy restricted protocol to promote weight loss. Based upon the

literature, increased consumption of whey protein may decrease ACE activity and may be related to maintenance of lean mass. Measures of changes in ACE activity, plasma renin activity, and aldosterone concentration were compared to changes in body composition for each diet group.

Methods

Study Design

A randomized, parallel design was used to compare the effects of diets containing varying protein quantities from different protein sources. After signing consent forms, subjects were randomized to a control diet (CD), mixed protein diet (MP), or whey protein diet (WP) **(Figure 4-1)**. Subjects were directed to maintain their daily activity habits and not increase physical activity during the study.

For the first 8 weeks, all the food to be consumed was provided to the subjects. Subjects came daily to the General Clinical Research Center (GCRC) of the University of Minnesota to pick up meals. At daily visits, each subject completed a compliance questionnaire, a satiety questionnaire, and was weighed.

On completion of the 8-week feeding phase, subjects continued on their assigned diet for an additional 12-week *ad libitum* phase. Subjects were instructed by a registered dietitian on how to continue the assigned diet. Those subjects assigned to WP were given additional Designer Whey® protein powder and Pure Protein® bars. Subjects were expected to report daily intake on a secure, personal dietary analysis web program (www.nutrihand.com), which the principal investigator could evaluate for compliance.

Every four weeks, each subject returned to the GCRC to be weighed and have blood drawn for fasting glucose and insulin levels. At baseline, 8, and 20 weeks, each subject came to the GCRC for evaluations, including indirect calorimetry to measure resting energy expenditure (REE), fasting glucose and insulin, RAAS activity, and a dual energy x-ray absorptiometry (DXA) scan to measure body composition. Concentrations of fasting glucose and insulin were used to calculate the homeostatic model assessment of insulin resistance (HOMA IR: = [fasting glucose (mmol/l) * fasting insulin (μU/ml)] / 22.5) (Muniyappa, Sihoon, Chen, & Quon, 2008) at these three clinical visits.

Approval of the study was obtained from the University of Minnesota Committee for the Use of Human Subjects and Research. The entire study was performed at the University of Minnesota GCRC.

Subjects

Healthy, midlife (40 – 60 yrs) adults were recruited from the Minneapolis-St. Paul metropolitan area through local posters and advertisement. Eligibility was determined by responses to a telephone interview and subjects were disqualified if they reported a current medical problem, were pregnant, or were using prescription medication. Subjects were asked about their willingness to comply with dietary treatments. Inclusion criteria included BMI 27 – 32 kg/m^2, no recent weight changes, a fasting blood glucose measure < 126 mg/dL, and a desire to lose weight. Of 85 individuals screened by telephone interview, 27 were eligible for further screening at the GCRC. A total of 18 participants consented to the protocol and were enrolled in the study.

Resting Energy Expenditure

At the screening visit, eligible subjects had their REE measured with the ParvoMedics True One Metabolic Monitor (Provo, UT). All REE measures were completed in a fasting state after the subject had rested in a supine position for about thirty minutes. Fifteen minutes of a stable, continuous measure were used to calculate the baseline REE for each subject (Rocha, Alves, & Fonesca, 2006). The baseline REE was evaluated along with the activity level reported by each subject to determine total energy needs. To determine the activity level, each subject was asked if they regularly engaged in specific activities and what duration of time was spent in each given activity. From this evaluation, a custom reduced energy diet was tailored to promote 0.75 kg weight loss weekly. Each subject's usual food consumption prior to starting the study was assessed by the Diet History Questionnaire (NCI, 2003).

Dietary Treatments

The macronutrient composition for each dietary treatment is shown in **Table 4-1**. All three diets were similar in dietary fiber, cholesterol, calcium intake, and fatty acid balance. *Ad libitum* additions of salt were not controlled with these diets. Diets were designed to differ in the percent of energy from protein and the protein source. The two high protein diets contained 30% energy from protein and 40% energy from carbohydrate compared to CD, which contained 15% energy from protein and 55% energy from carbohydrate. The CD and the MP diets contained protein from the same sources, but in different quantities. The WP diet contained the same quantity of protein by mass as the MP, but the entire increase of protein above the level of the CD came from the addition

of Designer Whey®, a commercial whey protein isolate. Daily calcium intake was balanced across groups by providing supplemental calcium tablets (Caltrate 600) to subjects in the CD and MP groups.

Experimental diets were formulated with commonly available food items, including whey protein, as shown in **Table 4-2**. A five day menu rotation was developed and then modified for each experimental group. The nutrient composition of the test diets was calculated with Nutritionist V nutrient analysis software (Hearst Corporation, 2005). The nutrient data for all food items used in the menus were obtained from the USDA standard reference database as included in Nutritionist V.

Dietary Compliance and Satiety Assessment

Subjects completed compliance questionnaires on a daily basis during the 8 week feeding phase. The first questionnaire asked the subject to report all quantities of provided food left uneaten, and to specify any additional food that had been consumed during the previous 24 hours. In the second questionnaire, satiety was evaluated by use of a Labeled Affective Magnitude scale (LAM) (Cardello & Schutz, 2004; Schutz & Cardello, 2001). Similar to a Visual Analogue Scale (VAS), this scale contained phrases describing different degrees of hunger or fullness on a 100 mm vertical line (0mm = 'Greatest Imaginable Hunger', 50mm = 'Neither hungry nor full', 100mm = 'Greatest Imaginable Fullness'). Subjects were instructed to mark the vertical line at the place that best described their current feeling of hunger or fullness.

During the 12 week *ad libitum* phase, compliance was monitored by daily online recording via the Nutrihand.com website. The online records were reviewed weekly by

investigators with feedback provided to each subject by email. Records were evaluated for maintenance of restricted energy intake and assigned protein intake. The average weekly intake for each subject was calculated from the selection of consecutive days in each week with three or more meals recorded each day. The mean intake per treatment group was calculated using the average weekly intake of each subject.

Blood Analyses

After fasting overnight, plasma samples were collected from subjects in EDTA vacutainer tubes for analysis of plasma renin concentrations. Serum samples were collected in vacutainer tubes for glucose, insulin, aldosterone, and ACE analysis. Samples were separated by centrifuge at 2000 x *g* and 5^{o} C for 20 minutes and then individually labeled and stored at -80^{o} C until analysis was completed.

Insulin was measured with a commercially available ELISA kit (LINCO Research, St. Charles, MO) with a range of 2-200µU/mL. The procedure used microtiter plates coated with an antibody for human insulin. Samples were added to the prescribed wells and additional antibodies were added to sandwich the captured insulin. Quantification of the captured insulin was accomplished by measuring the activity of horseradish peroxidase in tetramethylbenzidine with an ELISA reader. Glucose was measured with a spectrophotometric protocol developed by Morin et al (Morin & Prox, 1973). A 2.5 mL glucose oxidase-preoxidase reagent was added in duplicate to 50 µL serum samples, vortexed, and then incubated two minutes before measuring absorbance in the spectrophotometer (Varian Cray 50 2.0). ACE activity was measured with a commercially available spectrophotometric assay (ALPCO Immunoassays, Salem, NH)

with a reference range of 27 – 68 U/L. The production of hippuric acid by ACE was stopped with the addition of HCl and the product then combined with cyanuric chloride to a measurable absorbance at 382 nm (Hurst & Lovell-Smith, 1981). Plasma renin activity was measured with a commercially available immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Webster, TX) with lower and upper detection limits of 5 and 500 pg/mL, respectively. The assay measured the renin that was attached to two antibodies in a non-competitive enzyme reaction (Miles, 1975). Aldosterone was measured with a commercially available radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) with lower and upper detection limits of 25 and 1600 pg/mL, respectively. This competitive radioimmunoassay measured the aldosterone bound to the antibody, which is inversely proportional to the aldosterone present in the original sample (Yalow, Berson, Odell, & Daughaday, 1971).

Body Composition Measures

Body weight was measured on an electronic scale (Scale-Tronix 5005 Stand-On Scale) to the nearest $\pm$ 0.1 kg with subjects wearing light clothing and no shoes. Height was measured to the nearest $\pm$ 1.0 cm on a stadiometer attached to the scale. BMI was calculated as kg/m^2. DXA measures of total body, lumbar spine, and dual femur were completed at baseline, 8, and 20 weeks by a trained technician. The DXA software (Prodigy, General Electric Medical, Madison, WI) calculated total tissue, total fat tissue, total lean tissue, percent body fat, and bone mineral density from the total body scan (Williams et al., 2006). In addition to total body measures, the DXA software identified five regions for additional analysis: trunk, arm, leg, gynoid, and android regions. The

android region is defined as the waist area starting from a longitudinal line directly above the pelvis and progressing upward a height that is 20% of the measured distance from the pelvis to the neck base. The gynoid region is defined from a longitudinal line at the top of the pelvis and progressing downward a total height twice as high as the android region (Campbell & Tang, 2010).

Statistical Analyses

All data are presented as means (+ SEM) of each dietary treatment group. The groups were compared using analysis of variance in Procedure General Linear Models (Proc GLM) (SAS version 9.2, SAS Institute, Inc. Cary, NC). Area under the curve (AUC) was measured for sodium and potassium intake during the *ad libitum* phase of the trial. The overall F-test is reported, with the results of pair-wise group comparisons and tests of within-group changes from baseline. Results were considered significant at $p < 0.05$.

Results

Subjects

Randomization of subjects to the three treatment groups was successful. At baseline, no statistically significant differences existed between treatment groups for age, weight, BMI, or percent body fat **(Table 4-3).** All subjects completed the 8 week feeding phase of the trial and one female from each group did not complete the 12 week *ad libitum* phase **(Figure 4-1)**. Follow up with non-completers indicated the reasons for not

returning for follow up clinical visits were life challenges and dissatisfaction with rate of weight loss.

Weight Loss and Body Composition

Significant weight loss within each treatment group was observed ($p < 0.05$ for CD, MP, and WP ranging from 6 to almost 10 kg). Differences in weight loss between all three treatments were not significant. However, there was a trend ($p = 0.08$) toward greater weight loss in the WP group compared to the CD group.

Body composition improved with a reduction in fat mass in all treatment groups. There was an observed trend of greater total fat mass loss ($p = 0.09$) in the WP group compared to the CD group, especially in the leg and gynoid regions (**Figure 4-2**). In addition, there were some regional differences in lean mass changes between treatments (**Figure 4-3**) with the WP group showing slightly greater loss of lean mass in association with greater total body weight reduction. In comparison by fat : lean ratio, the CD group showed a loss of 5.6 : 1g compared to a loss of 8.4 : 1 g with the WP group. The difference in total lean mass loss between groups at week 20 was not significantly different.

RAAS Activity, Blood Pressure, and Metabolic Measures

Plasma renin and ACE activities, and aldosterone concentration are reported in **Table 4-4**. Renin activity was not significantly different between groups and did not change over the five month protocol. ACE activity in the CD group was higher at baseline compared to the other treatment groups but the difference was not statistically

significant. By week 8, all the groups had similar ACE measures with a significant decrease within the CD group ($p < 0.05$). Over the 20 weeks, ACE activity decreased in the WP group, but was not statistically different from other groups. Aldosterone concentration was similar at baseline between treatments, and a trend toward decreasing aldosterone concentration ($p = 0.08$) was observed in the CD and WP groups at week 20.

Systolic blood pressure decreased in all three treatments during the first 8 weeks. At the conclusion of the study, there was a significant difference ($p < 0.04$) in change of systolic blood pressure between the CD and WP treatment. There were no significant changes in diastolic blood pressure between treatments.

No significant differences were observed between groups for fasting glucose or insulin measures at any time point in the study. HOMA IR values at baseline were similar between groups and the decreasing values observed with all treatments were not significantly different at weeks 8 or 20.

Dietary Results

Protein intake between diets was significantly different throughout the 20 weeks. As designed, protein intake during the feeding phase for the CD group averaged 0.8 g/kg/day, while protein intake of the MP and WP groups averaged 1.4 g/kg/day. A comparison of the essential amino acid composition average between the MP and WP treatment during the feeding phase showed an increase by mass in tryptophan (48%), threonine (63%), isoleucine (27%), leucine (45%), cystine (46%), and valine (21%) with daily intake in the WP treatment. This increase is based primarily on the additional whey protein consumed in the diet.

During the *ad libitum* phase, protein intake of the CD group increased to 1.1 g/kg/day and protein intake of the MP group was 1.3 g/kg/day, while the WP group maintained 1.4 g/kg/day. Subjects maintained a protein and carbohydrate intake that was significantly different ($p < 0.05$) between the CD treatment and both high protein treatments. No significant difference in AUC analysis for sodium and potassium intake was observed. However, the self-reported online dietary records did not ask subjects to report added salt. Ninety-three percent of the subjects reported *ad libitum* intake for more than six weeks, and 53% of the subjects reported all weeks of *ad libitum* intake.

Satiety

Average satiety responses during the feeding phase showed that even on a reduced calorie diet most subjects reported "fullness" (>50 mm). Average weekly responses did not show a significant difference between the groups. However, the WP group reported the least variability during the entire 8 weeks as shown in **Figure 4-4.** By the week 8, a trend ($p=0.08$) toward differences between the WP and the other treatments was observed at the 'Neither hungry nor full' level.

Discussion

In our study design incorporating custom reduced energy diets, all subjects showed similar weight loss and circulating hormone activity even though dietary composition was significantly different. Therefore, the hypothesis that a whey protein diet would result in greater weight loss and improved body composition compared to standard weight loss diets was rejected in this study. Although the measured outcomes

were not statistically different, there was a trend in the WP group toward greater weight loss, greater fat loss, and reduced blood pressure compared to the other treatment groups. There was no evidence to support a RAAS mediated effect as a possible mechanism to explain the blood pressure changes in the WP group.

Some previous weight loss studies (Harp, Henry, & DiGirolamo, 2002; Ho et al., 2007) have reported decreases in plasma renin and ACE activities as well as aldosterone concentrations when subjects have achieved greater than 3% weight loss. It is important to note that all of our subjects achieved greater than 3% weight loss, and showed no significant differences among the three diets in RAAS activity as assessed by renin and ACE activity, and aldosterone level. Therefore, our results fail to identify a weight loss mechanism related to a decrease in circulating ACE activity and the consumption of whey protein at the levels provided (15% of energy). In a previous study, when a high protein diet (2.0g / kg/day) was fed in a weight maintenance diet to normal weight subjects, increased plasma renin activity and aldosterone were observed (Daniels & Hostetter, 1990). Two of our treatments were high protein diets, which may have contributed to sustaining RAAS measures even during weight loss. However, the CD group did not show any significant decrease in RAAS with the comparatively lower protein levels. It is possible that the sample size of each group in the pilot study was not sufficient to identify a RAAS mediating effect. It is important to note that dietary sodium and potassium differences between groups may have confounded examination of this issue. Additionally, we only examined circulating RAAS biomolecules. Therefore, we

cannot rule out the possibility of RAAS related adipose and muscle-specific mechanisms related to weight loss under these conditions.

Consumption of prepared milk proteins in other studies has resulted in decreased blood pressure in moderately hypertensive subjects (Aihara et al., 2005; Hata et al., 1996; Seppo et al., 2003). All of the subjects in our study were normotensive; therefore, no elevated RAAS levels were observed at baseline, which might have negated the ability to demonstrate a RAAS mediating effect by whey protein during the course of the study. At the end of our study, subjects in the WP group showed a significant decrease in systolic blood pressure compared to the CD group with no significant change in RAAS activity.

Changes in circulating ACE activity did not correlate with the observed decrease in blood pressure. In contrast to our results, Harp et al reported a decrease in blood pressure and ACE activity with weight loss (Harp et al., 2002). However, Harp et al also observed that decreases in plasma renin activity and aldosterone were more strongly associated with weight loss than was ACE activity. A trend of decreasing aldosterone concentration ($p = 0.08$) was observed in the CD and WP groups compared to the MP group. However, the similar decrease in aldosterone concentration in the CD and WP groups was not associated with similar weight loss between groups.

High protein reduced energy diets have been shown to maintain lean tissue while promoting decreases in fat tissue (Layman et al., 2003). In this pilot study, there were no significant differences between groups for change in lean tissue. On average, the MP group gained lean tissue and the WP group lost lean tissue. Because of the high availability of essential amino acids and BCAA, the observed decrease in lean tissue for

the WP group was surprising. The measure of lean tissue loss in the WP group was comparable to the measure of lean tissue loss in the CD group, yet the WP group lost more weight than the CD group. Since exercise was not part of the protocol, these changes in lean tissue reflect the diet effect on lean tissue without an exercise program. If an exercise program were added to the weight loss protocol, a greater retention of lean tissue might be anticipated (Layman et al., 2005).

Trends of decreased fat tissue were observed with the MP and WP diets compared to the CD diet. These results agree with earlier studies by Layman et al that demonstrated improved body composition among those subjects assigned to a high protein diet (Layman et al., 2003; Layman et al., 2009). Differences in body composition between treatments were most clearly observed in measures of the leg and gynoid regions. It is not clear whether or to what extent the difference in amino acid composition between treatments was related to the differences observed in fat loss and lean tissue gain between the two high protein groups.

The strengths of this pilot study include the control feeding and parallel design, which provided opportunity to evaluate the effect of the different diets. The three treatments offered comparison between a standard diet and two variations of a high protein diet. The comparison between high protein diets allowed the assessment of outcome differences when different sources of protein are favored in the diet composition.

One limitation of this pilot study was small sample size. A parallel-arms design with 6 subjects in each arm was 80% power to detect an effect size larger than 1.8

between groups, at the .05 level. This corresponds to a difference between groups of at least 5.6 kg in weight, 5.6 kg in total body fat mass, or 6.5 ng/L in renin. Therefore, the hypothesis of this study was rejected since these differences were not observed.

These preliminary results based on our feasibility study establish the basis for further studies regarding the effectiveness of incorporating whey protein into custom reduced-energy diets for weight loss at midlife. The trend in the WP group toward greater weight loss, greater fat loss, and reduced blood pressure compared to the other treatment groups warrants further investigation in a larger sample.

Funding for this work was provided by grants from the National Center for Research Resources, National Institutes of Health, M01-RR00400, and from USDA National Needs Graduate Fellowship Competitive Grant No. 2005-38420-15786 from the National Institute of Food and Agriculture, and from Next Proteins, LLC.

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Figures and Tables

Figure 4 – 1

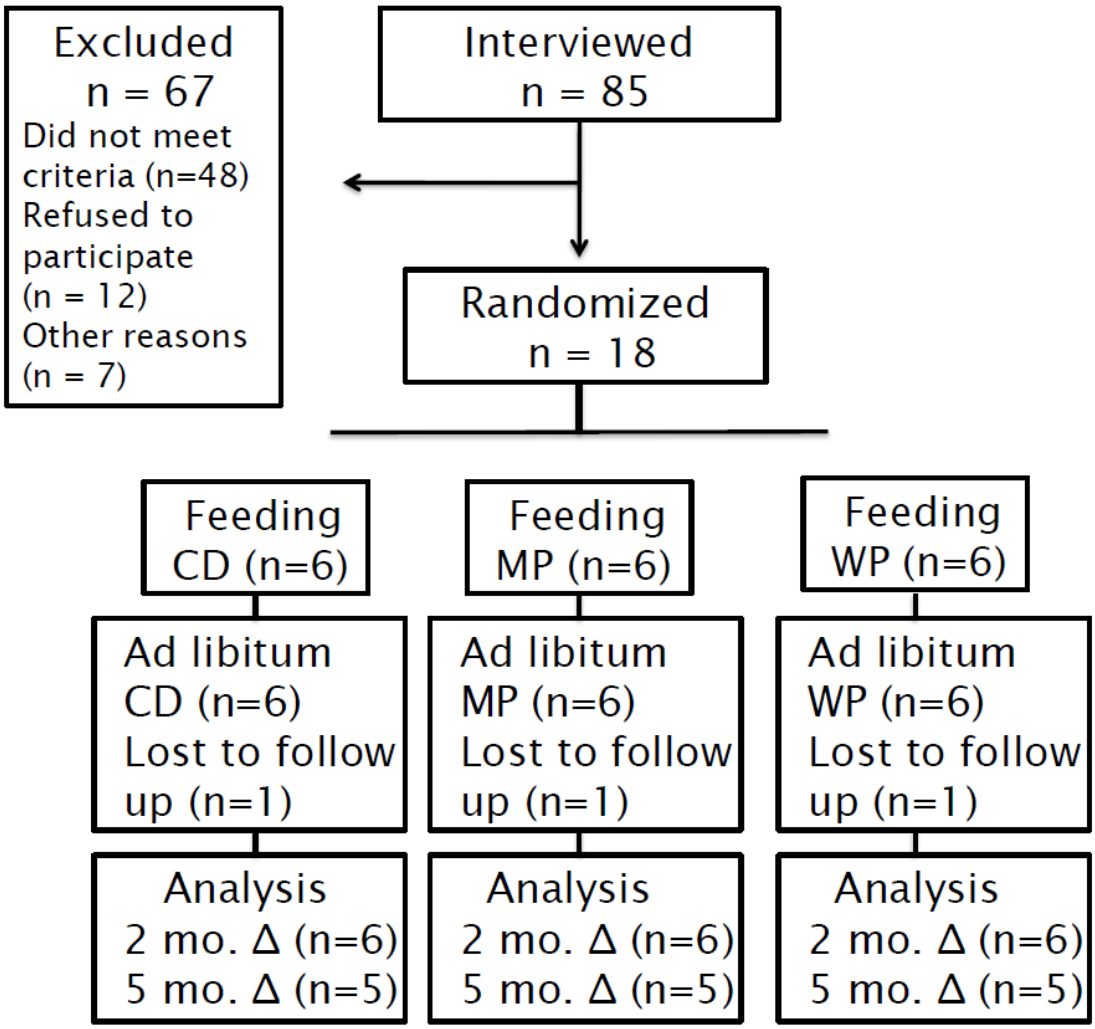

Figure 4 -1. Flow diagram for the study design and subjects
CD – control diet; MP – mixed protein diet; WP – whey protein diet

Figure 4 – 2

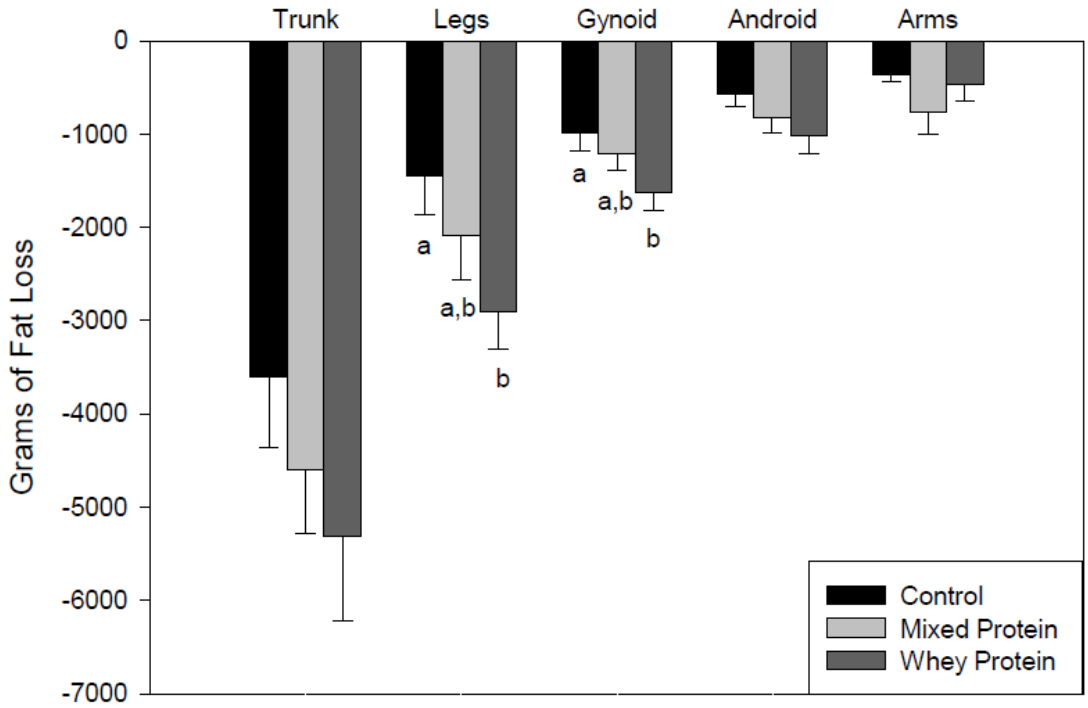

Figure 4 – 2. Fat loss by Region and by Diet at Five Months. Values measured by DXA and reported as mean (± SEM). Different lower case letter indicate significance between groups as determined by GLM.

Figure 4 – 3

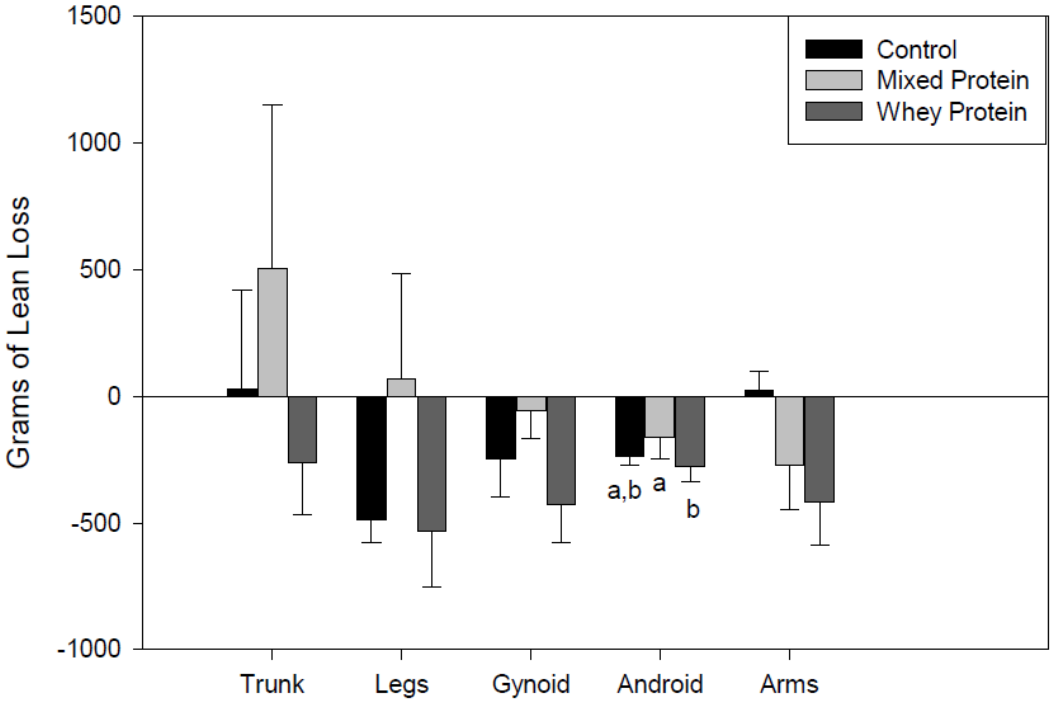

Figure 4 – 3. Lean Tissue Change by Region and by Diet at Five Months. Values measured by DXA and reported as mean (± SEM). Different lower case letters indicate significance between groups as determined by GLM.

Figure 4 – 4

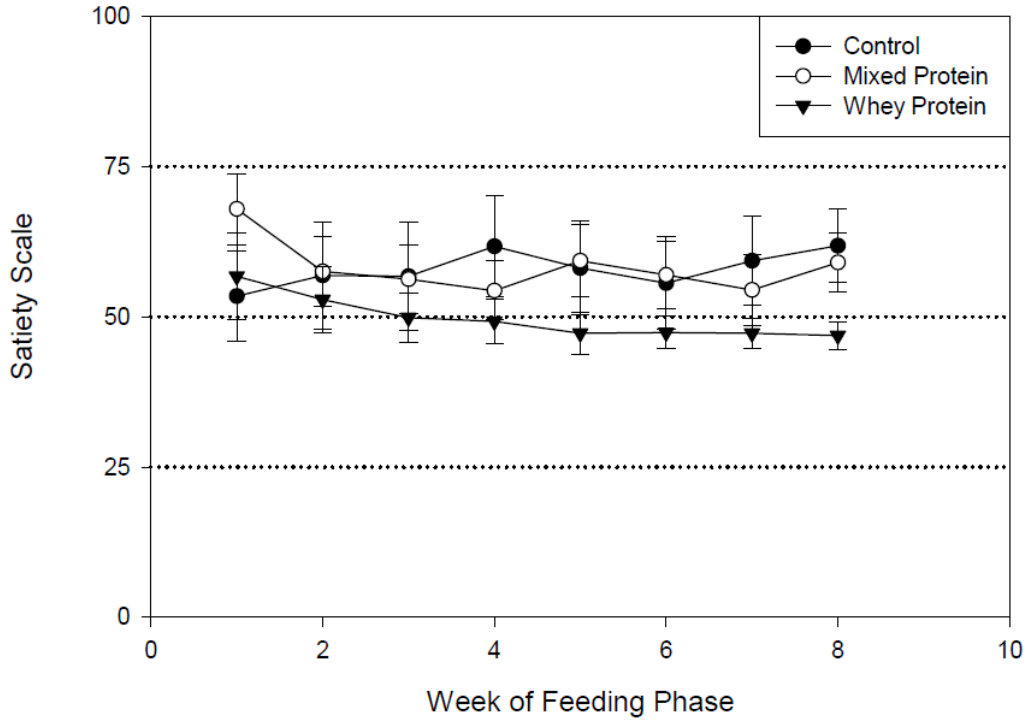

Figure 4 – 4. The weekly average satiety score during the eight week feeding phase. The Labeled Affective Magnitude (LAM) Scale consisted of labeled terms on a 100 mm scale with 0 mm begin 'Extremely Hungry', 50 mm being 'Neither Hungry nor Full', and 100 mm being 'Extremely Full'. Data shown are means ($\pm$ SEM). Week 8 differences between groups p = .08.

Table 4 – 1

Macronutrient Composition between Diet Groups[a]

Macronutrient	CD	MP	WP
Kilojoules	6699 ± 8.4	6723 ± 2.0	6698 ± 11.7
Protein (g)	63.4 ± 0.2	124.1 ± 0.5	124.2 ± 0.4
Carbohydrate (g)	225.3 ± 0.2	163.9 ± 0.6	166.9 ± 0.5
Fat, Total (g)	54.9 ± 0.4	54.0 ± 0.2	54.7 ± 0.4
Saturated Fat (g)	16.1 ± 0.1	16.0 ± 0.2	16.2 ± 0.1
Monounsaturated Fat (g)	16.1 ± 0.1	16.1 ± 0.2	16.3 ± 0.1
Polyunsaturated Fat (g)	16.1 ± 0.1	15.9 ± 0.2	16.1 ± 0.1
Dietary Fiber (g)	25.0 ± 0.5	24.0 ± 1.0	23.2 ± 0.7
Cholesterol (mg)	239.8 ± 0.8	240.0 ± 1.6	268.5 ± 3.2
Calcium (mg)	1316.1 ± 41.0[b]	1668.0 ± 90.9[b]	1536.0 ± 47.8

Data are means (± SEM)

[a]Calculated by Nutritionist V software

[b] includes Caltrate 600 supplement

Table 4 – 2

Sample Menu (6699 kJ)

Meal	Menu Item	Diet	CD (g)	MP (g)	WP (g)
Breakfast	Apple Juice		200	100	70
	Wheat Chex Cereal		37	25	25
	2% Milk		162	180	200
	Whole Wheat English Muffin		62	23	30
	w/ Butter		10	3	0
	w/ Canola Margarine		0	0	6
	Jam – Low Sugar		4	3	3
	Egg Beaters		0	75	0
	w/ Butter		0	3	0
	w/ 2% milk		0	20	0
	Cheddar Cheese, Low fat		0	35	0
	Designer Whey – Chocolate		0	0	28
Lunch	Rye Bread		46	0	0
	Reduced Calorie Wheat Bread		0	46	46
	Corned Beef		44	70	35
	Mayonnaise, Regular		14	6	0
	Mayonnaise, Lite		0	0	20
	w/ Egg Yolk		10	1.5	0
	Romaine Lettuce		25	20	20
	Red Tomato		37	30	30
	Yellow Mustard		4	3	3
	Carrots, raw		25	20	25
	Celery, raw		25	20	25
	Blueberries		187	80	75
	GENISOY Deep Sea Soy Nuts		0	35	0
	Designer Whey – French Vanilla		0	0	28
Dinner	Chicken Breast, cooked		56	135	55
	Egg Noodles, cooked		187	75	0
	Rotini, cooked		0	0	85
	Chicken Gravy		75	50	75
	w/ Olive oil		3	4	4
	Green Beans, cooked		125	90	100
	Whole Wheat Dinner Roll		44	0	0
	Reduced Calorie Wheat Bread		0	25	30
	w/ Butter		0	3	8
	w/ Canola Margarine		5	0	0
	Mandarin Oranges, canned		175	90	70
Snack	Milk Chocolate Chips		30	0	0
	Walnuts		14	14	15
	Skim Milk		150	240	220
	Designer Whey – Chocolate		0	0	28

Table 4 – 3

Subject Characteristics by Group at Baseline and Change in Study Parameters at 2 and 5 Months

	CD	MP	WP	Overall *p value*
N (F/M)	6 (5/1)	6(5/1)	6(5/1)	
Age (years)	51.3 ± 2.1	49.6 ± 3.5	49.2 ± 1.6	0.824
Baseline				
BMI	29.9 ± 0.6	30.3 ± 0.7	30.6 ± 0.6	0.712
% Body Fat	43.0 ± 2.5	42.7 ± 2.5	45.2 ± 2.9	0.777
HOMA-IR	0.89 ± 0.18	0.96 ± 0.28	0.99 ± 0.26	0.954
Weight (kg)				
Baseline	81.6 ± 1.9	84.4 ± 1.2	85.2 ± 4.2	0.626
2 mo. Δ	- 5.3 ± 0.76*	- 5.9 ± 0.59*	- 7.3 ± 0.73*	0.167
5 mo. Δ	- 6.1 ± 0.82*	- 7.6 ± 1.72*	- 9.7 ± 1.27*	0.198
Systolic				
Baseline	117.8 ± 4.3	117.7 ± 2.7	116.2 ± 3.2	0.933
2 mo. Δ	- 2.2 ± 5.1	- 8.7 ± 3.9	- 3.3 ± 3.8	0.541
5 mo. Δ	3.2 ± 4.5[a]	- 3.6 ± 2.7[a]	- 7.2 ± 1.7[b]*	0.106
Diastolic				
Baseline	67.7 ± 2.9	66.8 ± 2.4	66.2 ± 3.4	0.936
2 mo. Δ	3.8 ± 4.2	- 4.3 ± 2.1	- 2.3 ± 3.8	0.255
5 mo. Δ	1.2 ± 3.2	- 2.6 ± 1.3	- 6.0 ± 4.0	0.289
Fasting REE				
Baseline	1627 ± 139	1582 ± 152	1452 ± 81	0.613
2 mo. Δ	- 269 ± 93*	- 160 ± 125	- 43 ± 69	0.296
5 mo. Δ	- 264 ± 237	- 247 ± 184	- 21 ± 88	0.583
Total Body Fat mass (kg)				
Baseline	34.65 ± 1.9	35.61 ± 2.1	37.78 ± 1.6	0.498
2 mo. Δ	- 4.29 ± 0.7*	- 5.17 ± 0.5*	-5.99 ± 0.7*	0.211
5 mo. Δ	- 5.45 ± 1.1*	- 7.54 ± 1.4*	-8.77 ± 1.3*	0.216
Total Body Lean mass (kg)				
Baseline	43.83 ± 2.6	45.67 ± 1.8	44.95 ± 4.9	0.927
2 mo. Δ	-1.12 ± 0.9	- 0.32 ± 0.8	-1.14 ± 0.4*	0.680
5 mo. Δ	-0.32 + 0.4	+0.43 + 1.1	-1.09 + 0.1*	0.300

Data are means (± SEM) Row values displaying different lowercase letters [a,b] are significantly different ($p < .05$, Proc GLM)

* marks significant within-group change from baseline ($p < 0.05$)

Table 4 – 4

RAAS Concentrations by Group

	CD	MP	WP	*p value*
N (F/M)	6 (5/1)	6(5/1)	6(5/1)	
Renin (ng/L)				
Baseline	7.2 ± 0.5	7.2 ± 1.3	7.7 ± 1.8	0.941
2 mo.	8.4 ± 0.8	8.5 ± 1.6	8.3 ± 0.7	0.984
5 mo.	9.5 ± 1.4	8.4 ± 1.8	9.0 ± 1.2	0.871
ACE (U/L)				
Baseline	61.2 ± 3.2	50.3 ± 4.4	50.0 ± 5.9	0.187
2 mo.	51.7 ± 2.1*	50.0 ± 5.4	48.0 ± 6.0	0.867
5 mo.	52.2 ± 7.0	54.4 ± 5.7	46.8 ± 4.6	0.651
Aldosterone (ng/L)				
Baseline	46.5 ± 6.4	53.8 ± 8.7	54.5 ± 11.1	0.789
2 mo.	48.9 ± 8.6	77.8 ± 21.5	73.1 ± 18.6	0.461
5 mo.	49.0 ± 4.4[a]	67.8 ± 11.1[b]	42.8 ± 4.4[a]	0.077

Data are means (± SEM) Row values displaying different lowercase letters [a,b] are significantly different ($p < .05$, Proc GLM)

* marks significant within-group change from baseline ($p< 0.05$)

Chapter Five: Perceived Importance of Dietary Protein to Prevent Weight Gain: A National Survey among Midlife Women

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Prepared for

Journal of Nutrition Education and Behavior, June, 2013

Objective: Evaluate reported use of the practice of "eating more protein" to prevent weight gain among midlife women.

Design: Cross-sectional national survey

Participation: One thousand eight hundred twenty-four midlife women (40-60 y) from the 9 United States geographic regions, primarily married (71%), white (76%), and well educated; half were premenopausal (49%).

Outcomes: Frequency of dietary practices to prevent weight gain, Weight Efficacy Lifestyle score, self-reported weight change and body mass index over the past 2 years, and current protein intake.

Analysis: Linear regression models determined associations between weight change, protein intake, and reported use of the practice of "eating more protein" to prevent weight gain.

Results: Most women correctly identified good protein sources, and the majority could indicate the daily percent dietary energy recommended from protein. "Eating more protein" to prevent weight gain was reported by 43% of women as a practice to prevent weight gain and was associated with weight loss over a 2-year period and with increased percent energy from protein.

Conclusions and implications: Reported use of the practice of "eating more protein" was associated with weight loss over 2 years. Education regarding dietary protein requirements may enhance the use of this practice.

Introduction

Successful weight loss and maintenance of lean mass with high-protein diets has been reported in several studies among adult men and women (Baba et al., 1999; Clifton, Keogh, & Noakes, 2008; Layman et al., 2003). These effects may be related to enhanced satiety (Apolzan, Carnell, Mattes, & Campbell, 2007), increased thermogenesis (Halton & Hu, 2004), and improvements in body composition (Westerterp-Plantenga, Nieuwenhuizen, Tome, Soenen, & Westerterp, 2009). Although many high-protein diets have reported significant results at 6 months, effectiveness in weight loss is comparable to low-fat/high-carbohydrate diets at 12 months (Hession, Rolland, Kulkarni, Wise, & Broom, 2009). Based on the reported short-term benefits of a high-protein diet for weight loss or control, interest in including more protein in consumer diets may be increasing. A national qualitative consumer survey indicated that many consumers were interested in including more protein in their diets (~50%), whereas 40% and 37% believed protein helps them feel full and helps with weight loss, respectively (International Food Information Council Foundation, 2010).

The Institute of Medicine Dietary Reference Intakes has established an Acceptable Macronutrient Intake Range (AMDR) for protein at 10%-35% of total calorie intake (Institute of Medicine, 2005). The 2010 Dietary Guidelines Advisory Committee addressed dietary protein in their policy document by emphasizing the AMDR because consumers had begun to use high-protein diets more commonly for weight control (Dietary Guidelines Advisory Committee, 2010; USDA and USDHHS, 2010). The Dietary Guidelines Policy Document emphasizes that individuals should evaluate current

intake to confirm that nutritional benefits of vitamins and minerals from nutrient-dense food are attained while maintaining energy balance.

More midlife (40-59 y) women (66%) are overweight or obese in the United States (US) compared to younger (20-39 y) women (55.8%) (Flegal, Carroll, Kit, & Ogden, 2012). Increased age has been associated with decreased activity, resulting in an energy imbalance that favors weight gain in women (Brown, Williams, Ford, Ball, & Dobson, 2005). Within the US, 66% of women and 48% of men wanted to lose weight, according to the 2003 Behavioral Risk Factor Surveillance System (Bish et al., 2007), representing a significant increase from 44% of women and 29% of men reported earlier (Kruger, Galuska, Serdula, & Jones, 2004; Serdula et al., 1999). In the US, healthy practices reported most often to promote weight loss have included smaller portions, increasing fruit and vegetable intake, decreasing fat intake, eliminating intake of sweetened beverages, and increasing exercise (Kruger, Blanck, & Gillespie, 2006; McGuire, Wing, Klem, Seagle, & Hill, 1998). Among midlife women in Australia (Williams, Germov, & Young, 2007), those successful at controlling weight over 2 years used a combination of practices involving decreasing the amount of food consumed and cutting down on fats and sugars. Previous studies have not specifically assessed the frequency that women report eating more protein as a weight control practice.

The purpose of this study was to (1) describe perceptions about protein sources and requirements, (2) identify the reported frequency of using the "eating more protein" practice to prevent weight gain, and (3) compare reported protein intake to reported frequency of using the "eating more protein" practice to prevent weight gain among a

national sample of midlife women. This information will assist those who counsel women about weight loss and maintenance to ensure that practical recommendations about dietary protein are appropriate based on current knowledge and practice.

Methods

This cross-sectional survey was part of a larger study involving midlife women to describe eating occasions and the rational, emotional, and contextual needs related to those occasions (Perry, 2011). A survey packet mailed to participants included a 1-day food record booklet, eating occasion questionnaires to assess needs related to eating occasions, and a general questionnaire booklet that included questions to assess anthropometric and physical characteristics (Mills, Perry, & Reicks, 2011), weight-related self-efficacy and practices, and perceptions about protein sources and requirements.

Participants

Participants were members of a national mail panel maintained by Taylor Nelson Sofres (TNS) Global. The database maintained by TNS Global includes 1.3 million persons from 500,000 households in the US. The demographic and household information of the panel's participants is regularly updated. A sample population was selected by TNS Global that was balanced to the demographic characteristics of the US census data (US Department of Commerce, Bureau of the Census & US Department of Labor, Bureau of Labor Statistics, 2009) based on the 9 geographic regions, metropolitan and micropolitan statistical areas within the US (Office of Management and Budget,

2000), age, income, household size, race/ethnicity, and household composition. The study was approved with informed consent procedures by the University of Minnesota Institutional Review Board prior to data collection. Each participant who returned a completed packet received $6 from TNS Global.

In May, 2008, recruitment letters and consent forms were mailed by TNS Global to 8,000 households, and 2,713 women (33.9%) returned a signed consent form. These women were sent survey packets, and 1,634 (60.2%) completed packets were returned to TNS Global. To increase sample size, another 1,200 households were sent a survey packet and consent form in July, 2008; 292 (24.3%) completed packets were returned by August 2008, which yielded a total sample of 1,926 women. Data included in the general questionnaire were collected by TNS Global staff via machine-scannable questionnaires, reviewed where potential questions occurred as to respondent intent and corrected prior to providing them to the researchers in an Excel file (version 12, released 2007).

Physical Status

A written set of instructions was included in the survey packet directing participants to complete the general questionnaire after completing all other instruments. Two pilot-tests were completed to test the instructions with mail panel members who were not part of the larger group, and the tests were revised as needed. Current height, weight, age, menopausal status (Hislop et al., 2006), and weight 2 and 5 years ago were self-reported. Body mass index (BMI) was calculated as kg/m^2. Women were classified as normal weight (BMI< 25), overweight (BMI ≥ 25 and < 30), or obese (BMI ≥ 30) (World Health Organization, 9/1/2012).

Weight Gain Prevention Practices

Reported frequency of 13 practices was assessed with the question, "Have you used the following practices to prevent weight gain?" listed in a checklist format; response options were "Yes,, in the past 12 months," "Yes, more than 12 months ago," or "Never." The original list of practices was modified to add the practices of "eating more protein" and skipping meals, and to more clearly define supplement use to enhance satiety or to increase metabolism (Perry, 2011). Response to "Yes, in the past 12 months" and "Yes, more than 12 months ago" were both considered "Yes" for comparison of those who had ever used the practice to those who had never used the practice.

Self-Efficacy

The Weight Efficacy Lifestyle questionnaire (WELQ) was composed of 20 items, with 4 questions from each of 5 situational factors (negative emotions, availability, social pressure, physical discomfort, and positive activities) (Clark, Abrams, Niaura, Eaton, & Rossi, 1991). An example question from the negative emotions category was "I can resist eating when I am angry (or irritable)." Women responded on a scale of "0" (not confident) to "9" (very confident) for each question. A higher score indicated greater self-efficacy toward food-related situations. Previous studies using the WELQ have been conducted with weight-loss groups who were predominantly white with middle to upper incomes (Clark, Cargill, Medeiros, & Pera, 1996). The generalizability of the WELQ to

minority and low-income groups may be limited; however, the women in the current study were primarily white and fairly well educated.

Perceptions about protein

Four questions were developed to evaluate dietary protein perceptions (**Table 5-1**). The questions were pretested with 42 women from TNS Global's mail panel meeting the same recruitment criteria as those in the current study. Face validity was confirmed by several registered dietitians, who provided comments regarding suggested revisions. The first question assessed perceptions about good sources of protein. The second question assessed the frequency with which participants chose the various food types provided in the first question as a protein source. The third question asked participants to select the daily percent energy that should come from protein. The final question evaluated perceptions about why protein may be helpful for weight loss based on current nutrition concepts regarding protein and weight loss (Whitney & Rolfes, 2005).

Food Intake

Actual dietary protein intake, total caloric intake, and percent energy from protein were estimated from the self-reported date that participants provided in a 1-day food record booklet. Instructions, based on those used by other investigators, instructed women to describe all food items and beverages and amounts consumed immediately after eating (Kolar et al., 2005). Women were also instructed to record time eaten, type of occasion, and preparation methods/recipes over a 1-day period. The food record booklet included reduced-scale photographs of representative food items and serving

sizes (Kolar et al., 2005) and an example of a completed 1-day record that described the food items in adequate detail. An 11-minute instructional DVD accompanied the booklet and was also available online.

Instructions to complete a 1-day food record on a weekday (Monday through Thursday) were included in 57% of survey packets, and 43% included instructions to complete a 1-day food record on a weekend day (Friday-Sunday). Data from the food record booklet were entered into the Nutrition Data System for Research software program (NDSR; version 2008, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN 2008) by nutrition students trained in the use of NDSR. A registered dietitian certified in the use of NDSR software entered about 20% of the food records a second time to monitor data entry and provide regular feedback to standardize and improve quality of data entry. Data were excluded as part of the larger study when food intake information was missing, when meal times recorded on an eating occasion questionnaire did not match those on the food record booklet, and when implausible intakes were recorded. Intake data considered implausible (from about 20 women) were identified by researchers and mutual agreement within the context of the entire day's intake. The exclusions resulted in nutrient intake data from 1,633 women based on 5,556 eating occasions.

Data Analysis

All statistical procedures were performed with SAS (version 9.2, SAS Inc., Cary, NC, 2002-2008) software. Descriptive statistics were used for demographic and physical characteristics, WELQ total scores, and protein intake in grams/day from the 1-day food

record booklet. Primary outcome measures were tested for normality (SAS PROC UNIVARIATE) and found to be normally distributed. A generalized linear model (PROC GLM) was used to determine differences in variables by BMI group (normal, overweight, and obese) and by weight change groups. Chi-square tests were used to determine differences in categorical variables among BMI groups.

Separate multivariate regression models (SAS PROC REG) were used to identify factors associated with each of 3 dependent variables – reported weight change over 2 years, percent energy from protein, and frequency of reported use of the "eating more protein" practice to prevent weight gain. Demographic, anthropometric, and physical characteristics and the WELQ score were added to the models as explanatory variables. Weight change, percent energy from protein, and reported use of the "eating more protein" practice to prevent weight gain were also included as independent variables when the particular factor was not being modeled as the dependent variable. Family income (<$30,000; $30,000-49,999; $50,000-74,999; ≥ $75,000) was modeled as a 4-levvel categorical variable; marital status, menopausal status, and education (2+ years of college vs. no college) were dichotomized as 1 = yes and 0 = no, and race/ethnicity was modeled using indicator variables. Statistical analyses were 2-sided, and $P < .05$ was considered significant.

Results

Demographic characteristics are presented in **Table 5-2**. Of 1,926 returned questionnaires, 102 were excluded as a result of incomplete data regarding WELQ survey questions, or for self-reported age outside of 40-60 years. Analysis was completed with

the remaining 1,824 questionnaires. About 40% of all women were classified as normal weight. Normal weight participants tended to be younger and were more likely to be premenopausal and married, have a larger household size, and be more educated than those in the overweight and obese BMI groups. Among all participants, a significantly lower WELQ score was observed as BMI increased.

Participants who reported current weight and weight 2 years ago (n = 1,754) were classified by weight change group. Three groups were identified, a Maintenance group (n = 381) reporting $\leq$ 1 kg weight change (mean 0.0 kg $\pm$ 0.42 kg), a Lose group (n = 659) reporting >1 kg weight loss (mean -9.95 kg $\pm$ 10.83 kg), and a Gain group (n = 714) reporting >1 kg weight gain (mean +7.20kg $\pm$ 7.57kg).

Weight Gain Prevention Practices

The reported frequency of use of 13 weight gain prevention practices is presented in **Table 5-3**. The most common practices included cutting down portion size, exercising, and cutting down on fats and sugars, all reportedly used by >80% of all women. The fourth most common practice was "eating more protein" to prevent weight gain (42.5%). Frequencies of practices were significantly different between BMI groups except for following a vegetarian diet and smoking. In general, women in the normal BMI group reported using weight gain prevention practices less frequently compared to the overweight and obese groups. More obese women (~52%) than normal weight women (~34%) reported using the "eating more protein" practice to prevent weight gain.

Of the top 6 practices to prevent weight gain, 4 practices were significantly different between the Lose and Gain groups (**Table 5-4**). The Lose group reported using

"eating more protein" to prevent weight gain more frequently than the Gain group, and the Gain group reported skipping meals more frequently than the Lose group. Scores on the WELQ were significantly higher in the Lose group compared to the Gain group for all 6 practices. The Maintenance group WELQ scores were significantly higher and frequencies of using the practices were significantly lower for all 6 practices compared to the Lose and Gain groups, as might be expected from a group that may not face food control challenges (data not shown).

Perceptions about Protein

The frequency of responses to the protein questions is shown in **Table 5-1**. Most women were able to correctly identify good sources of protein, whereas 37% chose the "I don't know" response to the question, "How many of the calories you eat each day should come from protein?" Obese women reported using the practice of "eating more protein" to prevent weight gain more often than overweight or normal-weight women ($P < .001$). Those who reported using the practice of "eating more protein" to prevent weight gain were more likely to respond that a higher percentage of energy should come from protein and had appropriate perceptions of why protein was useful for weight loss ($P<.001$; Table 5-1). The majority of participants indicated that protein is helpful for weight loss because protein builds muscle; however, more than 40% of the women selected the response that protein provides more energy than carbohydrates or fat.

Reported use of the practice of "eating more protein" to prevent weight gain was related to estimated dietary intake based on a 1-day food record. Those who reported using the practice of "eating more protein" to prevent weight gain had greater total

protein intake (72.5 g ± 29.1g vs. 68.2 g ± 27.6 g; $P = .002$) and greater percent energy from protein (16.7% vs. 15.5%; $P < .001$) compared to those who never used this practice, respectively (data not shown).

Regression Models

Each of the 3 regression models demonstrated significant associations, but the overall fit of each model represented by R^2 was minimal, ranging from 0.02 to 0.16, which explains a limited proportion of variance (**Table 5-5**). Correlations between variables did not exceed $R^2 = 0.30$; therefore, all variables remained in each model as indicated (O'Rourke, Hatcher, & Stepanski, 2005). Current BMI and income were significant factors associated with reported use of the "eating more protein" practice to prevent weight gain. A significant association was observed between the calculated percent energy from protein and reported use of the practice of "eating more protein" to prevent weight gain ($P < .001$). The practice of "eating more protein" was positively associated with BMI and income, whereas weight change, household size, and WELQ scores were negatively associated.

Discussion

The results of the current study based on a national survey provide information regarding the reported frequency of "eating more protein" as a weight gain prevention practice and perceptions of factors that facilitate use of the practice, such as protein sources and dietary requirements. The relatively high proportion of women who reported using the practice of "eating more protein" to prevent weight gain (~43% for all women

and more than half of obese women) was consistent with the focus on protein by the 2010 Dietary Guidelines Advisory Committee, the increased interest in protein reported in another survey of adults (International Food Information Council Foundation, 2010), and clinical studies that have suggested positive effects when higher protein consumption is used as a weight control strategy (Halton & Hu, 2004; Westerterp-Plantenga et al., 2009).

In the current study, reported use of the "eating more protein" practice to prevent weight gain was associated with a negative weight change over 2 years, with more women in the Lose group reporting use of this practice compared to those in the Gain group. Reporting ever "eating more protein" to prevent weight gain was also associated with a greater percent energy from protein. Other studies regarding weight control strategies did not attempt to link reported use of strategies such as reducing calories/ amount of food or eliminating sweets, junk food, or snacks to dietary intake (Savage & Birch, 2010; Williams et al., 2007). Furthermore, in their questionnaires, these earlier studies did not include the option of "eating more protein" as a practice to control weight or prevent weight gain (Kruger et al., 2006; McGuire et al., 1998; Serdula et al., 1999; Williams et al., 2007). The addition of this option was helpful to further describe commonly used practices to prevent weight gain. However, the way that women manage to include more protein in their overall diet, including the types of food consumed and meal occasions affected, is still not known.

Although the majority of women were able to identify good sources of protein, a number indicated they usually / sometimes select from the vegetable group (46%) or fruit group (~43%) when choosing a protein source. In addition, although many participants

recognized soy powder, whey powder, and Ensure as good sources of protein, few (~17%) indicated they chose these food items as a protein source. Therefore, perceptions of select food items as good protein sources did not always correspond to choosing these food items as sources of protein.

About one third of women indicated, "I don't know" in response to the question about how much of daily calories should come from protein. More information about dietary protein is needed in highly visible national dietary guidelines for consumers, such as the Nutrition Facts Panel or MyPlate consumer materials. In support of this concept, a recent study among adolescents and their parents showed a lack of knowledge of calories contained in carbohydrates relative to protein and fat (Nelson, Lytle, & Pasch, 2009). Although this information is provided in the Dietary Guidelines policy document (USDA and USDHHS, 2010), consumers may be exposed to only selected messages and not read the entire document. The extent that consumers are aware of the AMDR outlined in the policy document is not known (USDA and USDHHS, 2010), yet they are encouraged to consume an appropriate number of calories within the AMDR to manage body weight. Some women in the current study (~43%) indicated that protein provides more energy than carbohydrates or fats, which is consistent with the premise that consumers may not be aware of the basic energy contributions of each macronutrient to the diet or that they felt that protein intake made them feel more energetic than when they eat carbohydrates or fats. Given that the AMDR for protein is quite broad, education about energy balance and macronutrient energy content may be important.

The measure to assess frequency of using the "eating more protein" practice to prevent weight gain was based on ever having used the practice, which may not be reflected in a 1-day record of food intake. Ever using the practice of "eating more protein" to prevent weight gain was not correlated with eating self-efficacy reflected in the WELQ score. Although increased self-efficacy has been associated with fiber (Hagler et al., 2007), and fruit and vegetable intakes (Shaikh, Yaroch, Nebeling, Yeh, & Resnicow, 2008), limited cross-sectional studies have examined the relationship between self-efficacy and protein intake.

The strengths of this study include the large number of midlife women from a national mail panel. Although requests to participate were sent to a sample balanced according to the US Census data for demographic characteristics, self-selection resulted in a nonrepresentative national sample, which is a limitation of the study. Other limitations were that a 1-day food record to assess dietary intake limits the ability to accurately estimate usual intake. Women (n = 161) who provided problematic food record data (missing, mismatched times, or implausible intakes) had lower levels of education ($P < .001$) and income ($P < .001$) and were less likely to be white ($P < .001$) than the remaining women, whereas BMI, age, household size, and employment status did not differ. Therefore, exclusion of data from these women may have lessened the likelihood of observing relationships between income / education and other variables. Data regarding current weight and weight history were self-reported, therefore accuracy was not confirmed. Recent studies have confirmed significant discrepancies between self-reported weight and measured weight (Gorber, Tremblay, Moher, & Gorber, 2007;

Isidoro et al., 2011). In these studies, weight was often underreported and height was overreported. Relying on recall of weight history from 2 and 5 years in the past is an additional limitation.

Implications for Research and Practice

The practice of "eating more protein" to prevent weight gain was the fourth most frequent practice reportedly used among a national sample of midlife women. Reported use of this practice was related to self-reported weight loss over 2 years. Two factors associated with effective use of this practice included the level of protein intake and self-efficacy toward weight management. Women may need more information regarding protein energy content and effective selection of protein sources to enhance protein intake as a weight management strategy. Additional research is needed to evaluate the change in self-efficacy that may be associated with information about protein. Given that the majority of Americans are overweight, identifying the most effective practices and related factors surrounding successful weight loss and prevention of weight gain are important.

This project was supported by national Research Initiative Grant no. 2007-55215-17907 from the United States Department of Agriculture national Institute of Food and Agriculture Human Nutrition and Obesity program.

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Table 5 -- 1 Responses to Protein Questions for All Women and by Reported Use of "Eating More Protein" Practice to Prevent Weight Gain

	All Women (%)	Reported Use of the "Eating More Protein" Practice %	Did Not Report Use of "Eating More Protein" Practice %	*P* [b]
1. "Which type(s) of foods do you think are good sources of protein?"(Mark all that apply)[a]	n=1824	n=775	n=1049	
Beef, chicken, fish, pork, lamb	94.1	94.7	93.7	0.37
Milk, yogurt, cheese, eggs	76.0	79.0	73.9	0.01
Margarine, olive oil, canola oil, butter	4.9	5.3	4.6	0.48
Wheat bread, corn meal, oatmeal, pasta	14.8	15.0	14.7	0.87
Baked beans, lentils, peanuts, walnuts	79.9	82.5	78.0	0.02
Lettuce, cabbage, broccoli, carrots, greens	14.7	15.2	14.3	0.58
Apples, oranges, bananas, grapes, prunes	11.7	13.7	10.3	0.03
Soy powder, whey powder, Ensure, Boost	43.4	47.1	40.6	0.006
2. "How often do you choose each type(s) of food as a protein source?" (usually/always; sometimes; or rarely/never – Mark only one response for each food type)[a,c]	n=1824	n=775	n=1049	
Beef, chicken, fish, pork, lamb	94.1	97.0	91.9	<0.001
Milk, yogurt, cheese, eggs	87.7	91.6	84.8	<0.001
Margarine, olive oil, canola oil, butter	29.3	30.5	28.4	0.34
Wheat bread, corn meal, oatmeal, pasta, rice	44.2	45.0	43.7	0.56
Baked beans, lentils, peanuts, walnuts	82.5	85.0	80.7	0.02
Lettuce, cabbage, broccoli, carrots, greens	46.1	49.8	43.4	0.006
Apples, oranges, bananas, grapes, prunes	42.6	46.1	40.0	0.01
Soy powder, whey powder, Ensure, Boost	16.8	22.5	12.7	<0.001

Table 5 - 1 continued

3. "How much of the calories you eat each day should come from protein?" (Mark only one response)	n=1774	n=762	n=1012	
5 – 10%	2.4	1.8	2.8	0.44
12 – 15%	12.0	8.2	15.0	0.68
20 – 25%	27.4	29.9	25.5	0.005
30 – 40%	21.3	30.7	14.2	<0.001
I don't know	36.9	29.4	42.5	0.23
4. "Protein is helpful for weight loss because ____?"(Mark all that apply)[a]	n=1824	n=775	n=1049	
Builds muscle, not fat	57.7	64.9	52.4	<0.001
(Extra) is not stored in the body	10.9	14.6	8.2	<0.001
Provides more energy than carbs or fat	42.7	50.1	37.2	<0.001
Helps you feel full	40.5	49.9	33.5	<0.001
I don't know	17.3	9.2	23.3	<0.001

[a]Frequency of responses for multiple choice questions

[b]Chi Square test (significance level $P = 0.05$)

[c]The percentage represents the proportion who selected the combined "usually/always" and "sometimes" response options.

Table 5 -- 2

Demographic and Personal Characteristics of Respondents to a National Survey about Protein Intake and Weight Gain for All Women and by BMI Group[a]

Characteristics	All women	BMI Category Normal 40% (n=731)	Overweight 25.1% (n=457)	Obese 34.9% (n=636)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*P* [b]
Age	49.5 ± 5.9 (n=1824)	48.9 ± 5.7^{x} (n=731)	49.7 ± 5.9^{y} (n=457)	50.0 ± 5.6^{y} (n=636)	0.003
Household size	3.0 ± 1.4 (n=1823)	3.1 ± 1.4^{x} (n=730)	2.9 ± 1.4^{x,y} (n=457)	2.9 ± 1.4^{y} (n=636)	0.02
BMI (kg/m^2)	29.0 ± 8.2 (n=1824)	21.9 ± 2.0^{x} (n=731)	27.2 ± 1.4^{y} (n=457)	37.6 ± 7.0^{z} (n=636)	<0.001
Weight change (kg) (2 yrs)	-0.8 ± 11.2 (n=1754)	-1.7 ± 7.5^{x} (n = 681)	-1.2 ± 9.9^{x} (n=451)	+0.5 ± 14.8^{y} (n = 622)	0.002
WEL Total Score	121 ± 38 (n=1800)	132 ± 33^{x} (n=711)	118 ± 38^{y} (n=456)	110 ± 40^{z} (n=633)	<0.001
	% (n)	% (n)	% (n)	% (n)	*P* [c]
Income > $50K	64.3 (1172)	74.2 (542)	68.9 (315)	49.5 (315)	<0.001
Married	71.6 (1306)	78.0 (569)	74.6 (341)	62.3 (396)	<0.001
White	76.5 (1395)	82.2 (600)	75.5 (345)	70.8 (450)	<0.001
≥ 4 yr college degree	53.6 (975)	59.9 (438)	57.6 (263)	43.1 (274)	<0.001
Employed full time	51.9 (945)	51.6 (375)	55.6 (254)	49.7 (316)	0.15
Premenopausal	48.9 (872)	52.9 (374)	49.2 (222)	44.2 (276)	0.005

[a]Normal weight = BMI < 25 kg/m^2, overweight = BMI ≥ 25 and < 30 kg/m^2, obese = BMI ≥ 30 kg/m^2

[b]Values with different superscript letters (x, y, z) in the same row are significantly different (General Linear Models procedure / Duncan) (significance level $P = 0.05$)

[c]Chi-square test (significance level $P = 0.05$)

Table 5 – 3

Frequency of Weight Gain Prevention Practices among Midlife Women by BMI Group[a,b]

Practice	All women % (n)	Normal % (n)	Overweight % (n)	Obese % (n)	*P* [c]
Cutting down portion size	86.0 (1569)	77.7 (568)	91.3 (417)	91.8 (584)	<0.001
Exercising	85.0 (1551)	81.5 (596)	87.1 (398)	87.6 (557)	0.003
Cutting down on fats/sugars	81.7 (1491)	75.1 (549)	86.0 (393)	86.3 (549)	<0.001
Eating more protein	42.5 (775)	34.1 (249)	43.3 (198)	51.6 (328)	<0.001
Skipping meals	37.3 (680)	28.6 (209)	40.0 (183)	45.3 (288)	<0.001
Commercial programs	24.4 (445)	14.5 (106)	25.0 (114)	35.4 (225)	<0.001
Meal replacement items	20.2 (368)	12.9 (94)	21.2 (97)	27.8 (177)	<0.001
Metabolism supplement	20.1 (366)	12.9 (94)	24.1 (110)	25.5 (162)	<0.001
Fasting	20.1 (366)	15.1 (110)	23.2 (106)	23.6 (150)	<0.001
Satiety supplement	13.8 (251)	9.2 (67)	13.8 (63)	19.0 (121)	<0.001
Vegetarian diet	13.4 (245)	13.7 (100)	14.7 (67)	12. 3 (78)	0.50
Laxatives/diuretics	12.2 (223)	9.9 (72)	11.4 (52)	15.6 (99)	0.005
Smoking	11.9 (217)	10.8 (79)	12.7 (58)	12.6 (80)	0.50

[a]Normal weight = BMI < 25, overweight = BMI ≥ 25 and < 30, obese = BMI ≥ 30.

[b]Weight gain prevention practices in descending order by frequency. Response to "yes – in past 12 months" and "yes – more than 12 months ago" were both considered as "yes" for this frequency table.

[c]Chi square test (significance level $P = 0.05$)

Table 5 – 4

Top 6 Most Frequently Used Weight Gain Prevention Practices[a] and WEL Scores[b,c] by Lose Group[d] and Gain Group[d]

	Lose Group (-9.95 ± 10.84 kg)[e]	Gain Group (+7.19 ± 7.57 kg)[e]	*P*
1. Smaller portions/fewer meals	91.9 (606)	88.2 (630)	0.02
WELQ Score	121 ± 36 (606)	112 ± 37 (630)	< 0.001
2. Exercising	89.1 (587)	86.3 (616)	0.12
WELQ Score	122 ± 37 (587)	111 ± 37 (616)	< 0.001
3. Cutting down on fats/sugars	87.7 (578)	81.8 (584)	0.002
WELQ Score	122 ± 36 (578)	112 ± 38 (584)	< 0.001
4. Eating more protein	50.4 (332)	41.5 (296)	0.001
WELQ Score	120 ± 37 (332)	109 ± 37 (296)	< 0.001
5. Skipping meals	37.5 (247)	43.6 (311)	0.02
WELQ Score	118 ± 37 (247)	110 ± 37 (311)	0.006
6. Commercial programs	28.8 (190)	25.8 (184)	0.20
WELQ Score	116 ± 37(190)	98 ± 38 (184)	<0.001

[a]Values represent % (n) and *P* value for Chi square test (*P* = 0.05)

[b]Values represent mean ± standard deviation (n) and *P* value from General Linear Models procedure (significance level *P* = 0.05)

[c]WEL Score for those reporting use of the particular practice by Lose and Gain Groups

[d]Lose group (n=659) reporting >1 kg weight loss over two years, Gain group (n=714) reporting >1 kg weight gained over two years.

[e]Mean ± standard error (kg weight change over two years)

Table 5 – 5

Multivariate Linear Regression Models for Weight Change, Reported Use of the "Eating More Protein" Practice to Prevent Weight Gain, and Percent Energy from Protein Among Midlife Women[a]

Variables	Y = weight (kg) change		Y = Reported use of the "eating more protein" practice		Y = % energy from protein	
	R^2 = 0.040, *P* <0.001		R^2 = 0.061, *P* <0.001		R^2 = 0.020, *P* = 0.002	
	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Demographic						
Weight change	--		-0.01 ± 0.00	<0.001		0.38
Current BMI	--		0.01 ± 0.00	<0.001		0.66
Household size		0.78	-0.03 ± 0.01	0.02		0.91
Education		0.58		0.56		0.30
Income	0.51 ± 0.23	0.03	0.03 ± 0.01	0.002		0.21
African American		0.23		0.92		0.85
Asian		0.83		0.68		0.46
Age		0.46		0.27		0.27
Menopausal status		0.43		0.18		0.84
Marital status		0.24		0.21		0.45
Dietary behavior						
% energy from protein		0.40	0.01 ± 0.00	<0.001	--	
WEL score	-0.05 ± 0.01	<0.001	-0.001 ± 0.000	0.008		0.10
Reported use of the "eating more protein" practice	-2.34 ± 0.56	<0.001	--		1.22 ± 0.28	<0.001

[a]Each model includes explanatory variables shown, unless omitted as indicated by a dash mark
Multiple regression analysis (significance level *P* = 0.05)

Chapter Six: Conclusions and Future Research

Conclusions

The two studies in this dissertation provided data on the efficacy of HP diets in a weight loss trial among midlife adults and the use of eating more protein as a practice to prevent weight gain among midlife women in the US. The primary objectives of the first study were to examine 1) the effect of a reduced-energy HP diet on body weight and composition, 2) the effect of a HP diet based on whey protein compared to a HP diet based on mixed protein, 3) the effect of a whey protein diet on ACE activity, and 4) the association of ACE activity to body composition changes. The primary objectives of the second study were to examine 1) the frequency of reportedly choosing "eating more protein" as a practice to prevent weight gain, 2) the association of using the "eating more protein" practice with weight maintenance, and 3) the association of a WEL score with the reported use of the "eating more protein" practice.

One of the key findings from the weight loss study was that a HP diet containing whey protein did not produce greater weight loss or statistically significant improvements in total body composition compared to a standard weight loss diet. However, while the weight loss differences between the CD and WP diet were not significant, a trend of greater weight loss was observed in the WP group compared to the CD group, and a significant loss of fat in the gynoid and android regions of the body were observed in the WP group compared to the CD group. The lack of significant difference compared to the standard diet may have been due to the small sample size of each group. Thus, we conclude the HP diet with WP produces comparable results in weight loss and body composition to the standard CD and did not produce any significant negative effects.

A second key finding from the weight loss study was that the concentration of RAS metabolites was not significantly changed due to the increased intake of WP. There was a significant decrease in systolic blood pressure in the WP group, but this change could not be attributed to a change in the RAS metabolites. Similar decreases in systolic blood pressure had been reported in earlier studies containing fermented milk proteins and an interaction between the milk peptides and ACE has been suggested (Seppo, Jauhiainen, Poussa, & Korpela, 2003; Pan, Guo, Zhao, & Cao, 2011) . However, while hypertensive subjects were recruited in these earlier studies, all of the subjects of the weight loss trial were normotensive. For the subjects in this weight loss study, blood pressure was not a significant symptom related to their weight and the RAS metabolite concentrations did not significantly change between groups. Since there was no significant change in ACE concentration, no association was found between ACE concentration and body composition. Thus, we conclude that within this controlled trial, the peptides of whey protein do not have a specific interaction with the RAS that might explain changes observed in body composition or blood pressure.

A key finding from the national survey was that "eating more protein" was the fourth most frequently reported practice for weight gain prevention among midlife women following 1) cutting down on size of meals or between meal snacks, 2) exercising, and 3) cutting down on fats and/or sugars. Though the increased number of weight loss studies and popular fad diets suggested "eating more protein" was a practice being used, this was the first national study to describe the frequency of this practice compared to other well-known practices. "Eating more protein" as a practice to prevent

weight gain was associated with improved weight maintenance. Therefore, this finding may help nutrition educators prioritize resources to better educate clients on the use of protein in weight maintenance.

Another key finding from the national survey was that an increased WEL score was not directly associated with use of the "eating more protein" practice. A high WEL score is related to successful weight maintenance, and those identified as weight "maintainers" based on survey results had the highest WEL scores. However, the maintainers did not report using the same practices to maintain weight because they may have had fewer challenges maintaining weight. Among those who reported losing or gaining weight, the reported practices chosen to prevent weight gain were similar between groups. The primary difference between these groups was that those who reported losing weight had significantly higher WEL scores compared to those who reported gaining weight. Therefore, between these two groups a higher WEL score was associated with reportedly using the "eating more protein" practice; however, the WEL score of those who lost weight was still lower than the WEL score of those who maintained weight. The WEL questionnaire may be a helpful tool for nutrition educators to use with weight loss clients to address food relationships and increase the effectiveness of weight gain prevention practices.

These two studies provide a micro- and macroscopic perspective on the use of protein in weight loss and maintenance. While the use of a reduced-energy HP diet was as effective as a standard reduced-energy diet, the WP diet induced some regional fat loss and a decrease in systolic blood pressure. At the national level, midlife women

infrequently chose whey protein powder as a source of protein. Perhaps additional education on the ease of using WP and its related benefits may provide the additional success many overweight individuals are seeking. While all the participants in the controlled trial lost weight, a controlled environment is not the norm. Additional factors such as self-efficacy, nutrition knowledge, and social factors will significantly influence the final outcome of a chosen practice.

Future Research Directions

Future studies incorporating whey protein into the diet should focus on an expanded hormone profile to identify other hormones that may be modulated by increased whey protein intake. Additional study of the effect of whey protein intake on satiety related and other hormones such as leptin, ghrelin, adiponectin, growth hormone, cortisol, and bradykinin may help provide a broader perspective of the metabolic effects resulting from increased protein intake. An effect on regional fat mass and systolic blood pressure was observed with increased intake of WP that was not the result of changes in the RAS metabolism; therefore, other hormones related to adipose metabolism and stress hormones may be responsible and should be evaluated with WP intake. These hormones are known to be affected by weight loss and dietary changes, but the current study was limited to RAS metabolites.

Additionally, further studies should incorporate a weight maintenance design, which may more effectively evaluate the effect of whey protein on hormone concentrations, adipose metabolism, and blood pressure. The effect of weight loss on reduction of blood pressure could not be completely ruled out in our clinical study;

therefore, a study design with weight maintenance as the model may be useful in determining the effect of whey protein apart from weight loss.

More research with the WEL questionnaire is necessary to establish how to increase self-efficacy among the overweight population. The national survey confirmed higher WEL scores were related to successful weight maintenance as has been reported in clinical trials (Linde, Rothman, Baldwin, & Jeffery, 2006; Richman, Loughnan, Droulers, Steinbeck, & Caterson, 2001). Establishing increased self-efficacy as an outcome for national weight loss programs may become a standard as knowledge is gained regarding the best ways to increase self-efficacy.

Knowledge regarding dietary protein has been a challenge to measure, and no appropriate evaluation tool has been developed. In the national survey, an attempt was made to evaluate the protein knowledge of midlife women, but the questions used introduced some possible biases making results difficult to interpret. Clear distinctions need to be made between what a person knows and what a person does with what they know. While it may seem that a person's knowledge should influence behavior, a definitive tool for measuring nutrition knowledge has yet to be applied to evaluate the relationship with behavior.

Finally, as "eating more protein" has been identified as a practice used for weight gain prevention among midlife women, more research is needed to identify how this practice is used by adults. A one-day food record was completed with the questions about frequency of weight gain prevention, but more in-depth analysis is needed to evaluate regularity of protein intake and diet composition. A comparison of men to

women, their weight maintenance practices and WEL scores, may provide additional assistance to nutrition educators addressing the global challenge of obesity.

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Appendices: Weight Loss Study

CONSENT FORM
Effects of Whey Protein on Weight Loss
and Metabolic Status of Midlife Adults

You are invited to participate in a pilot project studying the effect of protein in a reduced calorie diet and weight loss. You were selected as a possible participant because you meet the conditions that demonstrate you are a healthy volunteer who meets our eligibility requirements. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

This study is being conducted by Noel D. Aldrich, USDA National Needs Fellow, Susan K. Raatz, Ph.D., Assistant Professor in the Department of Medicine; Marla M. Reicks, PhD, Professor in the Department of Food Science and Nutrition; and Bruce Redmon, MD, Associate Professor in the Department of Medicine, of the University of Minnesota. Dr. Redmon will serve as the study physician by monitoring laboratory results and providing medical support as required.

Study Purpose

The purpose of the study is to evaluate the effectiveness of dietary regimes varying in protein content and source on weight loss in midlife adults. We will evaluate whether the percentage of dietary protein has an effect on weight control and whether the source of protein has an effect on weight control.

Study Procedures

If you agree to participate in this study, we would ask you to do the following:

Your first visit to the General Clinical Research Center (GCRC) will be for a screening process to determine whether you meet the eligibility requirements for the study. At this visit, we will draw a 28 ml (about 5 teaspoons) blood sample, and we will have you breath into a Metabolic Monitor for about 20 minutes after resting for 30 minutes, so your resting energy expenditure can be measured. Women of child bearing age will take a urinary pregnancy test. Pregnant women will not be allowed to proceed with the test measures of this study. A positive pregnancy test will result in the subject being removed from the study. This screening visit will take about 3 hours.

If you decide to be in the study, you will be randomly assigned to a reduced calorie diet treatment. Your meals will be prepared for you for 8 weeks, followed by 12 weeks of meal preparations that you complete with the directions we give you. The total study will take 20 weeks to complete. Blood samples will be taken to evaluate the effect of the dietary treatment on insulin levels and related compounds that may be related to weight loss stress. The blood samples obtained will allow us to determine if the diets have an effect on your metabolism that may benefit your heart and bone health. During the study, you will be asked to maintain your usual exercise patterns.

The study diets include a reference diet, a high mixed protein diet, and a high protein (whey) diet. Each of the diets is comprised of a 5-day menu rotation and prepared with food items considered to be normal in an American diet. You will be randomly assigned to one of the dietary treatments for 8 weeks. During these 8 weeks, you will need to come to the GCRC daily to pick up your meals. After completion of the 8 week diet, you will be instructed in meal preparations, so that you can continue to follow the diet at home on your own.

Once you are enrolled in the study, you'll be asked to come to the GCRC at week 1, week 8, and week 20 for a Mixed Meal Tolerance Test (MMTT). You will be provided a mixed meal and a nurse will insert an IV into your arm for the duration of the 2-hour MMTT. Blood samples will be taken during the two hours of the MMTT. A total of 18 ml (about 3 teaspoons) of blood will be taken at week 1, and a total of 46 ml (about 9 teaspoons) of blood will be taken at week 8 and week 20. You will be provided lunch after the blood draw. You will be asked to return to the GCRC for week 4, 12, and 16 for an additional 5 ml (1 teaspoons) of blood. By the conclusion of the study, a total of 150 ml (30 teaspoons) will have been drawn.

In addition, your body composition will be measured by DEXA (dual energy x-ray) densitometry. A scan with the DEXA equipment provides a minimal radiation dose with the total radiation equivalent of one-day exposure to the sun in Minnesota, or 1/100 of the radiation exposure of a chest x-ray. DEXA scans will be performed by staff who are licensed X-ray operators in the state of Minnesota. Your body composition will be measured by DEXA at week 1, week 8, and week 20.

At week 8 and week 20, you will breathe into a Metabolic Monitor again. Your visits at week 1, week 8, and week 20 will require additional time for the various measures to be completed. These visits will take about 5 hours.

If, for any reason, you wish to discontinue your participation in the study, please notify Noel D. Aldrich, USDA Fellow, by phone at 612-626-5107. We may use the partial data that was obtained from your participation in the evaluation of our study results. Early withdrawal from the study may be required if you are placed on any medications that affect the major metabolic pathways that we are studying. If anything changes in your medical status or medication, please inform the study staff. The study physician and primary investigator will review these changes to make an assessment regarding continued participation.

Risks of Study Participation

You may experience discomfort from hunger and feel inconvenienced by having to fast for blood draws. The risks associated with study participation are those of blood draws, which may cause bruising, or rarely, infections at the needle puncture site or you may feel some pain dizziness, or feel faint. We will minimize the risk of any problems with your blood draws by having them performed by trained phlebotomists, registered nurses, and certified medical assistants.

The diets in this study will contain nuts, soy, whey, corn, and artificial sweeteners. If you have allergies to any of the food items used in this study, there may be a risk associated with the consumption of the prescribed dietary treatments. In addition, you may find the scheduled menu to

be monotonous in the repeating sequence. You may feel hungry on the reduced calorie diet.

Breach of confidentiality with your laboratory data results will be minimized by having all of the samples coded and results available to the researchers only.

Benefits of Study Participation

There are no benefits to participation in this study participation. You may, however, lose a portion of the weight you desired to lose.

Study Costs/Compensation

Costs: No charges will be made for the General Clinical Research Center visits while you are a participant in this study.

Compensation: There is no monetary compensation for participation in this pilot study. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this research.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or to your insurance company. If you think that you have suffered a research-related injury let the study physician know right away.

Confidentiality

Confidentiality: The records of this study will be kept private. Only the investigators and sponsor will have access to identifying data. Your visits to the General Clinical Research Center and information from these visits will be recorded in your medical record at the Fairview University Medical Center. All study visits at the General Clinical Research Center are recorded in your medical record by the medical staff (dietitians, nurses and physician) who may be involved in your study visit. Specific research laboratory results will be analyzed in the investigators' laboratories and will not be included in the medical record. In any sort of report that is published, information that will make it possible to identify you will not be included. If records are reviewed by the study's sponsor or by researchers not directly connected with this study, your name and other important identifying information will be removed from the records before they are released.

Protected Health Information (PHI)

Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether to participate in this study will not affect your current or future relationship with the University of Minnesota Fairview University Medical Center. If you decide to participate, you are free to withdraw at any time without affecting this relationship.

Contacts and Questions

The researchers conducting this study are Noel D. Aldrich, USDA Fellow; Susan K. Raatz, PhD, RD; and Bruce Redmon, MD. You may ask any questions you have now, or if you have questions later, **you are encouraged** to contact them. You may contact Dr. Raatz at (612) 624-6642, Dr. Redmon at (612) 626-1960, or the pilot study Principal Investigator, Noel Aldrich, at (612) 626-5107. You will be given a brochure with these phone numbers.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), **you are encouraged** to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at University of Minnesota Medical Center, Fairview Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN 55454.

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

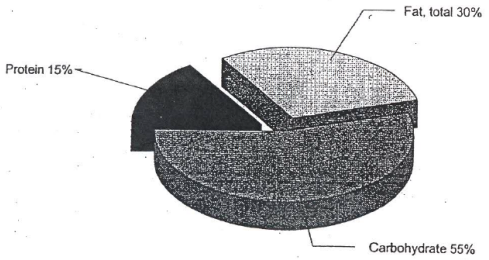
Control Diet Analysis

Diet Analysis

Control 2
P1215
ID:

10/5/07

Ht: 0 in. Wt: 0 lbs.



Macronutrients

Kilocalories	1599.926	kcal
Protein	63.449	gm
Carbohydrate	225.257	gm
Fat, total	54.528	gm
Alcohol	0.000	gm
Cholesterol	239.844	mg
Saturated Fat	16.110	gm
Monounsaturated Fat	16.049	gm
Polyunsaturated Fat	16.115	gm
Dietary Fiber, total	24.953	gm
Sugar, total	71.380	gm
Other Fats	6.254	gm

Minerals

Sodium	2208.990	mg
Potassium	2249.599	mg
Calcium	716.138	mg
Iron	15.682	mg
Phosphorus	1122.682	mg
Magnesium	296.604	mg
Zinc	8.841	mg
Copper	1.120	mg
Manganese	5.562	mg
Selenium	0.120	mg
Chromium	0.087	mg
Molybdenum	82.956	µg

Vitamins

Vitamin A (RE)	1293.493	RE
Vitamin C	140.788	mg
Vitamin D (ug)	3.045	µg
Vitamin E	7.684	mg
Thiamin	1.423	mg
Riboflavin	1.520	mg
Niacin	18.087	mg
Pyridoxine (Vitamin B6)	1.386	mg
Folate	422.447	µg
Cobalamin (Vitamin B12)	2.936	µg
Biotin	17.025	µg
Pantothenic Acid	3.757	mg
Vitamin K	84.642	µg

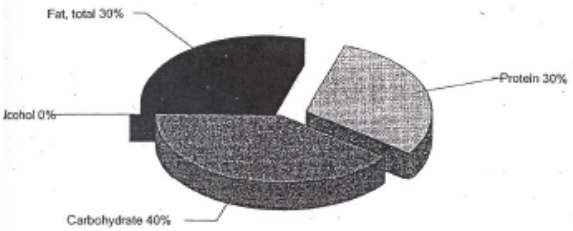
Mixed Protein Diet Analysis

Diet Analysis

High Protein
P1215
ID:

10/5/07

Ht: 0 in. Wt: 0 lbs.



Macronutrients

Kilocalories	1605.687	kcal
Protein	124.129	gm
Carbohydrate	163.866	gm
Fat, total	53.997	gm
Alcohol	0.138	gm
Cholesterol	239.996	mg
Saturated Fat	15.967	gm
Monounsaturated Fat	16.059	gm
Polyunsaturated Fat	15.909	gm
Dietary Fiber, total	23.981	gm
Sugar, total	56.421	gm
Other Fats	6.062	gm

Minerals

Sodium	2794.018	mg
Potassium	2633.283	mg
Calcium	1068.036	mg
Iron	17.126	mg
Phosphorus	1572.865	mg
Magnesium	278.934	mg
Zinc	12.219	mg
Copper	1.031	mg
Manganese	3.613	mg
Selenium	0.141	mg
Chromium	0.083	mg
Molybdenum	77.129	µg

Vitamins

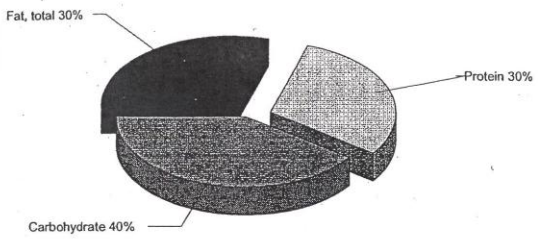
Vitamin A (RE)	1505.371	RE
Vitamin C	102.025	mg
Vitamin D (ug)	4.290	µg
Vitamin E	8.544	mg
Thiamin	1.307	mg
Riboflavin	3.044	mg
Niacin	27.298	mg
Pyridoxine (Vitamin B6)	1.963	mg
Folate	390.076	µg
Cobalamin (Vitamin B12)	6.026	µg
Biotin	16.850	µg
Pantothenic Acid	5.397	mg
Vitamin K	120.504	µg

Whey Protein Diet Analysis

Diet Analysis

Whey
P1215
ID: 10/5/07

Ht: 0 in. Wt: 0 lbs.



Macronutrients

Kilocalories	1599.650	kcal
Protein	124.167	gm
Carbohydrate	166.865	gm
Fat, total	54.725	gm
Alcohol	0.000	gm
Cholesterol	268.549	mg
Saturated Fat	16.234	gm
Monounsaturated Fat	16.269	gm
Polyunsaturated Fat	16.058	gm
Dietary Fiber, total	23.221	gm
Sugar, total	60.528	gm
Other Fats	6.164	gm

Minerals

Sodium	2536.333	mg
Potassium	2417.914	mg
Calcium	1536.002	mg
Iron	13.149	mg
Phosphorus	1498.146	mg
Magnesium	578.608	mg
Zinc	25.056	mg
Copper	0.845	mg
Manganese	3.749	mg
Selenium	0.094	mg
Chromium	0.076	mg
Molybdenum	63.619	µg

Vitamins

Vitamin A (RE)	1382.792	RE
Vitamin C	101.419	mg
Vitamin D (ug)	3.194	µg
Vitamin E	6.635	mg
Thiamin	5.344	mg
Riboflavin	6.803	mg
Niacin	16.637	mg
Pyridoxine (Vitamin B6)	7.377	mg
Folate	342.925	µg
Cobalamin (Vitamin B12)	10.724	µg
Biotin	13.997	µg
Pantothenic Acid	30.493	mg
Vitamin K	77.509	µg

Daily Questionnaire

P1215 Effects of Whey Protein on Weight Loss and Metabolic Status

Subject ID: _____ **Date:** _____

1. Was there any food provided with your diet over the past 24 hours that you did not eat?

_____ YES _____ NO

If yes, please list the foods or beverages you did not consume and the proportion of the item that you did not eat:

2. Did you eat or drink any foods in addition to the diet you were provided?

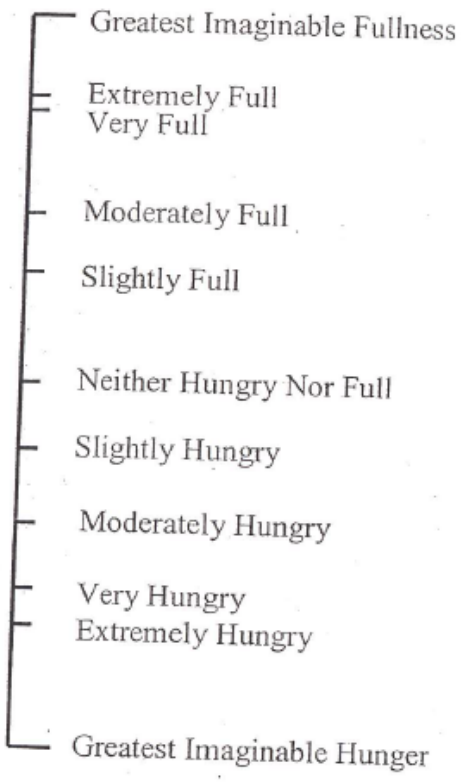
_____ YES _____ NO

If yes, please list the foods or beverages you consumed, the amount of the item, and the time that you ate it.

Food / Beverage	**Amount**	**Time of Day**

Additional questions on the back side.

3. Rate the degree of hunger/fullness you felt over the past 24 hours. Show your rating by placing a slash mark somewhere on the line below.



Appendices: National Survey

P.O. Box 474 Toledo, OH 43654
Toll-Free Number: **1-800-537-4097**
Mon – Fri, 8:00 AM to 11:00 PM EST
Sat, 10:00 AM to 6:00 PM EST
Contact Us: http://mysurvey.com/contactus.cfm
Privacy: http://mysurvey.com/privacy.cfm

174075

General Questionnaire

Please complete the following questionnaire after you have completed the Breakfast, Lunch, Dinner and Snack Questionnaires and the Food Record Form over your assigned 24 hour period.

Please X one box for questions 1 through 7. **(X ONE BOX FOR EACH)**

	Usually / Always	Sometimes	Rarely / Never
1. When you eat bread or rolls, how often do you add butter or margarine?	1 ☐	2 ☐	3 ☐
2. When you cook vegetables, how often do you add oil, margarine or butter?	1 ☐	2 ☐	3 ☐
3. When you eat vegetables, how often do you add oil, butter or margarine at the table?	1 ☐	2 ☐	3 ☐
4. When you eat potatoes, how often do you use butter, margarine, or sour cream?	1 ☐	2 ☐	3 ☐
5. How often do you use milk or cream in coffee or tea?	1 ☐	2 ☐	3 ☐
6. When you eat chicken or turkey, how often do you eat the skin?	1 ☐	2 ☐	3 ☐

	Yes	No
7. Do you eat in restaurants and/or purchase take-out food more than three times per week?	1 ☐	2 ☐

What type of milk, spreads and cooking oils do you usually use?

Please specify only the type you use most often.

8. Milk: (X ONE Box)

1 ☐ Whole cow's milk 2 ☐ 2 % cow's milk 3 ☐ 1 % cow's milk 4 ☐ Skim cow's milk 5 ☐ Do not use
6 ☐ Another type of milk **(Write In)** _____

9. Margarine: (X ONE Box)

1 ☐ Regular 2 ☐ Diet/low-fat 3 ☐ Fat-free 4 ☐ Spray 5 ☐ Do not use
6 ☐ Brand name **(Write In)**_____

10. Real Butter: (X ONE Box)

1 ☐ Regular 2 ☐ Whipped 3 ☐ Light 4 ☐ Do not use

11. Salad Dressing (X ONE Box)

1 ☐ Regular 2 ☐ Diet/low-fat 3 ☐ Fat-free 4 ☐ Do not use
5 ☐ Brand name **(Write In)**_____

12. Oil: (X ONE Box)

1 ☐ Canola oil 2 ☐ Corn oil 3 ☐ Olive oil 4 ☐ Safflower oil 5 ☐ Soybean oil
6 ☐ Other oil 7 ☐ Do not use

13. Mayonnaise: (X ONE Box)

1 ☐ Regular 2 ☐ Diet/low-fat 3 ☐ Fat-free 4 ☐ Do not use
5 ☐ Brand name **(Write In)**_____

14. Have you used the following practices to prevent weight gain? **(X ONE Box For EACH)**

	Yes, in the last 12 months	Yes, more than 12 months ago	No, never
Commercial weight loss programs (e.g. Weight Watchers, Jenny Craig)	1 ☐	2 ☐	3 ☐
Meal replacements or slimming products (e.g. Herbalife)	1 ☐	2 ☐	3 ☐
Exercise	1 ☐	2 ☐	3 ☐
Cutting down on size of meals or between meal snacks	1 ☐	2 ☐	3 ☐
Cutting down on fats and/or sugars	1 ☐	2 ☐	3 ☐
Laxatives or diuretics	1 ☐	2 ☐	3 ☐
Supplements to burn fat or boost metabolism	1 ☐	2 ☐	3 ☐
Supplements to feel full	1 ☐	2 ☐	3 ☐
Fasting	1 ☐	2 ☐	3 ☐
Vegetarian diet	1 ☐	2 ☐	3 ☐
Smoking	1 ☐	2 ☐	3 ☐
Skipping meals	1 ☐	2 ☐	3 ☐
Eating more protein	1 ☐	2 ☐	3 ☐

15. How tall are you? **(Write In #)** _____ feet _____ inches

16. How much do you weigh now? **(Write In #)** _____ lbs

17. What did you weigh 2 years ago? **(Write in #)** _____ lbs

18. What did you weigh 5 years ago? **(Write in #)** _____ lbs

19. Have you had a menstrual period during the previous 12 months? **(X ONE Box)**

1 ☐ Yes 2 ☐ No

20. Are you currently taking hormone replacement therapy? **(X ONE Box)**

1 ☐ Yes 2 ☐ No

21. Did you take a vitamin and mineral supplement, such as Centrum® or One-A-Day®, today? **(X ONE Box)**

1 ☐ Yes 2 ☐ No

22. Please select a number from 0 to 9 where 0 is not confident and 9 is very confident. **(X ONE Box For EACH)**

	Not Confident 0	1	2	3	4	5	6	7	8	Very Confident 9
I can resist eating when I am anxious (nervous)	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can control my eating on the weekends	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when I have to say "no" to others	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I feel physically run down	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am watching TV	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am depressed (or down)	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when there are many different kinds of food available	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when I feel it's impolite to refuse a second helping	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when I have a headache	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am reading	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am angry (or irritable)	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when I am at a party	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when others are pressuring me to eat	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am in pain	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating just before going to bed	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I have experienced failure	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating even when high-calorie foods are available	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I think others will be upset if I don't eat	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I feel uncomfortable	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐
I can resist eating when I am happy	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐

23. Which type(s) of foods do you think are good sources of **protein**? **(X ALL That APPLY)**

1 ☐ Beef, chicken, fish, pork, lamb
2 ☐ Milk, yogurt, cheese, eggs
3 ☐ Margarine, olive oil, canola oil, corn oil, butter
4 ☐ Wheat bread, corn meal, oatmeal, pasta, rice
5 ☐ Baked beans, lentils, peanuts, walnuts, chickpeas
6 ☐ Lettuce, cabbage, broccoli, carrots, greens
7 ☐ Apples, oranges, bananas, grapes, prunes
8 ☐ Soy powder, whey powder, Ensure, Boost

24. How often do you choose each type(s) of food as a **protein** source? **(X ONE Box For EACH)**

	Usually / Always	Sometimes	Rarely / Never
Beef, chicken, fish, pork, lamb	1 ☐	2 ☐	3 ☐
Milk, yogurt, cheese, eggs	1 ☐	2 ☐	3 ☐
Margarine, olive oil, canola oil, corn oil, butter	1 ☐	2 ☐	3 ☐
Wheat bread, corn meal, oatmeal, pasta, rice	1 ☐	2 ☐	3 ☐
Baked beans, lentils, peanuts, walnuts, chickpeas	1 ☐	2 ☐	3 ☐
Lettuce, cabbage, broccoli, carrots, greens	1 ☐	2 ☐	3 ☐
Apples, oranges, bananas, grapes, prunes	1 ☐	2 ☐	3 ☐
Soy powder, whey powder, Ensure, Boost	1 ☐	2 ☐	3 ☐

25. How much of the calories you eat each day should come from **protein**? **(X ONE Box)**

1 ☐ 5% – 10%
2 ☐ 12% – 15%
3 ☐ 20% - 25%
4 ☐ 30% - 40%
5 ☐ I don't know

26. **Protein** is helpful for weight loss because _____. **(X ALL That APPLY)**

1 ☐ protein builds muscle, not fat.
2 ☐ extra protein is not stored in the body.
3 ☐ protein provides more energy than carbs or fat.
4 ☐ protein helps you feel full.
5 ☐ I don't know

27. Which of the following protein supplement products have you seen in stores or in advertisements? **(X ALL That APPLY)**

1 ☐ Protein water (such as Special K_20, Stacker 2)
2 ☐ Whey protein beverage (such as Naked Juices)
3 ☐ Soy protein beverage (such as Odwalla, Bolthouse Farms, Silk)
4 ☐ Protein bars (such as Genisoy, Detour)
5 ☐ Soy protein powder mix (such as Genisoy, Soytein)
6 ☐ Whey protein powder mix (such as Designer Whey)
7 ☐ Amino acid tablets (such as Lysine, Carnitine, Arginine)

28. How often do you buy the following **protein** supplement products? **(X ONE Box For EACH)**

	Never	Once A Year	Once A Month	Once A Week	Every Day
Protein water (such as Special K_20, Stacker 2)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Whey protein beverage (such as Naked Juices)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Soy protein beverage (such as Odwalla, Bolthouse Farms, Silk)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Protein bars (such as Genisoy, Detour)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Soy protein powder mix (such as Genisoy, Soytein)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Whey protein powder mix (such as Designer Whey)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Amino acid tablets (such as Lysine, Carnitine, Arginine)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

29. Have you ever eaten more protein than you usually eat to help you lose weight? **(X ONE Box)**

1 ☐ Yes 2 ☐ No

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The following questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

30. Do you currently have a job or do any unpaid work outside your home? **(X ONE Box)**

1 ☐ Yes → **(Continue)**

2 ☐ No **(Skip To TRANSPORTATION PHYSICAL ACTIVITY)**

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

31. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

(Write In #) _____ days per week → **(Continue)**

1 ☐ No vigorous job-related physical activity → **(Skip To Qu. 33)**

32. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

33. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

(Write In #) _____ days per week → **(Continue)**

1 ☐ No moderate job-related physical activity → **(Skip To Qu. 35)**

34. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

35. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

(Write In #) _____ days per week → **(Continue)**

1 ☐ No job-related walking → **(Skip To TRANSPORTATION PHYSICAL ACTIVITY)**

36. How much time did you usually spend on one of those days **walking** as part of your work? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

37. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No traveling in a motor vehicle → **(Skip To Qu. 39)**

38. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

39. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No bicycling from place to place → **(Skip To Qu. 41)**

40. How much time did you usually spend on one of those days to **bicycle** from place to place? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

41. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No walking from place to place → **(Skip To HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY)**

42. How much time did you usually spend on one of those days **walking** from place to place? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

43. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No vigorous activity in garden or yard → **(Skip To Qu. 45)**

44. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

45. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No moderate activity in garden or yard → **(Skip To Qu. 47)**

46. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

47. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No moderate activity inside home → **(Skip To RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY)**

48. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

49. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No walking in leisure time → **(Skip To Qu. 51)**

50. How much time did you usually spend on one of those days **walking** in your leisure time? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

51. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No vigorous activity in leisure time → **(Skip To Qu. 53)**

52. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

53. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

(Write In #) _____ days per week → **(Continue)**

1 ☐ No moderate activity in leisure time → **(Skip To TIME SPENT SITTING)**

54. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

TIME SPENT SITTING

These questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

55. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

56. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**? **(Write in # of hours and/or minutes)**

_____ hours per day _____ minutes per day

57. Below are a number of statements about food. Using a 6 point scale, where **"1" means "Strongly Disagree"** and **"6" means "Strongly Agree,"** please indicate how much you agree or disagree with each statement. If the statement does not apply to you, please select Strongly Disagree.
(X ONE Box For EACH Statement)

Statements	Strongly Disagree 1	2	3	4	5	Strongly Agree 6
Deciding what to serve for dinner is stressful	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I can usually eat what I want, and I never seem to gain weight	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I am always looking for ways to make meals more interesting and varied	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Meals are family time in my household	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I put a lot of effort in looking for coupons and/or finding products on sale	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Having to plan meals is a hassle	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I really feel guilty when I overeat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Meals in my household can be very stressful times	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Eating something indulgent helps me relax	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I am a real food lover	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I will pay more for higher quality products	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
On most days food takes a back seat to other activities/responsibilities	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I avoid cooking as much as possible	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
When I am feeling bored I usually have something to eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
The more authentic an ethnic restaurant the better	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I can't watch television without having a snack	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I try to eat well, but it doesn't usually work out	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I'm tired of hearing what is and isn't healthy	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Others show their appreciation for the foods I serve	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Having something to eat helps me deal with stress	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Even when we eat together, people in my household frequently eat different foods	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I make a point to eat foods that are natural/organic	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I get a lot of satisfaction in seeing others enjoy foods I have made	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I only buy foods I know people in my household will eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
If it is just me eating, I never bother to cook	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I always compare prices on the foods I buy	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Frequently I eat just to have something to do	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I am finding it harder and harder to maintain my weight	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I consider myself to be an adventurous eater	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Taking care of others usually comes before my meal needs	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I will always buy one brand over another if it is on sale.	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

Question 57 Continued On Next Page →

Question 57 Continued

	Strongly Disagree 1	2	3	4	5	Strongly Agree 6
I would eat differently if it weren't for the influence of others I eat with	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I frequently eat certain foods because they remind me of the past	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I tend to take comfort in eating the same foods regularly	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Seeing advertisements for food makes me hungry	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I carefully read nutrition/ingredient labels on the foods I buy	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
On most days I am so busy that I need to force myself to eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I will pay more for foods that are more healthful	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I like to cook, but never get around to it.	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I am careful to balance the foods/calories I eat throughout the day	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I usually don't have time to plan meals	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
It always seems that I am being tempted to eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Cooking is a real chore	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I take the time to prepare good meals most nights of the week	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I watch my fat intake carefully	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I have to admit I live to eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I know more about nutrition than most people	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
It is hard not to eat when I smell food	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I prepare special dishes that I am known for	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
If I have a disappointing meal, I will make up for it the next time I eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
It is important to use food up before it goes bad	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Certain foods I eat connect me with my cultural heritage	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I cook certain dishes because they remind me of my mother/grandmother	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I can't go to bed without having something to eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
When it comes to food, I tend to buy the best	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I really only have time to cook on weekends	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I always reward myself with a treat when I have had a stressful day	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I love to try new recipes and new food products	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
It seems I am always trying to lose or maintain weight	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
When it comes to eating, I never just let myself go	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I am a very creative cook	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I derive a great deal of pleasure from the food I eat	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Each meal I serve is well balanced across all food groups	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I eat certain foods when I am angry or sad	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
I really wish I had more time to cook for my household	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
It seems that I am always feeling guilty about what I ate	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

Thank you for your help with this study. Please return your completed questionnaires in the enclosed postage-paid envelope as soon as possible.

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