MURRAY, COLEMAN, M.S. Protein Level and Source in Single Meal Influence Voluntary Intake During Subsequent Meal (2017)
Directed by Dr. Joseph L Beverly. 60pp.

Dietary protein has been shown to be more satiating than other macronutrients. The objective of this present study was to assess the acute effect of a meal differing in protein level and source on subsequent intake. Diets included 15%, 20%, 35%, and 50% levels with same and mixed protein sources over a series of five feeding trials. Protein sources included casein, egg white, and wheat gluten. Rats were adapted to a feeding schedule consisting of a 30 minute breakfast meal (20% of daily calories) provided 60 minutes into the dark period. On test days, ground chow was made available 60 minutes after the breakfast meal. The amount consumed during this test period was measured as an index of the satiating effect of the breakfast meal. A 5 hour ad libitum period concluded the dark period. During each study, each rat received all of the test diets without consuming the same diet on back to back days. There were no differences in the amount of diet consumed during the breakfast meal, nor were there differences in the total calories consumed during the entire 24 hour period on test days. When fed a normal protein level (15% or 20%) there was no effect of protein source on subsequent intake during the test period. When rats consumed a breakfast of 35% wheat gluten protein an increase in subsequent intake resulted; however, when 35% egg white protein was consumed there was a significant decrease in intake indicating that egg white was more satiating than wheat gluten. Mixed diets composed of a 15% or 20% casein protein base and an addition of 20% or 15%, respectively, egg white or wheat gluten protein, totaling 35% of calories as protein, were also assessed. Similar results were seen when rats

consumed these mixed egg white/casein or wheat gluten/casein breakfast meal at a total of 35% of calories as protein. At the 50% protein level there was a decrease in subsequent intake, indicating the higher protein level was more satiating. Additionally, the different mineral profile of the 35% egg white diet was assessed showing that this was not a factor affecting the rat's consumption as the diet of 35% of calories from casein protein with added minerals did not result in a decreased subsequent intake as was seen with the 35% egg white diet. These data support a difference between protein sources in their apparent satiating effect on a subsequent meal when fed to rats at 35% of calories. The three protein sources produced different effects on subsequent intake with egg white decreasing intake, wheat gluten increasing intake, and casein having no effect. All of these effects are a result of an acute response to a randomly assigned breakfast meal at a limited amount of calories. The duration of the effect was short, as total caloric intake on test days was not affected by diet fed at the breakfast meal.

PROTEIN LEVEL AND SOURCE IN SINGLE MEAL INFLUENCE VOLUNTARY INTAKE DURING SUBSEQUENT MEAL

by

Coleman Murray

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____
Committee Chair

APPROVAL PAGE

This thesis written by COLEMAN MURRAY has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Committee Chair ______________________________

Committee Members ______________________________

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Date of Acceptance by Committee

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Date of Final Oral Examination

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Appetite is what causes a human or animal to locate and ingest food, leading to satiation as one eats until comfortably full(1). Satiety, or the feeling of fullness, lasts until hunger builds up which leads to the next eating occasion. This next eating occasion is dependent on internal factors and external environmental factors. These factors include feelings of hunger from the hypothalamus, satiety hormone levels, and the location and people present at the time of an eating occasion. Knowledge about satiety can help develop an index to measure the efficiency of foods and can help researchers understand the mechanism behind food intake and energy balance. Protein has been shown to be more satiating than other macronutrients and a moderate increase in protein at the expense at other macronutrients may increase satiety. High protein meals have been shown to be effective in treating obesity through reductions in body weight and preservation of lean mass due to their satiating effects(2, 3).

Although, high protein meals have been repeatedly shown to increase satiety, there is limited research surrounding the protein source and level necessary to see the benefits of increased satiety. Vander Wal et al concluded that an egg diet increased satiety and decreased subsequent intake; however, Lang et al found that there was no effect of type of protein on satiety after considering seven different protein source including egg, casein, gelatin, and wheat gluten(4, 5). Higher quality protein sources, like egg, are both easier to digest and have more of the essential amino acids that cannot be made by the human body. Lower quality protein sources are missing one or more essential amino acids. Furthermore, the effect of source is also dependent on type of meal, as liquid meals have been shown to be less satiating than a solid meal(6).

Satiety effects have been tied to various mechanisms including quality of the protein source, plasma amino acid composition, bioavailability, hormones present, or induction of a ketogenic state. There is no consensus in the research of a satiety mechanism or index of satiety for various sources and the methodologies vary greatly making results difficult to compare across studies. Gannon et al showed that consumption of gelatin, like whey protein, produces a rapid rise in plasma amino acids while egg white, beef, and fish give a very slow increase in plasma amino acids(7). Similar to casein protein, sources like cottage cheese, soy, and turkey induce a moderate, gradual increase in plasma amino acids. These results show that satiation may be determined by multiple things include digestion rates and differences in amino acid composition.

Even less research is available on satiety in response to a certain protein source fed as breakfast, the first feeding occasion after fasting(8). The objective of these current feeding studies was to examine subsequent consumption and satiety after a breakfast meal of varying protein sources and levels. Protein sources included egg white protein, wheat gluten protein, and casein protein. Mixed protein meals or single protein meals were provided at 15%, 20%, 35%, and 50% of calories from protein

CHAPTER II

LITERATURE REVIEW

Proteins are essential for proper nutrition and adequate health status throughout the life cycle. As early as the 1800's, research was conducted to determine the necessary protein and energy needs (9). Researchers believed that people with ample resources instinctively consume a diet with a sufficient amount of protein to maintain their health. At this time, an estimate of 125g of protein for an American man doing moderate work was the standard which was later challenged by other researchers who believed that 62g of protein was sufficient for maintaining a healthy physical condition. Protein sources were assumed to be of equal quality where protein from beans could be substituted for meat in a diet. Upon the discovery of amino acids, the building blocks of protein, analysis of these different protein sources revealed their difference in quantities of different amino acids. In a study with mice consuming zein, a plant based protein, the mice lived longer if their diet was supplemented with tryptophan, showing that this amino acid had an essential function and that there was a limited quality of different protein sources(9).

Exogenous and endogenous proteins provide the body with amino acids to use for the synthesis of tissues, enzymes, hormones, transporters, neurotransmitters, and countless other molecules critical for health. The human body requires 20 amino acids for protein synthesis. Dietary sources of protein are the primary provider of the 9 essential amino acids which cannot be synthesized in adequate amounts by the human body and therefore must be provided through food sources. There are 11 nonessential amino acids which can be made by the body in sufficient quantity. Some amino acids including arginine, cysteine, and tyrosine may be considered conditional, meaning they are only essential during certain life situations.

Protein sources differ in their ability to meet metabolic requirements for nitrogen and the 9 essential amino acids (10). To determine the quality of a protein source, the digestibility and amino acid content is considered. Higher quality proteins, complete proteins, contain all of the indispensable amino acids in the proper proportion. Examples of these complete proteins include milk, eggs, and meat. Lower quality proteins, incomplete proteins, are limited in one or more of a particular indispensable amino acid. Most often plants foods like grain or vegetable products are incomplete proteins. The amount of amino acids that are absorbed after consuming protein determines the bioavailability of that protein source. Animal proteins tend to have a higher bioavailability than plant proteins. Overall protein quality is assessed through a Protein Digestibility Corrected Amino Acid Score (PDCAAS). This score is calculated by comparing the amount of the limiting amino acid in a food in comparison to the amount of that amino acid in 1 g of a reference protein (egg or milk). This ratio is then corrected for the digestibility of the protein. The PDCAAS scores can range from 0 to 1. A score of 1 indicates that 100% or more of the essential amino acids is provided per 1 gram of protein. Milk protein (casein), egg white, ground beef, and several other animal products have the highest score of 1 while wheat gluten has a score of 0.25. PDCAAS scores were developed by FAO/WHO and are considered the gold standard for determining protein quality (11).

Protein turnover results in the formation of amino acid pools which consist of amino acids from dietary sources and those from the breakdown of body tissues. Amino acid pools are often found in the liver, intestines, and skeletal muscle(12). These amino acids are used to synthesize new proteins in the body which are used for growth and repair, production of non-protein nitrogen containing compounds, and for synthesis of glucose, ketone bodies, and fatty acids. To determine protein needs, nitrogen balance and indicator amino acid oxidation studies are used. Nitrogen balance evaluates the amount of dietary nitrogen intake versus losses from the body. Nitrogen accounts for approximately 16% of protein. It is constantly lost from the body in urine, feces, and skin. Moodiness, fatigue, various skin conditions, and edema may result when an insufficient amount of total protein or of essential amino acids is consumed. Protein must be consumed regularly to maintain nitrogen balance and available amino acids to meet the body's needs. Age, body size, and physiological state influence the human requirements for protein. The Estimated Average Requirement for protein for men and women age 19 or older is 0.66 grams of protein per kilogram of body weight. This recommendation is the lowest intake of dietary protein needed to reach nitrogen equilibrium. The Recommended Dietary Allowance (RDA) is 0.8 grams protein per kilogram of body weight for healthy adults.

Digestion of Dietary Protein

Digestion of dietary protein begins in the stomach where pepsins start the process of proteolysis. Upon entry to the small intestine, pancreatic proteolytic enzymes (trypsin, chymotrypsin, and carboxypeptidases A and B) further break apart the fragments of dietary protein. On the brush boarder membrane of the small intestine, peptidases from the mucosal cells break down the peptides into amino acids. The rate at which amino acids appear in circulation after dietary protein has been broken down depends upon intestinal motility, digestion, and absorption in the small intestine. Furthermore, dietary proteins differ in their amino acid content and therefore may affect the gastric emptying rate and overall transit time of the protein source through the intestine. The accessibility of the protein to various digestive enzymes and the secretion of hormones may also play a role in how quickly the amino acids appear in circulation.

Like dietary carbohydrates, dietary proteins differ in their speed of digestion and absorption to impact the metabolic and hormonal response to a meal(13). This concept of “slow” and “fast” proteins was shown in a study on whey and casein proteins. Whey proteins resulted in a rapid and high increase for a shorter duration in plasma amino acids classifying it as a “fast” protein. Casein resulted in a slower rise to a plateau of a moderate level of amino acids classifying it as a “slow” protein. The difference in these rates was predicted to be a result of different rates of gastric emptying. Because whey protein remains soluble it can empty more rapidly while casein, which lumps up in the stomach, has a delay in gastric emptying and therefore a delay in appearance of amino acids.

Gastric emptying is a complex process which is influenced by metabolic, neuronal, and hormonal signals. Many of these signals work to slow the rate of gastric emptying. In a study by Gannon et al, (7) postprandial total amino acid levels were identified for seven common protein sources including lean beef, turkey, gelatin, egg white, cottage cheese, fish, and soy. Similarly to casein, a moderate increase in plasma amino acids was seen from cottage cheese, soy, and turkey protein sources; while similarly to whey, a sharp increase was seen from gelatin. A slow and mild increase in plasma amino acids was seen by egg white, beef, and fish. Various other studies have shown similar results suggesting that many dietary proteins are slower than both whey and casein.

As the rate of gastric emptying increases there is a faster increase in plasma amino acid concentrations after ingesting specific proteins which may increase satiety(14). Some proteins are a stronger stimulant on gastrointestinal hormones including cholecystokinin and glucagon-like peptide-1. Caseinomorphins, a peptide from casein, is known to decrease gastric motility leading to lower concentrations of postprandial amino acids, therefore reducing the satiating effect of higher plasma amino acid concentrations. The opposite effects occur with caseinomacropeptide which increases the production of cholecystokinin which has been known to increase satiety in many studies; although, decreasing satiety in others. These benefits may be altered or hidden when a mixture of proteins and other macronutrients are ingested.

Protein, Weight Management, and Satiety

Overweight and obesity plague the United States population and lead to numerous health risks including type 2 diabetes, heart disease, high blood pressure, and some types of cancer (15). Generally, this excess weight comes from an energy imbalance as people tend to consume calorie laden food and beverages resulting in a greater caloric intake than output. According to the 2009-2010 National Health and Nutrition Examination Survey (NHANES), more than 2 in 3 adults are considered to be overweight or obese and more than 1 in 20 adults are considered to have extreme obesity. Approximately 33% of children between ages 6 and 19 are overweight or obese. These numbers have dramatically increased in the past 30 years, making obesity a growing problem in the United States. Many people look to fad diets or quick fixes to prevent weight gain or lose the excess pounds they have put on. Extensive research has been conducted on protein source and level and its connection to weight management.

The solution to obesity requires weight loss and long-term weight management(3). With body weight gain being a common pitfall, it is important to approach weight loss and maintenance considering several conditions to the solution. Despite being in negative energy balance, individuals must maintain basal energy expenditure and fat-free mass. Consumption of dietary protein can help meet these conditions to help those with weight issues lose the body weight once and for all since protein is composed of amino acids which act on influence satiety, energy expenditure, and limit the loss of fat free mass. Several mechanisms of weight management as a result of higher protein intake are described below. In addition, the link between protein and satiety is explored.

A high protein diet refers to diets with a minimum of 25-30% of their energy from protein(3). High protein diets for weight loss or maintenance requires a protein intake of 0.8 – 1.2 grams of protein per kilogram of body weight while decreasing the total energy intake. If energy restrictions are in place, this results in a certain amount of protein that is about the same as the amount of protein in a normal protein diet without energy restrictions. A normal protein diet generally ranges from 10 – 15% energy from protein in adults. When in energy balance, dietary intake equals the energy requirement as related to energy expenditure. In a weight management diet, designed to maintain energy balance, the amount of protein from a higher protein diet will then be higher than the amount in a normal protein diet. However, if the diet was designed for weight loss with a limited amount of calories, then a higher protein diet may have a comparable amount of protein to then normal protein level in a weight management diet due to the difference in caloric intake.

A variety of protein sources and levels have been shown to have an effect on appetite sensations. First, dietary protein has stronger satiety effects than dietary fat or carbohydrate. This means that an increased consumption of dietary protein may lead to decreased daily energy intake. Second, it is hypothesized that there is a homeostatic regulation of dietary protein intake in order to meet amino acid requirements(2). There are both a protein-specific appetite and a nonspecific appetite. Nonspecific appetite refers to the interaction between homeostatic, reward, and behavioral or environmental influences. Hormonal signals also have a large influence on energy intake and dietary protein can upregulate or downregulate the release of many of these peptides. For example, when elevated, ghrelin is known to increase hunger and the desire to eat(16). Ghrelin's actions may differ with the consumption of protein; however, this is still under study. Peptide YY and glucagon-like peptide 1 (GLP-1) are both associated with satiety and a decrease in appetite and have both been shown to increase post ingestion of protein. These hormonal signals may be a mechanism behind protein consumption, satiety, and weight management.

The protein-specific appetite helps to prevent overconsumption of protein. The protein leverage hypothesis suggests that a result of protein-specific appetite leads to increased food consumption when the diet has a lower protein density, meaning a lower ratio of protein to fat and carbohydrate(2). The opposite occurs when a diet is consumed that is higher in protein. This directly links dietary protein intake and energy balance. This protein-specific appetite effect has been noted in a variety of studies including an initial study by Gosby et al which looked at protein leverage in lean humans using diets that contained 10%, 15%, and 25% energy as protein(17). Results showed that when the percent of protein content in the diet was reduced from 15% to 10% there was an increase in caloric intake, mostly from savory snack foods between meals. However, when the protein content was increased from 15% to 25% there was no change in energy intake. Another study by Martens et al followed a similar experimental design looked at 5%, 10%, and 15% ad libitum protein diets over a 12 day period(18). Total energy intake was significantly lower in the 15% protein group compared to the lower protein groups.

Participants in the high protein group also noted that fluctuations in hunger and desire to eat were reduced, confirming the satiety value of protein.

Total energy expenditure equals the total of resting energy expenditure, the thermic effect of food, and energy expenditure from activity. Another mechanism by which higher protein diets have been shown to decrease subsequent intake is through increased thermogenesis during postprandial periods, as well as an increased resting metabolism(2). When metabolizing or storing protein, 20-30% of dietary proteins usable energy is expended versus only 5-10% required for carbohydrates and 0-3% required for fat. Furthermore, dietary protein has been shown consistently to induce a greater thermic effect of food when compared to carbohydrates and fats. A decrease in resting energy expenditure (REE) during weight loss can be attenuated by consumption of a higher protein diet. This preservation most likely accompanies the retention of lean body mass that results for a high protein diet. The increased thermogenic effect of protein is possible due to the higher protein turnover or the up-regulation of uncoupling proteins(14). It is also possibly due to the inability to store protein following increased protein intake. Specifically, the excess amino acids must be oxidized or eliminated from the body which leads to an increase in thermogenesis.

Most importantly, following ingestion of protein, an increase in satiety is noted as measured by a decrease in ad libitum consumption(2, 14). When compared to ingestions of isocaloric amounts of carbohydrate or fat, protein is well known to be the most satiating. It is believed that a slight increase in dietary protein may increase satiety and weight loss while decreasing energy consumption and increasing resting energy expenditure. This brings up the question about whether it is the increase in protein or the decrease in carbohydrate that leads to increased satiety and reduced body weight and body fat(3). Increased satiety has been shown in response to single meal studies and over a 24 hour time period. It is suggested that the satiety effect of a higher protein, lower carbohydrate, and moderate fat diet results from the ketogenic effect of the diet. The resulting ketone bodies, like β-hydroxybutyrate, lead to a reduced appetite and lower body weight in humans, while increasing energy expenditure. Furthermore, consumption of high protein diets with proteins that are primarily composed of ketogenic amino acids, like leucine and lysine, may results in increased plasma ketone body concentrations which may trigger increased satiety.

In a study by Weigel et al, an increase in protein content was studied while maintaining the carbohydrate content to examine if a low-carbohydrate diet decreases caloric intake and causes weight reduction in response to the lesser amount of carbohydrate or the increased amount of protein(19). Results showed that satiety was greatly increased in those on the isocaloric high protein diet (30% protein, 20% fat, and 50% carbohydrate) in comparison to the weight –maintaining diet (15% protein, 35% fat, and 50% carbohydrate). Researchers concluded that the increase in protein possibly contributed to the weight loss seen in low-carbohydrate diets.

Another study compared two energy-restricted weight loss diets(20). One was a high protein diet with 30% energy as protein and the other was a normal protein diet with 18% energy as protein in pre-obese and obese women. The postprandial feeling of fullness decreased in the normal protein diet group by almost three times as much as the high protein diet group, showing that the higher protein was more satiating. Overall, those women on the higher protein energy-restricted weight loss diet conserved more lean body mass, experienced a smaller reduction in satiety, and had greater overall global pleasure while losing body weight and body fat in comparison to those women consuming the normal protein diet.

Protein Sources and Satiety

Research has shown that different protein sources may influence satiety in different ways(14). In a study by Mikkelsen et al, a 2% greater energy expenditure was shown with consumption of an animal protein diet (29% energy as protein from pork) in comparison to a vegetable protein diet (28% energy as protein from soy)(21). A 3% increase in 24 hour energy expenditure was also noted when 17-18% energy as pork or soy protein was substituted for carbohydrate. Another study on healthy subjects, assessed satiety and food intake of six dietary protein sources including egg albumin, casein, gelatin, soy, pea, and wheat gluten protein(5). Contrary to other data reported, there was no variation in feelings of hunger or satiety, in subsequent energy intake, or in postprandial plasma glucose or insulin response to the meal.

Satiety is thought to increase with protein, fiber, and water content of foods, while foods are less satiating the more fat or greater palatability ratings(22). Egg is a food that has a satiety index that is 50% higher than white bread or breakfast cereal. In a study of overweight and obese participants, an isocaloric egg breakfast was compared to a bagel breakfast to determine which would increase satiety and reduce cravings and short-term intake. Those who ate the egg breakfast experienced increased satiety and decreased short-term subsequent intake for 24 hour after the egg breakfast(4). A follow up study was conducted in an overweight and obese population to assess the effects of a routine egg breakfast on weight loss(22). This study used a four different breakfast groups including a bagel, bagel diet, egg, and egg diet group. The egg diet and bagel diet groups were recommended to consume a 1000 kcal deficit while the regular egg and bagel groups maintained their normal energy intake. This study found that when not following a diet, the egg breakfast did not induce weight loss; however when following a reduced energy diet, the egg breakfast was shown to increase weight loss. These results were consistent with the literature which supports the satiating effect of eggs or a high protein breakfast in comparison with a higher carbohydrate breakfast.

A human study looked at short-term satiety when consuming a beverage with casein, whey, pea protein, egg albumin, or maltodextrin(23). These beverages were served as a starter, after having fasted for approximately 12 hours after ad libitum access to food. The results showed that food intake was significantly lower and satiety was increased following the preload consumption of casein or pea protein when compared to the plain water control beverage. There was no difference on ad libitum intake at 30 minutes post protein beverage preload. Overall, the limited research on different protein sources and satiety, in addition to the varying amounts consumed for a high versus low protein diet, leads to a range of results that do not allow for conclusions to be drawn about the probable satiety effects of the different types of proteins consumed.

Appetite and Subsequent Food Intake after High-Protein Meals

Most studies report either satiety improvements after consumption of higher-protein meals or limited differences in satiety between meals containing different protein levels or sources. There are several dietary factors that may contribute to these results. Consumption of a beverage instead of solid foods may result in a diminished satiety response and less reduction in intake at the next meal(2). Furthermore, the higher viscosity of the meal, the greater the delay in gastric emptying, leading to increased satiety(6).

Another consideration is whether or not there is a threshold effect for protein quantity during meals(2). Several studies suggested a protein meal threshold of 25-30 g protein required to increase net protein synthesis. It is unknown whether or not a threshold for satiety exists. Postprandial fullness was measured in preliminary trials comparing interventions of 15, 20, 25, and 30 g protein per meal showed that postprandial fullness was significantly increased only after a 30 g protein meal, which supports 30 g as a potential satiety threshold(24).

Lastly, there is a consideration of whether or not protein has a ceiling affect. This questions whether the consumption of additional protein during a meal may lead to increases in satiety. In a study by Belza et al, a normal protein diet (14% of energy from protein), a medium-high protein diet (25% of energy from protein), and a high protein diet (50% of energy from protein) were investigated to determine satiety level, presence of hormones, and appetite ratings(25). This dose response showed with increasing protein level there was an increase in postprandial fullness and GLP-1 and PYY presence while a decrease was noticed in ghrelin and subsequent hunger. Other studies found similar effects, allowing us to conclude that ranges of protein intake lead to graded postprandial satiety effects(2). Although there may be a moderate satiety effect when consuming higher protein meals, research is inconsistent in concluding that the satiety effect will reduce intake at the next eating occasion. It is unclear as to whether or not satiety resulting from protein-rich meals affect eating behaviors over the whole day or more. A recent study assessing the effects of a high-protein breakfast in overweight or obese, breakfast-skipping, late-adolescent girls found that the higher protein breakfast increased the feeling of daily fullness, decreased the energy consumed throughout the day, and decreased evening snacking on high-fat foods(26).

The Role of Breakfast and Satiety

Many people consider breakfast to be the "most important meal of the day" as it has been shown to immediately effect cognitive performance and feelings of wellbeing and overtime positively affect intake, diet quality, and weight management(8). Although consumption of breakfast has been shown to be beneficial, there is little consensus on how breakfast is defined in terms of food and nutrient composition, timing, and amount of energy. Breakfast literally refers to a meal that breaks the fast. Breakfast, as defined be research, is the first meal of the day that breaks the fast after the longest period of sleep(8). It is consumed at any location within two to three hours of waking and is composed of a food or beverage from a minimum of one food group. The proposed energy intake at breakfast is dependent on the number of eating occasions throughout the day; therefore, a range of 15-25% of total energy is recommended to allow for personal alterations according to eating habits. The definition of breakfast identifies food or beverage is consumed from a minimum of one food group, but a variety of foods from three or more food groups is ideal to improve nutrient intake and overall eating patterns. It is recommended that protein-rich foods are combined with nutrient-dense, carbohydrate-rich foods like grains, fruits, or vegetables to supply energy for the day and restock glycogen stores that were depleted during the fasting period. Although the optimal source and the ideal amount of protein is unknown, a protein-rich breakfast can increase satiety and improve overall diet quality.

Breakfast used to be considered a staple in the American diet; however, over the past 50 years there has been an increase in the number of individuals who skip or infrequently eat breakfast. During this same time frame, obesity has been on the rise in America. This has led to the question of whether or not breakfast plays a causal role in weight management(27). In a recent study by Leidy et al, a high protein and a normal protein breakfast were compared to continuous breakfast skipping in overweight or obese individuals to assess changes in appetite, food intake and body weight(28). The high protein diet, but not the normal protein diet, prevented an increase in fat mass, reduced daily intake, and decreased daily hunger when compared to breakfast skipping. Overall, the higher protein diet allowed for better weight management. Although these results showed approximately a 400 calorie decrease in daily intake over the 12 week study period in those individuals who consumed the high protein diet, while those who habitually skipped breakfast did not experience this reduction, rather they increased their intake by approximately 370 calories.

In a study of overweight or obese, “breakfast-skipping,” late-adolescent girls, an isocaloric high protein diet was compared to a normal protein diet and habitual breakfast skipping to determine how it affects appetite, satiety, and evening snacking(26). Consuming breakfast in general was found to decrease daily hunger and increased fullness when compared to breakfast skipping, but the high protein breakfast resulted in a greater increase when compared to the normal protein breakfast. Unlike the previous study, there was an increase of 290 calories consumed per day after a normal protein breakfast when compared to skipping breakfast, but there was no increase in intake after a high protein diet. Consumption of a high protein breakfast, but not a normal protein breakfast, resulted in decreased daily ghrelin levels, increased peptide YY levels, and reduced evening snacking when compared to breakfast skipping. In conjunction with the results from this study, other trials have also shown that a high quality protein breakfast can significantly influence satiety and those associated hormones. Bayham et al, assessed subsequent intake and hormone levels in 20 healthy overweight or obese individuals who consumed either an egg or a cereal breakfast(29). Results showed that despite consuming breakfasts of similar caloric content and macronutrient levels, the higher quality egg protein breakfast increased the feeling of fullness and peptide YY and decreased ghrelin. These changes were seen on day one, but only the increase in peptide YY lasted throughout the seven day trial.

The Possible Mechanisms Behind Protein-Induced Satiety

There is a range of protein amounts and types where satiation and reduced subsequent intake is achieved. This has been supported by slightly elevated concentrations of plasma amino acids and hormones or altered energy expenditure that is noticed by the central nervous system. A satiety signal noted in an early study by Mellinkoff, showed that food intake decreases when there are elevated levels of plasma amino acids which cannot be used for protein synthesis(30).

The quality or type of protein may also be involved in appetite suppression. As discussed earlier, the quality of protein sources are determined by the amino acid composition and whether or not the protein source is missing any essential amino acids. Gelatin is an incomplete protein because it is missing tryptophan and has lower amounts of other essential amino acids(3). Gelatin and gelatin supplemented with tryptophan were shown to suppress hunger more so than casein, soy, or whey protein sources. This was attributed to the indispensable amino acid deficiency theory which states that an animal may detect an incomplete protein source and stop eating it. Therefore, the satiation seen with the gelatin protein may be better attributed to a signal for hunger suppression rather than a satiety signal.

The induction of a ketogenic state is another possible mechanism for protein-induced satiety. The increase in fat oxidation with a high protein, low carbohydrate diet leads to a greater concentration of ketone bodies and a decreased appetite(3). Lastly, changes in concentrations of hormones may also play a role in the satiety effect from different types of protein. Hormones including amylin, cholecystokinin (CCK), ghrelin, glucagonlike peptide 1 (GLP-1), insulin, leptin, and peptide YY (PYY) have roles in central appetite regulation. Amylin, GLP-1, and CCK work to delay gastric emptying, making you feel fuller for a longer period of time. CCK, GLP-1, glucagon, and PPY all have roles in suppressing the appetite while ghrelin tends to trigger hunger.   Ghrelin increases gastric motility and decreases insulin secretion. GLP-1 promotes insulin release which helps to lower blood glucose and stimulates glycogen synthesis. CCK influences PYY release which increases satiety. The central nervous system notices changes in concentrations of these different hormones which could then affect food intake.

CHAPTER III

MATERIALS AND METHODS

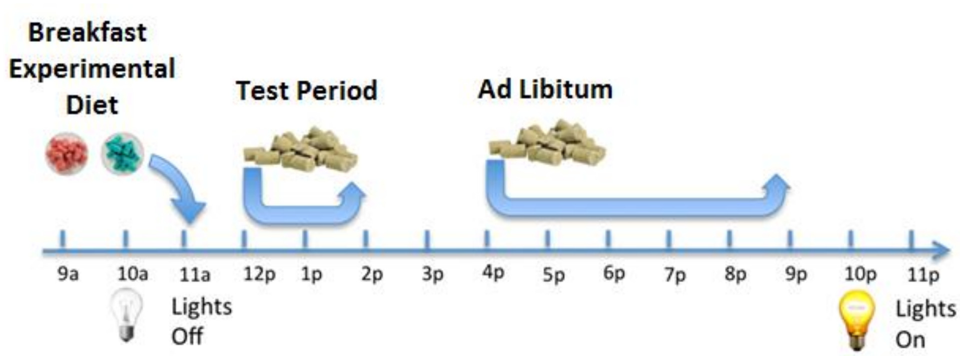
Animals

Male Sprague Dawley rats (Charles River; Raleigh, NC) between 150-200 grams were used to conduct five feeding study trials with 12 rats per trail. Rats were housed individually in hanging wire cages in a temperature controlled room. Rats were acclimated to a reversed 12 hour light cycle schedule upon arrival, with lights on at 10:00pm and off at 10:00am, and were provided with ad libitum access to water. Body weights of the rats were measured each day of the feeding schedule (Figure 1). The use of the animals was approved by The University of North Carolina at Greensboro's Institutional Animal Care and Use Committee (IACUC).

Feeding Schedule

Rats were acclimated to the feeding study schedule for a minimum of three days prior to beginning the study. During this time, rats were exposed to all of the different diets and became adapted to a reverse 12 hour light cycle. The feeding study did not begin until all rats consumed the entire 4g breakfast diets (approximately 20% of daily caloric intake). Lights went off at 10:00am which began the rats active period after an overnight fast. Each rat randomly received a 4 gram breakfast experimental diet at 11:00am and were given 30 minutes to consume the diet. From previous research, 30 minutes was sufficient time for the rats to consume all of their breakfast portion. During each feeding, white computer paper, referred to as spill sheets, were placed under the hanging wire cages to catch spillage. The amount each rat consumed was determined by subtracting the amount of food remaining in the cup and the spillage from the 4 gram amount initially in the food cup. On test days, 30 minutes after the breakfast experimental diet test period (12:00pm), ground chow was provided and the amount consumed over the next two hours constituted the test meal. Ad libitum chow was available to the rats for five hours each day, from 4:00pm to 9:00pm, providing time for the rats to consume necessary caloric intake. Each rat used got multiple exposures to the same diet, and were tested for satiety in response to the breakfast experimental diet consumed by the rat. The test period did not occur every day and animals received diets in a random order to ensure they did not receive the same meal on consecutive days. Rats were excluded from statistical analysis if they did not consume a minimum of 3g of the breakfast ration or if they did not consume 80% or more of their normal daily intake.

Figure 1. Animal Feeding and Light Schedule



Diet Preparation

A variety of isocaloric diets, based on AIN-93G, were made using ingredients purchased from Dyets, Inc (Bethlehem, PA) or Envigo (Madison, WI). Diets contained either 15%, 20%, 35%, or 50% of calories from casein protein, egg white protein, wheat gluten protein, or a combination of two proteins. These diets met all of the nutrient requirements of the rats. Each diet contained the appropriate mineral and vitamin mix to normalize the difference between the levels in the egg white powder and the wheat gluten powder. All diets contained the same amounts of fat and carbohydrate. All diet compositions are provided for the casein (Table 1), wheat gluten (Table 2), and egg white protein diets (Table 3).

Table 1. Composition of Casein Test Diets

	Casein - 15%		Casein - 20%		Casein - 35%		Casein 50%		Casein - 35% + minerals	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	15	14%	19	18%	33	33%	50	49%	35	34%
Carbohydrate	74	66%	67	61%	49	45%	34	29%	51	45%
Fat	9	19%	9	20%	9	21%	9	21%	9	20%
	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
Casein	175	609	220	764	385	1337	585	2033	409	1421
Corn Starch	434	1738	397.5	1590	286	1145	179	718	290	1160
Maltodextrin	144	577	132	528	92	369	58	232	93	372
Sucrose	109	437	92.3	369	58	233	48	193	78	312
Cellulose	50	0	50	0	50	0	50	0	50	0
Soybean Oil	90	810	90	810	90	810	90	810	90	810
Mineral Mix (Casein)	35	31	35	31	35	31	35	31	35	31
Vitamin Mix	10	39	10	39	10	39	10	39	10	39
Choline bitartrate	2.5		2.5		2.5		2.5		2.5	
L-Cystine	2.3	9	3	12	5.3	21	7.5	30	5.3	21
Potassium Sulfate									1.0	
Sodium Chloride									0.5	
	1053	4211	1032	4143	1014	3985	1065	4085	1064	4166
kcal/g		**4.0**		**4.0**		**3.9**		**3.8**		**3.9**

Table 2. Composition of Wheat Gluten Test Diets

Diet	Wheat - 15%		Wheat - 20%		Wheat - 35%		Wheat - 50%		20 **Cas**	15 **WG**	15 **Cas**	20 **WG**	15 **Cas**	35 **WG**
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	15	15%	20	20%	35	35%	50	51%	34	34%	35	35%	50	44%
Carbohydrate	71	65%	66	60%	49	44%	32	27%	48	43%	47	43%	55	43%
Fat	9	20%	9	20%	10	21%	10	22%	9	21%	9	21%	10	19%
	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
Casein									220	764	175	608	175	608
Wheat Gluten Powder	196.5	724	262	966	458.5	1690	655	2415	197	726	262	966	458.5	1408
Corn Starch	410	1639	367	1467	238	952	109	437	265	1061	252	1008	270	1078
Maltodextrin	132	529	118	473	77	307	35	141	86	342	81	325	87	348
Sucrose	83	333	75	298	48	194	22	89	54	216	51	205	72	290
Cellulose	53.5	0	53.5	0	53.5	0	53.5	0	50	0	50	0	50	0
Soybean Oil	90	810	90	810	90	810	90	810	90	810	90	810	90	810
Mineral Mix (Cas)									20	18	15	13	10.5	18
Mineral Mix (WG)	35	26	35	26	35	26	35	26	15	13	20	13	24.5	13
Vitamin Mix	10	39	10	39	10	39	10	39	10	39	10	39	10	39
Choline bitartrate	2.5		2.5		2.5		2.5		2.5		2.5		2.5	
L-Cystine									3	12	3	12	3	12
L-Lysine	2.9	12	3.9	16	6.8	27	9.7	39	2.9	12	3.9	16	6.8	27
L-Threonine	0.8	3	1.0	4	1.8	7	2.6	10	0.8	3	1.0	4	1.8	7
	1016	4115	1018	4099	1021	4052	1025	4006	1016	4016	1017	4019	1262	4658
kcal/g		**4.0**		**4.0**		**4.0**		**3.9**		**4.0**		**4.0**		**3.7**

Table 3. Composition of Egg White Test Diets

Diet	Egg White - 15%		Egg White -20%		Egg White - 35%		20 **Cas**	15 **EW**	15 **Cas**	20 **EW**	15 **Cas**	35 **EW**
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	15	15%	20	20%	35	36%	34	34%	35	36%	36	34%
Carbohydrate	71	65%	65	60%	48	44%	48	44%	47	42%	51	44%
Fat	9	20%	9	20%	9	20%	9	21%	9	21%	9	20%
	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
Casein							220	764	175	608	175	608
Egg White Powder	184.5	641	246	855	430.5	1496	185	643	246	855	250	922
Corn Starch	418	1670	377	1509	256	1025	273	1092	263	1050	277	1108
Maltodextrin	135	539	122	487	83	331	88	352	85	339	89	358
Sucrose	85	340	77	307	52	209	56	222	53	214	74	298
Cellulose	53.5	0	53.5	0	53.5	0	50	0	50	0	50	0
Soybean Oil	90	810	90	810	90	810	90	810	90	810	90	810
Mineral Mix (Cas)							20	18	15	13	10.5	18
Mineral Mix (EW)	35	56	35	56	35	56	15	13	20	18	24.5	13
Vitamin Mix	10	39	10	39	10	39	10	39	10	39	10	39
Choline bitartrate	2.5		2.5		2.5		2.5		2.5		2.5	
L-Cystine							3	12	3	12	3.0	12
	1012.7	4095	1012.7	4063	1012.7	3966	1012	3966	1012	3957	1057	4186
kcal/g		**4.0**		**4.0**		**3.9**		**3.9**		**3.9**		**4.0**

Statistical Analysis

Statistical analyses were conducted using Prism software. One and two-way Analysis of Variance with Tukey's post-hoc tests were conducted for each study to assess amount of ground chow consumed during the test period as related to experimental breakfast diet. Results shown with similar superscripts are not statistically different ($p>0.05$). A $p<0.05$ (*) or $p<0.01$ (**) were considered to be statistically significant in comparison to 15% protein diets. All studies used a one-way ANOVA with additional groups tested due to an unbalanced design with the main effect of experimental diet. Additionally, for study 1 and 5 a repeated measures ANOVA was used with each rat as a measure, as every rat got every diet. Mean test period consumption was reported as grams consumed +/- the standard error of the mean.

Study 1: 15% Casein Base Study

A crossover experimental design was used to assess the effect of protein level versus protein composition. Rats (n = 12) were given one of the experimental diets which included 15% casein protein, 35% casein protein, 15% casein protein + 20% egg white, or 15% casein protein + 20% wheat gluten protein diet as the breakfast experimental diet. The goal of this experiment was to evaluate the subsequent intake after a breakfast experimental diet at the higher level of protein in a mixed meal. At 30 minutes post consumption of the breakfast experimental diet (12:00pm), a second meal composed of ground chow was given to the rats for a two hour time period.

Consumption and spillage were quantified by measuring the remaining contents in the cup and the spillage on the sheet of paper under the rats hanging wire cage, respectively.

Study 2: 20% Casein Base Study

A crossover experimental design was used to assess the effect of protein level versus protein composition in various single and mixed protein source diets when given to rats (n = 12). Diets included a 20% or 35% protein level for casein, egg white, or wheat gluten proteins. Additionally, two mixed protein source diets of 20% casein protein + 15% egg white or 20% casein protein + 15% wheat gluten were used. The purpose was to determine whether the type of breakfast protein or the higher percentage of protein caused a change in subsequent ground chow consumption during the test period. At 30 minutes post consumption of the breakfast experimental diet (12:00pm), a second meal composed of ground chow was given to the rats for a two hour time period. Consumption and spillage were quantified by measuring the remaining contents in the cup and the spillage on the sheet of paper under the rats hanging wire cage, respectively.

Study 3: Wheat Gluten Mixed Diet Study

A 2 x 3 crossover experimental design was used to compare two protein sources (casein and wheat gluten) at three protein levels (15%, 35%, or 50%) plus an additional mixed protein source diet. Diets given to the rats (n = 12) included 15%, 35%, and 50% casein or wheat gluten protein, or 15% casein + 35% wheat gluten protein diet as the breakfast experimental diet. The goal was to determine if the mixed protein source breakfast resulted in an orexigenic response as seen in previous studies with the 35%

wheat gluten protein diet. At 30 minutes post consumption of the breakfast experimental diet (12:00pm), a second meal composed of ground chow was given to the rats for a two hour time period. Consumption and spillage were quantified by measuring the remaining contents in the cup and the spillage on the sheet of paper under the rats hanging wire cage, respectively.

Study 4: Egg White Mixed Diet Study

A 2 x 3 crossover experimental design was used to compare two protein sources (casein and egg white) at three protein levels (15%, 35%, or 50%) plus an additional mixed protein source diet given to rat subjects (n = 12). Diets included 15% or 35% casein or egg white protein, 50% casein protein, or a 15% casein + 35% egg white protein diet as the breakfast experimental diet. A 50% egg white protein diet was not used because the rats would not consume at least 3 g of the diet to be included in the study. The goal of the study was to assess rat's subsequent intake in a mixed protein source meal in comparison to a single protein meal. At 30 minutes post consumption of the breakfast experimental diet (12:00pm), a second meal composed of ground was given to the rats for a two hour time period. Consumption and spillage were quantified by measuring the remaining contents in the cup and the spillage on the sheet of paper under the rats hanging wire cage, respectively.

Study 5: Casein + Minerals Study

A crossover experimental design with two protein sources (casein and egg white) and two protein levels (15% and 35%) with one additional 35% casein + minerals. The purpose was to evaluate the high potassium and sulfur levels in the 35% egg white diet. Therefore, a casein diet was made with the mineral profile of the egg white diet at the same 35% protein level to assess if the mineral profile of the specific protein caused the reduced intake. The addition of sodium chloride and potassium sulfate normalized the mineral content between the 35% casein and 35% egg white protein test diets. Rats (n = 12) were given 4 grams (approximately 20% of daily intake) of 15% or 35% casein or egg white protein, or a 35% casein protein + potassium sulfate diet as the breakfast experimental diet. At 30 minutes post consumption of the breakfast experimental diet (12:00pm), a second meal composed of ground chow was given to the rats for a two hour time period. Consumption and spillage were quantified by measuring the remaining contents in the cup and the spillage on the sheet of paper under the rats hanging wire cage, respectively.

CHAPTER IV

RESULTS

Study 1: 15% Casein Base Study

Eleven animals were included in analysis. One animal was excluded due to not meeting the minimum consumption level of 3g for the breakfast experimental diet. Animals that received a breakfast experimental diet of 15% casein + 20% wheat gluten protein consumed approximately 43% more ground chow during the test period than those animals which received 15% casein + 20% egg white protein ($P<0.01$) (Figure 2). Compared to the 15% casein protein bases, there was no apparent effect of increasing casein to 35% of calories on intake of test meal. Differences in consumption between 15% casein + 20% wheat gluten protein and the 15% casein diet were not statistically significant. Differences in consumption between the 15% casein + 20% egg white protein and the 15% casein diet were statistically different with a 29% decrease in consumption of ground chow during the test period by the animals consuming the 15% casein +20% egg white protein experimental diet ($P<0.01$). Mean test period consumption was 7.3 +/- 0.4 for the 15% casein protein diet, 6.9 +/- 0.3 for the 35% casein protein diet, 8.1 +/- 0.5 for the 15% casein + 20% wheat gluten protein diet, and 5.7 +/- 0.5 for the 15% casein + 20% egg white protein diet. Rat consuming the 15% casein + 20% wheat gluten protein diet consumed more during the test period than the

35% casein diet, while those consuming the 15% casein + 20% egg white protein diet for the breakfast ration consumed less during the test period.

Study 2: 20% Casein Base Study

All twelve animals were included in the analysis of the 20% casein protein diet and the 20% casein +15% egg white protein diet. Eleven rats were used for analysis of the 35% casein protein diet and 35% egg protein diet. Ten animals were used for the analysis of 20% casein + 35% wheat gluten diet and the 35% wheat gluten diet. Animals were excluded if they consumed less than 3 g of the breakfast meal or if they did not meet at least 80% of their daily caloric needs. Compared to the 20% casein base diet, rats consuming the 35% egg white diet ate 40% less during the test period while those consuming the 35% wheat gluten consumed about 10% more of the ground chow during the test period. Similar results were seen with the mixed diets as there was a 41% decrease in test period consumption for rats that consumed the 20% casein + 15% egg white protein diet and only a 5% increase in subsequent consumption for those rats that were given the 20% casein and 15% wheat gluten mixed diet. Mean test period consumption was 7.0 +/- 0.6 for the 20% casein protein diet, 6.5 +/- 0.6 for the 35% casein protein diet, 7.4 +/- 0.6 for the 20% casein + 15% wheat gluten protein diet, 4.1 +/- 0.5 for the 20% casein + 15% egg white protein diet, 7.2 +/- 0.8 for the 35% wheat gluten protein diet, and 4.19 +/- 0.5 for the 35% egg white protein diet.

Figure 2. Total Amount of Food Consumed During the Test Period in Study 1. Bars with Different Letters are Significantly Different as assessed by repeated measures ANOVA (n = 11; P<0.01). 15% casein (15 C), 35% Casein (35 C), 15% Casein + 20% Wheat Gluten (C-W20), and 15% Casein + 20% Egg White (C-E20).

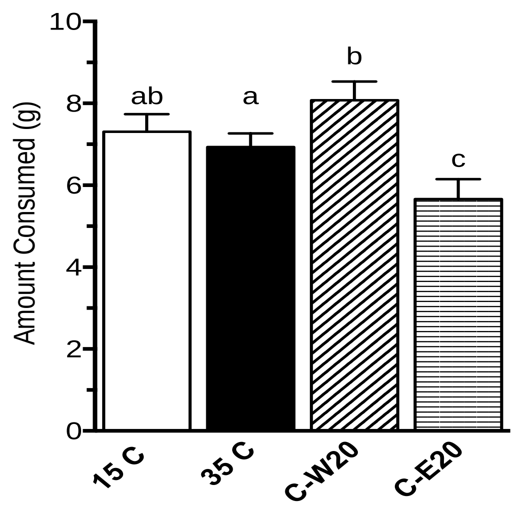
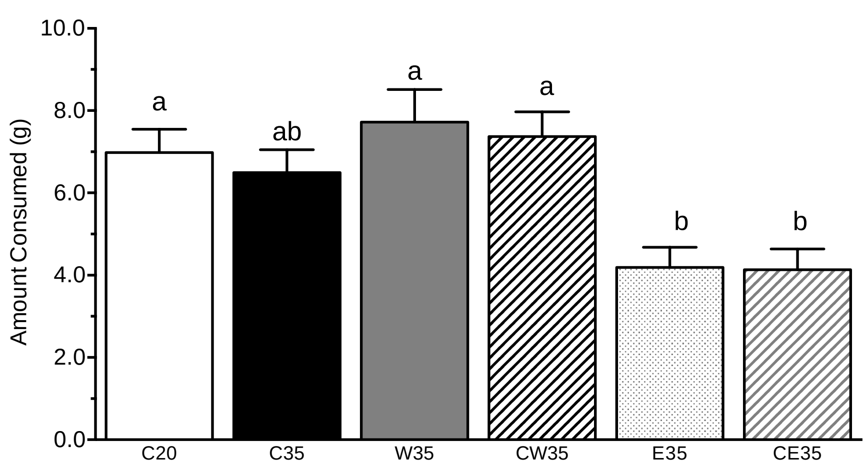


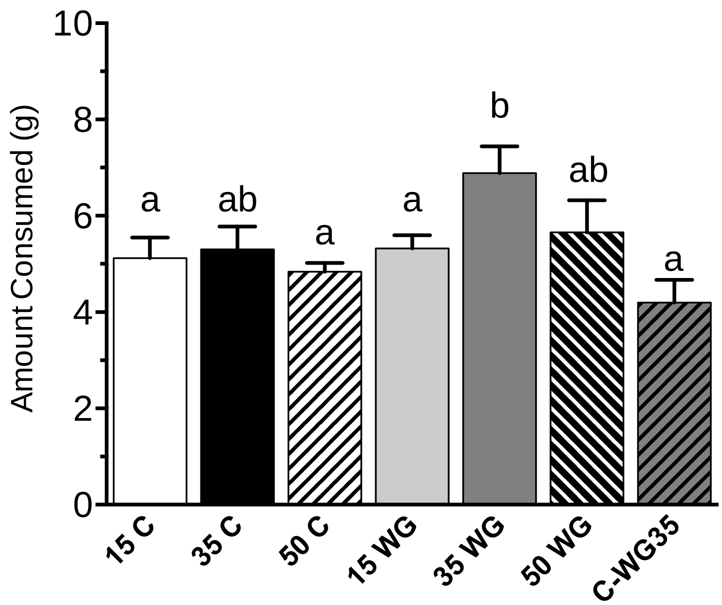
Figure 3. Total Amount of Food Consumed During the Test Period in Study 2. Bars with Different Letters are Significantly Different as assessed by repeated measures ANOVA (n = 10-12; P<0.01). 20% Casein (C20), 35% Casein (C35), 35% Wheat Gluten (W35), 20% Casein + 15% Wheat Gluten (CW35), 35% Egg White (E35), 20% Casein + 15% Egg White (CE35).



Study 3: Wheat Gluten Mixed Diet Study

Ten animals consuming the 15% casein protein diet, nine animals consuming the 15% wheat gluten diet, eight animals consuming the 35% casein protein diet, eight animals consuming the 35% wheat gluten diet, ten animals consuming the 50% casein protein diet, nine animals consuming the 50% wheat gluten protein diet, and ten animals consuming the 15% casein + 35% wheat gluten protein diet were included in analysis. Rats were excluded if they did not consume at least 3 g of their breakfast meal or a minimum of 80% of their daily caloric needs. Animals that received a breakfast of 35% wheat gluten protein consumed approximately 30% more than those that received a breakfast composed of the 15% wheat gluten protein diet ($P<0.01$). The increase in intake of the test meal after breakfast was not apparent when rats consumed the diet containing half of the calories from wheat gluten protein or casein + wheat gluten protein were not different from baseline diets. Mean test period consumption was 5.1 +/- 0.4 for the 15% casein protein diet, 5.3 +/- 0.3 for the 15% wheat gluten protein diet, 5.3 +/- 0.4 for the 35% casein protein diet, 6.9 +/- 0.6 for the 35% wheat gluten protein diet, 4.8 +/- 0.2 for the 50% casein protein diet, 5.66 +/- 0.7 for the 50% wheat gluten protein diet, 4.2 +/- 0.5 for the 15% casein + 35% wheat gluten protein diet.

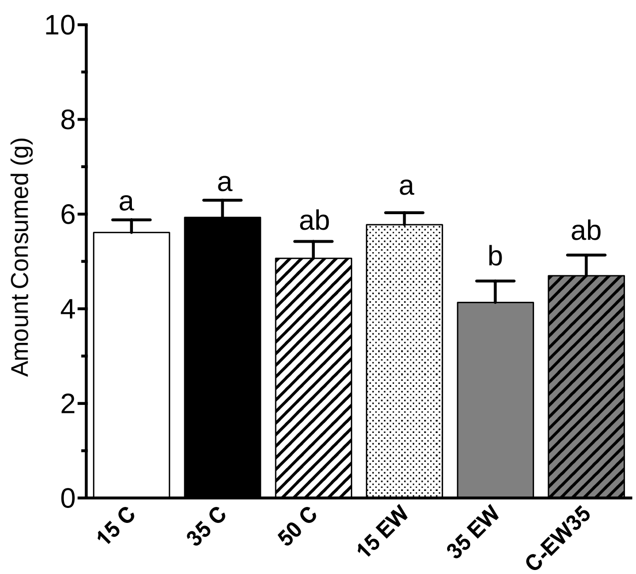
Figure 4. Total Amount of Food Consumed During the Test Period in Study 3. Bars with Different Letters are Significantly Different as assessed by one-way ANOVA (n = 8-10; P<0.01). 15% Casein (15 C), 35% Casein (35 C), 50% Casein (50 C), 15% Wheat Gluten (15 WG), 35% Wheat Gluten (35 WG), 50% Wheat Gluten (50 WG), 15% Casein + 35% Wheat Gluten (C-WG35).



Study 4: Egg White Mixed Diet Study

Ten animals consuming 15% casein diets, 15% egg white protein diets, and 35% casein diets were included in analysis. Nine animals consuming the 35% egg white diet and 50% casein diets were included in analysis. Seven animals consuming the 15% casein + 35% egg white protein diet were included in analysis. Animals were not included if they did not consume a minimum of 3g of the breakfast experimental diet or did not consume a minimum of 80% of their normal daily intake. There was not a significant difference in intake between rats that consumed the 15% casein, 35% casein, and 15% egg white protein experimental breakfast diets. Approximately a 30% decrease in consumption of ground chow during the test period was noted in rats that received the 35% egg white protein breakfast experimental diet in comparison to the 15% egg white or 35% casein breakfast experimental diet. That large of a decrease was not apparent when the rat consumed the mixed diet of 15% casein + 35% egg white protein where a portion of the calories were from the casein protein. Mean test period consumption was 5.6 +/- 0.3 for the 15% casein protein diet, 5.8 +/- 0.2 for the 15% egg white protein diet, 5.9 +/- 0.4 for the 35% casein protein diet, 4.1 +/- 0.5 for the 35% egg white protein diet, 5.1 +/- 0.4 for the 50% casein protein diet, and 4.7 +/- 0.4 for the 15% casein + 35% egg white protein diet.

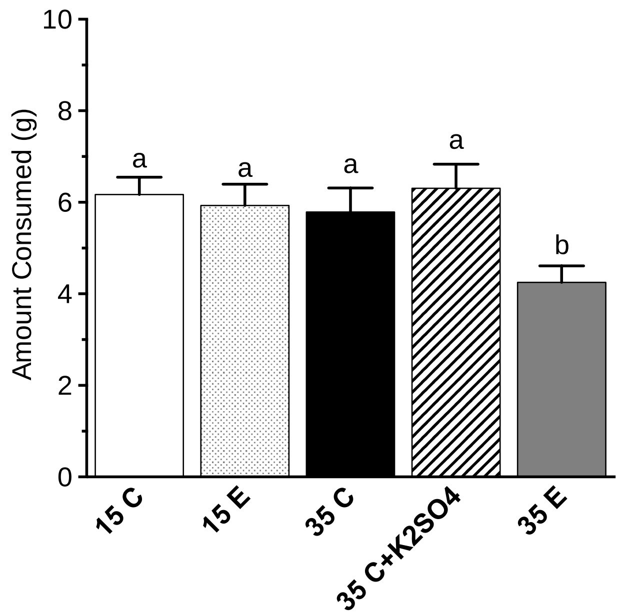
Figure 5. Total Amount of Food Consumed During the Test Period in Study 4. Bars with Different Letters are Significantly Different as assessed by one-way ANOVA (n = 7-10; P<0.01). 15% Casein (15 C), 35% Casein (35 C), 50% Casein (50 C), 15% Egg White (15 EW), 35% Egg White (35 EW), 15% Casein + 35% Egg White (C-EW35).



Study 5: Casein + Minerals Study

Ten animals were included in analysis of the 15% or 35% casein protein or egg white protein diets and a 35% casein diet with added minerals. There was no significant difference between the consumption of the 15% casein diet, 15% egg white diet, 35% casein diet, and the 35% casein + mineral diet. Those rats consuming the 35% casein protein diet with the additional minerals consumed approximately 33% more of the ground chow during the test period than the rats that consumed the 35% egg white protein. Mean test period consumptions were 6.2 +/- 0.4 for the 15% casein protein diet, 5.9 +/-0.5 for the 15% egg white protein diet, 5.8 +/- 0.5 for the 35% casein protein diet, 4.3 +/- 0.4 for the 35% egg white protein diet, and 6.3 +/- 0.5 for the 35% casein + minerals diet.

Figure 6. Total Amount of Food Consumed During the Test Period in Study 5. Bars with Different Letters are Significantly Different as assessed by one-way ANOVA (n = 10; P<0.01). 15% Casein (15 C), 15% Egg White (15 E), 35% Casein (35 C), 35% Casein + K_2SO_4 (35 C + K2SO4), 35% Egg White (35 E).



CHAPTER V

DISCUSSION

Single Protein Source Diets at 15%, 20%, 35%, and 50% Protein Levels

Subsequent intake was influenced by both the protein level and the protein source consumed by the rat during the breakfast period. Egg white protein was more satiating than wheat gluten protein and casein protein when provided to rats at a 35% protein level. Rats consumed less during a subsequent meal when they consumed a breakfast with 35% of calories from egg white protein (Figure 8). When rats were given a breakfast of 35% wheat gluten protein the opposite effect was noticed from that of the 35% egg white diet. The rats consumed more during a subsequent meal showing that the wheat gluten protein was less satiating at the 35% protein level than the casein or egg white protein sources. When the rats were given a breakfast of 50% calories from wheat gluten protein, intake during a subsequent meal was not higher, showing there was a limit to how much additional food the rats would consume. The rats did not consume enough of a 50% egg white diet during breakfast period, therefore a 50% pure egg white diet was not used. When rats were provided casein protein at 35% calories from protein there was no significant change in intake during a subsequent meal from those rats that had a breakfast of 15% casein protein. Similarly, when rats consumed a meal of 50% calories from casein protein the rats had reduced subsequent intake, even lower than that of the 15% casein diet. Regardless of the breakfast diet consumed or the amount consumed during the test meal, total calories consumed during the entire 24 hour period were not different (Figure 7).

Figure 7. Amount Consumed During 12 Hour Dark Phase. 20% Casein (C 20), 35% Casein (C 35), 35% Wheat Gluten (WG 35), 35% Egg White (EW 35), 20% Casein + 15% Wheat Gluten (C20/WG15), 20% Casein + 15% Egg White (C20/EW15).

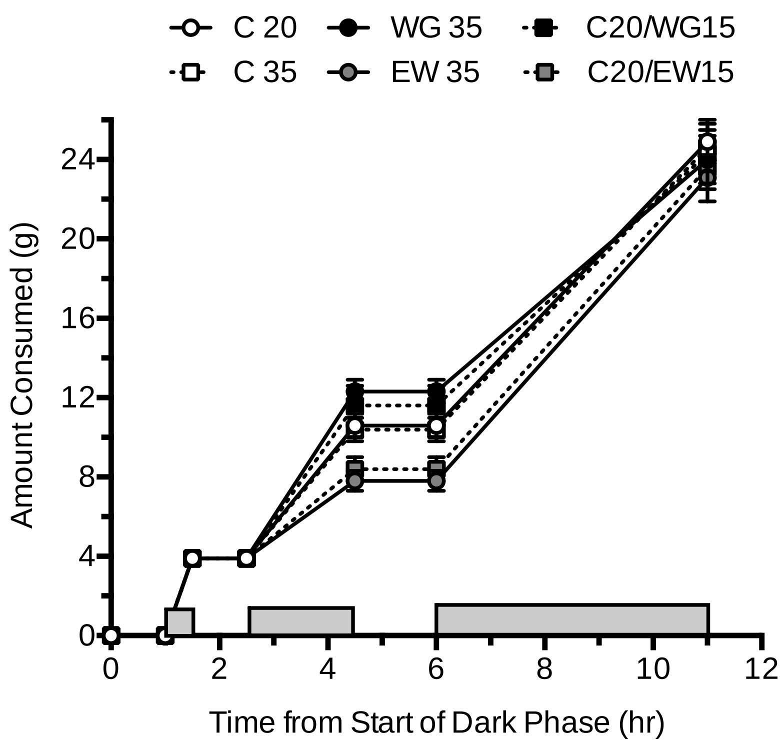


Figure 8. Percentage Change from the Control Diet for 35% and 50% Single Protein Source Diets. 35% Casein (35 C), 35% Wheat Gluten (35 WG), 35% Egg White (35 EW), 50% Casein (50 C), 50% Wheat Gluten (50 WG).

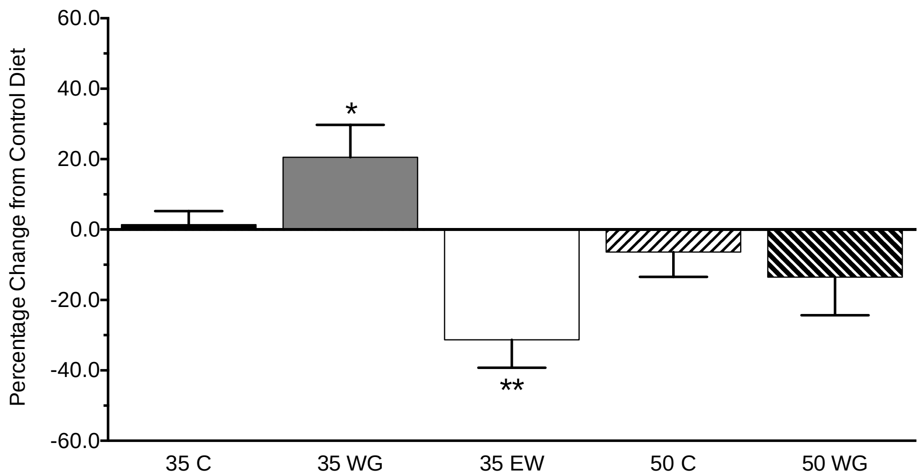
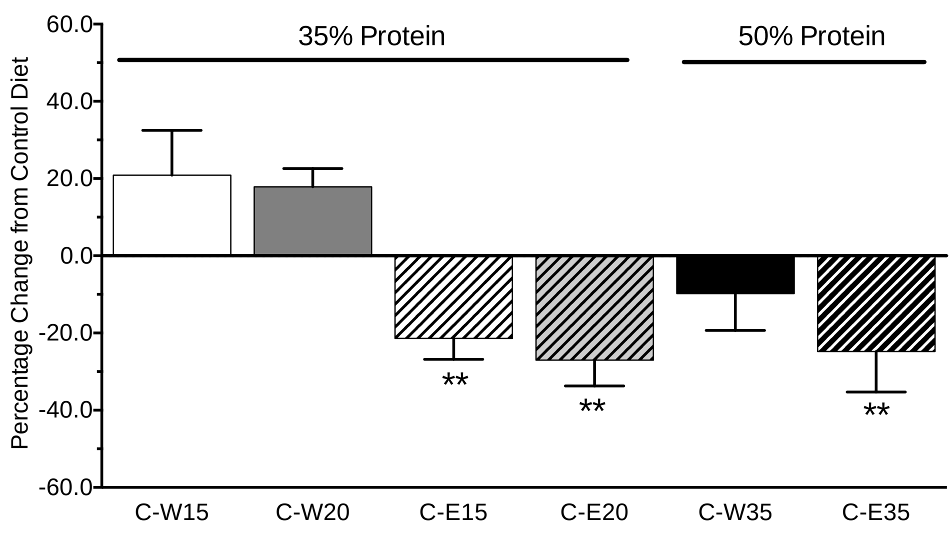


Figure 9. Percentage Change from Control Diet for 35% and 50% Mixed Protein Source Diets. 20% Casein + 15% Wheat Gluten (C-W15), 15% Casein + 20% Wheat Gluten (C-W20), 20% Casein + 15% Egg White (C-E15), 15% Casein + 20% Egg White (C-E20), 15% Casein + 35% Wheat Gluten (C-W35), 15% Casein + 35% Egg White (C-E35).



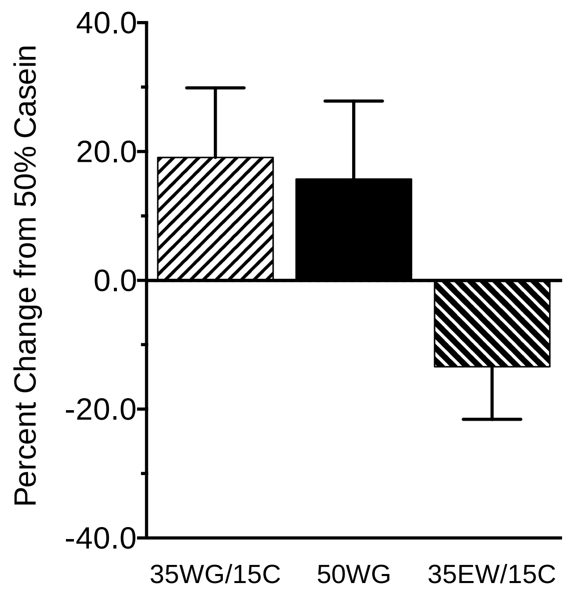
Mixed-Protein Source Diets at 35% or 50% Total Calories from Protein

There were multiple mixed-protein source meals used to further investigate the possible anorectic and orexigenic effects of egg white and wheat gluten protein sources, respectively. To assess whether the high protein level (35% of calories) or wheat gluten protein was inducing the increase in subsequent intake, a mixed diet of 20% wheat gluten protein and 15% calories from casein protein was provided to rats in study 1. As seen with the 35% wheat gluten diet, the mixed 15% casein + 20% wheat gluten diet also showed a significant increase in the amount consumed by the rats during a subsequent meal (Figure 9). Similarly, rats fed a breakfast of the mixed 15% casein + 20% egg white protein diet exhibited a significant decrease in intake like the pure 35% egg white protein diet. In study 2, the base casein protein level of the mixed diet was raised to 20% with 15% of calories from egg white or wheat gluten protein. Similar results were seen with an increase comparable to 35% wheat gluten protein diet in subsequent meal intake by those rats receiving 15% casein + 35% wheat gluten protein for breakfast and a decrease comparable to 35% egg white diet for those rats consuming the 15% casein + 35% egg white protein breakfast meal.

Single or Mixed-Protein Source Diets with 50% of Calories from Protein

Higher protein levels, to 50% calories from protein, were assessed in studies 3 and 4 to investigate if subsequent intake was altered due to the high protein level or the source of protein. The diet with a base of 15% casein and the additional 35% of calories from wheat gluten had a significant decrease in the amount consumed when compared to the 35% wheat gluten diet (Figure 10). The amount consumed was similar to the 15% base diets. Gluten protein, being less bioavailable, reached the threshold for satiety when a 50% protein level diet was consumed; however, the decrease in consumption was still greater than the consumption by rats consuming a 50% casein diet. The 15% casein + 35% egg white protein diet showed no further reduction in the amount consumed during the subsequent meal from that of the 35% egg white diet. Egg white protein is more bioavailable; therefore, the threshold for satiety can be reached more quickly as shown by the subsequent intake decreasing when a 35% egg white diet is consumed.

Figure 10. Percentage Change from 50% Casein for 50% Protein Level Diets. 15% Casein + 35% Wheat Gluten (35W/15C), 50% Wheat Gluten (50WG), 15% Casein + 35% Egg White (35EW/15C).



Effects of Diet Mineral Composition on Subsequent Intake

The difference in the mineral composition of the 35% egg white diet was not the cause of the reduced intake during a subsequent meal. A 4g meal of 35% egg white protein contains 29.31 mg of sulfur while both 35% casein and wheat gluten diets only contain 3.65 mg of sulfur. There is no research specifically concerning the relationship between sulfur and satiety to determine how this great of an increase in sulfur may influence satiety. With the addition of potassium sulfate we investigated this relationship in study 5. Our results showed that the addition of minerals to the 35% casein diet did not affect the consumption during the subsequent meal. This means that it was not the higher level of minerals (sulfur) in the egg white diet responsible for the decreased test period consumption. Although the 35% casein + mineral diet had higher amounts of elemental sulfur and lower amounts of sulfur containing amino acids, the sulfur content was the same.

Satiety Inducing Effects of Various Protein Sources

All rats consumed some amount of chow during the test period no matter what breakfast they consumed showing that complete satiety was not achieved by the 4 g test meal. Our lab group has shown that regardless of the breakfast meal provided, rats will consume chow immediately after removing the breakfast diet(31, 32). This could result from 4 g of breakfast (approximately 20% of daily caloric intake) being too limited of a serving to induce satiety or it could be due to a delay in satiety after consuming the breakfast meal. Furthermore, our research has shown that consumption of the chow differs between the different breakfast diets primarily during the first 30 minutes of the test period. When rats are given the choice between higher protein wheat gluten and egg white diets, they chose they wheat gluten diet, suggesting they have an aversion to the higher protein egg white diet. This was supported through behavioral analysis studies which showed that the rats took longer to consume the egg white diets than the wheat gluten diets.

Amino Acid Composition of Different Protein Sources

Rats consumed more during the test period after a breakfast with 35% of calories from protein with part or all of that being wheat gluten in comparison to diets of casein or egg white protein sources. This suggests that wheat gluten is unable to induce satiety to the level of other protein sources. This could be due to differences in amino acid composition. The wheat gluten diet only has 1.7 g tyrosine per 100 g protein while egg white has 3.9 g per 100 g protein. Tyrosine and phenylalanine are the precursor amino acids for dopamine production(33). With less tyrosine available there is increased competition with large neutral amino acids for tyrosine to cross the blood-brain barrier resulting on limited levels of circulating dopamine. Dopamine levels are important for controlling food intake and it has been shown that obese humans have fewer striatal dopamine receptors than lean humans, suggesting that those obese humans may over-eat and gain more weight(29, 33).

Digestibility of Protein Sources

The digestibility of the different protein sources may also play a role in their satiating ability. Wheat gluten protein may be digested so quickly that its satiating effect does not last long enough to keep the rat satiated through the test period, therefore resulting in their increased consumption. Limited research has been conducted on the plasma amino acid appearance rate and concentration after ingestion of a high protein meal. In a study by Gannon et al which looked at the plasma amino acid response after type II diabetics consumed various protein sources, it was concluded that consumption of egg white protein resulted in a slow increase in plasma amino acids(7). Egg white protein is a high quality protein with the highest PDCAAS score of 100 versus wheat which has a score of 25, signifying that wheat gluten has much lower digestibility than egg white. This difference may correspond to the appearance rate of the plasma amino acids, leading one to conclude that a the plasma amino acid concentration of a higher quality protein source will rise very slowly in comparison to that of a lesser quality protein like wheat.

Plasma Amino Acid Response to Different Protein Sources

Conflicting results have been seen in the limited research on plasma amino acid concentrations and protein sources. In a study of non-obese humans consuming a high protein diet (30% calories from protein), there was no increase in tryptophan (Trp) or the TRP to large neutral amino acid (LNAA) level although there was a significant increase in plasma LNAA levels(34). Furthermore, no relationship was found between the plasma amino acid changes and the decreased ad libitum intake. Contradicting results were found in a human study that looked at the plasma amino acid response to consumption of whey protein(35). The strong satiety response to whey protein was supported by a significant increase in total amino acid concentration and branched chain amino acid (BCAA: isoleucine, leucine, and valine) concentration between 15 and 120 minutes.

Hormone Response to Different Protein Sources

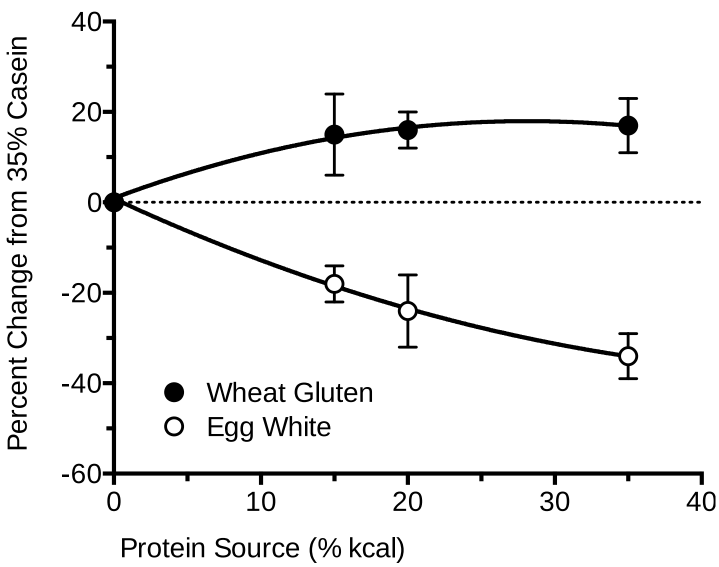
Much research suggests that the differing levels of gastrointestinal hormones in response to different protein sources may be influencing satiety. Elevated levels of cholecystokinin (CCK), glucagon, and amylin have been shown to inhibit meal intake and initiate a satiety sequence(16). The afferent vagus is responsible for inhibition of eating as CCK receptors in the stomach and proximal intestine, sensitive to the size composition of the gastrointestinal contents, send feedback to the brain. Glucagon like peptide-1 (GLP-1) and peptide YY (PYY) are both associated with long term reduction in food intake. Both GLP-1 and PYY peak after a meal has been completed and maintain higher levels between meals. Conversely, when ghrelin levels are high, food intake increases. As meals are consumed and satiation occurs, ghrelin levels decline. Bowen et al showed that ghrelin, CCK, insulin, and GLP-1 all exhibited post-prandial changes that could partially contribute to reduced energy intake and increased satiety(36). Differences were not found between the soy, whey, or gluten protein sources; however, this could be due to the use of a beverage rather than a solid food item. Consumption of a high protein egg-beef breakfast (35 g) led to reduced ghrelin and increased PYY daily concentrations when compared to those who skipped breakfast(26). The same result was not seen in a normal protein (13 g) cereal based breakfast. Bayham et al found ghrelin levels were lower and PYY levels were increased to greater levels after consumption of a higher quality egg breakfast in comparison to a lower quality cereal breakfast(29). The PYY effect was found to last throughout seven days while ghrelin had a more acute response.

Current Research and Gaps

Limited research has been published on the satiating effects of egg white protein versus wheat gluten protein sources, as much of the existing research focuses on whey, casein, or meat protein sources. An egg breakfast has been shown to decrease short term caloric intake and induce greater satiety when compared to a bagel breakfast(4). Another study concluded that there was no difference in intake behavior or satiating capacities of egg albumin, casein, gelatin, soy, pea, or wheat gluten protein sources(5). The difference in conclusions may be due to subject's consumption of mixed meals rather than consumption of only protein. Rats given a 40% pure protein diet of lactalbumin, egg white, or soy were found to decrease their intake while rats eating casein protein increased their intake after a mixed protein preload(37). These results were different on day two of the experiment with rats consuming the egg white or soy protein increased their intake and rats consuming casein ate less in comparison to day one.

More research is necessary to further determine how different protein levels and sources affect satiety. Analyzing plasma amino acids and hormone levels would be beneficial to determining the mechanism behind the changes in intake of different protein sources or levels. Results of these studies would enable us to make conclusions about what protein source and level is most effective at inducing satiety, decreasing subsequent intake, and allowing weight loss or management.

Figure 11. Percentage Change From 35% Casein of Wheat Gluten and Egg White Protein Sources at Varying Protein Levels.



Conclusion

The protein source of a breakfast-like meal affected the satiating effect on a subsequent meal. Our study focused on an acute response to a meal, rather than a chronic response like much of the literature. The duration of the satiation effect was short, as total caloric intake on test days was not affected by the diet fed at the breakfast meal. Overall, egg white was most satiating protein, casein had little effect on subsequent meal size, and what gluten showed an orexigenic effect as there was an increased consumption during the subsequent meal (Figure 11). Knowing what sources and level of protein can help mediate intake at the next meal can be beneficial to people who are trying to lose or maintain weight. To aid in reducing intake, someone may chose primarily egg white protein at a higher level, although this was only shown at an acute level. Further research is required to determine if chronic reduction in intake occurs with a certain protein source or level.

CHAPTER VI

EPILOGUE:
REFLECTION ON RESEARCH AND NEXT STEPS FOR FUTURE RESEARCH

Dietary protein has been repeatedly show to be the most satiating macronutrient. Our assessment of varying protein levels and protein sources provided to Sprague-Dawley rats as a breakfast allowed us to look at the effects on satiety and the underlying biology. Egg white, wheat gluten, and casein are all common breakfast protein sources for Americans and they all have varying ratios of tyrosine to large neutral amino acids. When reflecting on the five feeding studies, I would have considered adding 35% egg white and 35% wheat gluten protein diets to the first study to which I could compare the casein/egg white and casein/wheat gluten mixed protein diets. These two single source protein diets were added to study 2 for comparison. Research is time consuming and expensive. In retrospect, it would have been beneficial to take some blood samples throughout the various feeding trials to assess plasma amino acid composition. This would have been difficult to achieve due to our team's busy class and lab schedule as the feeding studies already required someone present at multiple time points in the day.

There are multiple areas that could be assessed for future research to determine which part of the protein source could be leading to changes in feeding intake during a subsequent meal. When considering what parts of the protein source could be leading to these changes in the feeding intake and the apparent satiety of the next meal I initially considered postabsorptive levels of amino acids. By titrating in a protein source, we have different levels of amino acids we can look to see what amino acid composition stands out a different time intervals after consumption of a certain breakfast protein. In particular, I would consider the presence of leucine, tryptophan, and tyrosine to assess bioavailability during the postabsorptive period. For this future study, blood samples will be taken at various time points pre- and postprandial to then analyze with the HPLC.

Additionally, possible indirect effects during the postingestive period could play a role in the resulting satiation from different protein sources. Proteins could possibly be affecting gut hormones, then gut hormones could be affecting intake levels causing some protein sources to be more satiating than others. Possibly ghrelin, a hunger hormone secreted when the stomach is empty, is more inhibited by egg white protein than wheat gluten protein. Maybe egg white has a greater CCK response if it is released more rapidly from the stomach. Amylin plays a role in glycemic regulation by slowing gastric emptying and promoting satiety. Perhaps more arginine is getting into beta cells resulting in an increase in release of amylin which could increase satiety.

Overall, by determining the hormonal profile and amino acid profile at the start of the 30 minute experimental breakfast diet and throughout the two hour test period where the amount of ground chow consumed is recorded could reveal differences between egg white and wheat gluten. The greatest difference is expected in the first 30 minutes of test meal. From our group's previous research, it is important to consider that animals on the wheat gluten diet tend to eat all in of the breakfast in the first 15 minutes versus animals on the egg white diet take 30 minutes for rat to eat all of the breakfast. It is important to consider if we see amino acid increase because its only 1 hour after egg white protein consumption versus the 1 hour 15 min after the wheat gluten protein consumption.

Aside from looking at the amino acid and hormone profile, translating the study into an appropriate population could help us understand satiety and cognitive function in human populations. Appropriate populations could include school aged children or athletes at the collegiate level to assess satiety and cognitive function. These diets of varying protein levels and sources could have effects on individuals with different backgrounds. An obese population could be used to assess the leptin response, perhaps resulting in a need for egg white protein consumption to reduce subsequent intake. The opposite could be true of a cachexic population who may benefit from wheat gluten consumption to help increase subsequent intake throughout the day.

In conclusion, I have learned a lot about the scientific process, what it takes to conduct genuine research, and how many dedicated members it takes to get a project done. I am very thankful for this experience, the teamwork from my lab mates, and the constant kindness and support from Dr. Beverly. I feel that I am finishing with skills that will help me as I pursue my next steps. I have become a stronger critical thinker and problem solver from this process preparing me for my future as a Registered Dietitian.

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APPENDIX A

ADDITIONAL TABLES

Table 4. Amino Acid Content of Different Diets. The amino acid contents for diets are provided according the amount consumed in a 4 gram meal. 15% Egg White (15%EW), 35% Egg White (35%EW), 15% Wheat Gluten (15%WG), 35% Wheat Gluten (35%WG), 15% Casein (15%Cas), 35% Casein (35%Cas), 15% Casein + 20% Egg White (15C 20 EW), 15% Casein + 20% Wheat Gluten (15C 35 WG)

	15%EW	35%EW	15%WG	35%WG	15%Cas	35%Cas	15C 20EW	15C 35WG
Arg	34.2	79.8	27.0	63.0	19.8	46.2	65.4	82.8
Lys	36.0	84.0	17.4	67.7	42.0	98.0	90.0	82.6
His	13.2	30.8	15.0	35.0	15.0	35.0	32.6	50.0
Met	22.8	53.2	7.2	16.8	13.8	32.2	44.2	30.6
Thr	25.8	60.2	17.4	44.8	22.8	53.2	57.2	63.4
Leu	50.4	117.6	41.4	96.6	48.0	112.0	115.2	144.6
Ile	35.4	82.6	21.0	49.0	30.0	70.0	77.2	79.0
Val	43.2	100.8	25.8	60.2	36.0	84.0	93.6	96.2
Phe	36.6	85.4	28.2	65.8	26.4	61.6	75.2	92.2
Trp	9.0	21.0	6.6	15.4	6.0	14.0	18.0	21.4
Tyr	23.4	54.6	10.2	23.8	27.6	64.4	58.8	51.4
BCAA	129.0	301.0	88.2	205.8	114.0	266.0	286.0	319.8
LNAA	198.0	462.0	133.2	310.8	174.0	406.0	438.0	484.8
EAA	244.8	571.2	230.4	537.6	223.2	520.8	549.6	760.8
Tyr/LNAA	0.12	0.12	0.08	0.08	0.16	0.16	0.13	0.11
Leu/LNAA	0.25	0.25	0.31	0.31	0.28	0.28	0.26	0.30
Trp/LNAA	0.05	0.05	0.05	0.05	0.03	0.03	0.04	0.04

Table 5. Mineral Content of Diets from Study 5: Casein + Mineral Study. The mineral compositions of all the diets from study 5, including the new 35% casein + K_2SO_4 diet, are provided according the amount consumed in a 4 gram meal. The potassium, sulfur, and chlorine content are now essentially the same between this new diet and the 35% egg white diet. 15% Egg White (15%EW), 35% Egg White (35%EW), 15% Casein (15%Cas), 35% Casein (35%Cas), 35% Casein + K_2SO_4 (35%Cas K2SO4).

	15%EW	35%EW	15%Cas	35%Cas	35%Cas K2SO4
Ca	25.15	26.03	30.56	30.73	30.73
P	33.33	34.20	22.63	29.33	29.33
Na	9.22	21.40	4.29	4.38	12.38
K	13.99	24.97	6.71	6.79	24.39
S	12.56	29.31	3.65	3.65	26.05
Cl	7.80	18.20	6.22	6.22	18.22
Zn	0.12	0.12	0.14	0.17	0.17
Fe	0.19	0.19	0.19	0.19	0.19
Cu	0.02	0.02	0.02	0.02	0.02
I	0.00	0.00	0.00	0.00	0.00
F	0.00	0.01	0.00	0.00	0.00
Mg	2.55	3.26	2.06	2.07	2.07
Mn	0.04	0.04	0.04	0.04	0.04