The Effect of Protein Quantity and Quality at Breakfast on Energy Metabolism, Appetite and Metabolic Health

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The Effect of Protein Quantity and Quality at Breakfast on Energy Metabolism, Appetite and Metabolic Health

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Science

by

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ABSTRACT

Obesity is a global health concern and, within the United States, the current obesity rate is 36% and projected to double within the next two decades. Obesity is linked to many chronic diseases such as cancer, heart disease and type 2 diabetes. In young females, weight gain (5-11 kg) between the ages of 20-30 years increases the risk of developing type 2 diabetes and cardiovascular disorders later in life. The cause of obesity is multifactorial in nature, however fundamentally weight gain occurs when energy intake is greater than energy expended (i.e. calories in > calories out). Therefore, identifying and validating nutritional intervention strategies to modulate energy balance is necessary in order to treat and prevent weight gain in the future. There is an abundance of scientific literature demonstrating diets higher in protein are beneficial for both weight loss and weight management. Higher protein intake is associated with increases in energy expenditure, decreases in hunger and improved glycemic response. What is less known is how protein quality of the diet impacts health outcomes. Protein quality is defined by the proportion of essential amino acids a protein contains relative to our body’s needs. Therefore, the quality of protein may also impact the ability of a protein to be beneficial for health. Metabolic health may also be influenced by the time of day protein consumption occurs, specifically the intake of protein at breakfast. Unfortunately, avoidance of breakfast consumption, as a whole, is inversely associated with body mass index. However, increasing protein intake in the morning has been supported as an effective strategy for weight loss by increasing energy expenditure, fat oxidation, and favorably altering appetite signaling. Yet, data is also lacking regarding protein’s adaptive metabolic response to habitual protein intake at breakfast. Therefore the objective of this thesis was to determine if protein quality and quantity consumed at breakfast influenced energy expenditure, appetite, and metabolic health in young females.
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DEDICATION

This thesis is dedicated to my parents, Wade & Renee Neumann, as without them I would not be where I am.
# TABLE OF CONTENTS

Introduction..........................................................................................................................01

Literature Review................................................................................................................08

  Obesity.................................................................................................................................08

  Dietary Protein...................................................................................................................11

  Importance of Protein at Breakfast................................................................................15

**Chapter 1:** Breakfast macronutrient composition influences postprandial energy expenditure and fat oxidation in young females who habitually skip breakfast........................................30

Appendix A...........................................................................................................................54

**Chapter 2:** Breakfast protein source does not influence postprandial appetite response and food intake in normal weight and overweight young females........................................55

Appendix B...........................................................................................................................75

Conclusion...........................................................................................................................76

Curriculum Vitae................................................................................................................77
FIGURES

Literature Review
Overview of energy balance ........................................................................................................20

Chapter 1
Figure 1: Postprandial energy expenditure and substrate oxidation following a PRO- or CHO- breakfast or continued breakfast skipping ........................................................................47
Figure 2: Ratings of appetite two hours postprandial (PP) following a PRO- or CHO-breakfast or continued breakfast skipping using visual analog scales .......................................................................48
Figure 3: Changes in glucose response over time following a PRO- or CHO-breakfast or continued breakfast skipping ........................................................................................................49

Chapter 2
Figure 1: Flow diagram of the participant screening and selection process .........................69
Figure 2: Appetite responses following test breakfasts .................................................................70
Figure 3: Glucose response to the test breakfasts .......................................................................71
TABLES

Chapter 1
Table 1: Dietary characteristics of test breakfast.................................................................50
Table 2: Participant characteristics.....................................................................................51
Table 3: Average energy and macronutrient composition of ad libitum food intake over 3 days following either a CHO- or PRO-based breakfast...............................................52
Supplemental Table 1: Postprandial metabolic variables following consumption of either CHO- or PRO- based test breakfast.................................................................53

Chapter 2
Table 1: Participant characteristics.....................................................................................72
Table 2: Dietary characteristics of test breakfasts.................................................................73
Table 3: Energy and macronutrient content of 24-hour food intake.....................................74
Chapter 1


Chapter 2

INTRODUCTION

Obesity is a global health concern and, within the United States, the current obesity rate is 36% among both male and female adults and projected to double within the next two decades [1, 2]. Obesity is linked to many chronic diseases such as cancer [3], heart disease [4] and type 2 diabetes [4]. Specifically, young females have an increased risk for becoming obese [5-7]. Between 20-30 years of age, females gain between 6.7-11.3 kg of body weight [7]. This added weight places them at a higher risk for developing chronic diseases, specifically type 2 diabetes [7]. Efforts to understand the causes of obesity can aid in the development of health interventions to reduce the growing rate of obesity and its associated chronic diseases.

Obesity is a multifactorial health disorder. Genetics [8], socio-economic status [6], and lifestyle factors [9] all contribute to the growing rate of obesity. However, fundamentally weight gain is a measurable result of chronic energy imbalance in which energy intake (i.e. calories consumed) is greater than energy expended (i.e. calories burned) [10]. One way to correct energy imbalance is to target energy expenditure (EE). Total EE encompasses resting EE (REE; i.e. energy needed to sustain body functions at rest), activity thermogenesis (i.e. energy associated with physical activity and sickness), and thermic effect of food (TEF; i.e. energy associated with the digestion, absorption, and assimilation of nutrients in the body) [11]. By increasing one or more of these components, total EE is expected to also rise [12]. Many obesity treatments focus on decreasing energy intake and increasing expenditure [6]. However, successful obesity treatments promote a comprehensive lifestyle change, including increased physical activity and reduced caloric intake while adjusting the macronutrient composition of the diet for optimal health benefits [13]. For example, higher protein diets have been shown to be a successful strategy for treating and preventing obesity and improving metabolic health [14-16].
In general, individuals following higher protein diets (≥ 25% caloric intake from protein) have reported short-term increases in EE [17-20] and demonstrated reductions in acute energy intake [17, 18]. For instance, numerous nutritional intervention studies comparing protein quantity have reported reduced food intake after a high level of protein consumption and increased postprandial satiety responses [12, 21-25]. Similar findings are reported in longer-term studies reviewed by Leidy et al [12, 26]. EE is also observed to increase within high protein diets, specifically TEF [6]. In acute studies (i.e. after one meal), protein elicits a greater TEF compared to either carbohydrates or fats [27, 28]. Therefore it is argued increasing protein intake will not only impact postprandial satiety beneficially, but also increase total EE [12, 19], thus aiding in weight loss. However some suggest, the source of protein may also be equally as important as the quantity of protein when trying to achieve optimal health [16].

Less is known regarding protein quality and timing (i.e. the time of day consumption of protein occurs) on body weight management. Protein quality is referred to as the essential amino acid composition of a protein in relation to its ability to achieved defined metabolic actions [29]. Recently, the quality of protein has emerged as being a central component in energy balance and appetite regulation [29-31]. High-quality protein is shown to increase EE [31] and produce larger and more sustained increases in fullness compared to lower quality proteins [32]. Additionally, the timing of protein intake has been identified as a key factor in metabolic regulation. For example, recent data demonstrate that protein intake distributed evenly throughout the day (25-30 g per meal) is associated with positive changes in muscle protein synthesis compared to skewed protein intake in which most protein is consumed with the evening meal (~ 60 g). Furthermore, skewed protein consumption results in a blunted protein synthesis response [33], however, consumption of 25-30 g protein in the morning elevates muscle protein synthesis.
attenuating the rate of protein breakdown [34] and promoting the preservation of lean muscle mass. However, more research is needed to define the optimal protein source to be consumed at breakfast to elicit maximum health benefits.

In general, breakfast consumption is associated with a healthy lifestyle [35], yet within young adults, 26% of males and 24% of females regularly skip breakfast [36]. A recent meta-analysis concluded habitual breakfast skipping is associated with weight gain [37]. Although no direct causation has been established between breakfast skipping and obesity, there is sufficient evidence to conclude skipping breakfast results in poor appetite regulation and increased energy intake at the following meal which may lead to overeating and weight gain [37-39]. However, additional research is needed to define the optimal breakfast macronutrient composition and to define the role of protein quality at breakfast for ideal health outcomes. Therefore the objective of this thesis is to determine if protein quantity and quality consumed at breakfast influenced EE, appetite, and metabolic health in young females.
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LITERATURE REVIEW

OBESITY

Obesity is a global health concern and, within the United States, the current obesity rate is 36% among both male and female adults and projected to double within the next two decades [1, 2]. Obesity is linked to many chronic diseases such as cancer [3], heart disease [4] and type 2 diabetes [4]. Over the past two years the obesity rate has stabilized among adults [2], yet the population of obese adults is still rising [2, 5]. Specifically, there is an increased risk of becoming obese in early adulthood, chiefly among females [6]. In 2010, 36% of young females were considered obese [2, 7]. Yet even females who gain between 8-11 kg of body weight in early adulthood are at a higher risk for developing type 2 diabetes, coronary heart disease, depression, polycystic ovarian syndrome, and infertility [8, 9]. Fortunately, obesity is one of the foremost preventable causes of death and disease in the US [10]. Therefore, understanding the underlying causes of weight gain can help prevent and treat the onset of obesity and its associated chronic diseases.

The development of obesity is multifactorial in nature with factors such as environment, socioeconomic status, genetics and lifestyle choices all influencing the risk obesity [11-13]. Traditionally, the rise in obesity has been attributed to environmental changes such as increasing portion sizes [14], increased access to nutrient-poor foods, and a diminishing importance of physical activity [15, 16]. For instance, over the last four decades the average portion sizes have dramatically risen both in and outside the home [14]. For example, Young et al [17] concluded between 1965 and 2005, the number of proprietary “larger-size” options available went from 14 available options to 140 which positively corresponds to the rise of obesity. Many studies have demonstrated increased portion sizes lead to increases in energy intake in a single sitting.
[17, 18]. Rolls et al [19] found greater portion sizes, independent of demographics and serving method, significantly increased energy intake in a single meal. Supporting findings were found by Dilibreti et al [20] in which increased portion size was positively associated with energy intake in an, uncontrolled, observational setting. Not only does overeating at a single meal increase energy intake, it is also associated with potential chronic overeating [21].

Second, low socioeconomic status has also been found to be associated with increased obesity rates [6, 10]. One explanation for this relationship maybe due to the lower cost of nutrient-poor foods, such as fast-foods, compared with the higher-cost of fruits and vegetables [6]. Also, the lack of access to recreational settings has been linked to an increased risk of obesity within low-socioeconomic families [22, 23]. Unfortunately, low-socioeconomic status in adolescents is highly correlated to obesity in young adulthood [6].

Third, in recent years, genetics has emerged as another contributor to obesity, predisposing some individuals to weight gain regardless of environmental and socioeconomic factors [16, 24, 25]. Polymorphisms within appetite and metabolic genes cause predisposition to obesity in some individuals [26] and estimates of 8-85% of the current obesity concern is thought to be due to a change in the genetic conditions [27]. Yet even with genetic predisposition, understanding the impact of lifestyle choices can mitigate the onset of weight gain.

Lifestyle choices, such as diet composition and physical activity are another contributor that can influence the onset of obesity [13]. Consequently, diet (i.e., energy intake; EI) and physical activity (i.e., energy expenditure; EE) are the two primary components of energy balance (Figure 1) [28]. Obesity is a result of chronic energy imbalance (i.e., EI > EE). As a whole, physiological energy balance constantly fluctuates, however when energy balance is in equilibrium, EI equals EE resulting in net zero weight gain [11, 29]. A net positive energy
balance indicates the body is storing more energy than expending (i.e., EI > EE) and weight gain incurs [11]. In contrast, a net negative energy balance indicates the body is expending more energy than storing (i.e., EI < EE) promoting weight loss [11]. Although EI is depended on caloric consumption, modulating EE is more complex.

EE can be divided into three components: resting EE (REE; i.e. energy needed to sustain body functions at rest), activity thermogenesis (i.e. energy associated with physical activity and sickness), and thermic effect of food (TEF; i.e. energy associated with the digestion, absorption, and assimilation of nutrients in the body) [30]. REE, activity thermogenesis, and TEF account for 60%, 32%, and 8% of total EE, respectively [30, 31]. Altering the rate of one or more of these components will affect total EE. Some researchers have speculated a reduced rate of TEF contributes to the obesity epidemic [32]. Thus, creating treatments that promote increases in total EE, specifically TEF, along with reducing EI intake are essential to treating and preventing obesity.

In previous years, the treatment of obesity was approached through general awareness, policy, and, as mentioned previously, environmental changes [33]. Still, the obesity rates were increasing so a push for innovated approaches was needed. Today, current treatment of obesity includes pharmacology, bariatric surgery, and clinical interventions focused on behavioral training, and modulating energy balance through increasing physical activity and reducing/modifying EI through dietary changes [10, 33]. Currently, nutritional interventions, focusing on altering the macronutrient composition (i.e., the ratio of protein, carbohydrates and fat) of the diet, have been proven successful for reducing EI and increasing EE. Specifically, diets higher in protein have been shown to be effective at weight management and promoting weight loss [34-38].
**DIETARY PROTEIN**

Consumption of dietary protein is necessary for proper growth and development [39]. The recommended dietary allowance (RDA) for protein is 0.8g/kg/day while the acceptable macronutrient distribution range (AMDR) states 10-35% of daily intake should be protein [40]. In the US, protein is not considered a lacking nutrient [40], however recent evidence has supported diets toward the upper end of the AMDR (30-35%) for protein may be more beneficial for weight management [39, 41, 42]. To accomplish this, reducing carbohydrate intake and increasing dietary protein intake has shown to be successful for weight loss and improved metabolic health [36, 43-45].

A recent comprehensive meta-analysis compared 24 randomized controlled clinical trials to determine if high protein diets (HPD; 1.25 ± 0.17 g protein/kg/day) were more beneficial for weight loss compared with the traditional standard-protein diets (SPD; 0.72 ± 0.09 g protein/kg/day) through manipulating the protein:carbohydrate macronutrient ratio [46]. Inclusion criteria required a mean study interval of 12.1 ± 9.3 weeks within adults (≥ 18 y) and a fat intake less than or equal to 10% of total EI. Health outcomes measured included body weight and composition, fat mass, fat free mass, blood lipid and glycemic levels. Those following a HPD saw greater declines in body weight, fat mass, and triglycerides with minute reductions in fat free mass compared with SPD. However, no differences were determined in total cholesterol and glycemic levels between the two diets. The exact mechanism for these changes is unknown, however within independent studies, three health outcomes found to be consistently present when comparing high-protein, low-carbohydrate diets (HP) are EE, appetite regulation, and glycemic control [38, 46-50].
Dietary protein requires more energy to be digested, absorbed and assimilated within the body compared to carbohydrates and fats [31, 38]. This unique property of protein is reflected in an increased postprandial thermogenic effect (i.e. TEF) thus increasing total EE [47, 48]. Halton et al [30] performed a meta-analysis on 15 studies summarizing the thermogenic effect of protein within a HP. Data measurements were collected anywhere from a two hour duration to 36 hours. The HP resulted in a higher TEF than the high-carbohydrate, low-protein diets (HC) with up to a 22% increase in total EE with the HP compared to the HC diets. Another study conducted by Martens et al [44] also found increased TEF within a HP over a 12-week dietary intervention. Furthermore, they observed a preservation of total EE over 12 weeks within the HP diet while a reduction in total EE was seen in those following the HC. A similar trend was observed in a four-week nutritional intervention study [43]. Baba et al [43] reported a ~252 kcal/day reduction in REE in subjects following the HC while the HP sustained their baseline REE. Collectively, a HP may be one nutritional strategy for increasing total EE.

Appetite and satiety responses are also influenced by the consumption of protein [38, 51-56]. In a recent comparison of 24, acute trials comparing HP to HC diets on satiety regulation and appetite signaling, Leidy et al [38] reported consistent findings of a decline in postprandial hunger response and increased fullness following consumption of a HP. In the same comparison study, orxyogenic-hormones such as ghrelin, were found to be reduced while anorxygenic-hormones, peptide YY and glucagon-like peptide 1, were increased. The shifting of appetite hormones is said to be attributed to protein’s presence in the gut and is amplified depending on the quantity of protein consumed [53], however the molecular mechanisms behind these changes are unclear [38]. Furthermore, the reduction in appetite observed with increased protein intake coincides with a reduction in food intake in the subsequent meal. Brennan et al [57] reported an
average reduction of 14% less caloric intake following a HP meal compared with a HC meal over 210 min. Rains et al [58] found similar results with a 108 kcal reduction in energy intake at the following meal within the HP compared with the HC diet.

Lastly, improved glycemic control has been observed when comparing HP to HC which is an important metabolic factor when discussing the prevention of chronic diseases, such as type 2 diabetes [59]. A recent study (2 week) conducted by Park et al [60] observed HP reduced postprandial glucose values by 10% compared to HC [60]. Acheson et al [47] concluded similar results with a 32% lower postprandial glucose response between HP and a HC over 5.5 hours. In the same study, when comparing within protein sources, Acheson et al [47] found the high-quality proteins were associated with a blunted blood glucose response compared with lower-quality proteins. Thus indicating protein quality, along with quantity, may also beneficially influence metabolic health.

Protein quality is an important consideration when determining optimal protein intake. Although equal quantities of different protein sources may have the same caloric content, the digestibility and composition of amino acids may impact EE and blood glucose regulation differently [47, 61]. Protein quality has traditionally been described as a protein’s essential amino acid composition’s ability to achieved specific metabolic actions [61]. Therefore, high-quality proteins usual contain a complete essential amino acid profile, while low-quality proteins do not contain all of the essential amino acids [62]. However, a review by Millward et al [61] mentions two primary aspects of protein quality that also need to be considered: 1) other nutrients being consume with the protein and 2) the physiological needs of the individual consuming the protein. To demonstrate this, Abou-Samra et al [63] tested four isolated protein sources (whey, casein, egg, and pea) on appetite and glycemic control. Casein, a high-quality
protein, and pea, a low-quality protein, exhibited greater satiation responses and reduced EI compared with egg and whey, higher-quality proteins. Yet, whey was observed to have a lower postprandial blood glucose response compared to the casein-, pea-, and egg-proteins. In contrast, Wheeler et al [64] found no difference in glycemic response between proteins differing in quality, however these protein were consumed within a mixed meal, supporting Millard’s argument regarding a protein’s quality is dependent on the complete nutrient profile of the food or meal. This is further supported in a recent study by Li et al [65] who found the quality of the protein, when consumed in a mixed meal, did not influence postprandial glycemic control or satiety over a four-week period.

The quality of protein may also be dependent on the rate of protein digestion [61]. Fast-proteins, such as whey, are quickly digested and broken down, compared to slow-proteins, such as casein, which are digested and broken down at a slower rate [61]. This opposing relationship could explain some of the differences observed between protein sources. For instance, in the study discussed previously by Abou-Samra et al [63], although whey was a high-quality protein, pea, a low-quality protein, exhibited a greater satiation response. On the scale of digestibility, pea is digested at 2.4g/hour compared with whey at ~9g/hour [66]. Thus pea would remain in the gut for an extended period of time, and increase protein within the gut is shown to stimulate appetite-regulating hormones [61] therefore promoting satiation to a greater extent than whey.

Yet simply the timing of daily protein consumption may also effect the observed health benefits of a higher protein diet [47, 67-70]. For instance, the time of protein intake during the day can influence the rate muscle anabolism [71] within the body as increased protein is known to stimulate muscle protein synthesis [68, 72, 73]. Evidence suggests in order to maximize muscle anabolism, a threshold of 30g of protein is required [72, 74, 75]. However, a majority of
protein ingestion typically is skewed toward the evening, with little ingestion at breakfast causing the body to remain in protein breakdown for a majority of the day [74]. Thus, Mamerow et al [68] concluded muscle anabolism was more effectively stimulated when protein was consumed evenly throughout the day (e.g. 30g at breakfast, lunch, and dinner) compared to skewing intake towards dinnertime (e.g. 0g at breakfast, lunch, 90g at dinner) [68]. This is an important finding as increased muscle mass is associated with increased energy expenditure [76], improved body composition [72], increased weight loss [72]. The data above indicates protein timing, in addition to protein quality and quantity, is an important factor in defining the role of dietary protein in metabolic health.

**IMPORTANCE OF PROTEIN AT BREAKFAST**

Daily breakfast consumption is considered an important part of a healthy diet [77-79], specifically breakfasts higher in protein [48, 60, 77, 80-82]. Breakfast can be defined as any meal eaten prior to 10:00 am [79] and a breakfast skipper can be anyone missing breakfast five or more times per week [79]. Currently 25% of US adults skip breakfast [78], and is linked to poor body composition [83], poor diet quality [84], and decreased satiety [85] when compared with adults who regularly eat breakfast [77, 84-87].

Although no direct causation between skipping breakfast and obesity has been established, studies have found adolescents who regularly omit breakfast are prone to having a higher body mass index (BMI) than those who regularly eat breakfast [82, 88, 89]. Affenito et al [88] reported ~ 20% reduction in breakfast eating within female adolescents as they enter young adulthood compared with their earlier adolescent years. The increase in breakfast skipping observed throughout adolescence mirrors the observed increase in BMI [88] and continues into
young adulthood [83, 90]. Within young adults, females have a 5% higher obesity rate than males [2] with nearly 24% of young females habitually skip breakfast [78].

Breakfast skipping is also associated with poor diet quality [84, 91]. Affenito et al [88] performed an longitudinal study among 2,379 girls observing their dietary and nutritional behaviors over 10 years (9-19 years). A reduction in calcium and fiber were found as the frequency of breakfast consumption decreased. Barton et al [89] found similar results within breakfast skipping in addition to a decline in other micronutrient intakes, such as iron and folic acid, compared with habitual breakfast consumption. In contrast, habitually eating breakfast is associated with improved blood lipid profiles, reduced abdominal obesity and lower blood pressure as reviewed by Barton et al [89].

Additionally, improved appetite response is associated with habitual breakfast consumption, however reduced subsequent food intake is less conclusive [39, 53-55]. First, Leidy et al [53] observed a decline in prospective hunger response in habitual breakfast skippers following breakfast consumption. This observation is further supported throughout literature [29, 38, 78, 92, 93]. Furthermore, McCrory [78] reviews three acute (<1 day) studies comparing breakfast consumption on appetite and EI. Eating breakfast was shown to consistently reduce postprandial hunger response, desire to eat, and prospective food consumption in all three studies. However, within the studies testing EI, only one demonstrated breakfast skippers consumed a higher caloric intake at the subsequent meal compared to those who regularly consume breakfast. Similar data is seen in longer-term studies (>7 day). For example, Leidy et al [93] found the consumption of breakfast, specifically breakfasts high in protein, over one week increased postprandial feelings of fullness compared to breakfast skipping with no difference in EI.
The macronutrient composition of breakfast may also influence the benefits associated with breakfast consumption [77, 84, 85, 91]. Multiple studies have focused on the effect of HP breakfasts on appetite regulation and EI in the short-term (≤ 1 week) [53-55, 92-95]. For example, Vander Wal et al [96] compared the response to consuming an egg versus bagel breakfast over a three hour postprandial period. They concluded that the egg-based breakfast exhibited greater satiation responses and was associated with reduced food intake when compared to the bagel breakfast. Leidy et al [53] also concluded HP breakfasts (35g protein) lead to greater feelings of fullness compared to a HC breakfasts (13g protein) or breakfast skipping. Observed reductions in evening snacking among those who consumed the HP breakfast was also reported.

As previously established, higher protein intakes has been shown to increase EE through raising the TEF. Studies have also demonstrated protein’s thermogenic effect at the breakfast hour. Leidy et al [56] measured acute appetite response and EE over a four hour period in female subjects and concluded a HP breakfast (30% energy from protein) lead to increases in fat oxidation and reductions in carbohydrate oxidation compared with a normal-protein breakfast (18% protein). Baum et al [48] concluded similar findings in children. They observed the HP breakfast (21% protein) had higher postprandial EE and fat oxidation rate compared with the HC breakfast (4% protein).

Finally, inclusion of a protein at breakfast has also been associated with improved glycemic control. Park et al [60] compared a HP (35% protein) and HC (15% protein) breakfast within diabetic adults and concluded the HP breakfast attenuated postprandial glucose response compared with the HC over one week. Ratliff et al [94] found similar data in adult men. The HP breakfast (23% protein) resulted in less peaks and incursions in postprandial glucose values.
compared with a HC breakfast (16% protein). The HC breakfast also resulted in significantly higher postprandially insulin levels. Comparable data from Alwattar et al [51] concluded the HP breakfast (32g protein) stabilized blood glucose values throughout the day compared with HC breakfast (12g protein) in adolescence females.

In both acute and chronic conditions, consumption of high-protein breakfasts exhibit the potential for reduced EI, increased EE, and improved metabolic health. However, additional information is needed regarding the effect of a high-protein breakfast in healthy young females. Furthermore, data is needed regarding protein’s adaptive response to EE, appetite, and glycemic control at the breakfast hour.

CONCLUSION

In conclusion, obesity is a growing epidemic in the US with specific concerns among young females [2]. Added weight in the early adult years can increase their risk of developing chronic diseases later in life [8]. Therefore, identifying novel strategies to treat and prevent weight is essential. One successful strategy for weight loss has been nutritional intervention using higher protein diets [38], which increase EE [56], improve appetite regulation [39, 53, 54, 56], and glycemic control [47, 97]. However, most research focuses on the effects of higher protein intake on weight loss or muscle function and less is known regarding the role of protein intake at breakfast.

Therefore, the objectives of this thesis were:

(1) To determine if breakfast macronutrient composition improved postprandial EE and appetite after a one-week adaptation in young females who habitually skip breakfast.
(2) To determine if protein source (animal protein versus plant protein) at breakfast influences satiety and glucose response and decreases daily food intake.

We hypothesized:

(1) A high-protein breakfast will increased 

2) Consume a breakfast with a high quality protein will improved satiety and glycemic response compared to breakfast with a low quality protein source.
Figure 1. Overview of energy balance.

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CHAPTER 1
Breakfast macronutrient composition influences postprandial energy expenditure and fat oxidation in young females who habitually skip breakfast

ABSTRACT

Purpose: The purpose of this study was to determine if breakfast macronutrient composition improved postprandial (PP) energy expenditure (EE) and appetite after a one-week adaptation in young females who habitually skip breakfast.

Methods: A randomized, controlled study was conducted in females (24.1 ± 2 y), who skip breakfast (≥ 5 times/week). Participants were placed into one of three groups for eight days (n=8 per group): breakfast skipping (SKP), carbohydrate (CHO; 351 kcal; 59 g CHO, 10 g PRO, 8 g fat) or protein (PRO; 350 kcal; 39 g CHO, 30 g PRO, 8 g fat). On days 1 (D1) and 8 (D8), EE, substrate oxidation, appetite and blood glucose were measured over 120 minutes. Three-day food records were also collected.

Results: There was an effect of breakfast, time and breakfast over time on EE and substrate oxidation. PRO had higher (P < 0.05) PPEE net incremental area under the curve (niAUC) compared to SKP niAUC and CHO niAUC on D1 and D8, with PRO having 29% higher PPEE than CHO on D8. On D1, PRO had 30.6% higher fat oxidation than CHO and on D8, PRO had 40.6% higher fat oxidation than CHO. There was an interaction (P < 0.0001) of time and breakfast on appetite response. In addition, CHO had a significant increase (P < 0.05) in PP hunger response on D8 versus D1. CHO and PRO had similar PP glucose responses on D1 and D8. There was no effect of breakfast on daily energy intake.
**Conclusions:** Consumption of PRO breakfast for eight days increased PPEE compared to CHO and SKP, while consumption of CHO for one week increased PP hunger response with no adaptive response of breakfast consumption or composition over eight-days.
INTRODUCTION

Obesity is a world-wide epidemic that continues to grow [1]. Added weight is a risk factor for a number of health concerns such as type 2 diabetes, hypertension, and heart disease, however the risk of developing a chronic health condition is amplified when weight gain occurs in early adulthood [2-5]. Thus, new approaches to reduce or prevent weight gain in this age group are essential for preventing the onset of obesity and chronic disease later in life.

Breakfast is considered an integral part of a healthy and balanced diet due to the associations demonstrating individuals who habitually skip breakfast have a higher body mass index (BMI) and an increased risk of developing chronic disease [6]. Furthermore, breakfast skipping is associated with an increased risk of weight gain and obesity in young adults as well as elevated cholesterol levels, overeating, and poor blood glucose control [7, 8]. Yet, nearly 40% of American adults skip breakfast on any given day [9], despite the known health benefits associated with eating breakfast such as increased feelings of fullness, reduced post-meal cravings [10-15], improved body composition [16], and a decreased incidence of overweight and obesity [7, 17].

Postprandial (PP) energy expenditure (EE) is a potential target for the treatment of obesity since it can be influenced by the macronutrient composition of the diet [18-21]. Meals higher in protein have a greater impact on PPEE than carbohydrates [19, 21], by increasing PPEE by up to 20% [22]. Recent research has also found that increasing protein consumption (20-30 g protein) at breakfast compared to a standard cereal-based breakfast (containing 10-15 g protein) may increase subjective feelings of fullness and satiety throughout the day [23, 24] and decrease caloric intake at lunch [24]. In addition, consumption of protein for breakfast results in less variation of PP glucose and insulin values [25], which is an important consideration for
reducing the risk of chronic disease. Most studies examining the effect of breakfast macronutrient composition are acute interventions, examining the effect of protein on PPEE, appetite and glycemic response after one test meal [15, 16, 25]. To our knowledge, the adaptive response of habitual breakfast consumption and composition has not been explored. Therefore, the purpose of this study was to determine if breakfast macronutrient composition improved PPEE and appetite after a one-week adaptation in young females who habitually skip breakfast.

METHODS

Participants. Females, ages 18-36, were recruited to participate in this study. Participants were recruited through the university daily newsletter, social media, and flyers. Participants were required to be habitual breakfast skippers (defined as skipping breakfast ≥ 5 days/week). Females who smoked, had dietary restrictions, were taking medication (excluding hormonal birth control), or had any pre-existing metabolic conditions (e.g. type 1 or 2 diabetes) that prevented them from consuming the test breakfasts were excluded from the study. Forty females were selected to participate in the study. However, only twenty-four females completed the study: sixteen participants dropped out of the study due to scheduling conflicts or failure to appear for the first study day. Ethical approval for the study was obtained from the Institutional Review Board at the University of Arkansas (Fayetteville, AR; Appendix A). Written consent was obtained from all participants prior to starting the study.

Study design. Participants (n=24) were assigned to one of three dietary interventions using a controlled, randomized design: protein-based breakfast (PRO; n=8), carbohydrate-based breakfast, (CHO; n=8) or breakfast skipping (SKP; n=8). All participants completed two visits to the laboratory with seven days between visits. Participants were instructed to fast overnight
and refrain from strenuous physical activity the day before testing. On study day 1 (D1), participants arrived at the Food Science Department at the University of Arkansas at 07:30. Upon arrival, body weight and height were measured. Fasting blood glucose levels, resting energy expenditure (REE), and baseline appetite assessments were also measured. Participants then continued to skip breakfast or were provided with either a protein- or carbohydrate-based breakfast. Participants eating breakfast were given 15 minutes to consume the test breakfast. Glucose and appetite assessments were collected at 15, 30, 60, 90, and 120 minutes PP. PEE was measured at 30, 60, 90, and 120 minutes following breakfast. At the end of D1, participants were provided with six breakfast meals corresponding to the breakfast group to which they were assigned. Participants were instructed to consume each breakfast prior to 10:00 for the following six days. Participants were also required to complete three, 24-hour food intake logs (self-selected two weekdays and one weekend) and maintain their typical physical activity level throughout the intervention period. On day 8 (D8), participants returned to the Food Science Department in a fasted state to repeat the same study protocol as D1.

**Test breakfasts.** Participants were assigned to one of three test breakfasts, which they consumed each day of the intervention period: a carbohydrate-based (CHO) breakfast, a protein-based (PRO) breakfast, or they continued to skip (SKP) breakfast. The CHO breakfast consisted of 1 English muffin (57g), low fat yogurt (170g), cream cheese (17g), and water (227ml). The PRO breakfast consisted of a proprietary breakfast sandwich (145g), Greek yogurt (150g), and water (227ml). Both test breakfast were similar in kilocalories and controlled for fat and fiber (Table 1). The SKP group was provided water (227 ml).
Anthropometric measurements. Body height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) with participants barefoot, in the free-standing position. Body weight was measured in the fasting state with participants barefoot to the nearest 0.01 kg using calibrated balance scales (Detecto, St. Louis, MO). BMI was calculated as weight (kg) divided by height (m) squared. Body composition was assessed by dual energy x-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Belgium) in the Human Performance Laboratory at the University of Arkansas.

Energy expenditure and substrate oxidation. Resting EE (REE; kcal/min) was measured with a TrueMax 2400 metabolic cart (Parvomedics, Sandy, UT) at 0, 30, 60, 90, and 120 minutes. Indirect calorimetry, using the ventilation hood technique, was measured in 30 second increments after a 20 minute rest period while in the supine, reclined position with only the last 15 minutes used for analysis from each time point [18]. PPEE (kcal/min) for each time point was determined by assessing the difference between REE at time 0 and times 30, 60, 90, and 120 minutes. Both REE and PPEE were controlled for fat free mass (FFM). Respiratory quotient (RQ), VO$_2$ (mL/min), VCO$_2$ (mL/min) were calculated from the rate of oxygen inhalation compared with carbon dioxide exhalation. Substrate oxidation rates were determined from RQ values [16].

Appetite and palatability ratings. Appetite and palatability were assessed using a traditional 100-mm visual analog scale (VAS) [26] with opposing anchors (e.g. “extremely hungry” or “not hungry at all”) at time points 0, 15, 30, 60, 90, and 120. Questions consisted of: “how hungry do you feel at this moment,” “how full do you feel at this moment,” “how strong is your desire to eat this moment,” and “how much food do you think you can eat at this moment.” Appearance (“how much do you like or dislike appearance of the breakfast foods”) and
palatability (“how much do you like or dislike the smell and taste of the breakfast foods”) of test breakfasts were assessed during breakfast consumption on D1 and D8 using a traditional 100-mm VAS with opposing anchors “dislike extremely” or “like extremely”.

**Blood glucose measurements.** One blood sample was collected in a capillary tube (Health Management Systems, Corp; Plano, TX) via finger stick at 0, 15, 30, 60, 90, and 120 minutes PP. Blood glucose levels were determined using a Lifescan One Touch UltraSmart System (New Brunswick, NJ). Each sample was measured in duplicate from the same capillary tube and the average was used in analysis [16, 17].

**Dietary assessment.** The energy and macronutrient composition of test breakfast meals and 24-hour food intake records were analyzed using Genesis R&D nutrient analysis software (ESHA Research, Salem, OR).

**Statistical analysis.** Summary statistics were calculated for all data (sample means and sample standard error of mean). Two-sample independent t-test were used to analyze breakfast palatability and appearance. Two-factor analysis of variance (ANOVA) was used to determine diet x time interaction for appetite ratings, glucose levels, REE, PPEE, RQ, VO$_2$, VCO$_2$, and substrate oxidation. If differences were found, two-factor, repeated measure ANOVA was used to determine differences. Where significance was found, the Bonferroni correction was applied and two-sample independent t-test was used to determine the degree of significance. Net incremental area under the curve (niAUC) was calculated for appetite ratings, REE, RQ, substrate oxidation, and glucose levels. One-factor analysis of variance (ANOVA) was used to compare demographics, dietary intake, and niAUC of diet groups for appetite ratings, glucose levels, REE, PPEE, and substrate oxidation. When significance or a trend was found, a two-sample independent t-test was used to determine the degree of significance or trend. Paired t-test
was used to determine within diet differences for appetite ratings, glucose levels, REE, PPEE, and substrate oxidation. All results reported as means ± SEM. All data was analyzed using GraphPad Prism Software v6.0 (La Jolla, CA). $P < 0.05$ was considered statistically significant.

RESULTS

**Participant characteristics.** Participant demographics are presented in Table 2. There was no significant difference in height, weight, BMI, body fat percentage, or FFM between diet groups. Age was significantly higher in SKP ($P < 0.05$) compared to PRO and CHO.

**Energy expenditure and substrate oxidation.** EE and substrate oxidation are presented in the line graphs (individual time points) and bar graphs (niAUC) in Figure 1. Overall, there was a significant ($P < 0.0001$) effect of time, breakfast, and breakfast over time on REE, PPEE, carbohydrate oxidation, and fat oxidation. There was no difference between D1 or D8 for REE, PPEE, carbohydrate oxidation, and fat oxidation. However, participants consuming PRO had significantly higher ($P < 0.05$) niAUC for both REE and PPEE compared to CHO and SKP. There was a significant effect ($P < 0.05$) of consuming breakfast on fat and carbohydrate oxidation, with no effect of breakfast type. In addition, PRO niAUC had 30.6% higher fat oxidation than CHO niAUC on D1 and a 40.6% higher fat oxidation than CHO niAUC on D8.

The results for RQ, VO$_2$, and VCO$_2$ are presented in Supplemental Table 1.

**Appetite & palatability ratings.** Results for perceived hunger, perceived fullness, prospective food consumption (PFC), and perceived desire to eat are presented in the line graphs (individual time points) and bar graphs (niAUC) in Figure 2. For each appetite response, there was an effect of time and breakfast over time ($P < 0.0001$ for each). There was a significant effect of breakfast consumption, not breakfast type, on perceived fullness ($P < 0.0001$).
was no difference in appetite response between D1 and D8 within diets. However, participants following the CHO breakfast reported increased hunger following consumption of the CHO breakfast on D8 versus D1 ($P < 0.01$). There was no difference in appearance or palatability between the CHO and PRO breakfast (Table 1).

**Blood glucose.** The results for blood glucose are presented in Figure 3. There was a main effect of time, breakfast and breakfast over time ($P < 0.0001$) on blood glucose levels. CHO and PRO lead to greater increase in glucose values compared to SKP at 30 and 60 minutes PP ($P < 0.01$). However, PRO had a 10% lower glucose levels compared with CHO at 30 minutes PP. There was no difference in niAUC values between PRO or CHO breakfasts or between D1 and D8 within diets.

**Ad Libitum dietary assessment.** Average daily energy intake is provided in Table 3. There was no significant effect of breakfast consumption or breakfast skipping on total energy (kcal) intake. However, participants consuming CHO had 25% lower energy intake compared to SKP and 33% lower energy intake compared to PRO.

**DISCUSSION**

To our knowledge this is the first study to examine the effect of breakfast macronutrient composition over an eight-day adaptation period on PP energy metabolism, appetite response, glucose response, and 24-hour food intake in breakfast skipping females. Breakfast consumption increased REE and PPEE compared to SKP and consumption of PRO increased REE and PPEE compared to consumption of CHO. Breakfast consumption also increased PP substrate oxidation, with a trend for PRO breakfast to increase fat oxidation compared to CHO. The macronutrient content of the breakfasts did not impact overall glucose response, however PRO had a lower
glucose peak at 30 minutes PP and a slower return to baseline values compared to CHO. There was no effect of the eight-day adaptation period on energy metabolism, substrate oxidation, glucose or appetite response, with the exception of hunger. CHO intake over the eight-day adaptation period significantly increased PP hunger. Collectively, this study demonstrates that habitual consumption of a breakfast higher in protein could increase PPEE and fat oxidation compared to a carbohydrate-based breakfast, and that breakfast consumption, in general, has more benefits than breakfast skipping in the short-term.

Breakfast is often recognized as the most important meal of the day [6, 18, 27]. However there is debate as to what defines the ideal breakfast meal [27], in addition to a lack of strong evidence to define which nutrients should be represented at breakfast [27]. A recent commentary published by the American Academy of Nutrition and Dietetics suggests that protein-containing foods (e.g. eggs, lean meat and low-fat dairy products) should be included in breakfast meals [27]. Literature supports diets higher in protein aid in the treatment of chronic, metabolic diseases such as obesity, type 2 diabetes and heart disease and have been shown to increase EE, improve satiety, regulate glycemic control and improve body composition (reviewed in [28-31]). However, the role of breakfasts higher in protein on metabolic health still needs to be defined.

The relationship between protein intake and increased PPEE is well-established [18, 21, 25, 32, 33]. However, very few studies have examined the impact of habitual breakfast consumption on PPEE. Furthermore, most protein intake studies conducted have use isolated protein sources, often consumed in liquid form, as the intervention rather than protein as part of a complete meal [25, 33-35]. For example, Acheson et al [25] administered whey-, casein-, soy-protein, and carbohydrate-based beverages for a breakfast meal, and demonstrated that the protein beverages, independent of protein source, increased PPEE to a greater extent than the
carbohydrate beverage in young men over a five-hour period [25]. In another study, both male and female young adults consumed either a high protein, low carbohydrate shake (30% energy from protein) or a low protein, high carbohydrate shake (5% energy from protein) over the course of 12 weeks [35]. At the end of the intervention period, the participants consuming the high carbohydrate shake had a significant reduction in PPEE compared to those consuming the high protein shake and compared to baseline values, which is in agreement with the findings from this study.

A majority of the breakfast literature is composed of acute meal studies, which make it difficult to make conclusions about the longer-term effects of breakfast interventions [9, 14-16, 18]. Interestingly, just consuming breakfast in the morning has been shown to only transiently suppress appetite (i.e. 4-5 hours) compared to skipping breakfast, without any different over the remaining-hour period [6]. This further supports the importance of protein consumption within the breakfast meal. Several acute studies have examined the effect of breakfast macronutrient composition on appetite regulation and energy intake. Leidy and Racki [15] demonstrated consuming breakfast increases feelings of fullness in breakfast skipping adolescents and breakfasts higher in protein decreases appetite to a greater extent than normal protein breakfasts. In another longer-term study (12-weeks), examining the impact of a high-protein breakfast versus a high-carbohydrate breakfast on appetite response, found an increase in 24-hour PP fullness and satiety following consumption of the high-protein breakfast for one-week compared to the high-carbohydrate breakfast, however this difference was not detected at the end of the 12-week intervention [35]. Although 24-hour appetite measurements were not taken in the current study, there was a suppression of appetite for two hours following breakfast consumption on both D1 and D8 of the intervention, with no impact of breakfast macronutrient composition.
These results are further supported by Leidy et al [36], who found a significant effect of breakfast consumption on appetite suppression in breakfast-skipping, late-adolescent females, but no effect of breakfast macronutrient composition after seven days of breakfast consumption.

There is an association between habitual breakfast skipping, higher BMI, and an increased risk of chronic disease [6]. Therefore, it is often argued that breakfast consumption could be an effective weight loss strategy since eating breakfast is often associated with reduced caloric intake and increased nutrient intake throughout the day when compared to habitual breakfast skippers [4, 8]. In the present study, although breakfast consumption increased feelings of fullness and decreased feelings of hunger, there was no effect of breakfast consumption or breakfast composition on 24-hour energy intake. This is supported by Leidy and Racki [15] who found that breakfast consumption and breakfast composition influenced energy intake at lunch, however total 24-hour energy intake was not different between groups.

Consumption of a high-protein diet has been linked to improved glycemic response, in both the short- [10, 18, 25] and long-term [30, 37, 38]. In this study, there was no effect of breakfast composition or breakfast adaptation on PP glycemic response. However, these results are consistent with findings from Alwattar et al [13], who found no difference in PP glycemic response between a high protein and high carbohydrate breakfast over time.

In conclusion, breakfast consumption decreased PP hunger and increased satiety compared to breakfast skipping, with no effect of breakfast composition, although 24-hour energy intake did not differ between groups. There was an increase in PPEE and fat oxidation with PRO, compared to CHO. In addition, consumption of CHO for eight days resulted in an increased hunger response. There was no impact of the eight-day adaptation period on any other outcomes. Taken together, these data suggest that increasing protein at breakfast has beneficial
effects on PPEE and satiety in habitual breakfast skipping females in the short-term, but a longer adaptation period may be needed.
REFERENCES


Figure 1. Energy expenditure and substrate oxidation following a PRO- or CHO-breakfast or continued breakfast skipping. Data are expressed as means ± SEMs; SKP n = 8, PRO n = 8, CHO n = 8. A. Resting energy expenditure (REE) over time per breakfast group and net incremental area under the curve (niAUC) for REE for each breakfast group. B. Postprandial energy expenditure (PPEE) over time per breakfast group and niAUC for PPEE for each breakfast group. C. Carbohydrate oxidation over time per breakfast group and niAUC for carbohydrate oxidation for each breakfast group. D. Fat oxidation over time per breakfast group and niAUC for fat oxidation for each breakfast group.
Figure 2. Ratings of appetite two hours postprandial (PP) following a PRO- or CHO-breakfast or continued breakfast skipping using visual analog scales. Data are expressed as means ± SEMs; SKP n = 8, PRO n = 8, CHO n = 8. A. Perceived hunger over time and net incremental area under the curve (niAUC) for perceived hunger for each breakfast group. B. Perceived fullness over time and niAUC for perceived fullness for each breakfast group. C. Prospective food consumption (PFC) over time and niAUC for PFC for each breakfast group. D. Perceived desire to eat over time and niAUC for perceived desire to eat for each breakfast group.
Figure 3. Changes in glucose response over time following a PRO- or CHO-breakfast or continued breakfast skipping. Data are expressed as means ± SEMs; SKP n = 8, PRO n = 8, CHO n = 8. Glucose response to the test breakfasts over time. *Difference between pooled (D1 + D8) SKP and pooled PRO, P ≤ 0.05; **difference between pooled SKP and pooled CHO, P ≤ 0.05. SKP, breakfast skipping; CHO, carbohydrate-based breakfast; PRO, protein-based breakfast.
Table 1. Dietary Characteristics of Test Breakfast.

<table>
<thead>
<tr>
<th></th>
<th>CHO</th>
<th>PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy content, kcal</td>
<td>351</td>
<td>350</td>
</tr>
<tr>
<td>Total protein, g</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Total carbohydrate, g</td>
<td>59</td>
<td>39</td>
</tr>
<tr>
<td>Total sugars, g</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

| Macronutrient composition, %   |       |       |
| Carbohydrate                   | 67    | 45    |
| Protein                        | 12    | 45    |
| Fat                            | 21    | 21    |

| Breakfast Appearance, mm²      | 69 ± 4| 64 ± 5|
| Breakfast Palatability, mm²    | 75 ± 3| 68 ± 5|

¹Values are means ± SEMs, n=16. CHO, carbohydrate-based breakfast; PRO, protein-based breakfast.
²Units are in millimeters (mm) according to a traditional 100-mm visual analog scale. Mean values are combined PRE & POST data.
Table 2. Participant Characteristics<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>SKP</th>
<th>CHO</th>
<th>PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants, n</strong></td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.1 ± 1.8*</td>
<td>21.9 ± 0.9</td>
<td>23.3 ± 1.3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.4 ± 2.1</td>
<td>162.1 ± 4.1</td>
<td>164.9 ± 2.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.9 ± 6.3</td>
<td>67.0 ± 7.0</td>
<td>72.6 ± 6.3</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8 ± 2.2</td>
<td>26.0 ± 1.9</td>
<td>26.6 ± 2.1</td>
</tr>
<tr>
<td>Fat Mass, %</td>
<td>45.3 ± 1.6</td>
<td>37.4 ± 3.1</td>
<td>40.5 ± 3.4</td>
</tr>
<tr>
<td>Fat Free Mass, kg</td>
<td>45.8 ± 3.4</td>
<td>43.6 ± 2.3</td>
<td>44.5 ± 1.5</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>3</td>
<td>6</td>
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<tr>
<td>Black</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEMs or n. *Different from CHO; P < 0.05. SKP, breakfast skipping; CHO, carbohydrate-based breakfast; PRO, protein-based breakfast.
Table 3. Average daily energy and macronutrient intake during adaptation period

<table>
<thead>
<tr>
<th></th>
<th>SKP Breakfast</th>
<th>CHO Breakfast</th>
<th>PRO Breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, kcal</td>
<td>2009 ± 193</td>
<td>1603 ± 127</td>
<td>2137 ± 349</td>
</tr>
<tr>
<td>Protein, g</td>
<td>88 ± 13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>57 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>234 ± 22</td>
<td>231 ± 19</td>
<td>246 ± 40</td>
</tr>
<tr>
<td>Fat, g</td>
<td>75 ± 9</td>
<td>54 ± 5</td>
<td>81 ± 17</td>
</tr>
<tr>
<td><strong>Macronutrient Intake, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>18</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>47</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td>Fat</td>
<td>35</td>
<td>29</td>
<td>35</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEM. Data obtained from 3-day food records. Labeled means in a row without a common letter differ, P < 0.05. SKP, breakfast skipping; CHO, carbohydrate-based breakfast; PRO, protein-based breakfast.

<sup>2</sup>Data expressed as percent energy of energy intake.
Supplemental Table 1. Postprandial metabolic variables following consumption of either CHO- or PRO- based test breakfast

<table>
<thead>
<tr>
<th>Time Following Breakfast, min</th>
<th>SKP</th>
<th>CHO</th>
<th>PRO</th>
<th>Effect of Time</th>
<th>Effect of Breakfast Type</th>
<th>Time x Breakfast Type Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POST</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RQ</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0</td>
<td>0.836 ± 0.019</td>
<td>0.850 ± 0.018</td>
<td>0.851 ± 0.013A</td>
<td>0.867 ± 0.023A</td>
<td>0.879 ± 0.019A</td>
<td>0.849 ± 0.018A</td>
</tr>
<tr>
<td>30</td>
<td>0.843 ± 0.017A</td>
<td>0.876 ± 0.016A</td>
<td>0.976 ± 0.012AB</td>
<td>0.912 ± 0.017abcB</td>
<td>0.916 ± 0.013bcB</td>
<td>0.902 ± 0.019acbB</td>
</tr>
<tr>
<td>60</td>
<td>0.843 ± 0.017A</td>
<td>0.851 ± 0.015ab</td>
<td>0.909 ± 0.015abcC</td>
<td>0.901 ± 0.018abAB</td>
<td>0.916 ± 0.019abB</td>
<td>0.906 ± 0.017abB</td>
</tr>
<tr>
<td>90</td>
<td>0.846 ± 0.014</td>
<td>0.852 ± 0.016</td>
<td>0.902 ± 0.013c</td>
<td>0.903 ± 0.019AB</td>
<td>0.899 ± 0.017AB</td>
<td>0.894 ± 0.017B</td>
</tr>
<tr>
<td>120</td>
<td>0.834 ± 0.015A</td>
<td>0.847 ± 0.015A</td>
<td>0.912 ± 0.0147bc</td>
<td>0.910 ± 0.016B</td>
<td>0.909 ± 0.013bAB</td>
<td>0.905 ± 0.016B</td>
</tr>
<tr>
<td>VO2, mL/min²</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>ns</td>
</tr>
<tr>
<td>0</td>
<td>4.861 ± 0.185AB</td>
<td>5.074 ± 0.144ab</td>
<td>4.685 ± 0.092Aa</td>
<td>4.819 ± 0.083bAa</td>
<td>5.914 ± 0.249B</td>
<td>5.610 ± 0.971ab</td>
</tr>
<tr>
<td>30</td>
<td>4.848 ± 0.163</td>
<td>5.128 ± 0.158</td>
<td>5.504 ± 0.113B</td>
<td>5.405 ± 0.115AB</td>
<td>5.914 ± 0.249B</td>
<td>5.610 ± 0.971</td>
</tr>
<tr>
<td>60</td>
<td>4.887 ± 0.155</td>
<td>5.163 ± 0.123</td>
<td>5.163 ± 0.129AB</td>
<td>5.540 ± 0.091B</td>
<td>5.931 ± 0.274</td>
<td>6.033 ± 0.371</td>
</tr>
<tr>
<td>90</td>
<td>4.967 ± 0.182</td>
<td>5.104 ± 0.132</td>
<td>5.104 ± 0.120AB</td>
<td>5.373 ± 0.112AB</td>
<td>5.759 ± 0.179</td>
<td>5.937 ± 0.306</td>
</tr>
<tr>
<td>120</td>
<td>4.975 ± 0.171</td>
<td>5.081 ± 0.177</td>
<td>5.081 ± 0.110AB</td>
<td>5.358 ± 0.105AB</td>
<td>5.660 ± 0.199</td>
<td>5.802 ± 0.251</td>
</tr>
<tr>
<td>VCO₂, mL/min²</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0</td>
<td>4.054 ± 0.147</td>
<td>4.308 ± 0.123</td>
<td>3.985 ± 0.085A</td>
<td>4.187 ± 0.157A</td>
<td>4.377 ± 0.207A</td>
<td>4.351 ± 0.303A</td>
</tr>
<tr>
<td>30</td>
<td>4.098 ± 0.181A</td>
<td>4.493 ± 0.153c</td>
<td>5.377 ± 0.156Ab</td>
<td>5.059 ± 0.120ncB</td>
<td>5.432 ± 0.262Ab</td>
<td>5.560 ± 0.331Ab</td>
</tr>
<tr>
<td>60</td>
<td>4.116 ± 0.151B</td>
<td>4.393 ± 0.131b</td>
<td>4.818 ± 0.141abc</td>
<td>5.005 ± 0.174B</td>
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<tr>
<td>90</td>
<td>4.198 ± 0.136A</td>
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<td>4.738 ± 0.135abc</td>
<td>4.867 ± 0.181bAb</td>
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<td>120</td>
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<td>4.776 ± 0.173abc</td>
<td>4.882 ± 0.141bB</td>
<td>5.150 ± 0.213bB</td>
<td>5.265 ± 0.249bcC</td>
</tr>
</tbody>
</table>

1Values are means ± SEMs. Labeled means within a treatment column without a common upper case letter differ, P < 0.05. If a value within a column does not contain an upper case letter, there is no difference within the column. Labeled means within a row without a common lower case letter differ, P < 0.05. If a row does not contain a lower case letter, there is no difference within that row. Respiratory Quotient, RQ.
2Controlled for Fat Free Mass
March 24, 2014

MEMORANDUM

TO: Jamie Baum
    Sun-Ok Lee
    Michelle Gray
    Stephanie Shouse
    Anne Okeyo
    Amy Dunn
    Christina Crowder

FROM: Ro Woodyraker
      IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 14-02-486

Protocol Title: The Importance of Breakfast Composition on Energy Metabolism and Metabolic Health in Breakfast-Skipping Young Women

Review Type: ☒ FULL IRB

Approved Project Period: Start Date: 03/24/2014 Expiration Date: 03/16/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 60 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.
CHAPTER 2

Breakfast Protein Source Does Not Influence Postprandial Appetite Response and Food Intake in Normal Weight and Overweight Young Females

ABSTRACT
Breakfasts higher in protein lead to a greater reduction in hunger compared to breakfasts higher in carbohydrate. However, few studies have examined the impact of higher protein breakfasts with differing protein sources. Our objective was to determine if protein source (animal protein (AP) versus plant protein (PP)) influences postprandial metabolic response in participants consuming a high protein breakfast (~30% energy from protein). Normal weight (NW; \( n = 12 \)) and overweight females (OW; \( n = 8 \)) aging 18–36 were recruited to participate. Participants completed two visits in a randomized, cross-over design with one week between visits. Subjects had 15 minutes to consume each breakfast. Blood glucose and appetite were assessed at baseline, 15, 30, 45, 60, and 120 minutes postprandial. Participants kept a 24-hour dietary record for the duration of each test day. No difference was found between NW and OW participants or breakfasts for postprandial appetite responses. AP had a significantly lower glucose response at 30 minutes compared with PP (−11.6%; 127 ± 4 versus 112 ± 4 mg/dL; \( P < 0.05 \)) and a slower return to baseline. There was no difference in daily energy intake between breakfasts. These data suggest that protein source may influence postprandial glucose response without significantly impacting appetite response in breakfast consumers.
INTRODUCTION

Early adulthood is a vulnerable life stage for weight gain, especially among females. The average weight gain for females between the ages of twenty and thirty is 12–25 lbs [1]. Weight gain during early adulthood increases the risk of developing a number of chronic health conditions such as type 2 diabetes mellitus, osteoarthritis, and some cancers [2, 3]. For example, after the age of eighteen years, females are 1.9 times more likely to develop type 2 diabetes if body weight increased 10–16 pounds and were 2.7 times more likely to develop type 2 diabetes if body weight increased 16–22 pounds [1].

Breakfast is often cited as the most important meal of the day for children, but this is also true for adults. There are many benefits associated with eating a healthy breakfast including improved micronutrient intake, decreased incidence of overweight and obesity, and lower cholesterol levels [4–7]. Several studies, in both adults and children, have shown that individuals who eat breakfast tend to weigh less than those who omit breakfast as eating a healthy breakfast can reduce hunger throughout the day [8, 9]. Consuming more protein (20–30 g) at breakfast than found in the standard cereal-based breakfast (10–15 g) may increase subjective feeling of fullness and satiety throughout the day [10, 11] and decrease calorie intake at lunch [11]. In addition, overweight females consuming sources of protein for breakfast five times a week for eight weeks lost 65% more weight and reduced their waist circumference by 83% more than those participants eating a carbohydrate-based breakfast [10].

The use of high protein diets to reduce the amount of food consumed at the next meal is a strategy used to help maintain negative energy balance during weight loss or to maintain weight equilibrium [12]. Protein-based breakfasts positively affect postprandial blood glucose homeostasis, of which tighter control is strongly associated with a lower risk of type 2 diabetes,
hypertension, and cardiovascular disease. Healthy participants as well as metabolically compromised individuals with type 2 diabetes both respond positively to high protein breakfasts, resulting in favorably altered biomarkers including reduced HbA1C%, postprandial glucose, postprandial insulin, and lower systolic blood pressure [13, 14].

Although several studies demonstrate positive effects of protein consumption at breakfast, very few have focused on the source or quality of the protein. Protein quality is important because although equal quantities of plant or animal protein may have the same caloric content, the digestibility and content of amino acids impact blood glucose regulation differently [15]. Therefore, the objective of this study is to determine if protein source (animal protein versus plant protein) at breakfast influences satiety and glucose response and decreases daily food intake.

MATERIALS AND METHODS

Participants. Female participants (n = 20; ages 18–36) were recruited using the university daily newsletter, social media, and word of mouth. Participants who were underweight (BMI ≤ 18.4), were smokers, were taking medication (with the exception of hormonal birth control), had food allergies and/or dietary restrictions (e.g., weight loss, vegetarian), disliked the foods served during the study, and/or had any known existing medical conditions that prevented them from eating the breakfasts were excluded from the study. Participants were recruited on a rolling basis and grouped based on their BMI score into normal weight (NW; BMI < 25; n = 12) or overweight (OW; BMI ≥ 25; n = 8) groups (Figure 1). A total of forty-seven females were screened and twenty-five participants started the study. Twenty-two of the females screened did not meet the study criteria. Twenty participants completed the study and were used in data analysis. Refer to Table 1 for participant characteristics. Females aged 18–36 were the focus of this study since this population is at a higher risk for weight gain [1] and there have been several
papers published using the population that are focused on breakfast [16, 17]. Ethical approval for the study was obtained from the Office of Research Compliance Institutional Review Board of the University of Arkansas (Fayetteville, AR; Appendix B). Written consent was obtained from all participants prior to beginning the study.

**Study Design.** The study was conducted using a randomized, crossover design in which each subject received two different breakfasts, animal protein-based (AP) and plant protein-based (PP), with at least a one-week washout period between each test day and no more than 14 days between testing days. Participants were instructed to fast overnight and limit their physical activity prior to each study day. Upon arrival, baseline measurements of blood glucose and appetite were collected. Food items for each breakfast were portioned, weighed, and labeled appropriately for each subject. Participants were then given 15 minutes to consume the test breakfast. Participants were asked to rate the appearance and taste of the breakfast using a visual analog scale (VAS) [18]. Blood glucose and appetite were analyzed at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial. In addition, participants were instructed to keep a 24-hour dietary food record for the remainder of each test day.

**Test Breakfast and Dietary Assessment.** The nutritional composition for the test breakfasts is described in Table 2. The AP had 29% protein, 29% fat, and 42% carbohydrates. The PP breakfast consisted of 27% protein, 26% fat, and 47% carbohydrates. The AP breakfast consisted of one commercially available breakfast sandwich (Jimmy Dean Delights Turkey Sausage, Egg White, Cheese and English Muffin Breakfast Sandwich), 85 g plain, nonfat Greek yogurt, 6 almonds, and 85 g fresh blueberries. The PP breakfast contained 2 vegan sausage patties (76 g; MorningStar Farms, Kellogg’s), 32.3 g of vegan country white bread (Rudi’s), 1 slice of vegan American cheese (19 g; Go Veggie, Galaxy Nutritional Products), 85 g of
blueberry soy yogurt (WholeSoy & Co.), and 28 g of fresh blueberries. Since we used commercially prepared products, we do not know the exact contribution of each protein source from each product. Participants were asked to record their food intake for the remainder of the test day using 24-hour dietary intake records. The participants were provided with detailed instructions and examples for completing the dietary intake records. The test breakfast composition and 24-hour dietary intake records were analyzed using the Genesis R&D diet analysis software package (Salem, OR).

**Anthropometric Measurements.** Body height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) with participants barefoot, in the freestanding position. Body weight was measured in the fasting state with participants barefoot to the nearest 0.01 kg using calibrated balance scale (Detecto, St. Louis, MO). BMI was calculated as weight (kg) divided by height (m) squared.

**Blood Glucose Measurements.** Blood glucose samples were measured using the fingerstick method at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial using a Lifescan One Touch UltraSmart System (New Brunswick, NJ). One blood sample per time point was collected in a capillary tube (Health Management Systems, Corp; Plano, TX). Samples were measured in duplicate from the sample collected in the capillary tube and the average was used in analysis [19, 20].

**Appetite and Palatability Ratings.** Participants were asked to rate their perceived hunger, fullness, desire for food, prospective food consumption, desire for something sweet, and desire for something savory using a 100 mm visual analog scale (VAS) [18]. The VAS is a validated questionnaire incorporating a 100 mm horizontal line scale with questions worded as
“how strong is your feeling of” and end anchors of “not at all” to “extremely.” Taste and appearance of test breakfasts were collected using the same method.

**Statistical Analysis.** Summary statistics were calculated for all data (sample means and sample standard deviations). Net incremental area under the curve (niAUC) was calculated for appetite ratings and glucose values and was used in analyses [21]. Two-sample independent -tests were used to determine initial differences between NW and OW participants and to analyze participant characteristics, breakfast appearance and palatability, and comparisons of niAUC between test breakfasts (AP versus PP). Twenty-four-hour energy and macronutrient intake were analyzed using one-factor analysis of variance (ANOVA). Two-factor, crossover, repeated measures analysis of variance (ANOVA) was used to examine significant differences between breakfast and weight groups over time for blood glucose and appetite ratings. The Bonferroni correction for multiple comparisons was applied when significance was observed within the analyses. Results are reported as means ± SEMs. All analyses were conducted using Prism GraphPad Software Version 6.0 (La Jolla, CA). \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Participant Characteristics and Compliance.** The physical characteristics of the participants are presented in Table 1. There was no difference in age or height between the NW and OW groups. Body weight and BMI were higher in the OW group \( (P < 0.05) \).

**Appetite and Palatability Response.** The results for perceived hunger, fullness, desire to eat, prospective food consumption, and food cravings are presented in Figure 2. There was no difference in appetite ratings or food cravings between NW and OW groups or between AP and PP breakfasts. However, there was an effect of time on both appetite and food cravings for both
group and breakfast \((P < 0.0001)\). The perceived taste and appearance responses to each breakfast were measured immediately following breakfast consumption. There was no difference in taste between AP or PP breakfasts (Table 2). Participants preferred the appearance of the AP versus the PP breakfast \((P < 0.05)\).

**Blood Glucose Response.** The results for postprandial glucose response are presented in the line graphs (individual time points) and bar graphs (niAUC) in Figure 3. Overall, there was an effect of time on postprandial blood glucose response \((P < 0.0001)\), with no effect of diet or weight group over time. Postprandial blood glucose was higher at 30 min following with PP breakfast compared to the AP breakfast, 126.8 ± 4.4 mg/dL versus 112.1 ± 3.9 mg/dL, respectively \((P < 0.05)\). Participants had a lower percent change in blood glucose response from the postprandial peak at 30 min to 120 min postprandial following the AP breakfast versus the PP breakfast \((-26.9 ± 4.3% \text{ and } -46.5 ± 4.9\%, \text{ resp.; } P < 0.01)\).

**24-Food Intake Assessment.** Nutrient composition of the 24-hour food intake records is shown in Table 3. Overall, there was no difference in 24-hour nutritional intake between weight groups or breakfast type. However, there was a trend for participants to have a higher caloric intake following the AP breakfast compared to the PP breakfast \((P = 0.09)\). In general, the OW group ate an additional 133 kcal more than NW group. The OW group consumed on average 44% of kcals from carbohydrate, 38% of kcals from fat, and 17% of kcals from protein after each test breakfast, while the NW group consumed on average 53% of kcals from carbohydrate, 36% of kcals from fat, and 21% of kcals from protein.
DISCUSSION

This is one of the first studies to examine the effect of complete meals comparing plant protein and animal protein sources, on postprandial appetite and glucose response in NW and OW females. The present study suggests protein source within the context of a higher protein meal exhibits no difference in appetite response or total nutritional intake; however, protein source could play a role in regulating postprandial blood glucose levels by decreasing the postprandial peak in blood glucose levels.

No difference in postprandial appetite response between AP or PP was detected; however, these results are consistent with several studies in current literature that have tested isolated proteins that were not part of a complete meal. Several studies have compared the effect of protein source on appetite within a mixed meal [22–24], demonstrating equal appetite responses to plant and animal proteins within higher protein meals (>22% protein). When whey protein was compared to casein and soy at 10% energy of a test breakfast, whey exhibited a greater satiating response; however, this difference diminished when the protein level was increased to 25% energy of a test breakfast, which is similar to the higher protein breakfast composition used in this study [22]. Another study examined beef versus soy within a mixed meal and found no difference in hunger or fullness responses over seven hours [24]. The similar effect of protein sources on appetite response within a high protein diet may be attributed to an overall increased consumption of amino acids [25, 26].

Furthermore, fiber is known to influence appetite response [27]. Although PP breakfast had a slightly higher fiber content (1 g) compared to AP, there is evidence that fiber quantity may have little impact on satiety within a high protein diet. One study demonstrated that when mixed meals, matching in protein content with differing fiber amounts, were ingested, there was no
difference found hunger or fullness area under the curve analysis [28] suggesting that protein quantity may influence satiety to a greater extent than fiber content. However, additional research needs to be explored comparing high protein/fiber diets and their effect on appetite. An increase in protein intake throughout the day, starting with breakfast, may help an individual to feel more satisfied and respond to neural signals of satiety and blood glucose regulation [29]. Though not significant, OW participants consumed fewer calories following the AP breakfast. In general, OW participants consumed less protein and consumed more calories compared to NW participants over the 24-hour test period. The underlying mechanism is still unknown, but high protein diets appear to spontaneously reduce food intake in individuals which could be attributed to satiating effect of protein [30].

Despite there being no significant differences in glucose response between breakfasts or weight groups over the 120 min postprandial period (niAUC), there was a trend for a more stable postprandial glucose response following AP breakfast for both NW and OW groups. The control of postprandial glucose levels is important for HbA1C% levels and diabetes risk [31, 32]. Both eucaloric and hypocaloric diets with increased protein lead to more stable postprandial glucose levels with lesser peak excursions and incremental area under the curve [33–36]. The higher postprandial glucose levels for both NW and OW following the PP breakfast could be attributed to the disparity in breakfast carbohydrate content or differing amino acid profiles of the test breakfasts. It has been observed that healthy individuals and those with higher postprandial glucose levels may do better with a high animal protein-based breakfast compared to a lower protein, carbohydrate-based breakfast [17]. Another possibility is that the lower blood glucose observed, following the AP breakfast, could be due to an increase in insulin production; however, insulin response was not measured in this study and needs to be further explored.
Limitations. The first limitation of this study is the short postprandial data collection period following breakfast consumption. Two hours postprandial may not be enough time to fully capture the postprandial appetite and glucose response, as meals are generally four to five hours apart and initiated by habit or hunger [37]. Many studies take postprandial measurements for four hours or longer following the test meal to ensure that appetite responses and metabolic measurements (e.g., glucose) return to baseline [16, 24]. Therefore, we may not have captured the entire postprandial breakfast response. Since there were no differences in postprandial appetite responses niAUC, we do not think measuring over a longer period would change our results. Additionally, the discrepancy in caloric and carbohydrate values and fiber content of the test breakfasts may have contributed to the differences observed in postprandial glucose response. The AP breakfast had lower postprandial glucose response at 30 min, which could be due to the lower carbohydrate and fiber content of this breakfast. However, since our conclusions are consistent with current literature, they do not warrant dismissal [22, 26, 38]. Finally, blood glucose was measured via fingerstick, not via intravenous blood draw, which limited the number of postprandial analyses conducted.

CONCLUSION

There was no difference in postprandial appetite response or 24-hour food intake after consumption of breakfasts higher in protein with differing protein sources, AP versus PP, in either NW or OW females. However, consumption of PP generated a higher postprandial glucose peak compared to AP. Taken together, these data suggest that protein source, as part of breakfast higher in protein, does not differentially affect appetite response but may differentially affect postprandial metabolism.
REFERENCES


Figure 1. Flow diagram of the participant screening and selection process.
Figure 2. Appetite responses following test breakfasts. Values expressed as means ± SEM. Data are depicted as appetite rating over time per weight group and breakfast type and net incremental area under the curve (niAUC). (a) Perceived hunger. (b) Perceived fullness. (c) Perceived desire to eat. (d) Prospective food consumption. (e) Desire for something sweet. (f) Desire for something savory. AP: animal protein; NW: normal weight; OW: overweight; PP: plant protein.
Figure 3. Glucose response to the test breakfasts. (a) Glucose response to the test breakfasts over time. (b) Glucose net incremental area under the curve (niAUC). Values expressed as means ± SEM. * indicates that blood glucose values for AP were significantly different than PP ($P < 0.05$). AP: animal protein; NW: normal weight; OW: overweight; PP: plant protein.
Table 1. Participant characteristics$^1$.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NW</th>
<th>OW</th>
</tr>
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<tbody>
<tr>
<td>Participants ($n$)</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Age (y)</td>
<td>$25 \pm 1^a$</td>
<td>$25 \pm 1^a$</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>$61.3 \pm 2.1^a$</td>
<td>$87.8 \pm 7.8^b$</td>
</tr>
<tr>
<td>Height (m)</td>
<td>$1.66 \pm 1.2^a$</td>
<td>$1.65 \pm 1.8^a$</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>$22.2 \pm 0.6^a$</td>
<td>$31.9 \pm 2.7^b$</td>
</tr>
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<td>Ethnicity</td>
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</tr>
<tr>
<td>Caucasian</td>
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<td>6</td>
</tr>
<tr>
<td>Indian</td>
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<td>1</td>
</tr>
<tr>
<td>Latina</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

$^1$Age, weight, height, and BMI are expressed as means ± SEM. NW: normal weight participants; OW: overweight participants. Means in a row without a common letter are significantly different ($P < 0.05$).
Table 2. Dietary characteristics of test breakfasts.

<table>
<thead>
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<th>Animal protein (AP) breakfast</th>
<th>Plant protein (PP) breakfast</th>
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</thead>
<tbody>
<tr>
<td>Total kcal</td>
<td>368</td>
<td>387</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>38</td>
<td>46</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Breakfast appearance, mm^1</td>
<td>74.8 ± 3.6^a</td>
<td>63.6 ± 3.5^b</td>
</tr>
<tr>
<td>Breakfast palatability, mm^1</td>
<td>73.1 ± 3.5^a</td>
<td>65.9 ± 3.8^a</td>
</tr>
</tbody>
</table>

^1Values are expressed as means ± SEM, n = 20. CHO: carbohydrate-based breakfast; PRO: protein-based breakfast. Means in a row without a common letter are significantly different (P < 0.05).
Table 3. Energy and macronutrient content of 24-hour food intake.

<table>
<thead>
<tr>
<th></th>
<th>AP-NW</th>
<th>AP-OW</th>
<th>PP-NW</th>
<th>PP-OW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2327 ± 141</td>
<td>2417 ± 251</td>
<td>2041 ± 161</td>
<td>2218 ± 269</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>271 ± 13.3</td>
<td>275.6 ± 22.9</td>
<td>308.18 ± 55.6</td>
<td>237.6 ± 35.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>93.5 ± 11.4</td>
<td>100.4 ± 13.7</td>
<td>83.1 ± 19.8</td>
<td>95.6 ± 13.7</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>123.1 ± 20.9</td>
<td>107.3 ± 20</td>
<td>107.4 ± 10.5</td>
<td>93.4 ± 14.1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. AP: animal protein; NW: normal weight; OW: overweight; PP: plant protein.
Appendix B

MEMORANDUM

TO: Jamie Baum  Stephanie Shouse  Dallas Johnson  Amy Dunn  Christina Crowder  Shelby Payne  Brianna Neumann  Muna Abdulrhida

FROM: Ro Windwalker  IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 14-07-028

Protocol Title: The Effect of Breakfast Composition on Satiety, Glucose Response and 24-Hour Food Intake

Review Type: [ ] EXEMPT  [ ] EXPEDITED  [X] FULL IRB

Approved Project Period: Start Date: 09/02/2014 Expiration Date: 08/12/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 20 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

The IRB determined and documented that the risk is no greater than minimal and this protocol may be reviewed under expedited review procedure for future continuing reviews.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.
CONCLUSION

In conclusion, breakfast consumption has shown to increase energy metabolism and improve appetite response, but these benefits are amplified within a high-protein breakfast, however no adaptive response was seen. Additionally, animal-based protein at breakfast may be beneficial for glycemic control, yet the source of protein does not seem to impact appetite response.
Curriculum Vitae

Brianna L. Neumann
Department of Food Science
University of Arkansas
Fayetteville, AR

SCHOLARLY ACTIVITIES, GPA 4.0/4.0

MS, May 2016
University of Arkansas, Fayetteville, AR
Food Science
Thesis Advisor: Jamie I. Baum, PhD
Thesis Title: The effect of meal-timing and macronutrient distribution on energy metabolism and metabolic health

BS, December 2013
Truman State University, Kirksville, MO
Major: Exercise Science
Minor: Business Administration

RESEARCH: SIGNIFICANCE AND IMPACT

Over 65% of American adults are considered overweight or obese. It is known added weight can lead to health issues such as heart disease, renal dysfunction, pulmonary disease, arthritis, and hormonal problems, specifically type II diabetes. However, it has been supported that the consumption of protein may reduce the risk of weight gain and improve blood lipid profiles, body composition, and increase energy metabolism. Yet, it is inconclusive as to whether it is the quality of protein, the quantity of protein, or the timing or protein ingestion that leads to these health improvements. Therefore the aim of my research is to determine 1) Can increasing protein intake at breakfast improve blood glucose response and energy metabolism 2) Does the quality of protein impact blood glucose and subjective hunger response and 3) Does the quality of protein alter energy metabolism. The following publications and presentations reflect these three objectives.

Peer-Reviewed Publications

Neumann BL, Dunn AC, Johnson D, Adams JD, Baum JI. Consumption of a high protein breakfast for seven days increases postprandial energy expenditure but not appetite when compared to a high carbohydrate breakfast in young females who habitually skip breakfast. In review – Nutrition Journal.

Crowder CM, Neumann BL, Baum JI. (2016). Breakfast protein source does not influence postprandial appetite and food intake in normal weight and overweight young females. Accepted - Journal of Nutrition and Metabolism.

University of Arkansas Publications

Additional Publications


Abstract Submissions


Presentations


Honors and Awards

1st place, oral competition – Food Science Club Research Competition, University of Arkansas  April 2016

Outstanding Department Masters Student – Food Science Department, University of Arkansas  April 2016

2nd place, oral competition - 3-Min Thesis Competition  February 2016
University of Arkansas

2nd place, poster competition - Food Science Club Research Competition, University of Arkansas  April 2015

Graduate Travel Grant  December 2016 & March 2015
University of Arkansas Graduate School

Premier Young Investigator - Arkansas Biosciences Institute  Fall 2014
Recognizes various student scientist throughout the state of Arkansas

TEACHING: SIGNIFICANCE AND IMPACT

Having the opportunity to work with undergraduate science students has been one of the most rewarding experiences during my graduate program. As a prior undergraduate student, I relished the wisdom of older students who had gone before me and had patience and understanding with my constant questions. Therefore, other than having the opportunity to teach, I love getting to know students and their academic and life goals, hoping to provide encouragement and support for them as a person both in and outside the classroom setting.

Teaching Experience

Tutored undergraduate students in Fundamentals of Nutrition, including answering emails and meeting with students inside and outside of office hours. Department of Human and Environmental Sciences, University of Arkansas, Spring 2015 – Spring 2016.


FDSC 4304L: Food Chemistry Laboratory: taught 4 labs: “Carbohydrates: reducing sugars, starch morphology, and gelatinization” and “Lipid Characteristics”. Department of Food Science, University of Arkansas, September 29 and October 1, 2015 and October 6 and 8, 2015.


SERVICE IN AND BEYOND THE UNIVERSITY OF ARKANSAS

University Service

Volunteer for University of Arkansas, Division of Agriculture Cooperative Extension Service, January 2016
- Recorded 2 audio lectures for an online protein education program

Volunteer product developer, a collaborative work between the Department of Food Science, University of Arkansas and University of Arkansas Medical School, January 2016.

Volunteer apple butter sales coordinator, Food Science Club, University of Arkansas, December 2015 – present.

Volunteer Holiday party coordinator, Food Science Club, University of Arkansas, December 2015.

Volunteered to help with high school agriculture day, Department of Food Science, University of Arkansas, December 2015.

Volunteer treasurer, Food Science Club, University of Arkansas, June 2015 – present.

Volunteered to help with a high school FFA career day, Department of Food Science, University of Arkansas, May 2015.

**Active Professional Memberships**

Institute of Food Technologists  
June 2015 – present

American Society for Nutrition  
October 2014 - present

**Outreach**

Volunteer with the Arkansas Food Innovation Center, Spring 2016
- Help with current food productions and equipment cleaning

Product Development Intern, May 2015 – August 2015
Treat Division, Simmons Pet Food, Siloam Springs, AR
- Formulated 25 various pet food treats for nationally recognized pet food companies
- Exposed to plant environment and HACCP protocols
- Researched product storage techniques on food safety, specifically water activity