THE EFFECTS OF A PROTEIN-RICH BREAKFAST ON GLUCOSE METABOLISM IN OVERWEIGHT/OBESE LATE ADOLESCENT GIRLS

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Background: Skipping breakfast has been associated with an increased risk of type II diabetes and obesity. **Purpose:** To examine the effects of normal protein vs. higher protein breakfast meals on pre- and post-lunch glycemic control in overweight/obese adolescents who either habitually skip breakfast vs. adolescents who habitually consume high CHO breakfast meals. **Methods:** Thirty-five overweight/obese teen girls participated in the following randomized crossover-design study. The participants were grouped according to habitual breakfast frequency. The habitual breakfast skipping group randomly completed the following breakfast patterns at home for 3 days: 1) Breakfast Skipping (BS); 2) Normal Protein (NP) breakfast; and 3) High Protein (HP) breakfast. The habitual breakfast consuming group randomly completed the NP and HP breakfast patterns at home for 3 days. On day 4 of each pattern (for both groups), the participants complete the respective 8-h testing day. The respective breakfast was provided at the beginning of the day and a NP lunch was provided 4h post-breakfast. Blood samples were collected at specific times throughout the day for plasma insulin and glucose responses. **Results:** The addition of breakfast led to increased morning glucose and insulin responses vs. BS (both, $P < 0.05$). When comparing the normal protein vs. high protein breakfast meals, the post-lunch glycemic response was different between the meals but was significantly modulated by the frequency of habitual breakfast consumption. The breakfast skippers experienced lower afternoon and total glucose concentrations following the normal protein breakfast but higher afternoon and total glucose concentrations following the high protein breakfast compared to the breakfast consumers (both, $P < 0.05$). Minimal differences in afternoon and/or total insulin were detected between meals or between groups. **Conclusion:** These data suggest that the addition of breakfast, regardless of protein content, has very little effect on post-lunch glycemic control in individuals who habitually skip the morning meal but illustrates novel differences in the glycemic response to high versus normal protein breakfast meals which appears to be influenced by habitual breakfast frequency.

INTRODUCTION

The obesity epidemic is negatively impacting the lives of approximately 25 million children and adolescents in the United States alone [1, 2]. With this increased prevalence, more young people are suffering from what were previously considered to be adult-onset conditions including, but not limited to, Type 2 diabetes mellitus (T2DM), cardiovascular disease, and metabolic syndrome [3, 4]. Although the prevalence of these conditions and/or diseases in young people remains small, many children and adolescents display pre-disease symptoms which, over time, may lead to early-onset [5]. Thus, it is imperative to identify the potential factors that contribute to the pre-disease conditions as well as to develop dietary strategies to prevent/and or treat the progression of these conditions.

Several cross-sectional studies illustrate a strong association between skipping breakfast and greater body weight, BMI, and/or increased risk of obesity in children and adolescents [6-9]. Breakfast skipping has also been negatively associated with poor glucose control and an increased risk of T2DM [10-13]. Although these studies identify strong associations between breakfast consumption, obesity makers, and T2D, they do not indicate causality.

Jovanovic et al. [13, 14] completed two randomized crossover-design studies in normal weight, healthy adults and in obese individuals with T2DM to examine the effects of breakfast skipping on markers of glucose control and T2DM risk factors. Compared to skipping breakfast, the consumption of breakfast lowered the post-lunch

rise in plasma glucose by 73% in healthy, normal weight participants and 95% in the obese participants with T2DM [13]. These data suggest that the consumption of a breakfast meal decreases the rise in glucose after the consumption of the lunch meal in both healthy and T2DM individuals [13, 14].

A key dietary factor to consider within the breakfast meal includes the macronutrient composition of the meal. Postprandial glucose concentrations are altered by both the amount and type of carbohydrate (CHO) consumed within the meal [15]. Jenkins et al. reported improvements in CHO metabolism and CHO tolerance during the lunch meal following the consumption of low-glycemic index (LGI) breakfast meals containing either fiber or slowly absorbed CHO [16, 17]. Specifically, there was a blunted glucose and insulin response following the consumption of LGI breakfast meals compared to a high-glycemic index (HGI) breakfast meals [16, 17].

Increased dietary protein has also been proposed to reduce postprandial glucose concentrations, since protein consumption is known to stimulate insulin secretion [18, 19]. However, the data is inconclusive with some [20-26], but not all studies [27-33], reporting blunted post-prandial glucose and insulin responses following the consumption of high vs. normal protein breakfast meals. The discrepant findings may be due to several key dietary factors including, but not limited to protein type (i.e., animal vs. plant) or food form (i.e., beverages vs. solids) which varied within and across studies. Furthermore, the majority of the studies did not screen and/or control for habitual breakfast frequency of consumption; thus, it is plausible that habitual breakfast skippers may respond differently to a breakfast meal than habitual breakfast

consumers. For example, Farshchi et al. found that breakfast skippers were more likely to have poor glycemic control compared to breakfast consumers [34].

Therefore, the purpose of this study was to examine the effects of the normal protein vs. higher protein breakfast meals on pre- and post-lunch glycemic control in overweight/obese adolescents who either habitually skip breakfast or habitually consume high CHO breakfast meals. We also sought to compare the normal protein vs. high protein meals between the breakfast groups.

EXTENDED REVIEW OF LITERATURE

I. Obesity

By the year 2030, obesity is estimated to cost the U.S. 900 billion dollars annually [35]. According to the Centers for Disease Control and Prevention (CDC), there has been an alarming increase in obesity in the U.S. over the past 20 years [36], and is defined based on the sex-specific body mass index (BMI) for age growth charts provided by the CDC [2, 37]. The obesity epidemic is negatively impacting the lives of approximately 25 million children and adolescents in the U.S. alone [1]. It is estimated that 35.7% of U.S. adults and 17% of U.S. children and adolescents (aged 2-19 years) are considered to be obese [36]. The CDC reported that the prevalence of obesity among children and adolescents has almost tripled since 1980 [36] with little evidence that the prevalence is decreasing [1].

Childhood obesity has nearly tripled in the past 30 years, increasing from 7-18% between 1980 and 2010 [2, 36]. It is critical to address this issue considering that obesity may lead to both short and long-term health consequences not only in the U.S., but globally as well [2]. The prevalence of childhood and adolescent obesity may be attributed to urbanization, unhealthy diets, and sedentary lifestyles [38]. With this increased prevalence, more young people are suffering from what were previously considered adult-onset conditions including, but not limited to: T2DM, cardiovascular disease, various forms of cancer, hypertension, and metabolic syndrome [1, 39]. Although the prevalence of these conditions and/or diseases in young people remains

small, many children and adolescents display pre-disease symptoms, which over time, may lead to early-onset [40, 41]. In particular, overweight and obese children and adolescents – specifically with fat storage in the abdominal region – display poor glucose control compared to their normal weight counterparts, which has been identified as the main factor in the etiology of T2DM [38, 42, 43]. Urakami et al. has confirmed that a strong association exists between obesity and T2DM in children over the past few decades [44].

Prevalence of Type 2 Diabetes Mellitus

The prevalence of T2DM continues to increase with severe consequences on morbidity and premature mortality [45]. In fact, T2DM is now considered to be the sixth leading cause of death in the U.S. [46]. Alberti et al estimated that by 2025, the global prevalence of T2DM is predicted to increase from 5.0-6.2% [46]. Although type 1 diabetes is the main form of diabetes among children, T2DM is estimated to be the major form within the next decade – particularly in various ethnic groups [47]. It is important to note that data related to T2DM incidence and prevalence in children and adolescents is less common than it is in adults [47]. However, several studies do exist and shed some light into the development and complications of this growing phenomenon.

Prior to considering the data of previous studies, it is important to understand the definition and diagnosis criteria of pre-diabetes and T2DM. The World Health Organization defines pre-diabetes as impaired fasting glucose (fasting plasma glucose ≥ 6.1 mmol/l and < 7.0 mmol/l), or impaired glucose tolerance (2-h plasma glucose after

an oral glucose tolerance test measurement ≥ 7.8 mmol/l and < 11.1 mmol/l) or both. T2DM is defined as having a fasting plasma glucose ≥ 7.0 mmol/l or 2-h plasma glucose ≥ 11.1 mmol/l. Velasquez-Mieyer et al. defined glucose homeostasis as a balance between insulin sensitivity and secretion [48]. The disruption of glucose homeostasis may therefore lead to impaired pancreatic beta-cell function and decreased insulin sensitivity – the two main contributors to the etiology of T2DM [48].

Link Between Obesity and T2DM

Holst-Schumacher et al. conducted a cross-sectional study to determine the prevalence of insulin resistance and impaired glucose tolerance in 214 overweight (38% of participants) and obese (62% of participants) Costa Rican schoolchildren (9.14 ± 0.80 years) [49]. Overweight and obesity were defined by their BMI. They found that over 20% of the children exhibited hyperinsulinemia, almost 50% were insulin resistant, and 6.5% had impaired glucose tolerance. These findings were greater in the obese children than they were in the overweight children [49]. Overall, glucose intolerance and insulin resistance in the Costa Rican schoolchildren that were studied is more common than what was previously thought. Holst-Schumacher et al. recommended lifestyle interventions and early screening for glucose intolerance and insulin resistance in overweight and obese children to prevent the advancement and ramifications of T2DM [49]. A limitation of this study is that insulin resistance was estimated rather than measured due to the fact that a hyperinsulinemic-euglycemic clamp, the gold standard of measuring insulin sensitivity, was too invasive and expensive.

Similar findings were also observed in a more recent cross-sectional study of 3173 schoolchildren (aged 7-18 years) in China to determine the prevalence of pre-diabetes and T2DM [50]. The prevalence of overweight and obesity in this study was 13% and 15.4%, respectively. Overweight and obesity were also defined by BMI. Zhu et al. found that the prevalence of pre-diabetes and T2DM in overweight and obese children was comparable to other population-based studies in other countries [51]. The occurrence of pre-diabetes and T2DM in overweight or obese children was 0.28% and 3.30%, respectively. However, this prevalence is significantly lower when compared to studies that were clinical in nature [52]. Another important finding from this study is that children going through puberty had a greater risk of developing pre-diabetes or T2DM compared to pre- or post-pubescent children. This may be attributed to physiological insulin resistance in children experiencing puberty – which may advance into T2DM in this population [53]. Overall, the findings of this study illustrate that overweight and obese children had more T2DM-related factors compared to their normal weight counterparts [50]. Zhu et al. recommended preventive strategies to combat the development of obesity, and subsequently, T2DM, as the incidences of pre-diabetes and T2DM continues to shift into a public health crisis. A limitation of this study was the inability to evaluate the temporal sequence between risk factors and progression of pre-diabetes and T2DM. Another limitation was utilizing the fasting capillary glucose as a screening tool, which is not very effective in pinpointing glucose intolerance.

Conversely, a review by Goran et al. challenges the overwhelming majority of studies that reported a high prevalence of pediatric T2DM exists [54, 55]. Pinhas-Hamiel et al. conducted a clinical trial in Cincinnati, Ohio that observed the prevalence of T2DM in adolescents increased by a disturbing 10% from 1982-1994 [55]. These studies have led to the understanding that there is a T2DM epidemic in the U.S. Nevertheless, these findings may be biased due to the population of children being diagnosed with pre-diabetes or T2DM and the absence of duplicated testing to determine if T2DM truly exists [54]. Goran et al. stated that sparse data from studies exist regarding the incidences of T2DM in children and adolescents [22]. It is also articulated that to infer an epidemic exists without sufficient confirmation may result in inappropriate changes and effects, such as accidental harm. Therefore, Goran et al. advocates for reason and balance in order to appropriately direct the approach in tackling this increasing health concern [54]. With childhood obesity and diseases related to obesity exponentially growing and turning into an epidemic in the U.S., other interventions besides weight loss should be considered to attenuate and eventually eliminate the risk of pre-diabetes and T2DM in overweight and obese children. Although T2DM may not be an epidemic, many children do exhibit pre-diabetic symptoms – one of which is poor glucose control.

II. Breakfast Skipping and Glucose Control

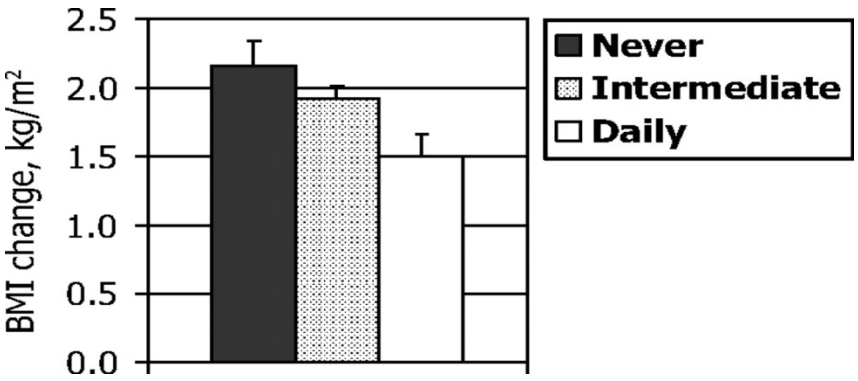
Based on the serious complications stemming from obesity, it is critical to identify contributors and develop successful dietary strategies to combat this disease. Although the etiology of obesity is multi-faceted, one specific dietary factor – breakfast

skipping – has been found to have a strong correlation with both obesity and T2DM. Several cross-sectional studies report that skipping breakfast is associated with greater body weight, BMI, and/or increased risk of obesity in children and adolescents [6, 8, 9, 56]. Other studies mention that skipping breakfast is also associated with increased snacking and a sedentary lifestyle [57]. Although these studies identify a strong relationship between breakfast consumption and obesity markers, they do not indicate causality.

However, Timlin et al. conducted a 5- year prospective study to examine the association between breakfast frequency and body weight change in 2216 middle and high school American adolescents [58]. In 1998-1999, students (aged 14.9 ± 1.6 years) were given the Project Eat 1 survey to assess personal and behavior factors and their anthropometric measures were recorded. They were also presented with the Youth and Adolescent Food Frequency Questionnaire. After five years, a longitudinal follow-up study- Project Eat 2- was conducted on the same students (now aged 19.4 ± 1.7 years). Timlin et al. found that the frequency of breakfast was inversely associated with BMI in a dose-response manner **(Figure 1)** such that those who never ate breakfast displayed a greater BMI compared to those who consistently consumed breakfast [58]. It was also reported that females were more likely to skip breakfast than males. In fact, it was noted that skipping breakfast transpired as a means to lose weight. It is important to note that while those who ate breakfast regularly tended to have greater daily energy intake compared to breakfast skippers, breakfast consumers had less total daily fat and saturated fat intakes – providing a possible explanation to the improvement of energy

balance and weight control in the breakfast consuming populations. A limitation in this study was the self-reporting of height and weight, which may have resulted in incorrect BMI assessments. Due to the observational nature of this study, causality between skipping breakfast and BMI cannot be established. Timlin et al. recommended interventions in adolescents to promote the healthy habit of consuming breakfast [58]. Lastly, other studies also report that obese and overweight adolescents are twice as likely to skip breakfast when compared to their normal weight counterparts [57]. Thus, increasing evidence exists supporting that daily consumption of breakfast has a protective effect against weight gain and obesity.

Figure 1 [58]
Time to breakfast and BMI change (adjusted for baseline BMI and breakfast category, age, and gender).



Glycemic Control

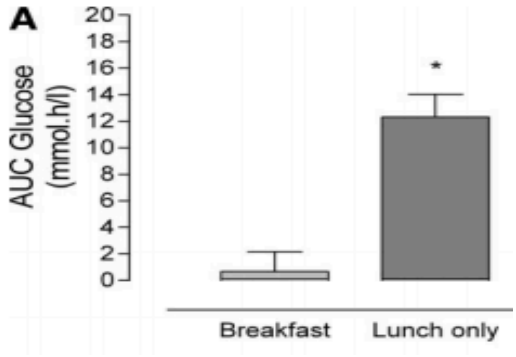
Breakfast skipping has also been negatively associated with poor glucose control and risk of both T2DM and cardiovascular disease in cross-sectional studies [10, 13]. Mekary et al. prospectively analyzed the Health Professionals follow-up study of 29206 men (aged 40-75 years) that was conducted in 1986 [10]. These male health professionals were followed for several years throughout the study and received

biennial questionnaires regarding their medical history, lifestyle, and health-related behavior. Mekary et al. discovered that breakfast consumption was inversely associated with the risk of developing T2DM in men [10]. Pereira et al. stated that consuming breakfast may better modulate blood glucose and appetite control throughout the day in both children and adults [59]. A limitation of the Mekary et al. analysis was that the composition of breakfasts consumed was unknown [10]. However, the daily consumption of breakfast – regardless of composition – is important in decreasing the risk of T2DM.

Jovanovic et al. conducted two randomized crossover design studies in normal weight, healthy adults and in obese individuals with T2DM [13, 14] to examine the effects of skipping breakfast on markers of glucose control and T2DM risk factors. In the first Jovanovic et al. study, they compared the post-lunch metabolic alterations in eight T2DM (aged 56.1 ± 2.8 years) obese (BMI 36.0 ± 2.5 kg/m^2) patients on 3 days in no particular order: breakfast consumption, breakfast skipping, and breakfast skipping with an arginine infusion 1 hour before lunch [13]. Compared to skipping breakfast, the consumption of breakfast lowered the post-lunch rise in plasma glucose by 73% in healthy, normal weight subjects and 95% in those with Type 2 diabetes **(Figure 2)**. This phenomenon is referred to as the second meal effect (SME) [13]. It was observed that the post-lunch plasma insulin levels remained unchanged during all three testing days. Jovanovic et al. observed a decrease in post-lunch free fatty acid (FFA) responses in those who ate breakfast compared to those who skipped breakfast, and discovered a

positive correlation to exist between FFA concentrations prior to lunch and the post-lunch rise in plasma glucose [13].

Figure 2 [13]
*Incremental change in plasma glucose after lunch. *P < 0.0001area under the curve (AUC; 4-8h).*



In the second Jovanovic et al. study, they attempted to understand the metabolic action behind the SME ten in healthy (aged 46.7 ± 3.9 years), normal weight (BMI 26.1 ± 1.1 kg/m^2) subjects on 2 days in no particular order: breakfast consumption and breakfast skipping [14]. They found that when breakfast was consumed, there was a 50% increase in postprandial plasma glucose conversion into muscle glycogen after lunch, in comparison to skipping breakfast. Again, a positive correlation was shown between FFA concentrations prior to lunch and the post-lunch rise in plasma glucose and a negative correlation was shown between FFA concentrations prior to lunch and the post-lunch increase in muscle glycogen synthesis [14]. A potential explanation to improved glycemic control is that prolonged elevation in FFAs, which occur during prolonged fasting periods (i.e., breakfast skipping), results in the inhibition of net hepatic glycogen utilization, increases in gluconeogenesis, and reductions in glucose utilization [14, 15, 60]. This is important due to several studies indicating that increased

plasma FFA concentrations leads to insulin resistance and T2DM [61, 62]. Because muscle glycogen synthesis is defective in T2DM patients, the findings in these studies may be applied as a therapeutic strategy. Based on these findings, it is possible that breakfast consumption leads to decreased circulating FFAs which in turn stimulates net hepatic glycogen utilization, decreases gluconeogenesis, and increases glucose utilization.

III. Breakfast Consumption

Glycemic Index

Another dietary factor to consider regarding the consumption of breakfast includes the macronutrient composition of the morning meal. Postprandial glucose concentrations are altered by both the amount and type of carbohydrate (CHO) consumed [15]. Jenkins et al. conducted a study to compare the effects of either a low glycemic index (LGI) breakfast (lentils) or a high glycemic index (HGI) breakfast (whole meal bread) of identical CHO content on a standard lunch in seven healthy subjects (aged 26 ± 3 years) [16, 17]. Jenkins et al. reported that subjects showed improved CHO metabolism/tolerance during the lunch meal following the consumption of either a LGI meal containing fiber or slowly absorbed CHO. The consumption of LGI foods in one meal reduces the glycemic response (meaning blood glucose is more regulated) to a subsequent meal [16, 63].

LGI foods have a slower delivery rate than do HGI foods, which may be the cause of the reduced glycemic response to a subsequent meal [63]. Numerous studies also

indicate that LGI foods enhance lipid profiles. Liljeberg et al. conducted a study on ten healthy subjects (aged 25-51 years) to assess the response of a LGI breakfast on lipaemia and glucose tolerance at a subsequent meal [64]. Liljeberg et al. illustrated a lowered glucose and insulin response along with reduced serum triglyceride levels at the subsequent lunch meal when the LGI spaghetti was consumed at breakfast compared to the HGI white wheat bread [64]. Elevated serum triglyceride levels and reduced insulin sensitivity are established risk factors for cardiovascular disease and T2DM. Thus, Liljeberg et al. demonstrated that an LGI breakfast is beneficial in reducing the risk of these diseases [64]. Moreover, Schulze et al. prospectively analyzed a follow up with the Nurses' Health Study II, a prospective cohort study involving 91249 U.S. female nurses (aged 24-44 years) to investigate the association between the risk of T2DM and glycemic index, glycemic load, and dietary fiber [65]. They found that HGI foods were strongly associated with the increased risk of T2DM and that high fiber foods were strongly associated with the decreased risk of T2DM. This study also mentioned that HGI diets lead to elevated FFA concentrations in both diabetic and healthy persons [65, 66]. A limitation of the Liljeberg et al. analysis was that the composition of breakfasts consumed was unknown. Alternately, the consumption of rapidly absorbed HGI foods lead to a large increase in blood insulin followed by a quick decrease in blood glucose, and ultimately stimulated FFA release. Over time, this may lead to insulin resistance and impaired CHO tolerance [63]. Therefore, it is important to understand the quality of CHO to help prevent the etiology of T2DM. Although the incorporation of

slow releasing CHO are dietary fiber are effective in reducing GI, the replacement of CHO with dietary protein also reduces GI, thus exerting similar alterations.

As the rise in meal frequency continues to increase in the U.S., so too does the proportion of CHO content in meals [67]. Ludwig et al. provided a synopsis of events that arise following a high CHO meal [68]. Once a high CHO food is ingested, it is promptly absorbed and results in hyperglycemia. Insulin is secreted, allowing muscle and adipose tissue to uptake the glucose in an effort to return glucose levels back to normal (80-100 mg/dl). Due to the elevated levels of insulin in circulation, a period of hypoglycemia occurs which leads to the increased feeling of hunger. Consequently, a high CHO meal may result in less glycemic control – a greater increase and decrease in postprandial blood glucose [69, 70]. This rapid rise and fall in blood glucose may lead to an increase in caloric intake [71].

High CHO

Chandler-Laney et al. conducted a study to examine the effects a high CHO/low fat meal has on glycemic control in 64 overweight but otherwise healthy adults (aged 21-50 years). The participants were assigned either the isocaloric low CHO/high fat breakfast meal or the high CHO/low fat breakfast meal. Participants who consumed the high CHO/low fat breakfast meal had greater postprandial plasma insulin, lower 3- and 4-hour plasma glucose, earlier glucose peak, and reported hunger sooner. The earlier onset of hunger reported by the participants in the high CHO/low fat group might be attributed to the earlier increase and decrease in postprandial glucose concentrations. The high CHO/low fat breakfast also resulted in the reduction of glucose concentrations

below fasting concentrations, most likely due to higher insulin concentrations. This finding suggests that slowly digested foods with higher content of fiber, protein, and fat may result in a longer time to peak glucose concentrations and a slower decline in glucose concentrations. The limitation of this study was the sample size used, therefore causing the inability to differentiate between the effects of total CHO content from total fat content.

High Protein

Several randomized cross-over design studies have been completed examining the glucose and insulin responses to normal vs. high protein breakfast meals (**See Table 1**). Below is a summary of the state of the research regarding the effects of acute higher protein meals on glycemic control.

Table 1: *Acute studies examining the effects of protein quantity in mixed-macronutrient, solid meals. ✓: Difference between High Protein vs. Normal Protein Meals; Ø: No Differences were observed.*

Reference	Acute Meals				Hormonal Response	
	Normal Protein (g)	High Protein (g)	Energy Content (kcal)	Meal	Glucose ↓	Insulin ↓
Belza, 2013 [21]	24	88	700	Breakfast	Ø	✓
Blom, 2006 [22]	19	57	400	Breakfast	✓	Ø
Acheson, 2011 [23]	1	57	450	Breakfast	✓	✓
Leidy, 2010 [72]	26	46	700	Breakfast	Ø	Ø
Al Awar, 2005 [24]	20	35	400	Breakfast	Ø	✓
Veldhorst, 2009 [28]	15	38	600	Breakfast	✓	✓
Veldhorst, 2009 [20]	15	38	600	Breakfast	✓	✓
Veldhorst, 2009 [29]	15	38	600	Breakfast	✓	✓
Leidy, 2007 [73]	17	28	400	Breakfast	Ø	Ø
Makris, 2011 [31]	12	24	350	Breakfast	Ø	Ø
Ratliff, 2010[25]	16	23	400	Breakfast	✓	✓
Agus, 2000[26]	17	30	450	Breakfast	✓	✓

Ratliff et al. conducted a randomized crossover trial to examine the effects of two isocaloric breakfast meals (high protein/low CHO egg-based breakfast vs. a normal protein/high CHO bagel-based breakfast) on post-prandial glucose and insulin responses in 21 men (aged 35 ± 16 years; BMI 25.4 ± 3.39 kg/m^2) [25]. The high protein, egg-based breakfast substantially suppressed post-breakfast (i.e., 3 h) insulin and glucose AUCs concentrations compared with the bagel-based breakfast **(Figure 3 & Figure 4)**[25].

Figure 3 [25]
*Comparisons between postprandial glucose responses between EGG (diamonds) and BAGEL (squares). * Indicates significantly (P < 0.05) different at a given point of time. Area under the curve was significantly lower in the EGG compared with the BAGEL (P < 0.01).*

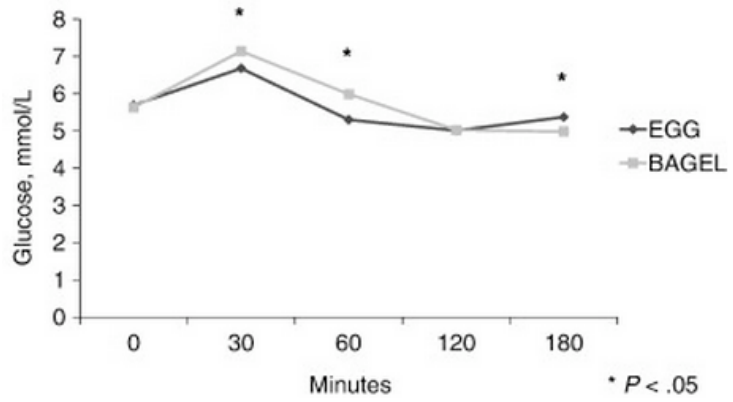
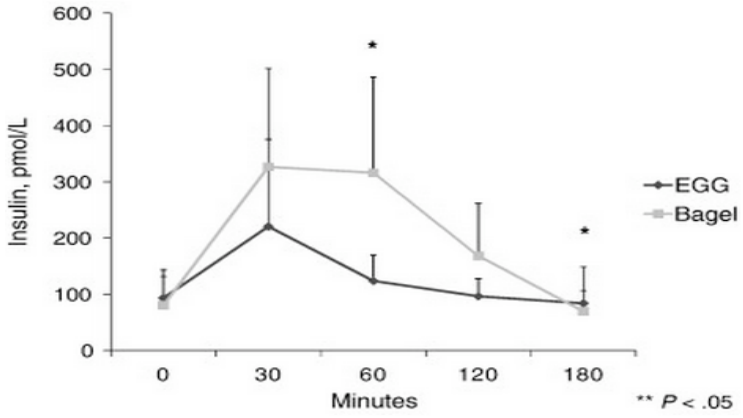


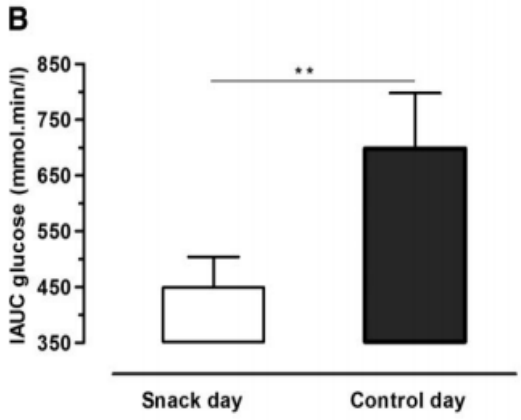
Figure 4 [25]
*Comparisons between postprandial insulin responses between EGG (diamonds) and BAGEL (squares). * Indicates significantly (P < 0.05) different at a given point of time. Area under the curve was significantly lower in the EGG compared with the BAGEL (P < 0.01).*



However, as shown in Leidy et al., the 4-hour postprandial glucose and insulin responses were not different following the consumption of HP vs. NP breakfast meals [30]. These findings were further confirmed in a 12 hour testing day including HP vs. NP breakfast, lunch, and dinner meals [27]. However, one point to consider is that neither study examined the glucose and insulin responses at the subsequent meals.

Chen et al. examined the effects of a HP pre-load (prior to breakfast) on the post-breakfast glucose, insulin, and FFA response in a randomized crossover study involving 10 men and women (aged 61 ± 2.3 years) with T2DM (BMI 28.8 ± 1.0 kg/m^2) [74]. They found a 40% reduction in post-breakfast glucose concentrations with the pre-breakfast, pre-load compared to no pre-load **(Figure 5)**. No differences in post-breakfast insulin or FFA concentrations were observed [74].

Figure 5 [74]
Metabolic changes between the 2 study days in 10 subjects with type 2 diabetes: incremental area under the plasma glucose curves during the 5-h post-breakfast period. P < 0.02.



Belza et a. conducted a 3-way, randomized crossover, double-blind controlled study to examine the mechanistic effects three isocaloric test meals of varying protein content (NP 14%; medium-HP 25%; HP 50%) have on plasma glucose and insulin levels in twenty-

five men (aged 30.0 ± 8.7 years; BMI 25.9 ± 4.7 kg/m^2) [21]. The plasma insulin response was decreased after consumption of the HP test meal compared the NP and medium-HP test meals, although there was no dose-dependent response difference between the 3 isocaloric test meals [21].

Blom et al. examined the effects HP (58% protein) vs. NP (19% protein) isocaloric breakfasts have on plasma glucose and insulin in a single-blind, crossover study involving fifteen lean, healthy men (aged 20.5 ± 2.5 years; BMI 21.6 ± 1.9 kg/m^2) [22]. The researchers observed that the HP breakfast had a lower increase in plasma glucose. However, no significant difference was observed in plasma insulin between the meals [22].

Acheson et al. conducted a randomized, double-blind, crossover, 5-treatment trial study in twenty-three lean, healthy subjects (aged 32 ± 6.3 years; BMI 22.7 ± 1.7 kg/m^2) to investigate the effects 4 isocaloric test meals (3 of the meals provided 50% of energy as whey, casein, or soy protein vs. a high CHO meal) have on plasma glucose and insulin levels [23]. They found that plasma insulin was increased in the HP meal compared to the NP meal. However, it was observed that glucose was decreased in the HP meal compared to the NP meal [23].

Al Awar et al. examined the postprandial effect of three isocaloric breakfast meals (low-protein = 10%, NP = 20%, and HP = 35%) on circulating levels of plasma glucose and insulin in eleven young, healthy women (aged 22.6 ± 0.7 years; BMI 20.4 ± 0.4 kg/m^2) in a randomized crossover study [24]. The investigators observed that the HP breakfast meal decreased the plasma insulin response compared to the low-protein and NP

breakfast meals. However, no significant differences were observed for plasma glucose levels.

Veldhorst et al. conducted three separate trials to investigate the effects different types of protein (casein; soy; whey) have on plasma glucose and insulin levels [20, 28, 29]. In the first study, Veldhorst et al. compared the effects normal (10%) and high (25%) whey protein isocaloric breakfast meals have on plasma glucose and insulin levels in twenty-five healthy subjects (aged 22 ± 1 years; BMI 23.9 ± 0.3 kg/m^2) in a randomized, single-blind trial [28]. It was observed that plasma glucose and insulin concentrations were higher in the high whey protein breakfast meal compared to the normal whey protein breakfast meal [28]. In contrast to the previously mentioned study, Veldhorst et al. compared the effects normal (10%) and high (25%) casein protein isocaloric breakfast meals have on plasma glucose and insulin in twenty-five healthy subjects (aged 22 ± 1 years; BMI 23.9 ± 0.3 kg/m^2) in a randomized, single-blind study [20]. They found that plasma glucose and insulin concentrations were lower in the in the high casein protein breakfast meal compared to the normal casein protein breakfast meal [20]. Similarly to the first Veldhorst et al. study mentioned above [28], Veldhorst et al. compared the effects normal (10%) and high (25%) soy protein isocaloric breakfast meals have on plasma glucose and insulin in twenty-five healthy subjects (aged 22 ± 1 years; BMI 23.9 ± 0.3 kg/m^2) in a randomized, single-blind study [29]. It was observed that plasma glucose and insulin concentrations were higher in the high soy protein breakfast meal compared to the normal soy protein breakfast meal [29]. The Veldhorst et al. studies described above illustrate that not only protein concentration, but the type

of protein (casein; soy; whey), must also be taken into consideration at the breakfast meal to more clearly analyze proteins' effects on plasma glucose and insulin [20, 28, 29].

Collectively, the data is inconclusive with some [20-26], but not all studies [27-33], reporting blunted post-prandial glucose and insulin responses following the consumption of high vs. normal protein breakfast meals.

IV. High Protein Diets and Health Outcomes Related to Glucose Control

Wycherley et al. conducted a meta-analysis consisting of 24 trials in overweight and obese adults to compare the effects of energy-restricted HP diets vs. isocaloric NP diets on health related outcomes, including glucose control [75]. Although isocaloric HP diets led to greater weight loss, improved body composition, increased resting energy expenditure, and decreased fasting FFA, no differences in fasting glucose concentrations were detected between diets. However, Dong et al. conducted a meta-analysis of nine randomized control trials to examine the effects HP diets on weight management and metabolic risk factors in T2DM subjects [76]. In this analysis, greater weight loss and considerably decreased HbA1C levels were detected following the HP diets despite no changes in fasting plasma glucose concentrations or lipid profiles [76].

Taken together these data suggest that there is inconclusive and sometimes conflicting evidence regarding the effects NP and HP meals have on glycemic control. Therefore, further studies should be conducted to better clarify the effects NP and HP meals have on glycemic control and whether HP meals may be more beneficial than NP meals in overweight/obese, habitual breakfast skipping adolescents.

III. Summary

- Obesity continues to significantly impact the lives of American young people
- Numerous, unhealthy dietary habits contributing to the obesity epidemic; in particular, breakfast skipping appears to be a primary factor due to the strong association with overeating, weight gain, obesity, poor glucose control and T2DM.
- Although the consumption of breakfast improves glucose control, several key dietary factors (i.e., glycemic index, macronutrient content) appear to modulate these responses
- Breakfast meals rich in dietary protein have been proposed to elicit immediate and sustained improvements in glucose control compared to high CHO meals. However, current evidence is conflicting and limited, particularly in overweight/obese, habitual breakfast skipping young people.

METHODS

I. Experimental Design

Thirty-five overweight and obese adolescent girls participated in the following randomized crossover-design breakfast study. The participants were grouped according to habitual breakfast frequency. The habitual breakfast skipping group randomly completed the following breakfast patterns at home for 3 days: 1) Breakfast Skipping (BS); 2) Consumption of Normal Protein (NP) breakfast meals; and 3) Consumption of Higher Protein (HP) breakfast meals. The habitual breakfast consuming group randomly completed the following breakfast patterns at home for 3 days: 1) Consumption of NP breakfast meals and 2) Consumption of HP breakfast meals. On the 4^{th} day of each pattern (for both groups), the participants came to the University of Missouri in the morning to complete the respective 8-h testing day. The participants began the testing day by completing their respective breakfast pattern. At 4- h post-breakfast, a NP lunch was provided. Blood samples were collected at specific times throughout the 8-h day for assessment of plasma insulin and glucose responses.

II. Study Participants

Adolescent girls were recruited from the Columbia, MO area through advertisements, flyers, and email list serves to participate in the study. Eligibility was determined through the following inclusion criteria: 1) age range of 13-20 y; 2) overweight to obese (BMI: 25-34.9 kg/m^2); 3) no metabolic or neurological diseases or other health complications; 4) not been clinically diagnosed with an eating disorder;

and, 5) not currently or previously on a weight loss or other special diet in the past 6 months; 6) documented regular menstrual cycles between 21-36 days in duration for the past 6 months. The 'breakfast skipping' group infrequently consumed breakfast (i.e., ≤ 2 breakfast occasions/wk), whereas the 'breakfast consuming' group frequently consumed a CHO-rich breakfast (≥ 5 eating occasions/wk). There was a 20% drop-out rate from the study due to fine constraints and non-compliance to the breakfast patterns.

Three hundred and fifty teens were interested in participating in the study. Twenty-five breakfast skippers and thirty-one breakfast consumers met the screening criteria, were available for the 8-h testing days, and began the study. Twenty breakfast skippers and 15 breakfast consumers completed all study procedures. Subject characteristics are presented in **Table 2**. All participants and their parents (if participant was <18 y of age) were informed of the study purpose, procedures, and risks and signed the consent/assent forms. The study was approved by the MU Health Sciences IRB. The participants received a stipend of $150/testing day.

III. Breakfast Patterns

The participants completed each breakfast pattern for a total of 4 consecutive days/pattern (i.e., 3 acclimation days/pattern and 1 testing day/pattern). For the BS pattern (for the habitual breakfast skippers only), the participants continued to skip the morning meal. For the NP and HP patterns (for both groups), the participants were provided with specific breakfast meals and asked to consume these at home (before school) between 7-9:30 am for 3 days. Throughout this period, the participants were

permitted to eat ad libitum throughout the remainder of each day. On Day 4, they completed the respective testing day. There was a 7-day washout period in between each of the breakfast patterns in which all participants returned to their previous habitual breakfast behavior.

IV. Breakfast and Lunch Meals

The dietary characteristics of the breakfast and lunch meals are shown in **Table 3**. The breakfast meals were 350 kcals which was approximately 18% of the total energy intake estimated from the energy expenditure equations specific for adolescents [77]. The macronutrient composition of the NP breakfast contained 15% protein, 65% CHO, and 20% fat, whereas the HP breakfast contained 40% protein, 40% CHO, and 20% fat. In addition to being matched for fat content, the breakfast meals were similar in energy density, dietary fiber, and sugar content.

The NP lunch meal, provided in each testing day (for both groups) was 500 kcal which was approximately 25% of the total energy intake [77]. The macronutrient composition of the lunch meals contained 15% protein, 65% CHO, and 20% fat.

V. Testing Day Procedures

The participants reported to the research facility between 6-9 am after an overnight fast to complete the 8-h testing day. Each participant was seated in a reclining chair and, for the next 30 min, was acclimated to the room and became familiarized with the testing day procedures. A catheter was then inserted into the antecubital vein of the non-dominate arm and kept patent by saline drip throughout the

remainder of the testing day. At time -15 min, a baseline (fasting) blood sample was drawn. At time 0 min, a meal including water was provided during the NP and HP days and only water during the BS day (for the breakfast skippers only). The participants consumed the meal and/or water within 30 min. Blood sampling was completed throughout the next 8 h. At +240 min (i.e., 4-h post-breakfast), the NP lunch was provided.

VI. Repeated Blood Sampling and Hormonal Analyses

Nineteen (19) blood samples (4 ml/sample; 76 ml/testing day) were collected throughout each 8-h testing day. Specifically, blood was collected at -15, +0, +30, +45, +60, +90, +120, +150, +180, +210, +240, +270, +285, +300, +330, +360, +390, +420, +450 min. The samples were collected in test tubes containing EDTA (ethylenediaminetetraacetic acid). Within 10 min of collection, the samples were centrifuged at -4°C for 10 min. The plasma was separated and stored in microcentrifuge tubes at -80°C for future analysis. Plasma glucose was measured through an in-house glucose oxidase assay (Thermo FIsher Scientific, USA). Plasma insulin was measured using the Milliplex MAP magnetic bead-based multi-analyte assay (Millipore Corporation; St. Charles, MO; HMHMAG-34K).

VII. Data and Statistical Analyses

The following outcomes were determined for the plasma glucose and insulin responses: 4h post-breakfast, 4h post-lunch, and total 8h net incremental Area Under the Curve (AUC) were calculated from the fasting (baseline) time point and the remaining points for each outcome.

A repeated measures ANOVA was performed to compare the main effects of breakfast on glucose and insulin responses in both groups. When main effects were detected, pairwise comparisons, using Least Significant Difference, were applied to compare differences between breakfast patterns. Additionally, a mixed factor ANOVA was performed to compare the glucose and insulin responses following the NP and HP meals between the breakfast skipping group vs. breakfast consuming group.

Analyses were conducted using the Statistical Package for the Social Sciences (SPSS; version 21.0; Chicago, IL, USA). $P < 0.05$ was considered statistically significant. All data are reported at mean ± SEM.

RESULTS

Figures 6-11 depict the glucose and insulin responses throughout each of the breakfast patterns in the breakfast skipping and breakfast consuming groups, respectively. The line graphs illustrate the time course of change, whereas the bar graphs illustrate the morning, afternoon, and total AUC measures.

Glucose

Within the breakfast skipping group, a main effect of breakfast was detected for morning and total glucose AUC (both, $P < 0.05$). Specifically, both breakfast meals led to increased morning glucose AUC (HP: 1283 ± 570 mg/dL*240 min; NP: 1645 ±626 mg/dL*240 min) vs. BS (-610 ± 514 mg/dL*240 min; both, $P < 0.05$; *Figure 6*). The HP breakfast, but not NP, led to increased total glucose AUC (HP: 5333 ± 944 mg/dL*450 min; NP: 4401 ± 1180 mg/dL*450 min) vs. BS (2651 ± 1134 mg/dL*450 min; $P < 0.05$; *Figure 6*). No other differences were detected between breakfast treatments.

Within the breakfast consuming group, the HP breakfast meal led to reduced morning, afternoon, and total glucose AUCs (536 ± 501 mg/dL*240 min; 3145 ± 667 mg/dL*240 min; and 3681 ± 1127 mg/dL*450 min, respectively) vs. NP (2002 ± 359 mg/dL*240 min; 4770 ± 548 mg/dL*240 min; and 6772 ± 770 mg/dL*450 min, respectively; all, $P < 0.05$; *Figure 7)*. No other differences were observed between treatments.

Lastly, the main effects of breakfast treatment, breakfast group, and treatment x group interactions were assessed (Figure 10). Regardless of group, the HP breakfast tended to reduced morning AUC vs. the NP breakfast (P = 0.065). In addition, a

treatment x group interaction was detected for afternoon and total glucose AUC (both, $P < 0.05$). Specifically, the breakfast skippers experienced lower afternoon and total glucose AUC following the NP breakfast but higher afternoon and total glucose AUC following the HP breakfast compared to the breakfast consumers (all, $P < 0.05$).

Insulin

Within the breakfast skipping group, a main effect of breakfast was detected for morning and total insulin AUC (both, *P < 0.05)*. Specifically, both breakfast meals led to increased morning insulin AUC (HP: 109299 ± 25245 pg/mL*240 min; NP: 181934 ± 26165 pg/mL*240 min) vs. BS (-11990 ± 31978 pg/mL*240 min; both, *P < 0.05; Figure 8*). However, the HP breakfast led to lower morning insulin AUC vs. NP ($P < 0.05$). The NP breakfast led to increased total insulin AUC (412090 ± 52894 pg/mL*450 min) vs. BS (242437 ± 60449 pg/mL*450 min; *P < 0.05*), whereas the HP breakfast did not (347728 ± 72301 pg/mL*450 min; Figure 8). No other differences were observed between treatments.

Within the breakfast consuming group, no differences in morning, afternoon, or total insulin AUCs were detected between breakfast meals (Figure 9).

Lastly, the main effects of breakfast treatment, breakfast group, and treatment x group interactions were assessed (Figure 11). Regardless of group, the HP breakfast led to reduced morning and total insulin AUC vs. the NP breakfast ($P < 0.05$). However, no other main effects or interactions were observed.

DISCUSSION

We sought to examine the effects of increased dietary protein at breakfast on glycemic control in overweight/obese adolescent girls who habitually skip breakfast as well as those who habitually consume a high CHO breakfast. As expected, the addition of breakfast, regardless of macronutrient content, led to increased morning glucose and insulin responses in the habitual breakfast skipping group. When comparing the normal protein vs. high protein breakfast meals, the post-lunch glycemic responses were different between the meals but were significantly modulated by the frequency of habitual breakfast consumption. For example, the breakfast skippers experienced lower afternoon and total glucose concentrations following the normal protein breakfast but higher afternoon and total glucose concentrations following the high protein breakfast compared to the breakfast consumers. Collectively, these data suggest that the addition of breakfast, regardless of protein content, has very little effect on post-lunch glycemic control in individuals who habitually skip the morning meal but illustrates novel differences in the glycemic response to high versus normal protein breakfast meals which appears to be influenced by habitual breakfast frequency.

Acute, post-prandial hyperglycemia elicits detrimental, hemodynamic effects on indices of cardiovascular function including increased heart rate, increased systolic and diastolic blood pressure, and increased plasma catecholamines in non-diabetic, healthy populations [78, 79]. In the last fifteen years, several observational studies have demonstrated that increased post-prandial glucose concentrations are associated with a threefold increased risk of various cardiovascular complications [80-83]. In addition, a

prospective study analyzing diabetes, obesity, and lifestyle in 10,000 Australians provided evidence of a dose-dependent relationship between post-prandial glucose concentrations and cardiovascular mortality [84]. In T2DM subjects, elevated post-prandial glucose concentrations (> 10 mmol/L) was associated with a 40% increased risk of myocardial infarction compared to those with post-prandial glucose levels < 8 mmol/L, particularly in women [85, 86]. Post-prandial glucose concentrations also appear to be the most important predictor of cardiovascular disease, even more so than fasting glucose levels or HbA_{1C} levels [78]. Thus, strategies to reduce post-prandial hyperglycemia might be beneficial in preventing the manifestation of T2DM and cardiovascular diseases. One such strategy includes the addition of breakfast, particularly one rich in protein.

Breakfast skipping has been negatively associated with poor glucose control and an increased risk of developing T2DM and cardiovascular disease [10, 13]. Thus, the daily consumption of breakfast may beneficially modulate blood glucose control throughout the day [59].

Jovanovic et al. conducted two randomized crossover design studies in normal weight, healthy adults and in obese individuals with T2DM to examine the effects of skipping breakfast on markers of glucose control and T2DM risk factors [13, 14]. Post-lunch metabolic responses were compared following the consumption of a normal protein (i.e., high CHO) breakfast, or no breakfast, in healthy, normal weight adults and obese adults with T2DM who were habitual breakfast consumers [13, 14]. Compared to skipping breakfast, the consumption of breakfast lowered the post-lunch rise in plasma

glucose by 73% in healthy, normal weight subjects and 95% in those with Type 2 diabetes. The reduced glycemic control during a subsequent meal (i.e., lunch) when the prior meal (i.e., breakfast) is skipped has been termed 'the second meal phenomenon' [13, 14] and has been postulated to occur as a result of an increased glucose conversion into muscle glycogen after lunch [14]. Specifically, Jovanovic et al. reported a 50% increase in postprandial plasma glucose conversion into muscle glycogen after lunch following breakfast compared to when breakfast was skipped [14].

Unlike Jovanovic et al. [13, 14], the current study did not detect improvements in post-lunch glucose or insulin concentrations following the addition of the normal protein (i.e., high CHO) breakfast. This discrepancy may be due to several factors such as age of the subjects, BMI differences, and habitual eating breakfast habits. For example, the average age of subjects in the two Jovanovic et al. studies [13, 14] was 56.1 ± 2.8 years and 46.7 ± 3.9 years, respectively, whereas the average age in the current study was 19 ± 1 years of age. This large difference in age could result in different physiological responses in glycemic control [87, 88].

Additionally, it is unknown whether the subjects in the Jovanovic et al. studies were habitual breakfast skippers or habitual breakfast consumers. In the current study, we observed that habitual breakfast skippers experience lower circulating afternoon and daily glucose concentrations after the consumption of a normal protein (i.e., high CHO) breakfast compared to the breakfast consumers. Thus, frequency of breakfast consumption appears to be an important factor in glycemic responses to CHO-rich breakfast meals. More studies are required to identify the mechanism-of-action by

which habitual breakfast patterns influence the physiological responses to CHO-rich meals.

The replacement of CHO with protein has been shown to decrease the postprandial glucose and/or insulin responses in some [20-26] but not all studies [28-33, 72]. Potential contributors for the conflicting findings might be due to the quantity of protein provided within the meals [89] as well as the type of protein and/or CHO included [20, 28, 29].

Regardless, the current study was the first to examine whether this response would extend into and contribute to the second meal phenomenon. In the current study, no differences in subsequent meal (i.e., lunch) glucose and insulin responses were observed with the high protein breakfast in habitual breakfast skippers. However, those that habitually consumed a CHO-rich breakfast exhibited greater reductions in post-lunch glucose following the high protein breakfast compared to the normal protein breakfast. Although speculative, these data would suggest an increased inability to optimally metabolize and/or utilize a large quantity of protein, at breakfast, in those who do not typically eat the morning meal. Further research is needed to confirm this concept.

Limitations

Several study limitations have been identified. One limitation involves the investigation of only two selected factors, plasma glucose and insulin. Other various

modulators such as circulating FFA may also be involved in the altered glycemic response to NP and HP breakfast meals.

Specifically, Jovanovic et al. observed a decrease in post-lunch free fatty acid (FFA) responses in those who ate breakfast compared to those who skipped breakfast, and discovered a positive correlation to exist between FFA concentrations prior to lunch and the post-lunch rise in plasma glucose [13]. Again, a positive correlation was shown between FFA concentrations prior to lunch and the post-lunch rise in plasma glucose and a negative correlation was shown between FFA concentrations prior to lunch and the post-lunch increase in muscle glycogen synthesis [14]. A potential explanation to improved glycemic control is that prolonged elevation in FFAs, which occur during prolonged fasting periods (i.e., breakfast skipping), results in the inhibition of net hepatic glycogen utilization, increases in gluconeogenesis, and reductions in glucose utilization [14, 15, 60]. This is important due to several studies indicating that increased plasma FFA concentrations leads to insulin resistance and T2DM [61, 62]. Because muscle glycogen synthesis is defective in T2DM patients, the findings in these studies may be applied as a therapeutic strategy. Based on these findings, it is possible that breakfast consumption leads to decreased circulating FFAs which in turn stimulates net hepatic glycogen utilization, decreases gluconeogenesis, and increases glucose utilization.

Another study limitation was that the varying sources of protein at the breakfast meals. Previous studies in a meta-analysis have reported that insulin concentrations are blunted after a meal containing slow-digesting protein, like casein, compared to fast-

digesting protein, like whey [23, 90]. Although the HP meals contained similar quantities of beef and egg protein between the breakfast groups in the current study, the NP breakfast meals were void of egg and beef and primarily contained dairy and wheat protein. Thus, the absence of significant insulin increases with the HP breakfast meals might have been due to the varied protein qualities within and between the breakfast meals.

Further research involving long-term intervention is necessary to confirm the present findings and identify long-term implications of glycemic control.

CONCLUSIONS

In summary, these data suggest that the addition of breakfast, regardless of protein content, has very little effect on post-lunch glycemic control in individuals who habitually skip the morning meal but illustrates novel differences in the glycemic response to high versus normal protein breakfast meals which appears to be influenced by habitual breakfast frequency. Thus, the addition of breakfast, particularly one rich in protein, may be an essential dietary approach to improve glycemic control in habitual high CHO consuming overweight/obese adolescents, but not in their habitual breakfast skipping counterparts.

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Table 2*: Subject characteristics*

Subject Characteristics	Breakfast Skippers (n=20)	Breakfast Consumers (n=15)
Age (y)	19 ± 1	19 ± 1
Height (cm)	167 ± 1	167 ± 2
Weight (kg)	79.6 ± 2.1	78.8 ± 2.6
BMI (kg/m^2)	28.6 ± 0.7	28.3 ± 0.7
Skips Breakfast (#/week)	6 ± 1	1 ± 1
First Eating or Drinking Occasion of the Day	12:30 ± 0:15 pm	8:15 ± 0:11 am
Fasting Glucose (mg/dL)	85.3 ± 3.3	86 ± 2
Fasting Insulin (pg/mL)	462.7 ± 230.3	577 ± 83.5

Table 3: *Dietary characteristics during the 8h testing day in Breakfast Skippers and Breakfast Consumers*

	Breakfast Skippers	Breakfast Skippers & Breakfast Consumers	Breakfast Skippers & Breakfast Consumers
	Breakfast Skipping Treatment	Normal Protein Breakfast Treatment	High Protein Breakfast Treatment
Breakfast			
Energy (kcal)	0	350	350
Total Protein (g)	0	12 ± 2	32 ± 2
CHO (g)	0	59 ± 2	38 ± 2
Fat (g)	0	8 ± 0	8 ± 0
Lunch			
Energy (kcal)	500	500	500
Total Protein (g)	17 ± 2	17 ± 2	17 ± 2
CHO (g)	83 ± 2	83 ± 2	83 ± 2
Fat (g)	11 ± 0	11 ± 0	11 ± 0

Figure 6: *Plasma glucose responses throughout the 8h testing day following the various breakfast patterns in habitual Breakfast Skippers. Different letters denote significance; P < 0.05.* ■ *Breakfast Skipping (BS);* ◆ *Normal Protein Breakfast (NP);* ○ *High Protein Breakfast (HP)*

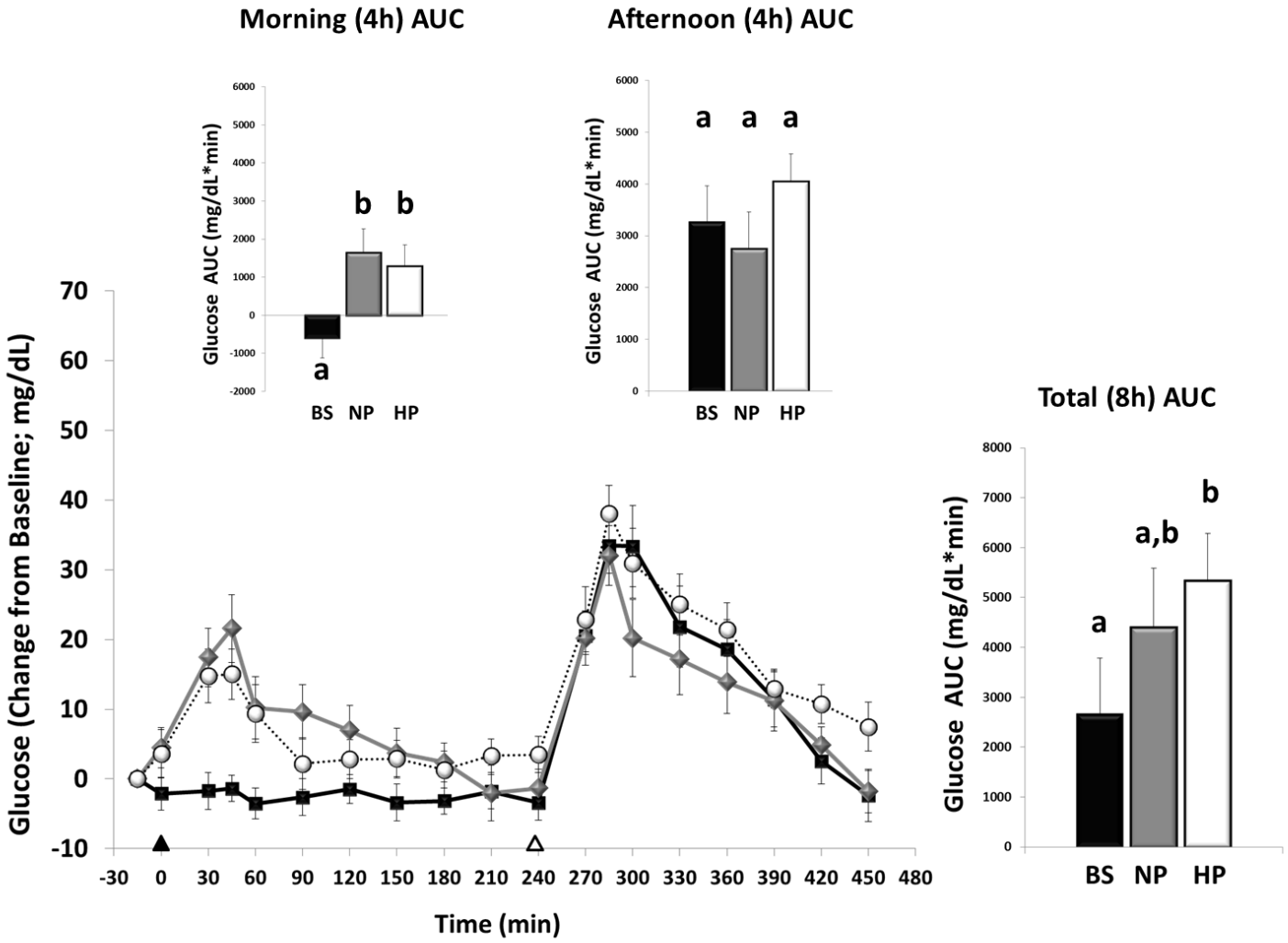


Figure 7: *Plasma glucose responses throughout the 8h testing day following the various breakfast patterns in habitual Breakfast Consumers. Different letters denote significance; P < 0.05.* ◆ *Normal Protein Breakfast (NP);* **O** *High Protein Breakfast (HP)*

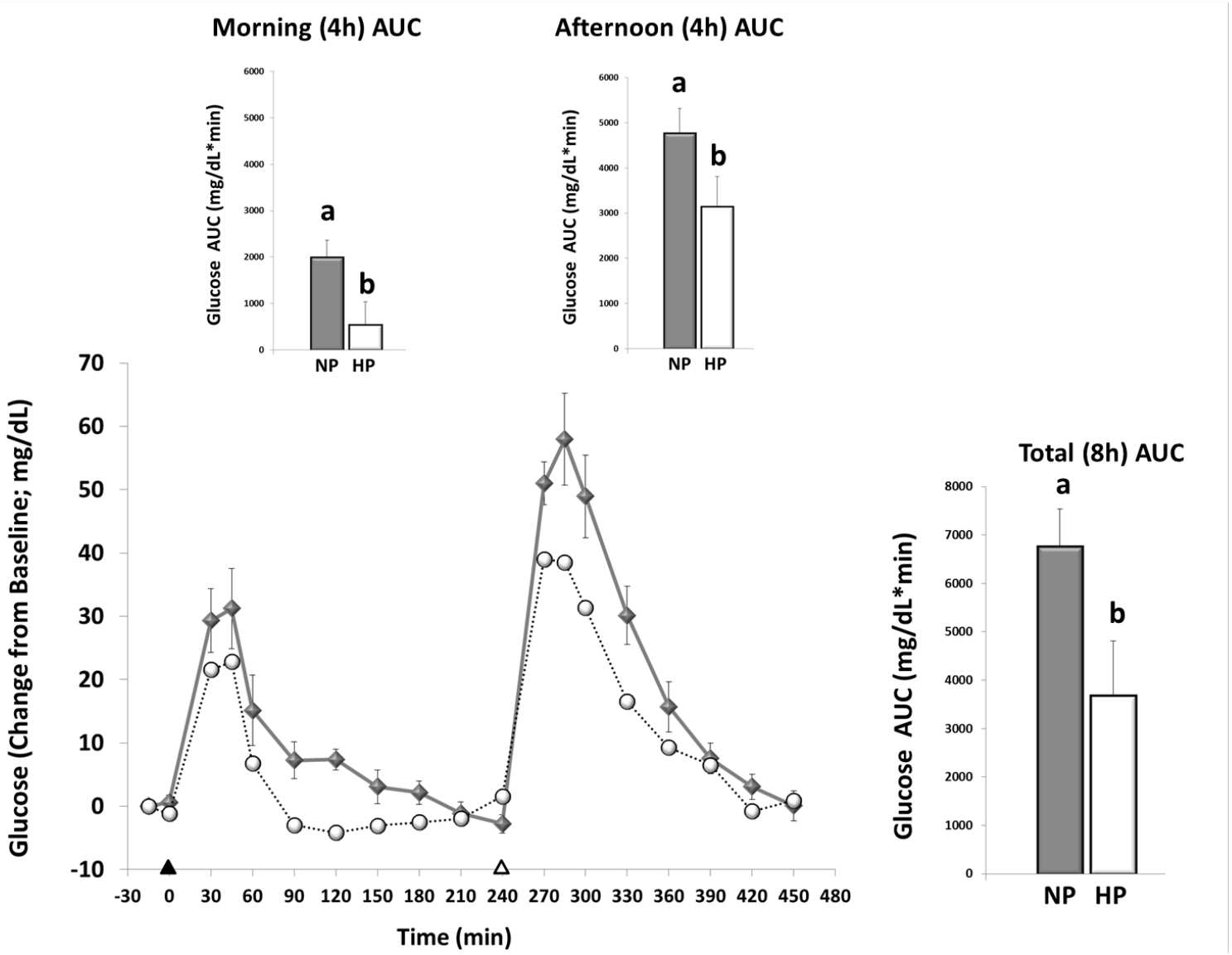


Figure 8: *Plasma insulin responses throughout the 8h testing day following the various breakfast patterns in habitual Breakfast Skippers. Different letters denote significance; P < 0.05.* ■ *Breakfast Skipping (BS);* ◆ *Normal Protein Breakfast (NP);* ○ *High Protein Breakfast (HP)*

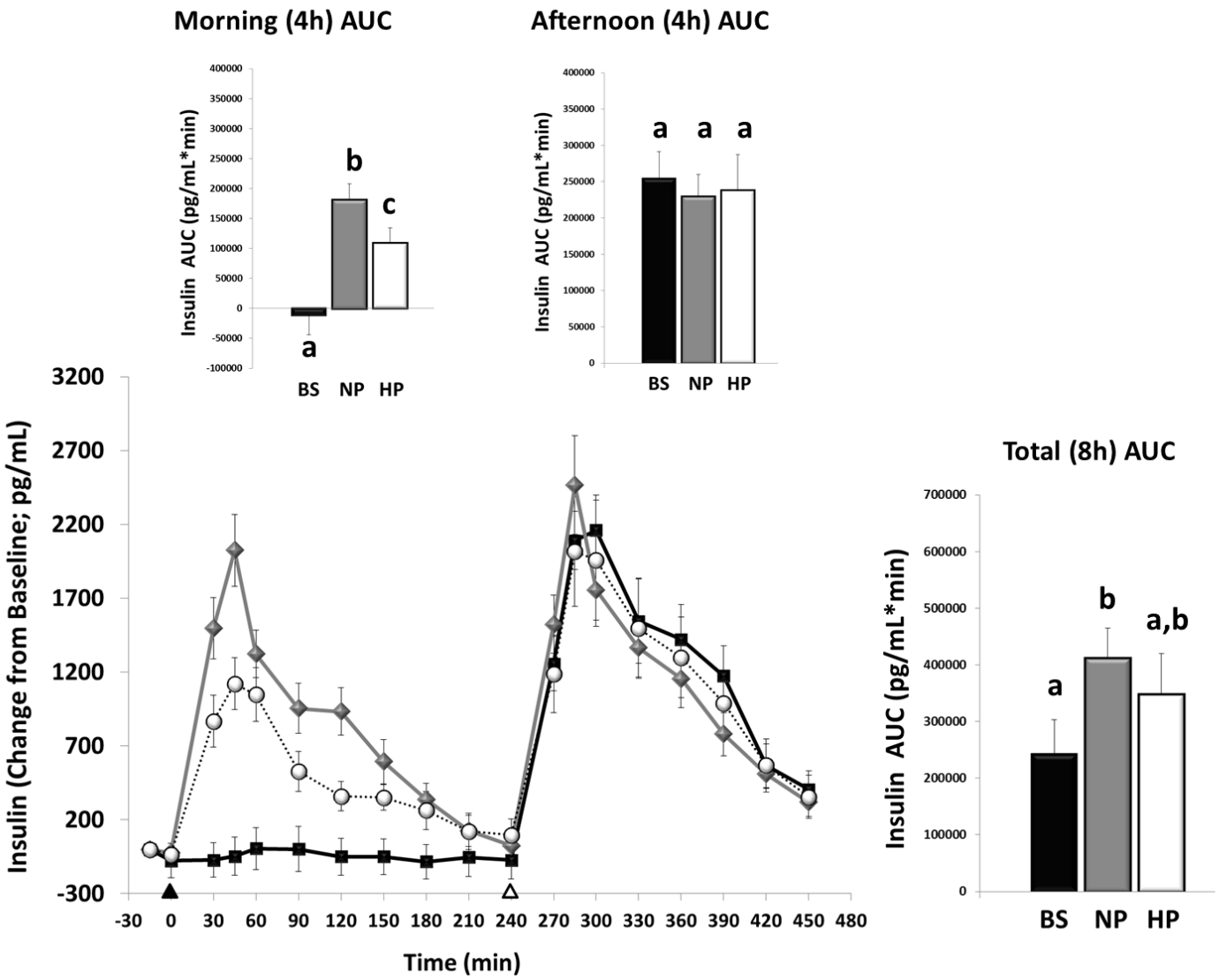


Figure 9: *Plasma insulin responses throughout the 8h testing day following the various breakfast patterns in habitual Breakfast Consumers. Different letters denote significance; P < 0.05.* ◆ *Normal Protein Breakfast (NP);* ○ *High Protein Breakfast (HP)*

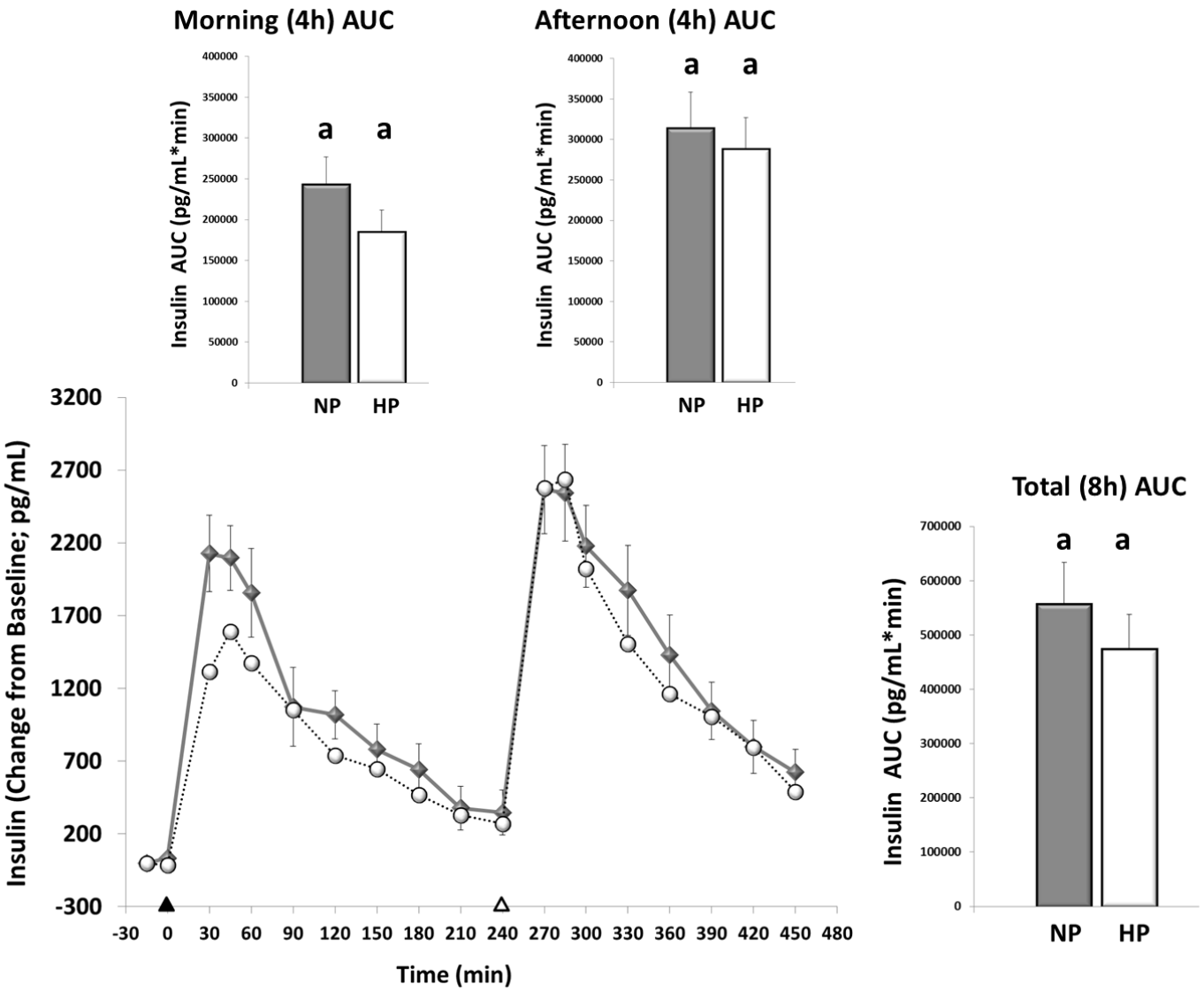


Figure 10: *Plasma glucose responses throughout the 8h testing day following the Normal Protein vs. High Protein breakfast meals in habitual Breakfast Skippers vs. Breakfast Consumers. Different letters denote significance; P < 0.05.* ☐ *Breakfast Skipping (BS)-Normal Protein (NP) Meal;* ■ *BS-High Protein (HP);* ○ *Breakfast Consuming (BC)-NP;* ● *BC-HP*

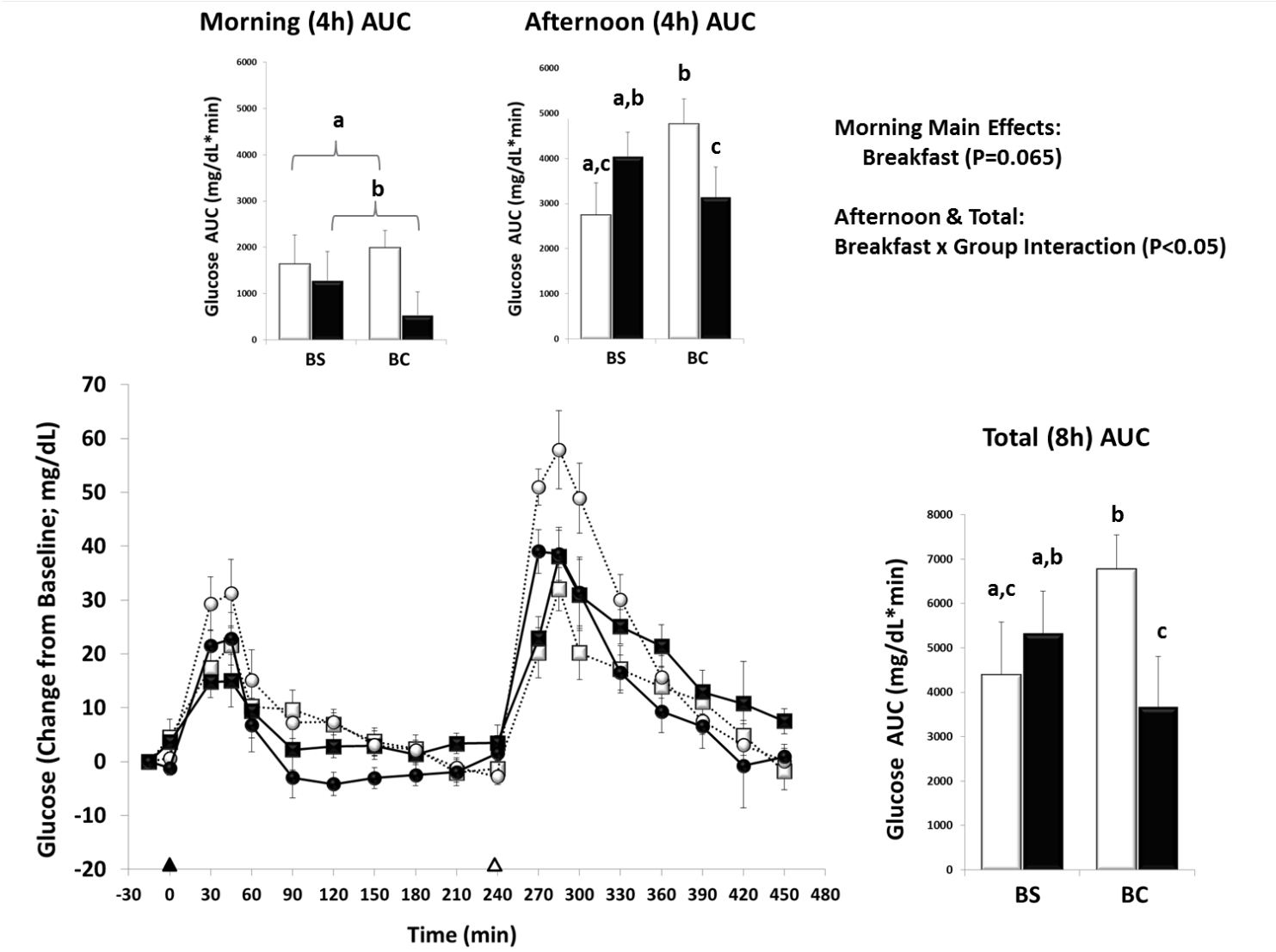
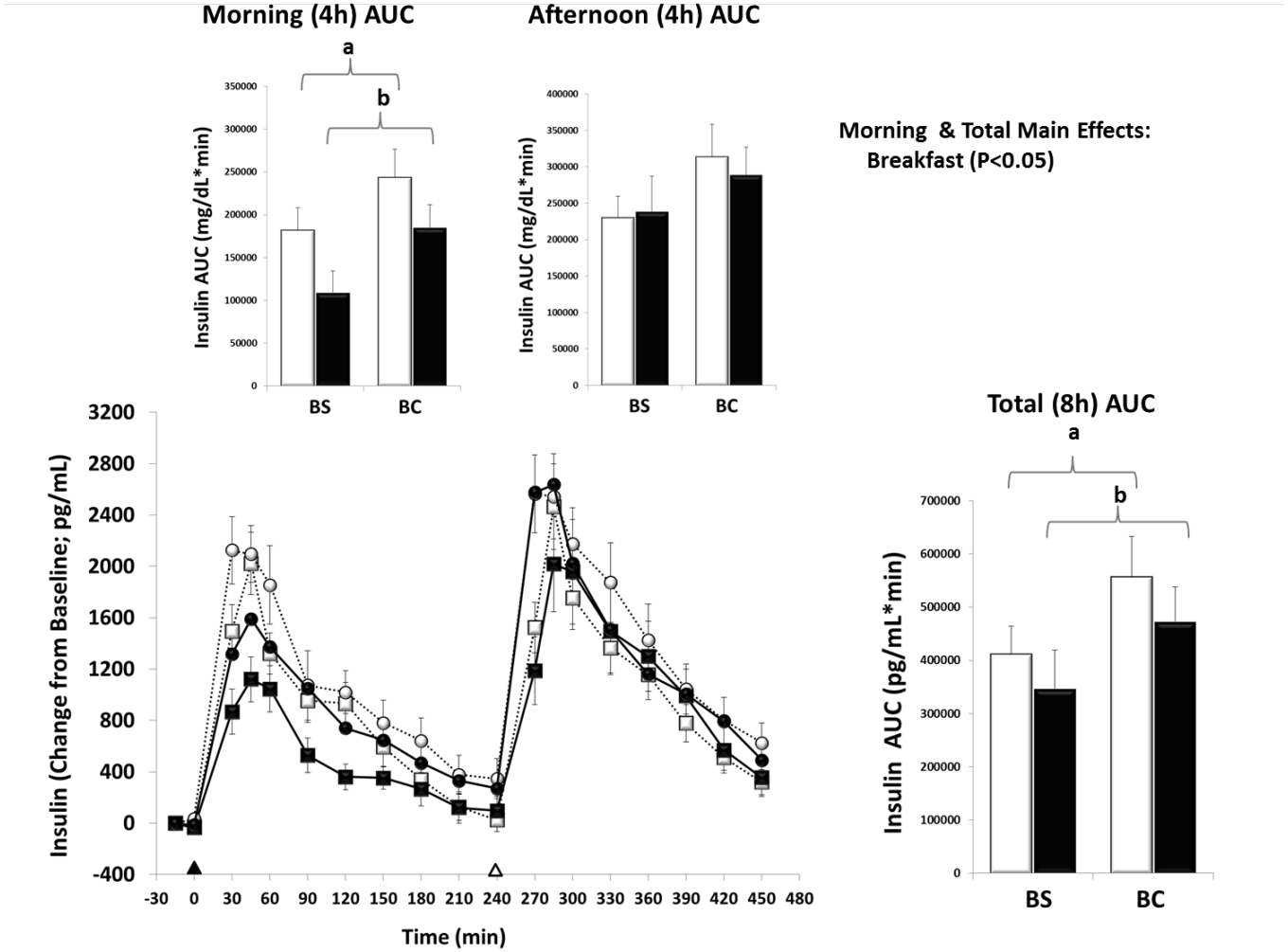


Figure 11: *Plasma insulin responses throughout the 8h testing day following the Normal Protein vs. High Protein breakfast meals in habitual Breakfast Skippers vs. Breakfast Consumers. Different letters denote significance; P < 0.05.* ☐*Breakfast Skipping (BS)-Normal Protein (NP) Meal;* ■*BS-High Protein (HP);* ○*Breakfast Consuming (BC)-NP;* ●*BC-HP*



APPENDICES

SCREENING CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATOR'S NAME: HEATHER J. LEIDY
PROJECT #: 1204820

Study Title: Power-up with Protein

INTRODUCTION

This consent may contain words that you do not understand. Please ask the Investigator or the s staff to explain any words or information that you do not clearly understand.

Note: If you are the parent of a potential participant (between the ages of 16 and 17 years), plea aware that the language in the consent is directly written for the study participant. Thus, we use and 'yours' to describe the procedures that the study participant will be completing.

This is a research study. Research studies include only people who choose to participate. As a stud participant, you have the right to know about the procedures that will be used in this research stud you can make the decision whether or not to participate. The information presented here is simply effort to make you better informed so that you may give or withhold your consent to participate in research study.

Please take your time to make your decision and discuss it with your family and friends.

You are being asked to take part in this study because you are a young people who regularly eat carbohydrate-rich breakfast and lunch meals.

This study is being sponsored by the Egg Nutrition Center/American Egg Board and National Cattle Beef Association.

In order to participate in this study, it will be necessary to give your written consent/assent.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to identify how the body and mind respond to the daily consumption o breakfast and lunch meals differing in normal and higher protein foods.

This research is being done because we currently do not know how the body and mind respond to specific combinations of normal vs. higher protein breakfast and lunch meals.

How Many People Will Take Part In The Screening Phase of the Study?

About 100 people will take part in the screening phase of this study at the University of Missouri, Columbia, MO.

What Is Involved in the Screening Phase of the Study?

If you participate in the screening phase of this study, you will complete the following items:

- Body Weight: This will be measured to the nearest 0.1 kg using a research scale in a self-contained room.

- Body Height: This will be measured to the nearest cm using a research stadiometer, which is a wall-mounted ruler.

- Pregnancy Test: This will consist of collecting a small urine sample to assess whether you are pregnant.

- Medical History, Dietary, and Physical Activity Questionnaire: The purpose of this questionnaire is to provide us with information concerning your past and current medical conditions, illnesses, diseases; medical surgeries; and medications. This is important for us to be aware of – as many health conditions, diseases, and medications can influence many of the outcomes we are measuring. Filling out this questionnaire will also allow us to know whether you have any food allergies, food intolerances, or if you are latex-intolerant. You will provide, to the best of your knowledge, a complete history of all of your medical disorders, diseases, medications, and medical procedures from birth to present. It is expected that no new medications, drugs, or supplements will be started during this study, nor will the dose of current medications change during this time. However, if any additions or changes occur, you will contact Dr. Leidy as soon as possible (so that she can assess whether these changes will influence the testing procedures—this is especially true with over-the-counter cough, cold, or allergy medicines). It is especially important for us to be aware of your day-to-day dietary practices, habits, and foods that you eat and avoid, to make sure you are a good candidate for this dietary study. We will also gain information about your day-to-day physical activity and exercise practices and habits, which we would like for you to maintain throughout the study.

- MRI Screening Questionnaire: The purpose of this questionnaire is to provide us with information concerning whether you would be permitted and/or are able to have an MRI scan performed (i.e., no pacemakers, metal implants, etc.).

How Long Will the Screening Phase of the Study Last?

The screening phase of the study will last approximately 1 hour.

You can stop participating at any time. Your decision to withdraw from the screening phase of the study will not affect in any way your medical care and/or benefits.

What Are the Risks of the Screening Phase of the Study?

There are no known risks with participating in any of the screening procedures.

Are There Benefits to Taking Part in The Screening Phase of the Study?

You may benefit from participation in this screening by gaining information about your body weight status as well as being informed of the effects of different breakfast and lunch meal combinations. You may also perceive benefits in learning about how eating healthy, protein-rich breakfast and lunch meals may improve your appetite control and unhealthy snacking.

What Other Options Are There?

An alternative is to not participate in this research study.

What about Confidentiality?

Information produced during the screening phase of this study will be stored in the Investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. If the Investigator conducting this study is not your primary, or regular doctor, she must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in the screening phase of the study.

It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the Food and Drug Administration (FDA), federal or state government agencies, or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by any of these agencies, the University of Missouri will use reasonable efforts to protect your privacy and the confidentiality of your medical information.

Some of the screening data collected will become part of the study data. Thus, the results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials.

If you do not meet the study criteria and/or for any reason decide not to participate in the study, all screening data will be shredded.

What Are the Costs?

There is no cost to you for completing the screening phase. The only cost you will have is the cost associated with traveling to and from the University of Missouri.

Will I be Paid for Participating in the Screening Phase of the Study?

You will not receive compensation for completing the screening procedures.

What if I am Injured?

It is not the policy of the University of Missouri to compensate human participants in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to participants who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

What Are My Rights as a Participant?

Participation in the screening phase of the study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate. If you decide to participate, you can change your mind and drop out of the screening phase at any time without affecting your present or future care in the institution. Leaving the screening phase will not result in any penalty or loss of benefits to which you are entitled. In addition, the investigator of this study may decide to end your participation in the screening phase of the study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed.

Whom Do I Call if I Have Questions or Problems?

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Heather J. Leidy, Primary Investigator, at 573-882-0654.

A copy of this consent form will be given to you to keep.

SIGNATURE

I confirm that the purpose of the research, the screening procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in the screening phase of the study.

Participant (if ≥ 18 years of age)	Date
Participant – Assent to Participate (if between the ages of 16-17 years)	Date
Legal Guardian/Advocate/Parent	Date
Additional Signature (if required); Identify relationship to Participant	Date

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the screening procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the screening phase of the study to the best of my ability.

Study Representative	Date

STUDY CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATOR'S NAME: HEATHER J. LEIDY
PROJECT #: 1204820

Study Title: Power-up with Protein

INTRODUCTION

This consent may contain words that you do not understand. Please ask the Investigator or the study staff to explain any words or information that you do not clearly understand.

Note: If you are the parent of a potential participant (between the ages of 16 and 17 years), please be aware that the language in the consent is directly written for the study participant. Thus, we use 'you' and 'yours' to describe the procedures that the study participant will be completing.

This is a research study. Research studies include only people who choose to participate. As a study participant, you have the right to know about the procedures that will be used in this research study so you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You are being asked to take part in this study because you are a young person who regularly eats carbohydrate-rich breakfast and lunch meals.

This study is being sponsored by the Egg Nutrition Center/American Egg Board and National Cattlemen's Beef Association.

In order to participate in this study, it will be necessary to give your written consent/assent.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to identify how the body and mind respond to the daily consumption of breakfast and lunch meals differing in normal and higher protein foods.

This research is being done because we currently do not know how the body and mind respond to specific combinations of normal vs. higher protein breakfast and lunch meals.

How Many People Will Take Part In The Actual Study?

About 30 people will take part in the actual study at the University of Missouri, Columbia, MO.

What Is Involved in the Study?

If you decide to take part in this study, there will be 4 different meal patterns to complete in any order. All of these meals include breakfast and lunch.

Each meal pattern will be followed for 4 days. For all patterns, we will provide different types of breakfast and lunch meals to consume at home. You will be expected to eat the breakfast meals between 6-9 am and the lunch meals between 10 am-1 pm for 3 days for each pattern.

Day 0 (Pre-testing day Brain Scan):
Teeth braces and/or retainers are of no risk during the testing day brain scans (which are described below). Braces and/or retainers do not generally exclude you/your child from this procedure. However, in a few instances, some types of braces or retainers cause disruption in the brain image (picture) that we receive during the scan. This disruption leads to unusable data. Thus, if you/your child have/has braces, a 5-minute pre-testing day brain scan will be performed one time (during Day 0). If no disruption in the brain image picture occurs, all testing day procedures (and brain scans) will be performed for each testing day. If disruption occurs, you/your child will continue with completing all other study procedures for all testing days but will not complete the brain scan procedures.

Days 1-3 (for each pattern):
Each meal will be prepared in a separate container and labeled as Pattern #, Breakfast Day 1-3 or Lunch Day 1-3. Each day, you will read the breakfast meal and lunch meal instruction sheets. These sheets include the directions for preparing each meal, a check-off log listing all of the foods to be consumed, and several short questionnaires regarding your feelings, thoughts, and mood towards the meal. You will be permitted to only eat the foods provided by the study for breakfast and lunch. However, after breakfast **and** lunch are completed, you can eat or drink anything else you choose to eat throughout the remainder of the day. All of the meals consist of normal foods commonly consumed by those who eat breakfast and lunch on a daily basis.

On Day 3 of each pattern, you will be given a dinner meal to eat between 3-6 pm. Once you eat this meal at the designated time, you will be expected to not eat or drink anything else until breakfast at our facility the next morning.

Day 4-Testing Day (for each pattern):
On Day 4 of each pattern, you will report to either McKee Gym Rm 10 on the MU Campus or the Clinical Research Center located on the 5th school of the School of Medicine on the MU Campus to complete the 10-hour testing days.

Here are the procedures that will be completed during each 10-hour testing day:

- Upon arriving, you will have your body weight measured and will then sit in a comfortable, reclining chair. All of the testing procedures will be explained one more time.

- You will be given an activity monitor to wear throughout the testing day. The monitor is a small device that looks like a pager, which measures your activity levels while you wear it. You will keep this on the entire day and will continue to wear it when you go home.

- When you are ready to begin the testing day, a catheter, which is a thin flexible, plastic tube, will be inserted into a vein located in the front (inside) of the elbow by one of the highly-trained research staff members using sterile techniques. The vein will be kept patent (i.e. 'cleared' or 'flushed') throughout the day by using a slow continuous dripping of sterile saline solution. We use the catheter so that we can take multiple blood samples without having to stick you multiple times.

- A small blood sample (~ 1 teaspoon) will be drawn from the catheter. The blood sample will be used to measure hormones (chemical messengers in the body) that respond to food intake. Also at that time, a questionnaire will be completed which asks about your hunger feelings, thoughts of food, and mood.

- We will provide breakfast, lunch and dinner at specific times. These will include common foods that young people typically eat on a daily basis.

- Right before dinner, the catheter will be removed and a brain scan will be completed using a brain imaging method known as Magnetic Resonance Imaging (MRI). This technique examines how water molecules in the brain behave in a strong magnetic field. MRI provides a detailed picture of what the brain looks like. We also use the functional MRI technique which provides information on blood flow and "brain activation." This is a non-radioactive (i.e., no x-rays), non-invasive technique. During this scan, you will lie on a table that "slides" into the scanner. Your head will be set in a specific testing position – making it difficult for you to move your head. During the scan, you will view numerous food, animal, scenery, and blurry pictures. This procedure lasts approximately 30 minutes.

- Throughout the day (when you are not completing the testing procedures), you will have "free time" to do the following things:

    - We will provide a laptop to play a number of "Hidden Objects/Seek and Find" computer games or check email, Facebook, etc.

    - We will provide a DVD player with movies to choose from.

    - You can bring magazines and/or books to read.

    - You will be permitted to use the restrooms in the facility at any time.

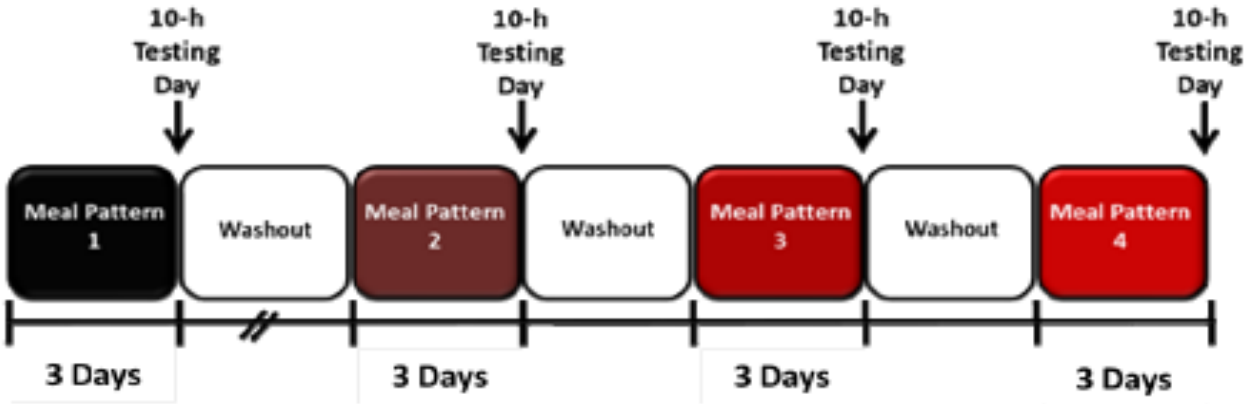
- At the end of the testing day we will provide a pack-out cooler containing numerous snacks to freely consume throughout the remainder of the evening.

You will repeat these procedures for each of the next 3 meal patterns.

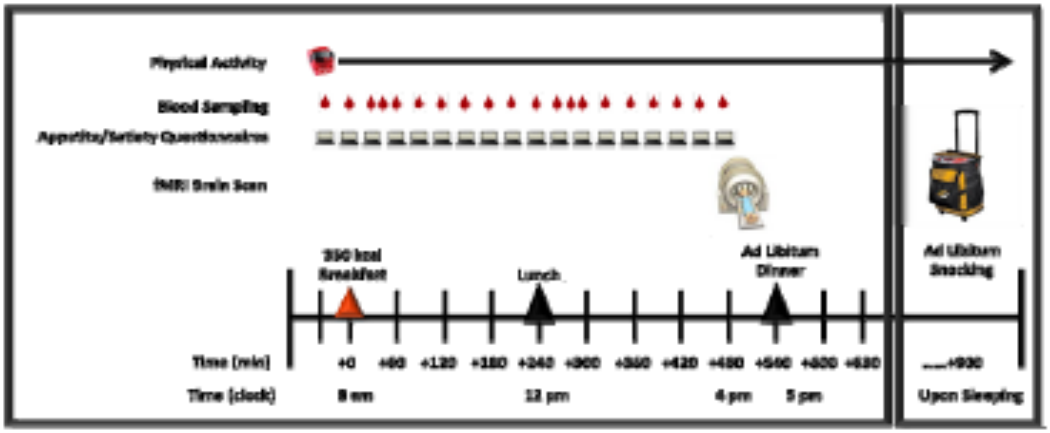
Each meal pattern lasts for 4 days (i.e., 3 acclimation days and 1 testing day/pattern). There will be a washout period in between each pattern. During this time, you will simply return to your normal breakfast and lunch meals. The washout periods can be a short as 7 days or as long as 3 weeks, depending on holidays, schedules, etc.

We have performed similar studies in this age group and have found that most young people easily tolerate and actually enjoy each part of the study.

The diagrams below show the entire study overview and the specific 10-hour testing day procedures:



Study Timeline



How Long Will I Be in the Study?

You will be completing 4 meal patterns which are 4 days/pattern in duration. Thus, the study procedures last for a total of 16 days. However, due to the washout periods in between the meal patterns, you will be enrolled in the study between 7 weeks and 16 weeks.

The Investigator may decide to take you off this study if new medication is prescribed by your doctor that would alter the study outcomes or if you are not correctly following the meal patterns.

You can stop participating at any time. Your decision to withdraw from the study will not affect in any way your medical care and/or benefits.

However, if you decide to withdraw from the study, we ask that all study forms and supplies be returned to our facility in a timely manner.

What Are the Risks of the Study?

While in the study, you are at risk for the side effects described below. You should discuss these with the Investigator and/or your doctor. There may also be other side effects that we cannot predict. Many side effects go away shortly after each testing day is completed, but in some cases side effects can be serious or long-lasting or permanent.

Risks and side effects related to the <u>study meals</u> include:

<u>Unlikely; with some Short-term Discomfort; Otherwise not Serious</u>:
Your stomach and/or bowels may become slightly upset due to the changes in your usual food and beverage intake. Any discomfort should stop within 1-2 days.

Risks and side effects related to the blood collection procedures include:

<u>Likely; with some Short-term Discomfort</u>
There may be some risks when having a catheter inserted into your arm. During the insertion, some pain may be felt which feels like a slight pinch. The pain will end within seconds after the insertion is completed.

There is a risk of developing a small bruise and/or infection at the catheter site. However, the catheter will be inserted by a highly trained technician or registered nurse using sterile techniques.

You may feel lightheaded and may faint at the sight of blood. Neither of these will occur due to the amount of blood being drawn. In fact, throughout each testing day, we will collect approximately 80 ml (2.7 oz.)/testing day; this is about 17% (for each testing day) of what would be taken if you donated blood through the American Red Cross. Thus, the amount of blood collected is small enough not to present any hazard to your physical well-being. However, you must agree not to donate blood for at least one month prior to, during, and for one month after the study.

There is a risk of developing a small rash or rash-like symptoms including redness, bumps and itching as a result of the adhesive used to secure the catheter and IV tubing to the arm during the testing day. This irritation generally clears up within 2 hours after removing the adhesive.

There are no substantial risks associated with this procedure.

Risks and side effects related to the brain scan (MRI) procedures include:

Less Likely; with some Short-term Discomfort

Unlike x-rays or CT-scans, MRI does not involve any ionizing radiation. However, the tasks may cause some fatigue similar to reading a book or doing homework. You may also experience discomfort from lying still. If this happens, please let us know and we will arrange for you to adjust your position. Additionally:

The safety of MRI has been evaluated over the past 20 years and no short-term effects have been observed. However, the long-term effects of MRI on the body are not fully known. Some individuals with claustrophobia (fear of closed or confining spaces) may find the MRI equipment too confining. In that case, you can request to be removed from the scanner and this will be done immediately. If you have any concerns about this, you can be placed in a MRI simulator to determine if the confining aspects and noises are too uncomfortable.

The MRI scanner makes sounds variously described as "thumping", "pounding", "banging", "chirping" and "buzzing"; these sounds can be loud. You will be required to wear protective earplugs and headphones during scanning to reduce the noise. However, you will be able to hear the Technologist and he/she can hear your voice when you respond.

The Investigators for this research project are not Licensed or Trained Diagnosticians or Clinicians. The testing performed in this project is not intended to find abnormalities, and the images or data collected do not comprise a diagnostic or clinical study. The Investigators and the University of Missouri are not responsible for failing to find abnormalities. However, on occasion the Investigators may perceive possible abnormalities. When this occurs, the Brain Imaging Center will consult with a Specialist. If the Specialist determines that additional inquiry is warranted, a staff person from the Brain Imaging Center will contact you. In such case, you are advised to consult with a Licensed Physician to determine whether further examination or treatment would be prudent. The Investigators, Specialist, Brain Imaging Center and the University of Missouri are not responsible for any decision you make with regard to examination or treatment. Because the images collected for this research project do not comprise a diagnostic or clinical study, the images will not be made available for diagnostic or clinical purposes.

No short-term effects to a fetus from this procedure have been observed. However, the long-term effects of MRI on the fetus are not fully known. Therefore, if you are sexually active and capable of becoming pregnant, you must use an effective method of birth control while participating in this research. If you are a subject in a multi-session study and become pregnant during the course of that study, you will no longer be able to participate in this MRI research study for the duration of your pregnancy.

You cannot have an MRI if you have any metal in or near your brain such as an aneurysm clip or a cochlear implant, or other contra-indicated implants such as a pacemaker for your heart or metal-containing prostheses (like a 'stent' or a heart valve, hearing aids, etc.). For example, welders and metal workers may be at risk for a MRI because they may have gotten small metal fragments in their eyes. This would be dangerous inside the magnet. There are also possible risks for participants if metal objects are drawn to the magnet while a participant is within or near the bore. Accordingly, you will be asked to leave all jewelry and metal objects outside of the testing area. No loose metal objects will be allowed near the magnet. Many items of clothing contain metal hooks, wires, etc. and some of these cannot be worn in the MRI device. We have clean garments that you can wear in this case.

For the reasons stated above the Investigator will observe you closely while giving the treatment described and, if you have any worrisome symptoms or symptoms that the Investigator or her associates have described, notify the Investigator immediately. The Investigator's telephone number is 573-882-0654. For more information about risks and side effects, please feel free to ask the Investigator.

Are There Benefits to Taking Part in the Study?

The need for healthy eating is an important part of everyone's lifestyle. With increasing concerns of obesity and other health related factors with food consumption, it is important to gain understanding in the quality of food that we consume on a daily basis. By examining different eating patterns, we are able to gain a better understanding surrounding the control of appetite and food intake regulation.

The knowledge gained from the results of this study will benefit society by providing insights as to whether a modest dietary strategy containing increased dietary protein at breakfast and/or lunch might lead to beneficial alterations in appetite control and subsequent food intake for better weight management.

You may individually experience benefits from this study by understanding the differences between different meal types and the associated effects on appetite control, food reward/cravings, and unhealthy snacking.

Great care will be taken in the planning and execution of this study to help ensure the safety and well-being of each participant. The risks associated with participation are no more than a minor increase over minimal risk; the potential gains for the participant, as well as society as a whole, are great. We believe this research is very important to combat adolescent obesity. Safe and effective ways to control body weight are important to prevent or delay the onset of complications from obesity, such as diabetes and heart disease. Overall, we believe this research protocol presents a very good risk-benefit ratio. Further, we are confident that this study will provide meaningful and useful information to aid in the development of recommendations to better manage energy balance and hopefully curb the increasing prevalence of abnormal weight regulation.

What Other Options Are There?

An alternative is to not participate in this research study.

What about Confidentiality?

Information produced by this study will be stored in the Investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. If the Investigator conducting this study is not your primary or regular doctor, she must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in this study.

It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the Food and Drug Administration (FDA), federal or state government agencies, or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by any of these agencies, the University of Missouri will use reasonable efforts to protect your privacy and the confidentiality of your medical information.

The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials.

In addition, if photographs are taken during the study that could identify you, you must give special written permission for their use. In that case, you will be given the opportunity to view the photographs before you give permission for their use if you so request.

What Are the Costs?

There is no cost to you for the meals, dietary information, blood analyses, and the brain scan images of your brain that are all part of this research study. Parking is also free of charge at McKee Gymnasium, Patient Parking at the Hospital, and the Brain Imaging Center. The only cost you will have is the cost with traveling to and from the University of Missouri.

Will I be Paid for Participating in the Study?

You will be compensated a total of $600 for completing all study procedures. Specifically, you will be paid $150 for completing each meal pattern which includes the 10-hour testing day and the 6 days of correctly following the specific meal pattern at home.

What if I am Injured?

It is not the policy of the University of Missouri to compensate human participants in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to participants who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk

Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

What Are My Rights as a Participant?

Participation in this study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate. If you decide to participate, you can change your mind and drop out of the study at any time without affecting your present or future care in the institution. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. In addition, the Investigator of this study may decide to end your participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed.

You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

Whom Do I Call if I Have Questions or Problems?

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Heather J. Leidy, Primary Investigator, at 573-882-0654.

A copy of this consent form will be given to you to keep.

SIGNATURE

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

Participant (if ≥ 18 years of age)	Date
Participant – Assent to Participate (if between the ages of 16-17 years)	Date
Legal Guardian/Advocate/Parent	Date
Additional Signature (if required); Identify relationship to Participant	Date

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

Study Representative	Date

BLOOD STORAGE

I agree to allow the use of my blood samples collected during this study to be used for future research that might be unrelated to this study. The blood samples will be stored for 10 years. These samples will likely be used for future analysis of food intake and appetite hormones that have not yet been identified or are currently unable to be measured. The use and disclosures of personal information listed in the consent form also apply to the saved blood samples. However, at any time, I can request that the blood samples be destroyed if I change my mind. If this occurs, I will provide a written request to Dr. Leidy at 204 Gwynn Hall; University of Missouri; Columbia, MO 65211. Lastly, I understand that Dr. Leidy can use and share information that was gathered before this request was received.

Participant (if ≥ 18 years of age) Date

Participant – Assent to Participate (if between the ages of 16-17 years) Date

Legal Guardian/Advocate/Parent Date

OR

I request my blood samples collected during this study to NOT be used for any future research that is unrelated to this study. I understand that I can still participate in this study if I refuse to have the blood samples retained.

Participant Date

Participant – Assent to Participate (if between the ages of 16-17 years) Date

Legal Guardian/Advocate/Parent Date

SCREENING CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATOR'S NAME: HEATHER J. LEIDY
PROJECT #: 1173258

FOR HS IRB USE ONLY
APPROVED
8/27/10
HS IRB Authorized Representative Date
EXPIRATION DATE: 8-27-2011

STUDY TITLE: **THE BENEFICIAL EFFECTS OF DIFFERENT BREAKFAST MEALS ON APPETITE CONTROL & COGNITION IN 'BREAKFAST SKIPPING' YOUNG WOMEN**

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.

This is a research study. Research studies include only people who choose to participate. As a study participant/parent of a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate/allow your daughter to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You/your daughter are being asked to take part in this study because you/your daughter follow the potentially unhealthy habit of skipping breakfast.

This study is being sponsored by the American Egg Board/Egg Nutrition Center and the National Cattlemen's Beef Association.

In order to participate in this study, it will be necessary to give your written consent.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to identify how the body and mind respond to the daily consumption of different breakfast meals in 'breakfast skipping' young women.

This research is being done because we currently do not know why breakfast is widely assumed to be "the most important meal of the day."

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 25 people will take part in this study at the University of Missouri, Columbia, MO.

WHAT IS INVOLVED IN THE SCREENING PHASE OF THE STUDY?

If you/your daughter take(s) part in the screening phase of this study, you/your daughter will complete the following items:

- Body Weight: This will be measured to the nearest 0.1 kg using a research scale in a self-contained room.

- Body Height: This will be measured to the nearest cm using a research stadiometer, which is a wall-mounted ruler.

- Medical History Questionnaire: The purpose of this questionnaire is to provide us with information concerning your/your daughter's past and current medical conditions, illness, diseases; medical surgeries; and, medications. This is important for us to be aware of as many health conditions, diseases, and medications can influence many of the outcomes we are measuring. Filling out this questionnaire will also allow us to know whether you/your daughter have any food allergies, food intolerances, or if you/your daughter are latex-intolerant. You/your daughter will provide, to the best of your knowledge, a complete history of all of your/your daughter's medical disorders, diseases, medications, and medical procedures from birth to present. It is expected that no new medications, drugs, or supplements will be started during this study nor will the dose of current medications change during this time. However, if any additions or changes occur, you will contact Dr. Leidy as soon as possible (so that she can assess whether these changes will influence the testing procedures—this is especially true with over-the-counter cough, cold, or allergy medicines).

- Dietary Questionnaire: The purpose of this questionnaire is to provide us with information concerning your/your daughter's day-to-day dietary practices, habits, and foods that you/your daughter eat and avoid. This is especially important for us to be aware of to make sure you/your daughter is a habitual 'breakfast skipper' and a good candidate for the dietary part of the study.

- Physical Activity Questionnaire: The purpose of this questionnaire is to provide us with information concerning your/your daughter's day-to-day physical activity and exercise practices and habits. We would like for you/your daughter to maintain these practices throughout the study.

- Acclimation to the MRI scanner: To rule out any potential discomfort, anxiety, and claustrophobia during the study brain scan, we will ask that you/your daughter lie in the mock (i.e., pretend) MRI

scanner to become familiar with the small space, air flow, head position, and limited head movement that is required.

- Generalized Study Procedures Questionnaire: The purpose of this questionnaire is to confirm that you/your daughter have appropriate comprehension of the purpose of the study as well as what procedures will be completed, potential risks involved, and what is asked of you/your daughter.

How Long Will the Screening Phase of the Study Last?

We think you will be in the screening phase for approximately 1 hour.

You can stop participating at any time. Your decision to withdraw from the screening phase of the study will not affect in any way your medical care and/or benefits.

What Are the Risks of the Study?

There are no known risks with participating in any of the screening procedures.

Are There Benefits to Taking Part in The Screening Phase of the Study?

You /your daughter may benefit from participation in this screening by gaining information about your/your daughter's body weight status as well as being informed of the negative effects of skipping breakfast. You/your daughter may also perceive benefits in learning about how eating breakfast on a daily basis may improve your/your daughter's appetite control and cognitive function.

What Other Options Are There?

An alternative is to not participate in this research study.

What about Confidentiality?

Information produced during the screening phase of this study will be stored in the investigator's file and identified by a code number only. The code key connecting your/your daughter's name to specific information about you/your daughter will be kept in a separate, secure location. Information contained in your/your daughter's records may not be given to anyone unaffiliated with the study in a form that could identify you/your daughter without your written consent, except as required by law. If the investigator conducting this study is not your/your daughter's primary, or regular doctor, she must obtain your permission before contacting your/your daughter's regular doctor for information about your/your daughter's past medical history or to inform them that you/your daughter are in the screening phase of the study.

It is possible that your/your daughter's medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the Food and Drug Administration (FDA), federal or state government agencies, or hospital accrediting agencies, in the course of carrying out their duties. If your/your daughter's record is inspected or copied by any of these agencies, the

University of Missouri will use reasonable efforts to protect your/your daughter's privacy and the confidentiality of your/your daughter's medical information.

Some of the screening data collected will become part of the study data. Thus, the results of this study may be published in a medical book or journal or used for teaching purposes. However, your/your daughter's name or other identifying information will not be used in any publication or teaching materials.

If you/your daughter do not meet the study criteria and/or for any reason, decide not to participate in the study, all screening data will be shredded.

WHAT ARE THE COSTS?

There is no cost to you for completing the screening phase. The only cost you/your daughter will have is the cost with traveling to and from the University of Missouri.

WILL I BE PAID FOR PARTICIPATING IN THE STUDY?

You/your daughter will not receive compensation for the completion of the screening procedures.

WHAT IF I AM INJURED?

It is not the policy of the University of Missouri to compensate human participants in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to participants who suffer injuries while participating in the research projects of the University of Missouri. In the event you/your daughter have suffered injury as the result of participation in this research program, you/your daughter are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

WHAT ARE MY RIGHTS AS A PARTICIPANT/PARENT OF A PARTICIPANT?

Participation in the screening phase of the study is voluntary. You/your daughter do not have to participate in this study. Your/your daughter's present or future care will not be affected should you/your daughter choose not to participate. If you/your daughter decide to participate, you/your daughter can change your/your daughter's mind and drop out of the screening phase at any time without affecting your/your daughter's present or future care in the institution. Leaving the screening phase will not result in any penalty or loss of benefits to which you/your daughter are entitled. In addition, the investigator of this study may decide to end your/your daughter's participation in the screening phase of the study at any time after she has explained the reasons for doing so and has helped arrange for your/your daughter's continued care by your/your daughter's own doctor, if needed.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you/your daughter have any questions regarding your/your daughter's rights as a participant in this research and/or concerns about the study, or if you/your daughter feel under any pressure to enroll or to continue to participate in this study, you/your daughter may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Heather J. Leidy, primary investigator, at 573-882-0654.

A copy of this consent form will be given to you to keep.

SIGNATURE

I confirm that the purpose of the research, the screening procedures, the possible risks and discomforts as well as potential benefits that I/my daughter may experience have been explained to me. Alternatives to my/my daughter's participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate/allow my daughter to participate in the screening phase of the study.

Participant (if ≥ 18 yrs of age)	Date
Participant-Assent to Participant (if between the ages of 13-17 yrs)	Date
Legal Guardian/Advocate/Parent	Date
Additional Signature (if required); Identify relationship to Participant	Date

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the screening procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the screening phase of the study to the best of my ability.

Study Representative	Date

A) Study Consent Form

STUDY CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATOR'S NAME: HEATHER J. LEIDY
PROJECT #: 1173258

FOR HS IRB USE ONLY
APPROVED
10/26/10
HS IRB Authorized Representative Date
EXPIRATION DATE: 8.27.2011

STUDY TITLE: **THE BENEFICIAL EFFECTS OF DIFFERENT BREAKFAST MEALS ON APPETITE CONTROL & COGNITION IN 'BREAKFAST SKIPPING' YOUNG WOMEN**

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.

This is a research study. Research studies include only people who choose to participate. As a study participant/parent of a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate/allow your daughter to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You/your daughter are being asked to take part in this study because you/your daughter follow the potentially unhealthy habit of skipping breakfast.

This study is being sponsored by the American Egg Board/Egg Nutrition Center and the National Cattlemen's Beef Association.

In order to participate in this study, it will be necessary to give your written consent.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to identify how the body and mind respond to the daily consumption of different breakfast meals in 'breakfast skipping' young women.

This research is being done because we currently do not know why breakfast is widely assumed to be "the most important meal of the day."

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 25 people will take part in this study at the University of Missouri, Columbia, MO.

WHAT IS INVOLVED IN THE STUDY?

If you/your daughter take(s) part in this study, there will be 3 different breakfast patterns to complete in any order. These include skipping breakfast or eating our "Rise & Shine" breakfast meals.

Each breakfast pattern will be followed for 7 days each. For the 'Breakfast Skipping' pattern, breakfast will be skipped as normal. However, for the other patterns, we will provide different types of meals to consume at home between 6-8 am for 6 days for each pattern.

Each breakfast meal will be prepared in a separate container and marked as Breakfast Day 1-6. Each morning, you/your daughter will read the breakfast meal instruction sheet. This sheet includes the directions for preparing each breakfast meal, a check-off log listing all of the foods to be consumed, and several short questionnaires regarding you/your daughter's feelings, thoughts, and mood towards the breakfast meal. You/your daughter will be permitted to only eat the foods provided by the study. However, after breakfast is completed, you/your daughter can eat or drink anything else you/your daughter chooses to eat throughout the remainder of the day. All of the breakfast meals consist of normal breakfast foods commonly consumed by those who eat breakfast on a daily basis. The next page lists the description of the breakfast meals.

Breakfast Menus

Rise Breakfast Meals		Shine Breakfast Meals	
Cheerios-style Cereal with Milk (Days 1,3,5,7)	Chex-style Cereal with Milk (Days 2,4, 6)	Waffles, Applesauce Topping, & Sausage (Days 1,3,5,7)	Breakfast Wrap & Yogurt (Days 2, 4, 6)
Cereal includes: Cheerios® Frosted Cheerios® Milk includes: Vitamin D (Whole) Milk Skim Milk Calorie Countdown-2% Hood® Milk	Cereal includes: Rice Chex Wheat Chex Multi-Brain Chex Sliced Almonds Milk includes: Vitamin D (Whole) Milk Skim Milk Calorie Countdown-2% Hood® Milk	Waffle Batter includes: Whole Wheat Flour Whole Ground Flaxseed Meal Ground Almond Meal Coconut Flour Country Crock® Spread Powdered Milk Fat Free Cheese Egg Whites Cinnamon Vanilla Extract No Calorie Sweetener	Sandwich: Lavash Bread Extra Lean Ground Beef Egg Whites Powdered Milk Sausage Spice Salsa Low-fat Cream Cheese 2% Pepperjack Cheese
		Applesauce Topping No sugar added applesauce Cinnamon No Calorie Sweetener	Yogurt: Low Fat Strawberry Yogurt Low Fat French Vanilla Yogurt Wild Blueberries No Calorie Sweetener
		Beef Sausage Extra Lean Ground Beef Sausage Spice	Yogurt Topping: Kellogg's All-Bran® Buds

On day 7 of each pattern (which will be on Saturdays), you/your daughter will report to the Melvin H. Marx Building on the MU campus to complete the 12-h testing day.

Here are the procedures that will be completed during thus day:

- Upon arriving, you/your daughter will have your/your daughter's body weight measured and will then sit in a comfortable, reclining chair. All of the testing procedures will be explained one more time.

- When ready to begin, a catheter, which is a thin flexible, plastic tube, will be inserted into a vein located in the front (inside) of the elbow by a registered nurse or trained research technician using sterile techniques. The vein will be kept patent (i.e. 'cleared' or 'flushed') throughout the day by using a slow continuous dripping of sterile saline solution. We use the catheter so that we can take multiple blood samples without having to stick you/your daughter multiple times.

- A small blood sample (~ 1 teaspoon) will be drawn from the catheter. The blood sample will be used to measure hormones, which are chemical messengers in the body, that respond to food intake. Also at that time, a questionnaire will be completed which asks about your hunger feelings, thoughts of food, and mood.

- Throughout the day, blood samples and questionnaires will be collected at specific times.

- We will provide breakfast, lunch, and dinner at specific times. These will include common foods that young people typically eat on a daily basis.

- Throughout the morning, several "Memory, Attention, & Reaction" tests will be completed on the computer. There are 2 sets of tests; each take approximately 45 minutes to complete.

- Right before dinner, the catheter will be removed and a brain scan will be completed using a brain imaging method known as Magnetic Resonance Imaging (MRI). This technique examines how water molecules in the brain behave in a strong magnetic field. MRI provides a detailed picture of what the brain looks like. We also use the functional MRI technique which provides information on blood flow and 'brain activation.' This is a non-radioactive (i.e., no x-rays), non-invasive technique. During this scan, you/your daughter will lie on a table that 'slides' into the scanner. Your/your daughter's head will be set in a specific testing position-making it difficult for you/your daughter to move your/your daughter's head. During the scan, you/your daughter will view numerous food, animal, scenery, and blurry pictures. You/your daughter will be asked to remember the pictures that you/your daughter saw during the scanning. This procedure lasts approximately 30 minutes.

- Throughout the day (when you/your daughter are not completing the testing procedures), you will have 'free time' to do the following things:

    - We will provide a laptop to play a number of "Hidden Objects/Seek and Find" computer games or check email, Facebook, etc.

    - We will provide a DVD player with movies to choose from.

    - We will also provide various card and board games to play.

    - You/your daughter can also bring magazines and/or books to read.

    - You/your daughter will be permitted to use the restrooms in the facility at any time.

- At the end of the day, we will provide a pack-out cooler containing numerous snacks to freely consume, at home, throughout the remainder of the evening.

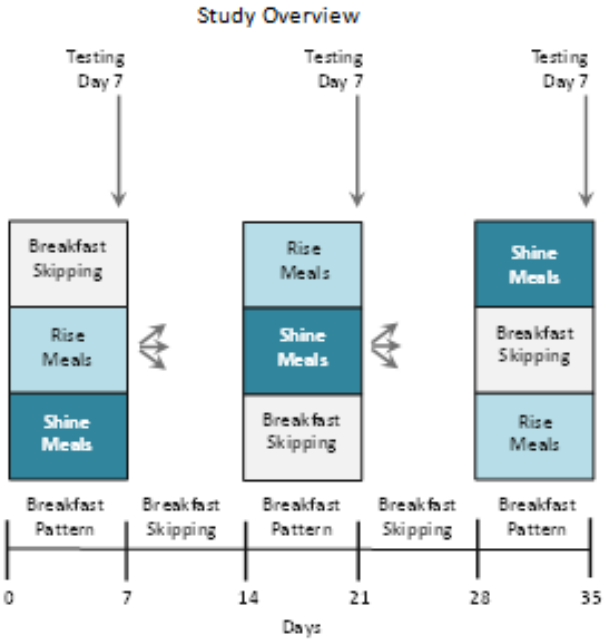
- Over the next 7 days, you/your daughter will go back to your normal pattern of 'Skipping Breakfast.' Sometime during this week, you/your daughter will return the pack-out cooler to us. At that time, we will provide the next breakfast pattern to you/your daughter.

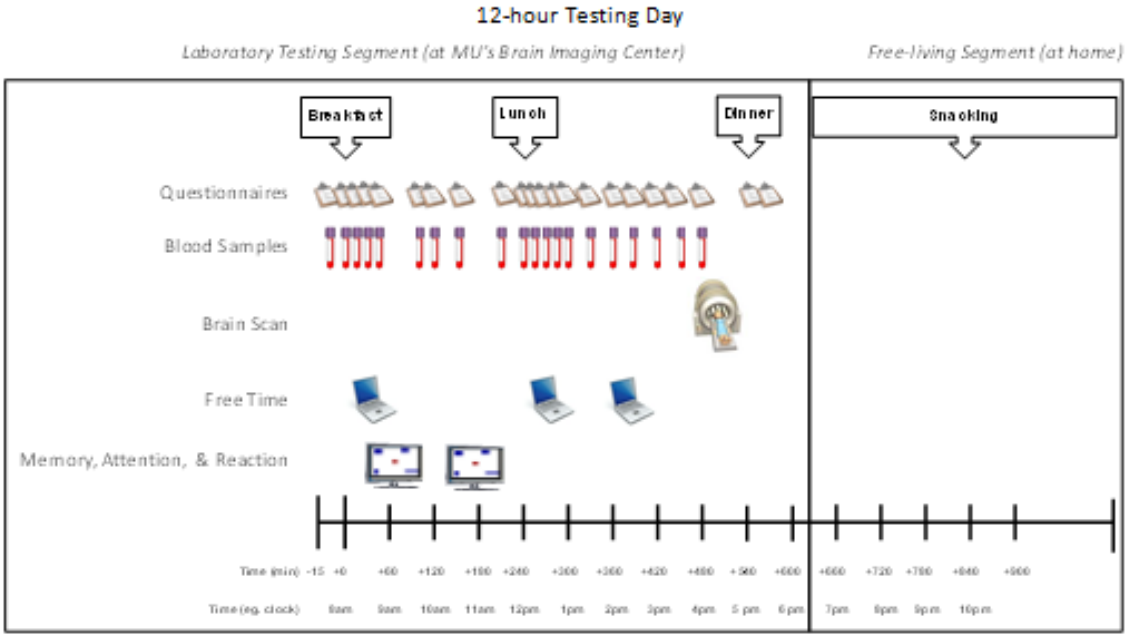
- You/your daughter will repeat these procedures for each of the 3 breakfast patterns.

- Each breakfast pattern lasts for 7 days. There will typically be a 7-day washout (i.e., 'Breakfast Skipping') pattern in between each one of these. However, depending on holiday and/or semester schedules, the washout period prior to or following the 'Breakfast Skipping' pattern may be deleted. Regardless, the entire study lasts a total of 35 days (i.e., 5 weeks).

- We have performed similar studies in this age group and have found that most young people easily tolerate and actually enjoy each part of the study.

Here are 2 diagrams of the entire study and the specific 12-hour testing day:





How Long Will I/My Daughter Be in the Study?

We think you will be in the study for 35 day (i.e., 5 weeks).

The investigator may decide to take you/your daughter off this study if new medication is prescribed by your/your daughter's doctor that would alter the study outcomes or if you/your daughter are not correctly following the breakfast patterns.

You can stop participating at any time. Your/your daughter's decision to withdraw from the study will not affect in any way your medical care and/or benefits.

However, if you/your daughter decide(s) to withdraw from the study, we ask that all study forms and supplies be returned to our facility in a timely manner.

What Are the Risks of the Study?

While in the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your/your daughter's doctor. There may also be other side effects that we cannot predict. Many side effects go away shortly after each testing day is completed, but in some cases side effects can be serious or long-lasting or permanent.

Risks and side effects related to the study breakfast meals include:

Unlikely; with some Short-term Discomfort; Otherwise not Serious:
Your/your daughter's stomach and/or bowels may become slightly upset due to the changes in your/your daughter's usual food and beverage intake. Any discomfort should stop within 1-2 days.

Risks and side effects related to the blood collection procedures include:

Likely; with some Short-term Discomfort
There may be some risks when having a catheter inserted into your/your daughter's arm. During the insertion, some pain may be felt which feels like a slight pinch. The pain will end within seconds after the insertion is completed.

There is a risk of developing a small bruise and/or infection. However, the catheter will be inserted by a highly trained nurse or technician using sterile techniques.

You/your daughter may feel lightheaded and may faint at the sight of blood. Neither of these will occur due to the amount of blood being drawn. In fact, throughout each testing day, we will collect approximately 105 ml (3.6 oz)/testing day; this is about 22% of what would be taken if you/your daughter donated blood through the American Red Cross. Thus, the amount of blood collected is small enough not to present any hazard to you/your daughter's physical wellbeing. However, you/your daughter must agree not to donate blood for at least one month prior to, during, and for one month after the study.

There are no substantial risks associated with this procedure.

Risks and side effects related to the brain scan (MRI) procedures include:

Less Likely; with some Short-term Discomfort
Although MR imaging is thought to be hazard free, you may feel physically uncomfortable or anxious when placed in the enclosed space of the MRI device. This is the same size space of the 'mock' scanner that you/your daughter laid down in during the screening meeting. You will be able to talk to a staff member by using a microphone and speaker system.

Noise from the MRI machine can also cause discomfort. Earplugs and/or earphones are provided to minimize this discomfort.

Reproductive Risks
The effects of the MRI procedures on a developing fetus (unborn baby) are unknown but could cause harm. For this reason, if you are pregnant or could become pregnant, then you must not participant in this study.

There are no substantial risks associated with this procedure.

For the reasons stated above the investigator will observe you/your daughter closely while giving the treatment described and, if you/your daughter have any worrisome symptoms or symptoms that the investigator or *her* associates have described, notify the investigator immediately. The *Investigator's* telephone number is 573-882-0654. For more information about risks and side effects, please feel free to ask the investigator.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. You may expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge. We hope the information learned from this study will benefit other 'breakfast skipping' individuals in the future.

You/your daughter may experience benefits from this study by understanding why your/your daughter's current dietary habits are unhealthy and potentially lead to reward-driven eating, increased motivation to eat, and overeating. This study will further show you/your daughter which dietary strategies might be the most beneficial in order to reduce these unhealthy and unwanted behaviors.

There is no guarantee that taking part in this research will result in any improvement in your/your daughter's eating habits.

WHAT OTHER OPTIONS ARE THERE?

An alternative is to not participate in this research study.

WHAT ABOUT CONFIDENTIALITY?

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your/your daughter's name to specific information about you/your daughter will be kept in a separate, secure location. Information contained in your/your daughter's records may not be given to anyone unaffiliated with the study in a form that could identify you/your daughter without your written consent, except as required by law. If the investigator conducting this study is not your/your daughter's primary, or regular doctor, she must obtain your permission before contacting your/your daughter's regular doctor for information about your/your daughter's past medical history or to inform them that you/your daughter are in this study.

It is possible that your/your daughter's medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the Food and Drug Administration (FDA), federal or state government agencies, or hospital accrediting agencies, in the course of carrying out their duties. If your/your daughter's record is inspected or copied by any of these agencies, the University of Missouri will use reasonable efforts to protect you/your daughter's privacy and the confidentiality of your/your daughter's medical information.

The results of this study may be published in a medical book or journal or used for teaching purposes. However, your/your daughter's name or other identifying information will not be used in any publication or teaching materials.

In addition, if photographs are taken during the study that could identify you/your daughter, you/your daughter must give special written permission for their use. In that case, you/your daughter will be given the opportunity to view the photographs before you give permission for their use if you/your daughter so request.

WHAT ARE THE COSTS?

There is no cost to you for the breakfast meals, dietary information, blood analyses, and the brain scan images of your/your daughter's brain that are all part of this research study. Parking is also free of charge at the Brain Imaging Center parking facility. The only cost you/your daughter will have is the cost with traveling to and from the University of Missouri.

WILL I/MY DAUGHTER BE PAID FOR PARTICIPATING IN THE STUDY?

You/your daughter will be compensated a total of ***$450*** for completing all study procedures. Specifically, you/your daughter will be paid *$150* for completing each breakfast pattern which includes the 12-hour testing day and the 6 days of correctly following the specific breakfast pattern at home.

WHAT IF I/MY DAUGHTER AM INJURED?

It is not the policy of the University of Missouri to compensate human participants in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to

participants who suffer injuries while participating in the research projects of the University of Missouri. In the event you/your daughter have suffered injury as the result of participation in this research program, you/your daughter are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

WHAT ARE MY/MY DAUGHTER'S RIGHTS AS A PARTICIPANT/PARENT OF A PARTICIPANT?

Participation in this study is voluntary. You/your daughter do not have to participate in this study. Your/your daughter's present or future care will not be affected should you/your daughter choose not to participate. If you/your daughter decide to participate, you/your daughter can change your/your daughter's mind and drop out of the study at any time without affecting your/your daughter's present or future care in the institution. Leaving the study will not result in any penalty or loss of benefits to which you/your daughter are entitled. In addition, the investigator of this study may decide to end your/your daughter's participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your/your daughter's continued care by your/your daughter's own doctor, if needed.

You will be informed of any significant new findings discovered during the course of this study that might influence your/your daughter's health, welfare, or willingness to continue participation in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you/your daughter have any questions regarding your/your daughter's rights as a participant in this research and/or concerns about the study, or if you/your daughter feel under any pressure to enroll or to continue to participate in this study, you/your daughter may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Heather J. Leidy, primary investigator, at 573-882-0654.

A copy of this consent form will be given to you to keep.

SIGNATURE

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I/my daughter may experience have been explained to me. Alternatives to my/my daughter's participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate/allow my daughter to participate in this study.

Participant (if ≥ 18 yrs of age)	Date
Participant-Assent to Participant (if between the ages of 15-17 yrs)	Date
Legal Guardian/Advocate/Parent	Date
Additional Signature (if required); Identify relationship to Participant	Date

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

Study Representative	Date

BLOOD BANKING

I agree to allow the use of my/my daughter's blood samples collected during this study to be used for future research that might be unrelated to this study. The blood samples will be stored for 10 years. These samples will likely be used for future analysis of food intake and appetite hormones that have not yet been identified or are currently unable to be measured. The use and disclosures of personal information listed in the consent form also apply to the saved blood samples. However, at any time, I can request that the blood samples be destroyed if I change my mind. If this occurs, I will provide a written request to Dr. Leidy at 204 Gwynn Hall; University of Missouri; Columbia, MO 65211. Lastly, I understand that Dr. Leidy can use and share information that was gathered before this request was received.

Participant (if ≥ 18 yrs of age)	Date
Participant-Assent to Participant (if between the ages of 15-17 yrs)	Date
Legal Guardian/Advocate/Parent	Date

OR

I request my /my daughter's blood samples collected during this study to NOT be used for any future research that is unrelated to this study. I understand that I/my daughter can still participate in this study if I refuse to have the blood samples retained.

Participant (if ≥ 18 yrs of age)	Date
Participant-Assent to Participant (if between the ages of 15-17 yrs)	Date
Legal Guardian/Advocate/Parent	Date