THE IMPACT OF A PROTEIN-RICH BREAKFAST ON FOOD CRAVINGS AND REWARD IN OVERWEIGHT/OBESE 'BREAKFAST SKIPPING' ADOLESCENT GIRLS

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By

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

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a candidate for the degree of master of science,

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

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ABSTRACT

This study examined whether the addition of a higher-protein (HP) vs. normal-protein (NP) breakfast leads to beneficial changes in perceived appetite, food cravings, and plasma homovanillic acid (HVA), which is an index of central dopamine production, in overweight/obese 'breakfast skipping' (BS) teen girls. A randomized crossover design was incorporated in which 20 BS girls (age 19±1y; BMI 28.6±0.7kg/m^2) consumed 350 kcal breakfast meals containing NP (13g protein) or HP meals (35g protein) for 7 days. On day 7, a 4h testing day was completed including the consumption of breakfast followed by perceived appetite, satiety, and food craving questionnaires and blood sampling for HVA concentration assessment throughout the morning. Breakfast, regardless of protein content, reduced perceived hunger and cravings for sweet and savory foods vs. BS (all, $p<0.05$). Breakfast also increased perceived fullness and overall pleasure/well-being vs. BS (all, $p<0.05$). Between meals, HP led to greater reductions in savory cravings vs. NP ($p<0.05$) and greater increases in fullness vs. NP ($p=0.08$). No other differences in perceived sensations were observed. Plasma HVA concentrations were greater following consumption of both breakfast meals vs. BS (both, $p<0.05$), with only HP exhibiting sustained increases prior to lunch vs. NP ($p=0.09$). Additionally, HVA concentrations were correlated with perceived fullness, breakfast palatability, and protein content at breakfast. In conclusion, these findings suggest that the daily addition of a protein-rich breakfast, containing 35g of protein, alters signals associated

with food motivation and reward, and might be a beneficial strategy to combat the modern food environment in young people.

INTRODUCTION

Adolescent obesity continues to be a growing public health concern in the United States, affecting the lives of over 25 million young people [1, 2]. Due to the fact that approximately 36% of adolescents are overweight or obese, and up to 80% will likely become overweight adults, it is essential to focus on this group to prevent this epidemic from perpetuating into further generations [3, 4]. Although the etiology of obesity is multifactorial, several behavioral and environmental factors have been shown to play a significant role. Of particular interest is the increasingly common dietary habit of breakfast skipping, which has closely mirrored the rise in obesity [5].

According to the most recent NHANES survey, 20% of children and 32% of adolescents skip breakfast on a daily basis [6]. The reason for concern stems from the strong relationship between breakfast skipping, weight gain, and obesity [6-8]. In teasing out the mechanism(s)-of-action underlying the breakfast skipping/obesity relationship, we recently reported that breakfast skipping leads to poor appetite control, reduced satiety, and greater energy intake at the next eating occasion compared to eating breakfast [9]. Because Americans generally eat (or stop eating) for reasons other than when experiencing physiological hunger or fullness, it is critical to extend the findings to explore the signals controlling food cravings and reward.

Dopamine is a powerful neural signal implicated in the regulation of food intake by stimulating reward-driven eating behavior [10-12]. Regarding the etiology of obesity, a negative correlation exists between dopamine and BMI such that obese individuals

display a blunted dopamine response occurring in proportion to BMI [13-15]. In obese animal models, central dopamine activity is reduced, and is accompanied by elevated preference for highly palatable, energy dense foods compared to normal weight control animals [16]. However, treatment with dopamine agonists reverses the excess body weight and obesity [15]. Collectively, these data suggest that strategies to stimulate dopamine activity may lead to significant improvements in obesity.

In general, dopamine is secreted in response to tasting highly palatable foods and evokes feelings of enhanced food reward, pleasure, and well-being [17-19]. Although much of the literature has focused on dietary fat and sugar as key stimulates of food reward [20, 21], dietary protein has also been speculated to elicit similar reward responses [17]. Data from our lab and others support the benefits of increased protein consumption, particularly at breakfast, for improved appetite control, satiety, and body weight management [9, 22-26]. We would like to extend these findings to examine the role of increased dietary protein at breakfast on signals controlling food reward.

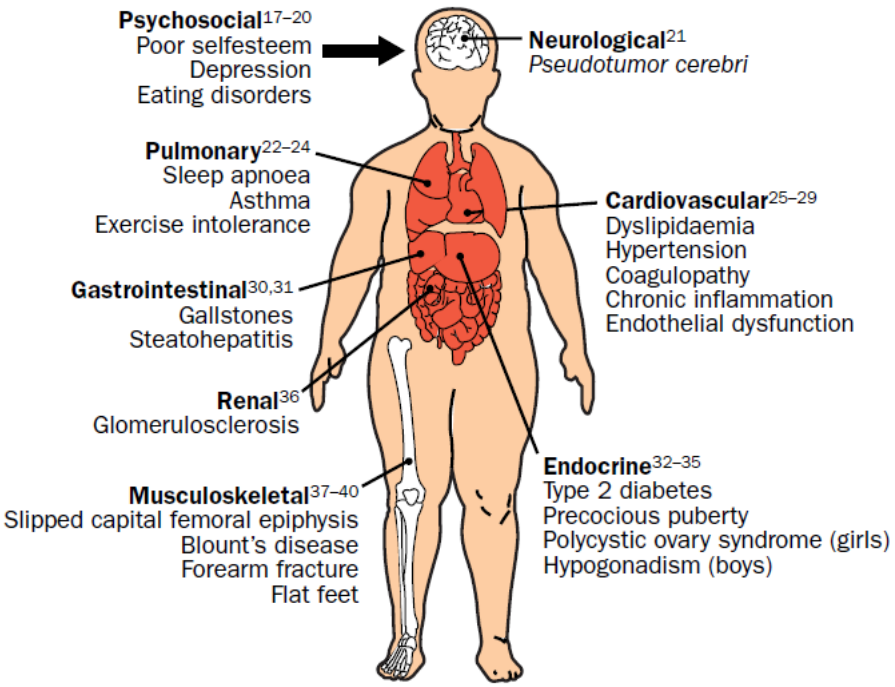
The primary aim of this study was to assess whether the daily addition of a normal vs. protein-rich breakfast alters perceived sensations, food cravings, and dopamine responses throughout the morning in overweight/obese 'breakfast skipping' adolescents. In addition, the relationship between dopamine and dietary protein, perceived sensations, and food cravings were also identified.

EXTENDED REVIEW OF LITERATURE

I. Obesity

The 2012 prevalence of obesity report by Ogden, et al. revealed that, as of 2010, 33% of children and adolescents remain overweight or obese and 17% are obese [27]. Although the current findings indicate that obesity is plateauing in young people, the U.S. continues to fall short of the Healthy People 2010 and 2012 objectives of reducing obesity to 15% [28]. The primary reason that obesity continues to be a major public health concern is due to the increased prevalence of many serious diseases and comorbidities which reduce quality of life and life expectancy [3, 29]. Though obesity is not as well studied in adolescents as adults, it is quite clear that the health complications in younger individuals are similar to that of older adults (Figure 1).

Figure 1: Complications of Obesity [30]



As shown in Figure 1, obesity in young people leads to a myriad of diseases and conditions. The more serious and more prevalent include the development of the following:

- Type 2 diabetes, which is increased from 1-2% in 1985 to 30-40% in 2005 [31];
- Cardiovascular disease, with 70% of obese young people having at least one risk factor [32]; and,
- Metabolic Syndrome, affecting 31% of obese young people [33].

Other common conditions include an increased risk of cancers, sleep apnea, bone and joint problems, and psychological problems stemming from poor self-esteem. With up to 80% of overweight adolescents likely to become overweight adults, and 36% of adolescents already qualifying as overweight or obese, it is vital to focus on this younger group of individuals in an effort to keep this epidemic from perpetuating in further generations [3, 4].

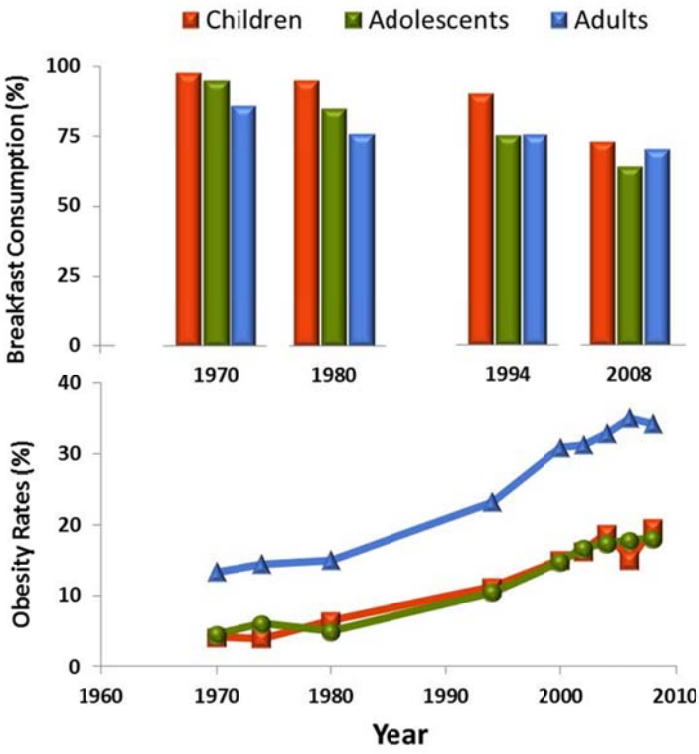
Dietary Habits Contributing to Obesity

The etiology of obesity is a complex web of behavioral and environmental facets comprised of unhealthy dietary habits, inactivity, and the food-centered society which young people are exposed to on a daily basis. Figure 2 illustrates the dietary factors that have the strongest association with weight gain and obesity, and shows the interaction between many of these unhealthy practices. Of particular interest is the increasingly common, yet unhealthy, dietary habit of breakfast skipping. As shown in Figure 3, there

has been a gradual decline in breakfast consumption which has closely mirrored the rise in obesity [5].

Figure 2: Dietary Habits Associated with Obesity [5]

Figure 3: Breakfast Consumption and Obesity in America [5]



The most recent NHANES 1999-2006 survey in 4,320 children and 5,339 adolescents indicates that approximately 20% of children and 32% of adolescents skip breakfast on a daily basis [6]. Furthermore, breakfast skippers had higher BMI-for-age z scores ($p<0.05$), greater waist circumference ($p<0.05$), and exhibited a greater prevalence of being obese (24% vs. 14%, $p<0.05$) compared to breakfast consumers. In a five year longitudinal study, Timlin et al. identified the relationship between adolescent weight and eating patterns. They found that an increase in BMI was inversely associated with the frequency of breakfast consumption [7]. This response occurred in a dose-dependent manner, with adolescents who ate breakfast regularly having the lowest BMI, and those who ate breakfast occasionally having BMIs lower than those who never ate breakfast. In teasing out the mechanism(s)-of-action underlying the breakfast skipping/obesity relationship, several potential factors exist.

Breakfast Skipping

According to the NHANES 1999-2006 data, breakfast skippers have lower intakes of micronutrients (including Vitamins A, C, E, B-6, B-12, thiamin, niacin, riboflavin, folate, phosphorus, magnesium, iron, zinc, and calcium) compared to breakfast consumers. Breakfast skippers also have diets lower in dietary fiber but higher in dietary fat compared to those who consume breakfast.

Along with poor diet quality, breakfast skippers also consume 40% more desserts, 55% more chips, 55% more soft drinks, and 40% more white bread vs. breakfast consumers. They also consume 45% less vegetables, 30% less fruit, 60% less

milk, and 65% fewer whole grains [34, 35]. Lastly, the likelihood of skipping breakfast was found to be greater in adolescents who frequently snack [36].

Breakfast skipping also negatively impacts appetite control and satiety. In a study by Leidy and Racki, breakfast skipping adolescents exhibited greater appetite and reduced satiety throughout the morning and mid-day hours; however, the addition of breakfast reversed these outcomes [9].

In a subsequent study by Leidy et al., breakfast skipping adolescent girls exhibited increased food motivation and food reward as shown by the heightened fMRI activation in the hippocampus, amygdala, anterior cingulate, and parahippocampus regions of the brain prior to lunch; however, the addition of breakfast reduced these activations[37]. In summary, breakfast skipping has been linked to a variety of unhealthy behaviors, including poor diet quality, poor food choices, reduced appetite control and satiety, and increased food reward which, over time, perpetuate the obesity trend.

II. Regulation of Food Intake

Physiological vs. Reward-Driven Eating

The human body produces a plethora of peripheral hormones that regulate energy homeostasis and physiological needs. Some of the more prevalent of these hormones include ghrelin, the hunger hormone, which is produced by the fundus of the stomach and acts centrally on the hypothalamus to increase perceived hunger and energy intake [38]. Others have been linked to satiety, including Peptide Y-Y_{3-36} (PYY), cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1). These hormones are

produced by the gut to also act on the hypothalamus, reducing food intake and intiating feelings of satiety [39]. Although these hormones clearly play an integral role in regulation of eating behavior, other factors must be taken into account, as not all eating occasions stem solely from physiological hunger or energy state.

Reward-driven eating is regulated by centrally-secreted neurotransmitters and appear to override our physiological regulatory system [40]. Traditionally, neural circuits involved in energy regulation were primarily in the hypothalamus, which included input from the gut hormones (ghrelin, PYY, CCK, and the like) through the vagus nerve and the brainstem [40]. However, in more recent years, other areas of the brain, specifically the corticolimbic pathways, have been included in food intake initiation through their regulation of learning and reward, memory, mood, and emotions, all of which factor into food reward and hedonic eating [40]. In the current food-centered environment, these non-homeostatic brain regions overcome the regulatory regions, ignoring our lack of physiological need. For example, day of the week, time of day, social eating, relationship to eating companions, food palatability, food convenience, and other such factors can cause dramatic variations in intake. Thus, it is imperative to focus on the mechanisms behind this eating behavior to overcome the challenges of obesity.

Liking versus Wanting

The concept of reward-driven eating can be broken down into two fundamental concepts, liking and wanting, which have recently been distinguished from one another. 'Liking' represents the collective perception of a food item which is primarily from the

objective, sensory properties of the food, including such things as the way the food looks, tastes, smells, and feels in the mouth [41]. The properties associated with each food stay consistent regardless of the energy state of the individual. Several neurotransmitters, including opioids, endocannabinoids, and GABAergic peptides, have been implicated in the 'liking' of various foods [41, 42].

Alternately, 'wanting' is the actual motivation behind seeking out and consuming a particular food and is representative of the amount of work an individual is willing to do to obtain that food. 'Wanting' also takes sensory properties of the food into account, but includes other factors as well, including the individual's level of hunger, the cost of the food, the time of day, and the social acceptability of the food. The dopaminergic neurotransmitters have been linked to food 'wanting' [41, 42].

It is important to note that these two phenomenon, though unique from one another, occur simultaneously in humans to more fully experience reward [41, 42]. Regardless, the two may be measured primarily through assessments of food palatability to determine 'liking' and by assessments of desire to eat and prospective food consumption to determine 'wanting'. Both measurements require the use of visual analogue scales and/or food stimuli [43].

Perceived Sensations/Visual Analogue Scales

One of the most common ways to assess perceived sensations involved with liking and wanting is through the use of Visual Analogue Scales (VAS) [44]. VAS typically consist of a 100 mm line, labeled by anchors at either end representing "Not at all" and "Extremely"; the individual is asked to place a mark along the line representing his/her

perceived feeling at the moment [44, 45]. Responses provide an indication of the magnitude of sensation which may be used as a measurement of that particular point in time, and can be compared to other time points to determine changes in these feelings [46, 47]. These tests represent a subjective measure of hunger or appetite, which has been correlated to feeding behavior in free-living studies [48]. VAS have been validated in young and older individuals, and have subsequently been frequently used in many studies [46].

Although the previous approach for the implementation of the VAS included paper questionnaires, newer methods of electronic administration of these tests have also been developed. In studies comparing the effectiveness of the two, the electronic version was shown to as accurate as the paper version [44, 45, 49]. Additionally, the electronic version was found to be easier and more convenient than the paper version, with a large majority of individuals preferring the electronic version [49]. A more recent study by Almiron-Roig et al., the electronic questionnaires were found to be faster and less error-prone [45].

Food Cravings

Food cravings are defined as an intense desire to eat a particular food. This 'desire' has been postulated to be an expression of a need by the body for a particular nutrient [50]; however, little research has supported these claims [50]. More recent evidence suggests that food cravings are a result of reward-driven behavior rather than a physiological need.

Commonly craved foods tend to be 'treat' foods such as cookies, ice cream, chocolate, and salty 'snack foods.' These foods also tend to be highly palatable and calorically dense 'snack foods' which are typically high in fat and sugar, and are often consumed in the absence of physiological hunger [51]. Palatable food has also been shown to cause cravings in the same regions of the brain as drugs, including the hippocampus, insula, and caudate regions [52]. Further, foods that are typically craved induce a dopamine release (see subsequent section) in response to their consumption [19].

Food cravings are extremely common [53, 54] and may be an important contributor to obesity due to their impact on snacking and binging behaviors [55, 56]. Self-reported cravings have been positively correlated with body mass index and caloric intake [57-59].

Studies in obese individuals have shown an increased preference for high-fat and high-sugar foods, with greater feelings of pleasure and subsequent increased intake in comparison to lean individuals [60, 61].

III. Dopamine

A more objective way to assess reward-driven eating behaviors is to identify and subsequently measure the signals controlling food motivation, reward, and cravings. One such signal implicated in motivation to eat and food reward is dopamine. Dopamine is a catecholamine, primarily secreted from the substantia nigra (SN) and ventral tegmental area (VTA) of the brain to act centrally as a neurotransmitter, and as an intermediate in the synthesis of norepinephrine and epinephrine [62, 63]. The amino

acid tyrosine is brought across the blood-brain barrier by a competitive, large neutral amino acid revolving door transporter. It is then hydroxylated by tyrosine hydroxylase to form dihydroxyphenylalanine, which is quickly decarboxylated by aromatic L-amino acid decarboxylase to synthesize dopamine (DA). The rate-limiting step in dopamine synthesis is tyrosine hydroxylase [64, 65]. Upon the arrival of an impulse at the neuron, dopamine is released to the synaptic cleft, where it binds to dopamine receptors; this activity is terminated by active reuptake into the nerve terminal, where the dopamine is then metabolized by monoamine oxidase to 3,4-dihydroxyphenylacetic acid, which is further metabolized by catechol-O-methyl transferase to form homovanillic acid (HVA) [66]. Any dopamine that was not reabsorbed by the neuron (about 20%) is metabolized by catechol-O-methyl transferase to 3-methoxytryramine, which is then acted on by monoamine oxidase, also forming HVA [66].

The neuronal circuitry of the human brain is a complex system composed of a tremendous number of neurons forming various connections between brain regions. Figures 4 & 5 illustrate the dopaminergic neurons that project into specific brain regions which regulate a number of functions in the body, including cognition, motor control, mood, reward, motivation, pain perception, food reinforcement, and sexual behavior [11, 67-71]. There are several subsets of dopaminergic neurons, each of which are distinct in function and anatomy [72]. The lateral A9 neurons of the SN project into the dorsal striatum (caudate putamen) and are associated with voluntary movement and potentially food reward-related learning [73]. Another set of dopaminergic neurons, termed tuberoinfundibular, originate in the hypothalamus and project into the pituitary;

these neurons regulate hormonal secretions, primarily prolactin [72]. The mesolimbic and mesocortical medial A10 neurons of the VTA innervate the ventral striatum and the limbic regions (nucleus accumbens, amygdala, and hippocampus); these have been associated with the regulation of feeding behavior, food reward, and food motivation [72-74]. Five dopamine receptors have been identified, with the D_1 and D_5 falling into the D_1-like receptors, and D_2, D_3, and D_4 falling into the D_2-like receptors. The D_1 receptor is found primarily in projections of the striatum, nucleus accumbens, and olfactory tubules; similarly, the D_2 receptor is also found in the striatum, the olfactory tubule, and the core of the nucleus accumbens [75]. D_3 receptors are found in the shell of the nucleus accumbens, the olfactory tubules, and the islands of Calleja [75]. D_4 receptors are located in the frontal cortex, amygdala, hippocampus, hypothalamus, and mesencephalon regions, and the D_5 receptors are also located in the hippocampus, as well as the lateral mammillary nucleus and portions of the thalamus [75].

Figure 4: Dopaminergic projections [72]

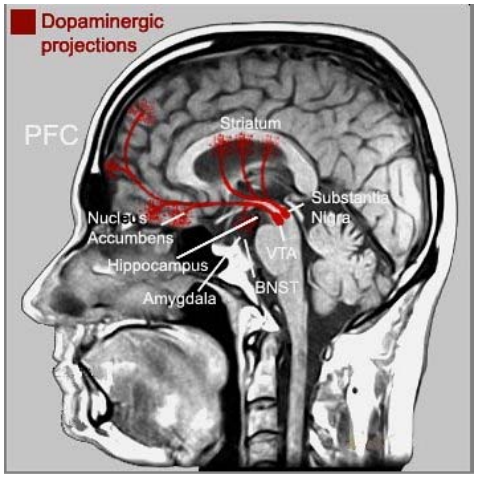
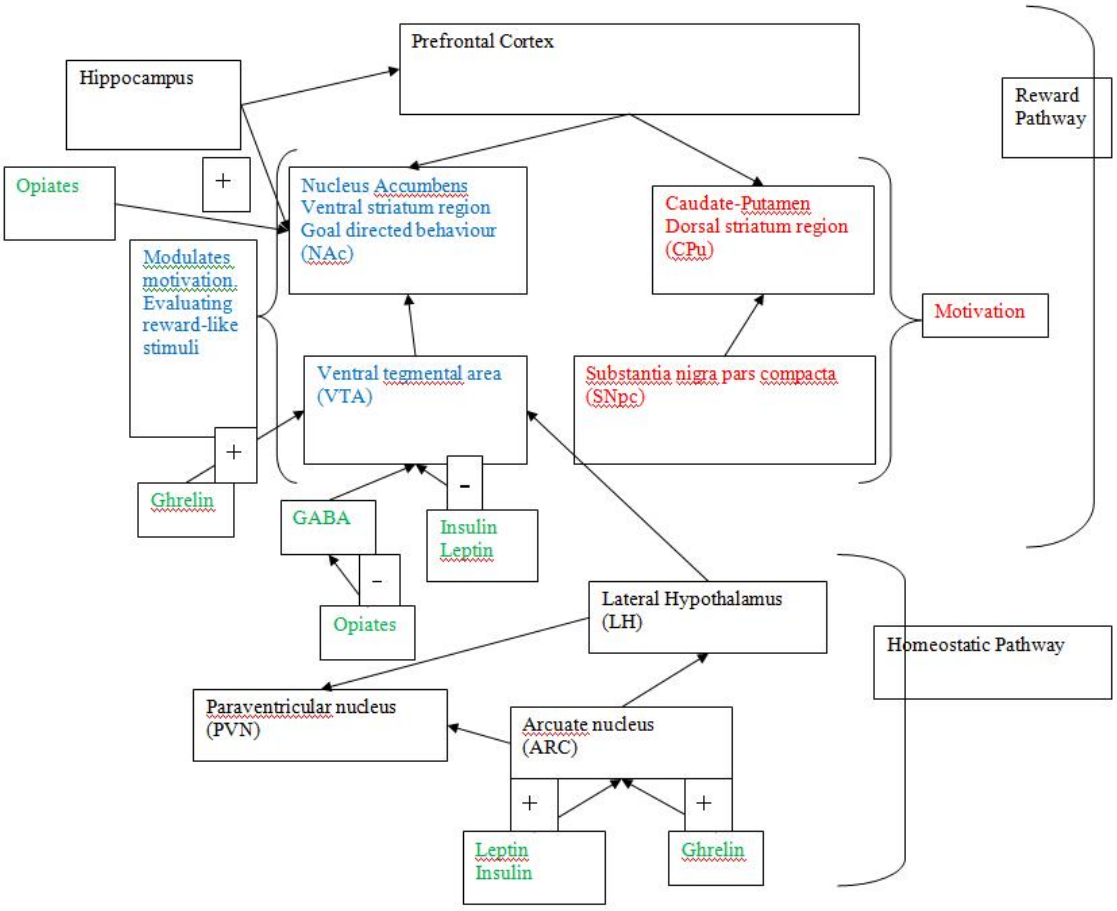


Figure 5: Mesolimbic Dopaminergic Reward System [72]



Effect of Dopamine on Feeding Behavior and Food Reward in Rodents

Dopamine has been shown to be essential for normal feeding behavior. In a study conducted by Zhou, et al., transgenic, dopamine-deficient ($DA^{-/-}$) mice exhibited severe hypoactivity, adipsia, aphagia, and subsequent weight loss by post-natal day 14. Approximately 65% of the $DA^{-/-}$ pups died within 3 weeks of birth, and 100% died within 4 weeks post-natal due to lack of intake and failure to thrive [76]. In a follow-up study, L-DOPA, a dopamine precursor, was administered two times per day beginning on post-natal day 14. This regimen temporarily restored activity, feeding behavior, and body

weight gain. However, without the continued treatment, the $DA^{-/-}$ pups would become hypoactive, adipsic, and aphagic within 12 h after the last injection. There was a 25% loss of body weight within the next 3 days and death occurred soon after if the injections were not administered [76].

In a similar study by Szczypka et al. [69], food intake and activity were examined in $DA^{-/-}$ pups following the daily injections of L-DOPA. Szczypka et al. found that food intake, activity, and body weight can be maintained $DA^{-/-}$ pups for up to a year with daily injections of L-DOPA along with energy dense, fed breeder chow, but not with standard chow. Additionally, the amount of food eaten was proportional to the dose of L-DOPA given [69].

In a follow up study, Szczpka et al. developed transgenic mice which lacked the ability to produce dopamine $DA^{-/-}$ and the long-term satiety hormone leptin [77]. Without the presence of dopamine, despite the removal of a potent satiety signal (leptin), the pups failed to consume enough food to maintain body weight [78]; however, L-DOPA injections restored normal feeding behavior in these pups. These findings suggest that dopamine is essential for maintenance of normal feeding behaviors in rodents by motivating the animals to seek out food; in the absence of dopamine, the drive to eat was absent, even if food was available and easily accessed.

Not only has dopamine been shown to play a pivotal role in maintenance of normal feeding behaviors in rats and mice [69, 78], it is also responsible for the rewarding properties of ingested food, particularly palatable foods. For example, as shown in Hajnal et al., dopamine secretion increased in the nucleus accumbens

following the consumption of increasing quantities of highly palatable sucrose in adult male Sprague-Dawley rats. This response occurred in a dose-dependent manner [79].

Another study conducted by Smith et al. examined the effects of a dopamine antagonist, pimozide, on ad libitum sucrose ingestion in rats. The dopamine antagonist led to decreased sucrose intake, regardless of sucrose concentration, suggesting that the reinforcing potency of sucrose had been blunted. Additionally, the administration of dopamine (D_1 and D_2) receptor antagonists similarly cause a decrease in ad libitum sucrose ingestion.

Collectively, these data indicate that dopamine is not only essential to normal feeding behaviors and motivation to eat, but also plays an important role in the reinforcing properties of food, particularly those which are highly palatable in nature.

Dopamine as a Potential Contributor in Obesity

The effects of psychostimulant drugs of addiction such as cocaine, nicotine, and methamphetamines have been well characterized, eliciting powerful states of euphoria, reward, and pleasure. These drugs elicit their hedonic responses through increased release and binding of dopamine throughout the corticolimbic system [15, 80]; however, with repeated usage, neurotransmitter adaptations occur, causing a down-regulation of the dopamine receptors and a decrease in dopamine release [15, 81]. These adaptations have been suggested to cause the increased motivation to seek out increasing quantities of drugs in drug addictions [82].

Recent evidence suggests that the consumption of palatable foods may work in a very similar manner to that of drug additions. Specifically, dopamine secretion initially

increases with the consumption highly palatably foods; however, chronic exposure to these foods leads to a down-regulation of dopamine receptors and decreased dopamine synthesis [13]. The blunted dopamine response potentially leads to subsequent overconsumption of palatable foods in an effort to compensate for the reduced dopaminergic reward responses [81].

Geiger and colleagues compared central dopamine concentrations in the nucleus accumbens of diet-induced obese female Sprague-Dawley rats vs. their normal weight counterparts. Compared to the normal weight rats, the diet-induced obese rats exhibited reductions in basal dopamine concentrations which were accompanied by an increased preference for highly palatable foods. Additionally, within the obese rats, ingestion of the highly palatable foods resulted in an increase in dopamine concentrations, whereas consumption of standard chow did not. Electrically-stimulated dopamine responses in obese rats were also significantly blunted [83]. Normal weight rats had a much greater electrically-stimulated dopamine response compared to obese rats in both the dorsal striatum and nucleus accumbens [83]. Thus, these data suggest that diet-induced obese rats potentially experience an increased drive to consume highly palatable, energy-dense foods to stimulate their chronically low production of central dopamine, leading to increased feelings of pleasure and reinforced food reward.

Using positron emission tomography (PET) in humans, Wang et al. compared the dopamine receptor availability between lean and obese subjects. They found that obese individuals have reduced dopamine receptor availability vs. lean subjects, with a negative linear correlation found between receptor availability and BMI [13]. Thus, it is

possible that the blunted dopamine and receptor availability may stimulate food intake in order to compensate for the decrease in reward; however, further research is warranted [13].

Further supporting the relationship between central dopamine production and food intake, administration of antipsychotic drugs, particularly atypical antipsychotics commonly used in the treatment of schizophrenia, leads to dramatic increases in body weight during the first few months of therapy and up through the first year of treatment [84]. Though the exact mechanism behind this has not yet been determined, the blockage of dopamine receptors by such drugs has been suggested as a potential mechanism for the alterations in eating behaviors [15, 84]. Conversely, drugs of abuse which act as agonists to dopamine receptors have been shown to have anorectic effects in both rodents and humans, resulting in weight loss [85].

Collectively, these data suggests that dopamine regulation plays an integral role in food reward and the regulation of energy intake, suggesting that strategies to increase dopamine secretion in obese individuals may be an important strategy in the management and prevention of obesity.

Relationship between Dopamine and Homovanillic Acid

Due to the fact that central dopamine is unable to cross the blood-brain barrier [86] and cannot be measured in a safe, non-invasive manner in humans, it is challenging to appropriately measure its activity. However, one alternative approach is to measure its metabolite(s) in body fluids; this technique has become an increasingly common and widely used practice to examine central catecholamine function [87]. Several dopamine

metabolites have been identified in the cerebral spinal fluid, urine, and plasma. The most predominant metabolite is homovanillic acid (HVA) [66, 88]. HVA, particularly in plasma, has been shown to be strongly correlated with central dopaminergic activity [89-91] and has been suggested to be the most appropriate indicator of central activity [66].

For example, in rats, plasma HVA was shown to be a direct metabolite of central dopamine; furthermore, changes in plasma HVA appear to parallel that of central dopamine [89]. In a study by Bacopoulos et al., male rats were given one of the following regimens: 1) 4-day injections of 6-hydroxydopamine, a neurotoxin designed to abolish dopaminergic neuron; 2) 4-day treatments with a pargyline, a monoamine oxidase inhibitor (MAOI-I); or 3) 4-day treatments with electrical stimulation of the nigrostriatal pathway. The abolishment of the dopaminergic neurons led to decreases in central dopamine, HVA, and plasma HVA concentrations. Similarly, administration of pargyline caused a rapid disappearance of central dopamine and HVA, which was mirrored by plasma HVA concentrations. In contrast, electrical stimulation resulted in significant increases in total plasma HVA. These data support that a significant portion of the circulating dopamine metabolites (namely, HVA) originate from central dopaminergic neurons [89].

We want to note that a portion of circulating, plasma HVA is produced in the periphery by the parasympathetic norepinephrine neurons, and a very small amount from peripheral dopaminergic nerves located in the kidney and liver [66]. However, several studies have been completed which provide additional evidence supporting the

use of plasma HVA to assess central dopamine production. As shown in Kendler et al., a cohort of rats were injected with debrisoquin, a drug known to inhibit peripheral HVA production only, or a combination of debrisoquin and haloperidol, a drug which increases central dopamine synthesis. With debrisoquin, although a decrease in plasma HVA was observed, no changes in central HVA were evident [90]. When both were injected, plasma HVA increased, despite the administration of debrisoquin. These data indirectly demonstrate the rise in central dopaminergic activity (as a result of haloperidol administration) and the formation of centrally-derived plasma HVA [90]. A study conducted by Sternberg et al. using both haloperidol and debrisoquin found similar results [91]. These studies demonstrate that a significant portion of plasma HVA originates from the activity of central dopaminergic neurons and can be an accurate, useful approach.

Homovanillic Acid Studies in Humans

Although limited data exist examining the concentrations of human plasma HVA in obesity and with dietary interventions, a number of studies have been conducted in anorexic and bulimic patients [92].

As shown by Castro-Fornieles et al., plasma HVA concentrations were elevated in patients with anorexia nervosa compared to controls [93]. However, a nine-month treatment program consisting of behavioral modification, biological management, and nutritional rehabilitation led to a significant reduction in plasma HVA in the anorexic patients (pre to post) which resembled that of the controls [93]. Increased plasma HVA was also shown in a similar study by Bowers et al. [94]. Individuals with bulimia exhibit

similar concentrations of plasma HVA to that seen in anorexia nervosa [95]. The altered dopamine responses shown in eating behavior disorders further support the link between normal dopamine signaling and eating behaviors in humans.

Dietary Protein and Central Dopamine

Numerous factors contribute to central dopamine production, with one potential factor being the diet. Dietary protein is a macronutrient essential for the health and nutrition of all individuals, and has been shown to be essential by providing amino acids needed by the cells of the body [96]. Daily protein intake recommendations in adult humans, according to the Food and Nutrition board, are 0.80 grams per kilogram of body weight per day, with a slight increase in adolescents (ages 15-19) to 0.85 grams of protein/kg/day [96]. The acceptable range of protein intake is set between 10% and 30% of overall energy intake per day [96]. However, numerous studies have been conducted exploring the effects of higher proteins meals and diets, with findings reflecting that a modest increase in protein intake leads to reductions in overall energy intake [97, 98], body weight [22, 99-101], and fat mass [22, 99-101], with an increased retention of lean body mass with weight loss compared to that of normal-protein diets [23, 102]. Higher protein intakes have also been shown to reduce feelings of hunger, and increase feelings of satiety and fullness, both with single protein-rich meals and an overall protein-rich diet [9, 23, 24, 103-105]. In examining the physiological, peripheral signals controlling appetite and satiety, research indicates that meals rich in dietary protein lead to a suppression of ghrelin, a peripheral hormone associated with hunger

[24, 98, 106], and an elevation in the satiety hormones Peptide Y-Y_{3-36} (PYY) and Glucagon-like Peptide-1 (GLP-1) [98, 104, 107].

However, as previously discussed, eating is not purely homeostatic in nature. Although there is strong evidence that dietary protein plays a definite role in regulation of physiological, energy-regulating hunger and satiety, limited data exists regarding whether increased dietary protein positively impacts non-homeostatic, reward-driving eating behavior [108]. In a study of overweight adolescent girls, the consumption of a high protein breakfast led greater reductions in brain activation in the anterior insula and middle prefrontal cortex compared to a normal protein version, suggesting that dietary protein positively influences reward driven eating behavior in humans. What is currently unclear is whether dietary protein plays a role in dopamine production and secretion. However, several hypotheses exist.

Tyrosine is an amino acid naturally found in protein-rich foods and is the precursor molecule to dopamine. Dietary protein is broken down during digestion processes, further providing tyrosine and phenylalanine to circulation. Tyrosine is the substrate for the rate-limiting enzyme (tyrosine hydroxylase) in the production of dopamine, and as such, the amount of tyrosine available to these enzymes directly impacts the amount of dopamine being produced [109].

A study by Fernstrom et al. examined the effects of varying quantities of dietary protein consumption on circulating and central tyrosine as well as central DOPA, which is a dopamine precursor [110]. Specifically, rats were fed a diet of 2%, 5%, 10% or 20% protein for a period of 14 days. Circulating tyrosine to LNNAs (large neutral amino acids)

ratio differed by four-fold between the 2% and 20% protein diet, and differed by a three-fold reduction between the 2% and the 10% protein diet. Further, central tyrosine also significantly differed between protein diets. The hypothalamus and prefrontal cortex exhibited a 90%-100% increase in tyrosine levels when comparing the 20% vs. 2% protein diet. As would be expected, DOPA synthesis followed a pattern similar to that of central tyrosine. There was a 50% increase in DOPA concentrations in the hypothalamus with the 10% vs. 2% protein diet. These data support that dietary protein does, in fact, impact central tyrosine levels and dopamine synthesis in rats. Whether or not this phenomenon is mirrored in human subjects has yet to be determined.

IV. Summary

- Obesity continues to be an on-going problem, particularly in adolescents.
- 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, and obesity.
- In America, many individuals, particularly adolescents, eat outside of energy need or 'physiological hunger'; in this group, the initiation of eating is primarily stimulated by the modern food environment, stimulating increased food reward and food cravings.
- A potent signal controlling food reward is the neurotransmitter dopamine, which increases in response to palatable foods and leads to feelings of pleasure and reward.

- Dopamine is blunted in obesity individuals and may be involved in the etiology of obesity.
- Since dopamine cannot be measured centrally in humans in a safe, non-invasive manner, other alternatives including measurement of homovanillic acid, which is the primary, peripheral metabolite of dopamine, have been examined and found to be well correlated to central dopaminergic activity.
- Although high fat, high sugar foods are highly palatable and elicit a dopamine response, dietary protein, which has well-established, beneficial effects on appetite control and satiety, might also play a role in food motivation and reward.

METHODS

I. Experimental Design

This study was a sub-study of a larger study designed to examine whether the addition of a normal protein versus higher protein breakfast reduces physiological and reward-driven eating behavior in overweight and obese 'breakfast skipping' teen girls. Primary outcomes for the parent study included perceived appetite and satiety assessments, metabolic and hormonal signals controlling appetite and satiety, neural activations in response to food stimuli (using fMRI), and evening and total energy intake (i.e., energy content).

Twenty overweight and obese 'breakfast skipping' adolescent girls participated in the following randomized crossover-design breakfast study. The participants randomly completed the following breakfast patterns at home for 6 days: 1) Breakfast Skipping (BS); 2) Consumption of Normal Protein (NP) breakfast meals; and 3) Consumption of Higher Protein (HP) breakfast meals (Figure 1). On the 7th day of each pattern, the participants came to the University of Missouri Brain Imaging Center (MU-BIC) in the morning to complete the respective 4-h testing day. The participants began the testing day by either skipping breakfast or consuming their respective breakfast meal. Blood samples and assessments of perceived appetite, satiety, and food cravings were completed at specific times throughout the morning. There was at least a 7-day washout period between each pattern.

II. Study Participants

Adolescent girls were recruited from the Columbia, MO area through advertisements, flyers, and email listserves 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) no clinical diagnosis of an eating disorder; 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; 7) infrequent breakfast consumer (i.e., ≤ 2 breakfast occasions/wk); and 8) right-handed (required for parent-study fMRI outcome).

One-hundred and forty-seven teens were interested in participating in the study. Twenty-five met the screening criteria, had three available Saturdays to complete the 10 hour testing days, and began the study. Twenty completed all study procedures. Subject characteristics are presented in Table 1. All participants and their parents (if participant is <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 total of $450 for completing all study procedures.

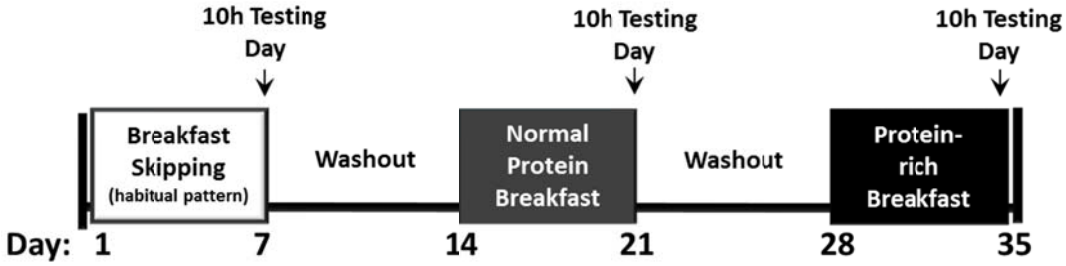
Table 1: Subject Characteristics

Subject Characteristics	Mean ± SEM
Age (y)	19 ± 1
Height (cm)	167 ± 1
Weight (kg)	79.6 ± 2.1
BMI (kg/m^2)	28.6 ± 0.7
Skips Breakfast (#/week)	6 ± 1

III. Breakfast Patterns

The participants completed each of the three breakfast patterns for seven consecutive days (Figure 5). For the BS pattern, the participants continued to follow their habitual practice of skipping breakfast and completed the Day 7 testing day accordingly. For the NP and HP patterns, the participants were provided with specific breakfast meals and asked to consume these at home (before school) between 7-9:30 am for 6 days. Throughout this period, the participants were permitted to eat ad libitum throughout the remainder of each day. On Day 7, 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 'breakfast skipping' behavior.

Figure 6: Randomized Cross-over Design (example)



IV. Breakfast Meals

The breakfast energy intake for the breakfast meals was comprised of 18% of the total energy intake (~350 kcal) as estimated from the energy expenditure equations specific for adolescents [96]. The macronutrient composition of the NP breakfast contained 15% protein (~13 g of dietary protein), 65% CHO, and 20% fat whereas the HP breakfast contained 40% protein (~35 g of protein, 60% of protein from egg and beef sources), 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. (See Table 2).

Table 2: Breakfast Characteristics

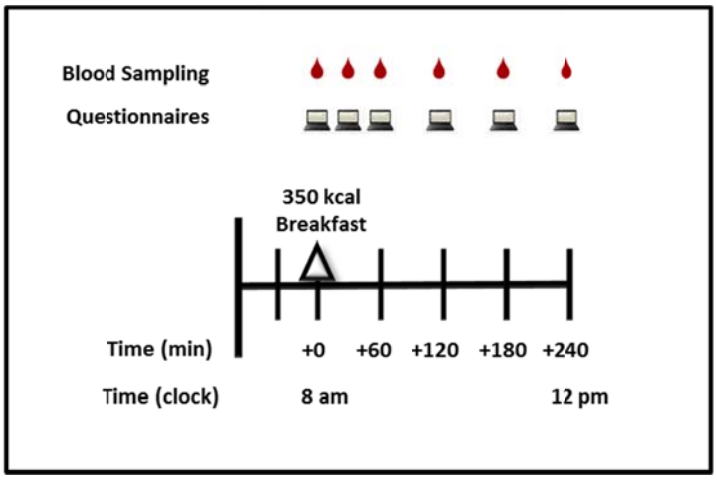
	Breakfast Skipping (BS)	Normal Protein (NP)	Higher Protein (HP)
Energy Content	0	350	350
Energy Density	0	1.33 ± 0.01	1.37 ± 0.03
Total Protein (g) (% of meal)	0	13.0 (15%)	35.1 (40%)
Egg (g)	0	0	11.0
Beef (g)	0	0	11.0
Dairy (g)	0	7.0	7.0
Plant-based (g)	0	6.0	6.0
Total Carbohydrate (g) (% of meal)	0	57.0 (65%)	35.1 (40%)
Sugar (g)	0	18.0	18.0
Fiber (g)	0	6.1	6.1
Total Fat (g) (% of meal)	0	7.8 (20%)	7.8 (20%)
Palatability*	N/A	67.8 ± 6	80.0 ± 4

*Palatability was assessed using the 100 mm VAS with end anchors of 'extremely' and 'not at all'

V. Testing Day Procedures

On a Saturday morning of Day 7 of each breakfast pattern, the participants reported to the MU Brain Imaging Center research facility between 6-9 am after an overnight fast to complete the 4 hour testing day (Figure 6). Each participant was seated in a reclining chair and, for the next 30 min, they simply 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 and a set of computerized questionnaires were completed. At time 0 min, a meal including water was provided during the NP and HP days and only water during the BS day. The participants consumed the meal and/or water within 30 min.

Figure 7: Testing Day Procedures



VI. Perceived Appetite, Satiety, and Food Cravings Questionnaires

Computerized questionnaires, which assessed perceived appetite sensations (i.e., hunger, fullness, desire to eat, prospective food consumption) and food cravings (i.e., salty, sweet, savory, thirst) were completed every 30 min throughout each of the 4-

hour testing days (Figure 6). The questionnaires contained visual analog scales incorporating a 100 mm horizontal line rating scale for each response. The questions are worded as "how strong is your feeling of" with anchors of "not all" to "extremely." The Adaptive Visual Analog Scale Software was used for data collection (Neurobehavioral Research Laboratory and Clinic; San Antonio, TX).

VII. Repeated Blood Sampling and Hormonal Analyses

Six blood samples (4 ml/sample) were collected throughout the 4 hour testing day (Figure 6). The samples were collected in test tubes containing EDTA ethylenediaminetetra-acetic acid). Protease inhibitors (pefabloc SC and DPP-IV) were added to the samples to reduce protein degradation. 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 homovanillic acid (HVA) was measured using the Eagle Biosciences, Inc. enzyme-linked immunosorbent assay.

VIII. Adherence and Compliance to the Breakfast Intervention

To document adherence to each breakfast pattern, the participants returned any uneaten food and containers. They also completed daily food check-off logs which included the list of the provided breakfast food items and quantities to be consumed. They were instructed to eat all the food items provided and mark those that were consumed. Eating foods at breakfast not provided by the study was highly discouraged. If this occurred, extra food was recorded on the food log and energy and macronutrient composition was calculated. Any participant who consumed <80% of the breakfast meal

provided each day for 5 of the 7 days or who consumed 20% of the total breakfast meal from non-study foods was excluded from further participation in the study.

IX. Participant Compensation

The participants were paid a total of $450 for completing all study procedures. Specifically, they received $150 for completing each breakfast pattern.

X. Data and Statistical Analyses

Summary statistics (sample means and and/or 4-h area under the curve (AUC) using the Trapezoidal rule were computed for all study outcomes. A repeated-measures ANOVA was performed to determine main effects of time, breakfast, and protein content for perceived sensations, food cravings, and HVA concentration outcomes. When main effects were detected, post-hoc analyses including pairwise comparisons were performed using Least Significant Difference procedures to identify differences among treatments. Data was expressed as mean ± SEM. Pearson correlations were conducted to determine the any significant relationships between plasma homovanillic acid concentrations and the following factors: dietary protein, perceived appetite and satiety; and food cravings. Analyses were conducted with the latest version of SPSS (Chicago, IL). $P<0.05$ was considered statistically significant.

RESULTS

As shown in Figures 8-11, the line graphs illustrate the responses assessed every 30 min throughout the Breakfast Skipping (BS), Higher Protein (HP), and Normal Protein (NP) breakfast testing days; the bar graphs depict the 4-hour AUC analyses across the testing period.

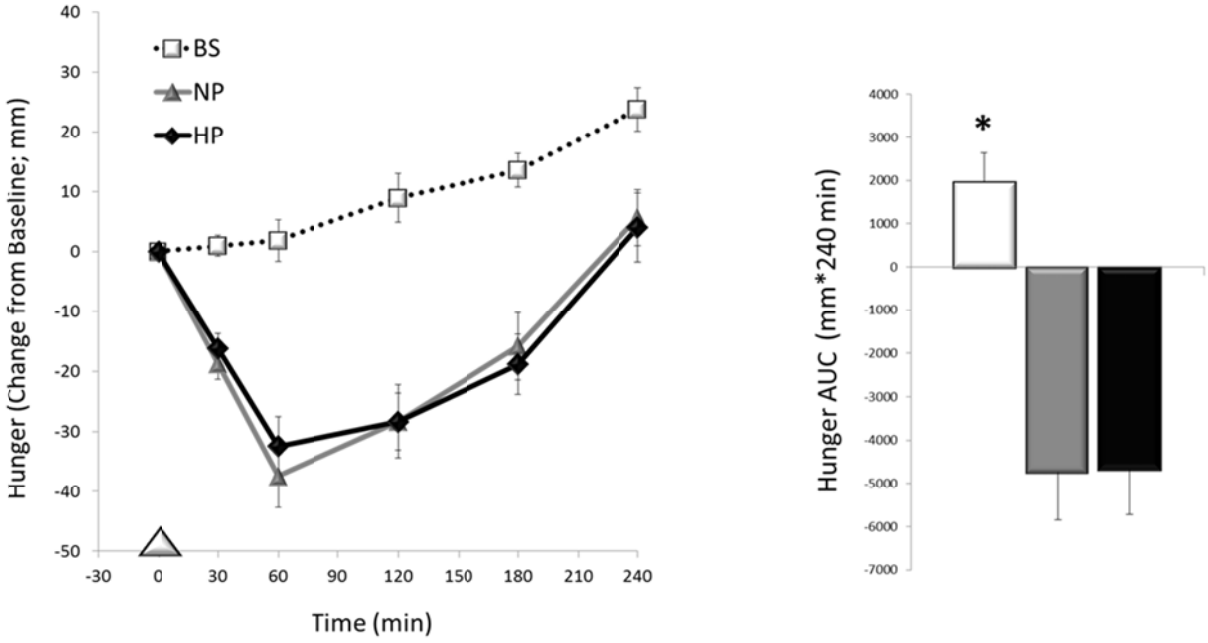
Perceived Appetite and Satiety

As shown in Figure 8a, skipping breakfast led to increased hunger throughout the morning, whereas the consumption of either breakfast meal led to an immediate decline in hunger followed by a gradual rise throughout the post-breakfast period. Statistical comparisons reveal that 4-hour hunger AUC was greater following BS (1956 ± 725 mm*240 min) compared to NP (-4728 ± 1188 mm*240 min, $p<0.05$) or HP (-4663 ± 1109 mm*240 min; $p<0.01$). No significant differences were observed between breakfast meals.

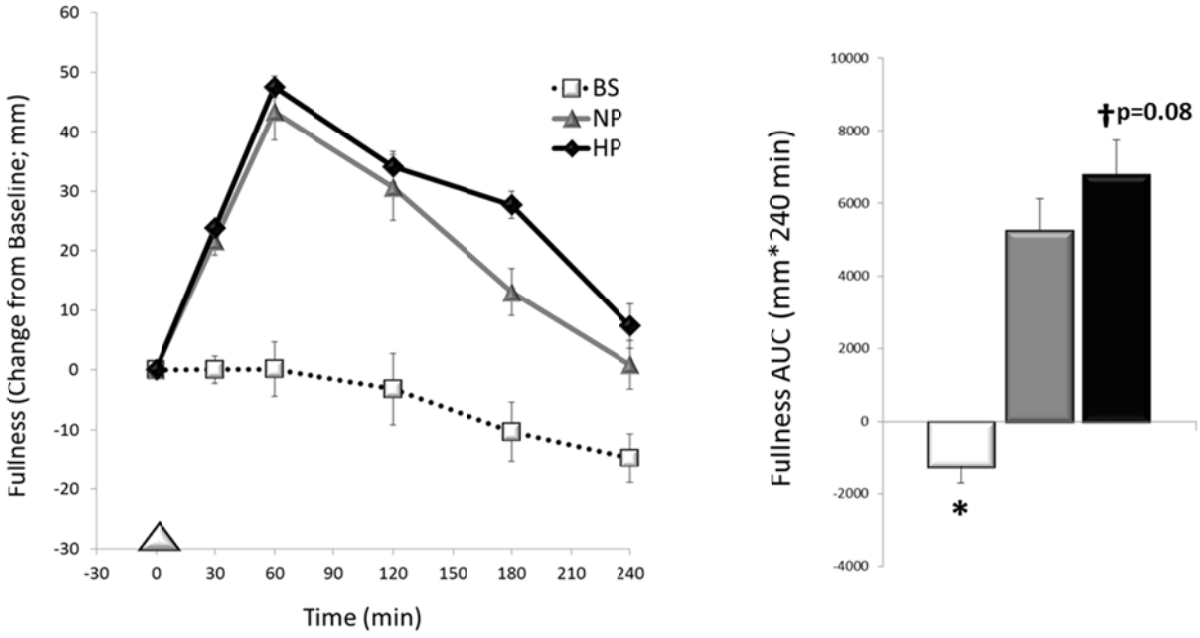
With respect to perceived fullness, skipping breakfast led to reduced fullness throughout the morning, whereas the consumption of either breakfast meal led to an immediate increase in fullness followed by a gradual decline throughout the post-breakfast period. Four-hour fullness AUC was lower following BS (-1245 ± 496 mm*240 min) compared to NP (5250 ± 936 mm*240 min, $p<0.05$) or HP (6783 ± 1015 mm*240 min; $p<0.01$). Additionally, the HP breakfast led to greater fullness vs. NP (trend, $p=0.08$).

Figure 8: Perceived Appetite and Satiety Responses

a) *Hunger responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, p<0.05*



b) *Fullness responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, p<0.05; †NP vs. HP, p=0.08*



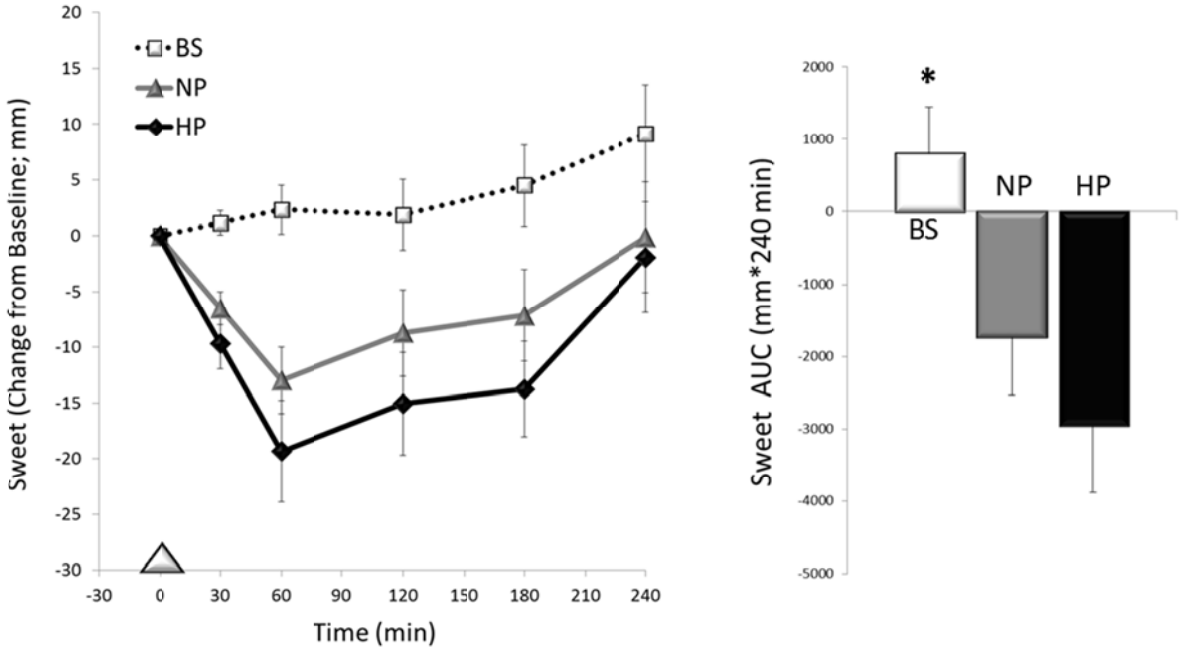
Perceived Food Cravings

As shown in Figure 9a, skipping breakfast led to increased desire for sweet foods throughout the morning, whereas the consumption of either breakfast meal led to an immediate decline in sugar cravings followed by a gradual rise throughout the post-breakfast period. Statistical comparisons reveal that 4-hour sweet AUC was greater following BS (798 ± 689 mm*240 min) compared to NP (-1732 ± 848 mm*240 min, $p<0.01$) or HP (-2949 ± 995 mm*240 min; $p<0.01$). Although 4-hour AUC was not different between breakfast meals, the HP meal led to visibly lower sweet cravings at each post-breakfast time point.

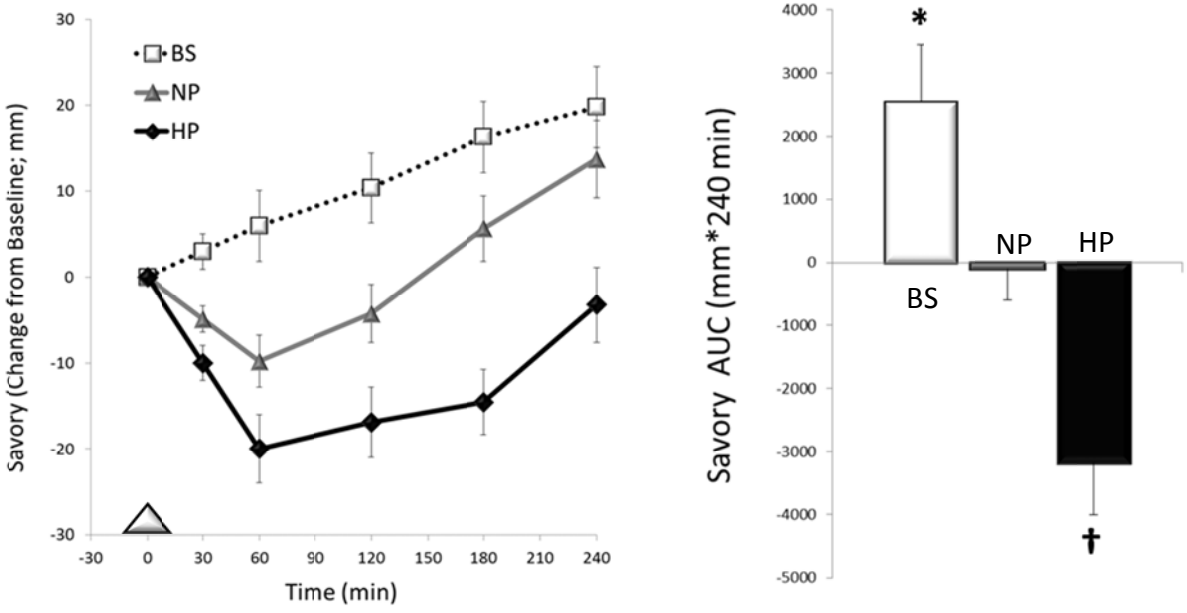
With respect to cravings for savory foods, skipping breakfast led to greater savory cravings throughout the morning, whereas the consumption of either breakfast meal led to an immediate decline in these sensations followed by a gradual decline throughout the post-breakfast period. Four-hour savory AUC was greater following BS (2550 ± 954 mm*240 min) compared to NP (-93 ± 766 mm*240 min, $p=0.07$) or HP (-3185 ± 867 mm*240 min; $p<0.01$). Additionally, the HP breakfast led to reduced savory cravings vs. NP (trend, $p<0.05$).

Figure 9: Food Craving Responses

a) *Sweet responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, p<0.05*



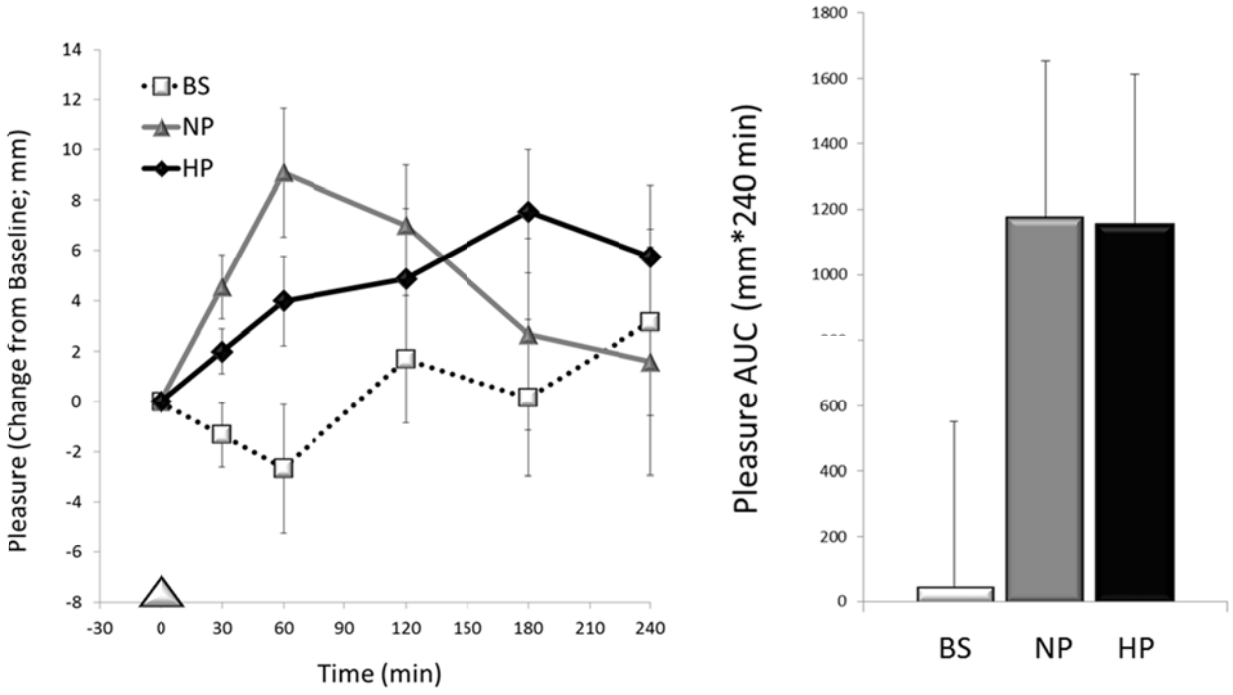
b) *Savory responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, p<0.05*



Perceived Pleasure

Figure 10 displays the perceptions of overall pleasure over the 4-h post-breakfast period. Skipping breakfast led to reduced pleasure throughout the morning, whereas the consumption of either breakfast meal led to gradual increases in pleasure. Although BS appears to reduce 4-h pleasure compared to consuming the breakfast, the differences were only significant shortly after breakfast (i.e., at 60 min post-breakfast, BS vs. NP and HP, $p<0.05$, data not shown), not with 4-h AUC. No differences were observed between NP vs. HP.

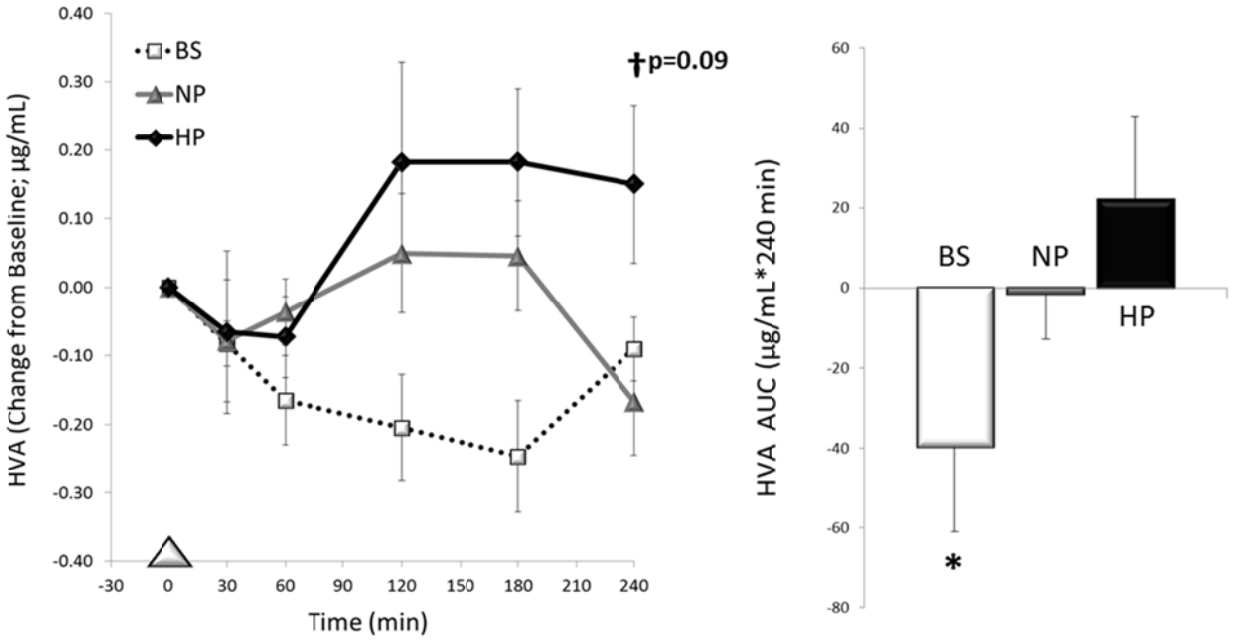
*Figure 10: Pleasure responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, $p<0.05$*



Dopamine metabolite, Homovanillic Acid (HVA)

Figure 10 depicts the changes in plasma HVA concentrations over the 4-h post-breakfast period. Skipping breakfast led to reduced HVA concentrations throughout the morning, whereas the consumption of either breakfast meal led to gradual increases in HVA. Statistical comparisons reveal that 4-hour HVA AUC was lower following BS (-39.64 ± 15.4 ug/mL*240 min) compared to NP (-1.48 ± 12.14 ug/mL*240 min, p=0.07) or HP (21.97 ± 22.66 ug/mL*240 min; p<0.05). Although 4-hour AUC was not different between breakfast meals, the HP meal led to increased 4-hour HVA AUC, whereas NP led to decreased HVA over the 4-hour period. Further, the pre-lunch (i.e. 240 min) HVA response tended to be greater following HP vs. NP (0.150 vs. -0.167 µg/ml; p=0.09)

*Figure 11: HVA responses over the 4 h post-breakfast period comparing breakfast skipping (BS), normal protein (NP), and high protein (HP) patterns; *BS vs. NP & HP, p<0.05*



Pearson's Correlational Analyses

To identify the relationship between peripheral HVA concentrations, study outcomes, and study treatments, Pearson's correlational analyses were performed and are shown in Table 3. Four-hour HVA AUC was associated with fullness AUC, breakfast protein content, and breakfast palatability.

Table 3: Correlational Analyses

4 h AUC, HVA	Pearson's Correlation Coefficient (r)	P-value
Perceived Sensations		
Hunger AUC	-0.262	NS
Fullness AUC	0.309	0.03
Food Cravings		
Sweet AUC	-0.148	NS
Savory AUC	-0.203	NS
Pleasure AUC	0.004	NS
Dietary Components		
Protein	0.340	0.02
CHO	0.252	NS
Breakfast Palatability	0.275	0.05

DISCUSSION

The addition of breakfast led to reductions in appetite and food cravings which were accompanied by increases in satiety and the dopamine metabolite HVA, with slight improvements observed following the higher vs. normal protein meals. Collectively, these data suggest that the daily addition of breakfast, particularly rich in protein, might serve as a beneficial strategy to reduce food cravings and food reward in overweight/obese young people.

Dietary protein has been well documented to improve appetite control through physiological reductions in hunger and increases in satiety [23, 24, 26, 111, 112]. It has recently been implicated as a potential modulator of dopamine synthesis since tyrosine, an amino acid found in protein sources, is the substrate required in the rate-limiting step of dopamine synthesis [64, 65]. Therefore, the consumption of higher protein meals provides greater quantities of available tyrosine for dopamine synthesis. This has been demonstrated in a study of rats fed diets varying in protein content from 2 to 20% of the diet as protein. The higher protein diet led to a substantial increase in central tyrosine which was accompanied by an increase in dopamine synthesis [110]. This demonstrates that dietary protein has the ability to modulate dopamine synthesis and can be an important regulator of both physiological and hedonic food intake.

Another dietary factor which has significant effects on both aspects of food intake is the unhealthy, but common, practice of breakfast skipping. In a pilot study from our lab, we showed that breakfast skipping adolescents exhibit greater appetite and

reduced satiety throughout the morning, leading to increased energy intake; however, the addition of breakfast, particularly breakfast containing increased dietary protein, reversed these outcomes [9]. In a subsequent study, we incorporated functional MRI to identify the neural responses to visual food stimuli prior to lunch in overweight/obese 'breakfast skipping' teen girls [37]. We found that breakfast skipping led to increased neural activation in brain regions controlling food motivation and food reward (i.e., hippocampus, amygdala, anterior cingulate, and parahippocampus) prior to lunch; however, the addition of a protein-rich breakfast led to reduced activation in these regions. The current study extended our previous findings to include another marker of food motivation and reward, that being homovanillic acid, which is the primary dopamine metabolite.

We found that the addition of breakfast led to increased HVA concentrations throughout the morning with greater increases in HVA, albeit not significant, following the higher protein breakfast vs. the normal protein version. These data, along with the positive correlation between HVA concentrations and breakfast protein quantity, suggest that the consumption of increased dietary protein potentially stimulates the formation, secretion, and/or utilization of dopamine. The increased HVA concentrations throughout the morning were also accompanied by reduced feelings of appetite and food cravings, further supporting the role of dopamine on food reward.

Although central dopamine regulates a number of pathways in the body that impact cognition, motor control, mood, pain perception, and sexual behavior [11, 67-71], it has also been shown to be involved with food motivation and reward by

reinforcing the rewarding properties of food [76]. Although dopamine is typically secreted in response to highly palatable foods, chronic exposure to these foods, particularly in obese individuals, leads to neural adaptations including reductions in dopamine receptor expression and decreased dopamine secretion [13]. In diet-induced obese rodents, this reduced dopamine response leads to an overcompensation of foods high in fat to potentially re-establish normal dopamine concentrations [81]. In humans, pyschostimulant drugs such as amphetamines and cocaine increase dopamine secretions and are known to have anorexigenic effects [113]. Further, the administration of dopamine agonists such as bromocriptine and methylphenidate have been shown to significantly reduce body fat and body weight in obese humans [114, 115]. Although the exact mechanisms behind this phenomenon have not been determined, these studies report reduced daily energy intake with the administrations of these agonists. However, other potential mechanisms (i.e., metabolism, energy expenditure, appetite, etc.) to explain the weight and fat loss were not examined in these studies.

Taken together, these data suggest that dopamine appears to play a critical role in modulating the reinforcing value and reward of food. Further, the dopamine pathway is blunted in obesity due to chronic exposure to highly palatable foods but can be re-established with pharmaceutical agents and potentially dietary factors including breakfast and increased dietary protein.

Strengths of this study include analysis of both hedonic (pleasure, cravings) and physiological sensations (hunger, fullness) which influence food intake, providing a better understanding of the effects of protein on both aspects of energy intake. This is also the first study to identify whether a modest dietary intervention (i.e., breakfast) would significantly alter plasma HVA concentrations in healthy, but overweight/obese individuals. This study may have been limited in sample size. Though an n=20 was adequate to detect differences between skipping breakfast vs. breakfast, a larger sample may have led to an increased ability to detect differences, particularly in HVA concentrations, between breakfast meals. However, further research, involving increased sample sizes, longer testing durations, and assessments of subsequent food intake are key in assessing the role of increased dietary protein at breakfast on dopamine, food motivation, and reward in overweight/obese teens.

CONCLUSIONS

In conclusion, the addition of breakfast led to beneficial changes in both physiological and hedonic aspects of food motivation in habitual 'breakfast skipping' teens. Further, the protein-rich breakfast led to additional benefits compared to the NP meal, resulting in increased fullness and plasma HVA. These data suggest that the daily addition of a high protein breakfast might be an important dietary strategy to reduce reward-driven eating and combat obesity in young people.

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APPENDICES

A) Telephone/Email Screening Form

Initial Email Screening Info & Form

Thank you for your interest in the Benefits of Breakfast study! As you are aware, the purpose of this study is to determine the benefits of eating breakfast in young women who typically skip breakfast. However, before I send more information about the study, let's see if you meet the study criteria:

Where did you hear about this? _____

Your Name: _____

Age: _____yrs

Date of Birth: _____mo/day/year

Weight: _____lbs

Height: _____ft _____inches

Do you have diabetes? Yes or No

Have you been clinically diagnosed with an eating disorder? Yes or No

Do you have any other metabolic, neural, or hormonal conditions? Yes or No

If yes, please list: _____

Are you following a specific weight loss or other special diet? Yes or No

If yes, please list: _____

Do you have any allergic reactions or intolerances to dairy or other foods? Yes or No

If yes, please list: _____

Are you right handed? Yes or No

Are you pregnant? Yes or No

How many times per week do you typically eat breakfast? _____

B) Screening Consent Form

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

C) 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
HS IRB Authorized Representative — Date: 10/26/10
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.

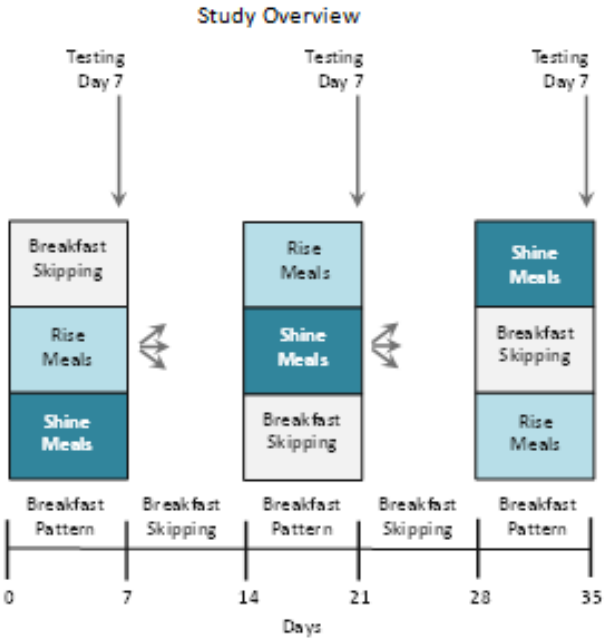
- Over the next 7 days, you/your daughter will go back to your normal pattern of 'Skipping Breakfast.' Sometime during this week, you/your daughter will return the pack-out cooler to us. At that time, we will provide the next breakfast pattern to you/your daughter.

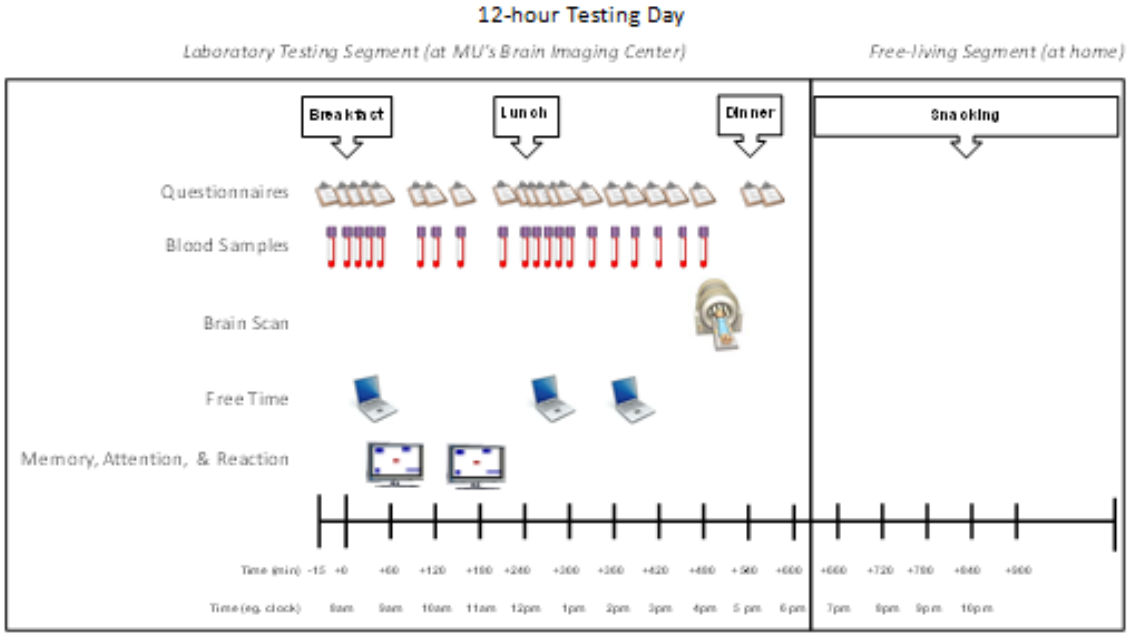
- You/your daughter will repeat these procedures for each of the 3 breakfast patterns.

- 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

D) Perceived Sensations

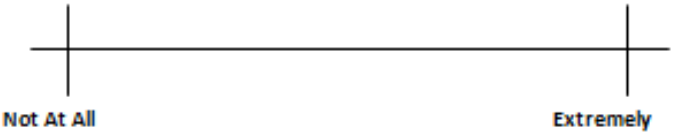
Appetite Questionnaire

ID: _____ Date: _____Testing Day: A, B, C

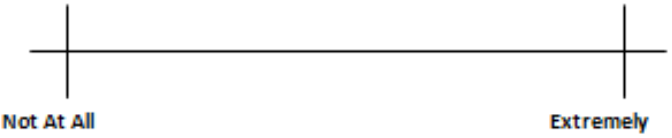
Timepoint: A1 Time:

Directions: Place 1 vertical mark " | " on the scale to reflect how your feel.

1. How strong is your feeling of hunger?



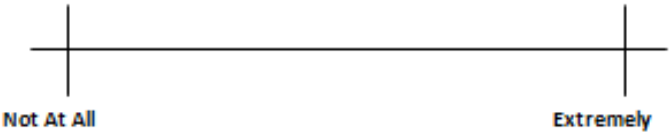
2. How strong is your feeling of being full?



3. How strong is your desire to eat?

4. How much could you eat right now?

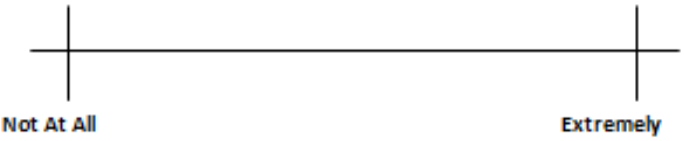
5. How thirsty are you?



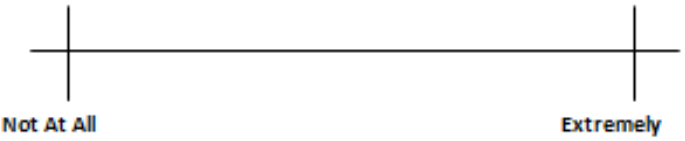
Timepoint: A1 **Time:**

6. How much would you like to have something sweet?

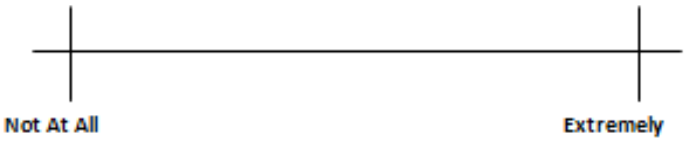
7. How much would you like to have something salty?



8. How much would you like to have some meat?



9. How strong is your overall feeling of pleasure?



10. How pleasant is the taste of the meal you just had?