THE ACUTE EFFECTS OF DIETARY PROTEIN AT BREAKFAST ON REWARD DRIVEN NEURAL ACTIVITY PRIOR TO LUNCH

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Chapter 1:

The prevalence of overweight and obesity among adolescents has tripled in the United States from the 1970s to 2008 [1]with approximately 32% identified as overweight and 17% as obese[2]. Adolescence is a critical life stage as unhealthy weight gain can lead to both immediate and long term physiologic and psychological consequences[3]. In fact, obese adolescents were found to have arteries similar to those of middle-aged adults, suggesting "advanced" vascular age[4] and increased risk of a heart attack, stroke, and premature cardiovascular disease[5]. There is also strong evidence that children with higher BMIs often become obese adults, potentially developing adult morbidities associated with chronic disease [3,6].

Excessive weight gain is inevitably the result of energy dysregulation occurring through reduced energy expenditure and/or eating beyond daily energy needs. Many environmental factors can contribute to this imbalance including those affecting both energy expenditure and energy intake[7]. However, there has been increased emphasis on the environmental effect on energy intake due to the increased availability of highly palatable, energy dense food as well as the increased presence of powerful food stimuli (i.e. billboards, commercials, vending machines) over the past three decades [8]. Collectively, these factors provide the foundation for the 'obesogenic environment', ultimately leading to desensitization to the physiologic, homeostatic signals [9] and an increase in non-homeostatic, reward-driven eating (i.e. eating regardless of physiologic need).

Overweight and obese young people appear to be at an increased risk to these environmental factors. Specifically, they are more susceptible to diets high in sugar sweetened drinks[10], less likely to compensate for 'fast food' calories[11], and show increased consumption of energy-density foods (sweets, chocolate, and crisps) following exposure to food advertisements [12]. However, effective dietary recommendations to combat these detrimental, environmental factors are currently lacking.

In the past, obesity researchers had a difficult time assessing the effectiveness of dietary interventions to reduce non-homeostatic, reward driven eating behavior. Although the limbic system

had been implicated with this type of eating, it was not until the recent advancements in neuroimaging that allowed us to systematically identify the specific brain regions involved. Initial studies examined the brain activation patterns when varying acute, extreme energy states (fasting (i.e., hunger) vs. fed (i.e., satiated))[13] and/or gustatory stimuli. These studies began forming the foundation of knowledge necessary to understand neural encoding of food reward[14-16]. Because visual food cues have become increasingly important in terms of their effects on reward driven eating, current neuroimaging studies have begun to focus on this modality.

Processing visual food stimuli requires the integration of both acute and chronic energy state of the individual as well as the memories and past experiences of the individual. As a result, research has been completed identifying the brain regions responsive to appetizing and appealing visual food cues that vary in motivational value (nutritional content; i.e. high calorie vs. low calorie)[17-21]. Furthermore, researchers have also characterized the effect of varying the motivational state (fasted vs. fed) [18,19,22]of subjects during exposure to these visual food cues. These findings have also been extended to adolescents, which appear to be especially susceptible to this phenomenon[19].

It has been proposed that overweight/obese individuals are especially vulnerable to visual food stimuli, significantly contributing to overconsumption in the current food environment. Studies comparing the limbic brains responsiveness to visual food cues in obese and lean individuals, have found heightened activity in the obese[19,23,24]. These findings further support the need to develop dietary recommendations specifically designed to address the current food environment. The identification of specific dietary factors that can increase feelings of satiety, reduce appetite, and reduce caloric intake is of importance to public health professionals, clinicians, and researchers alike. This appears to be the circumstance with two particular dietary components: breakfast and increased dietary protein[19,23-27].

Using nationally representative data from NHANES III, eating breakfast everyday has been associated with a 4-fold increase in the odds of having a healthful body weight compared to rarely or

never consuming breakfast in adolescents with one obese parent[28]. Further, breakfast skippers also tend to overeat throughout the remainder of the day, especially the evening hours[28]. One alarming trend is the larger amounts of unhealthy foods such as soft drinks, foods high in saturated fat, and energy dense snacks consumed by skippers when compared to breakfast consumers. This is especially disconcerting as these foods are wide spread in the current environment, and many of these types of foods elicit weak appetitive and compensatory responses[29,30]. In addition, dietary habits such as the consumption of breakfast become solidified during adolescents and will continue throughout adulthood further impacting body weight, health and overall wellbeing[3].

With respect to protein consumption, several studies have shown that increasing the amount of dietary protein consumed during an energy-restriction diet results in reduced hunger [31], increased satiety[32] and more satisfaction[31] compared to a normal protein energy-restriction diet. Furthermore, this effect has been substantiated with ad-libitum feeding protocols in which consuming a higher protein diet led to a reduction in daily energy intake which was accompanied by a greater amount of weight and fat mass loss compared to consuming a normal protein diet[32,33]. As a result, manipulating the quantity of protein prescribed in dietary interventions appears to be a promising strategy to combat the obesity crisis.

Recent data from our laboratory examined whether the daily addition of a protein-rich breakfast would lead to beneficial changes in the homeostatic signals surrounding appetite, satiety, and the regulation of energy intake in 'breakfast skipping' adolescents[25]. We demonstrated that, compared to skipping breakfast, the incorporation of breakfast leads to reductions in perceived appetite, increases in perceived fullness, and increases in the homeostatic, satiety-hormone, peptide YY (PYY). Energy intake at the next eating occasion was also reduced following breakfast vs. breakfast skipping. Additionally, the macronutrient composition of the breakfast meal further influenced the beneficial effects of the morning meal. The consumption of the protein-rich breakfast meal resulted in greater reductions in

appetite and energy intake at lunch[25]. These data suggest that the homeostatic signals surrounding appetite control and the regulation of energy intake are modulated with the daily consumption of a protein-rich breakfast. However, it is currently unclear as to whether these dietary factors decrease the susceptibility to environmental food cues and reward driven eating.

Specific Aim 1: To assess whether the daily incorporation of breakfast influences activation of specific brain regions associated with reward-driven eating in 'breakfast skipping' adolescent girls.

Hypothesis 1: Compared to skipping breakfast, the addition of a breakfast meal will result in:

- Attenuation of neural responses to food cues in brain regions associated with food motivation and reward. The regions of interest include the following:
  - Amygdala
  - Hippocampus
  - Cingulate
  - Parahippocampus
  - Insula
  - Orbitofrontal cortex
  - Pre-frontal cortex

Specific Aim 2: To assess whether a protein-rich breakfast results in greater changes in activation of specific brain regions associated with reward-driven eating in 'breakfast skipping' adolescent girls.

Hypothesis 2: Compared to a normal protein breakfast, a protein-rich breakfast will result in greater:

- Attenuation of neural responses to food cues in brain regions associated with food motivation and reward. The regions of interest include the following:
  - Amygdala
  - Hippocampus
  - Cingulate
  - Parahippocampus
  - Insula
  - Orbitofrontal cortex
  - Pre-frontal cortex

Specific Aim 3: To identify whether the activation in the brain regions of interest correlate with perceived appetite and satiety prior to lunch.

Hypothesis 3: Perceived appetite will be correlated with amygdala, hippocampus, parahippocampus, and insula, whereas perceived fullness will be inversely correlated with amygdala, hippocampus, parahippocampus, and insula.

Chapter 2: Literature Review

This review provides scientific evidence supporting the role of food reward on appetite, satiety, and motivation to eat through the incorporation of neuroimaging techniques to identify reward-driven brain activation patterns in response to acute and chronic energy states.

Components of Ingestive Behavior and Food Motivation

Hunger vs. Appetite

The distinction between hunger and appetite has been a topic of debate for decades. While the difference between the two components is difficult to ascertain, both are critical factors underlying the initiation of eating. In general, hunger is the strong need for food arising from the discomfort, weakness, or pain caused by a prolonged lack of food consumption [34]. Alternately, appetite is the desire or craving for a particular food or drink arising from the self-established pleasurable/rewarding properties of that food/drink. Based on these definitions, hunger reflects the physiological metabolic needs/energy state of the individual, whereas appetite reflects the psychological desire to eat. Although these components by definition act independently, intense hunger has also been shown to increase the appetizing, rewarding properties of food suggesting a synergistic association between these factors [35]. Regardless of the differing, underlying principles associated with these factors, both result in the overall increased motivation to eat. As our knowledge of ingestive behavior continues to grow, the intimately related nature of hunger and appetite becomes more apparent. However, in the current obesogenic environment, appetite-driven motivation to eat might be the stronger driving force. An example of this is seen in developed counties where motivation to eat arises more from habitual eating schedules, cultural customs, and readily available, highly palatable/highly rewarding foods rather than from energy depletion [36]. As a result of the paradigm shift in ingestive behavior (i.e., eating for reward, not physiologic need), a greater emphasis has been placed on understanding appetite-derived motivation to eat, with special emphasis on the brain regions encoding reward driven response.

Satiation and Satiety

While hunger and appetite are integral components of the initiation of eating, satiation and satiety are fundamental components in the cessation of eating. Satiation refers to the sensation of fullness that develops during the course of a meal and brings about the cessation of eating. Satiety is the sensation of fullness beginning at the end of an eating episode and serves to inhibit the initiation of the next eating occasion[37]. Just as the initiation of eating is influenced by food reward, satiety is also subject to the sensory properties of the food, showing a more pronounced decrease in motivation for a previously eaten food than that to an un-eaten food[38]. This effect has been termed sensory-specific satiety [38] and further emphasizes the effects of the rewarding properties of food on ingestive behavior.

Liking vs. Wanting

Two fundamental concepts surrounding food motivation and reward have emerged, liking and wanting, and are now partially discernable. The term 'liking' represents the collective perception of a particular food item based on objective sensory properties of the food (e.g., sight, taste, smell, flavor, temperature, and mouth-feel) which remain relatively unchanged regardless of energy states (i.e., fasting, fed, energy restriction, etc.). Alternately, 'wanting' is the actual seeking potential (motivation) for a particular food and represents how hard someone is willing to work to obtain the particular food. Similar to 'liking', 'wanting' includes the sensory properties of food but also includes other factors such as energy state, the degree of hunger or satiety, social acceptability of the food, food cost, and time of day.

Brain Systems involved with Ingestive Behavior and Food Motivation

Ingestive behavior is a complex process involving specific brain regions which act alone or in tandem to regulate the initiation and/or cessation of eating. The hypothalamus and brain stem have classically been considered the regions responsible for 'homeostatic' energy regulation. However, as our knowledge of energy regulation continues to grow, homeostatic and reward driven (non-

homeostatic) mechanisms have become less distinguished. Specifically, peripheral appetite-regulating hormones, previously thought to only act through the hypothalamus have now been shown to alter activation in other brain regions [39,40].

It is believed that emotions evolved in response to beneficial/harmful feelings underlying stimuli, and serve as a mechanism to reinforce or suppress behavior [41]. Thus, the drive to eat is intimately related to underlying feelings and emotions from eating experiences throughout life in addition to the energy state of the individual.

The 'limbic brain' has been identified as the center for emotional and motivational processing, including reward driven eating[42] and includes the limbic and para-limbic systems as well as components of the midbrain and higher order, multimodal regions of the frontal lobe [16]. The 'limbic brain' is capable of encoding unimodal senses from both internal and external sources, as well as combining sensory information with subjective stimuli (cognitive restrain, memories/past experiences, social context, etc) in multimodal processing. The functional interconnectivity of 'limbic brain' regions ultimately serves to encode all aspects of ingestive behavior (see Table 1 for regions and general functions).

Table 1: Brain Regions Involved with Food Motivation and Reward

Category	Region	Function	Experiment
Limbic	Amygdala	Memory and emotion	6,13,17,19,20,24,40,43
	Hippocampus	Memory/emotion/sensorimotor	13,24,40,44-47
	Striatum		
	Dorsal	Habit/memory/motivation	13,24,40,45,46,48
	Ventral	Reward	17,24,48-50
Para-limbic	Anterior Cingulate	Reward/decision	13,24,40,47,50
	Parahippocampus	Memory	5,13,40,43,47
	Fusiform	Object recognition	8,40,43,44,51
	Insula	Emotion/gustatory/desire/craving	19-21,24,40,45,47,52-54
	Orbitofront	Emotion/punishment/appetitive	19-21,24,40,54,55
	Lateral	Emotion/reward/appetitive value	
	Medial		20,24,50,55,56
Frontal	Prefrontal cortex	Executive function	19,20,24,40
Midbrain	Ventral teg. Area/ Substantia Nigra	Pleasure/reward/addiction	17,24,57
Homeostatic	Hypothalamus	Energy balance	13,20,44

Adapted from Van Vaugt et al

Techniques Developed to Measure Brain Activation

The advancements in neuro-imaging techniques have greatly increased our understanding of reward (hedonic) driven eating. The two most widely used techniques that are used to assess neural activity driven by food reward are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), both of which use change in blood flow resulting from neural activity as a proxy of neural activity.

PET

PET measures the emissions of radioactively labeled isotopes that have been injected intravenously. The most common radioactive isotope used in research surrounding food motivation and reward is H_20^{15O} as it readily crosses the blood brain barrier and thus can be used to measure cerebral blood flow (CBF). In general, as neural activity increases, blood flow increases. This results in an increased uptake of the H_20^{15O} in brain tissue, leading to a localized increase in gamma rays detected.

Thus, the local hemodynamic changes accompanying neural activity can be measured with a PET scanner[58]. Given that the half-life of H_20^{15O} is ~2 min, images are acquired every 7-8min in experiments with a spatial resolution of ~4-5mm [59]. The limitations of this method are therefore related to the low spatial resolution and the need for a radioactive tracer.

fMRI

fMRI, technically known as blood oxygen level dependent fMRI (BOLD fMRI) is used in combination with structural images produced by MRI. The structural MRI image is aligned with BOLD fMRI image to identify the location of activation with in a standardized brain space. During MRI, the subject is placed in a strong magnetic field that serves to magnetize tissues, aligning atoms nuclear spins (most notably hydrogen) as magnetic dipoles. The aligned hydrogen nuclei are then excited using radio frequency pulses, causing the dipoles to assume high energy (oriented against the magnetic field) states. As the nuclei relax from the excited state they emit radio frequencies that are detected by the receiver coil of the MRI machine. Since the time course at which tissues return to the lower energy state differs among tissues, it serves as the source of contrast for an MRI image. In BOLD fMRI, the blood oxygen level is used as an endogenous contrasting agent. Neural activity ultimately results in increased oxygenation of the blood which thereby decreases the concentration of deoxyhemoglobin. The paramagnetic properties of deoxyhemoglobin serve to distort the magnetic field, affecting the relaxation process. Thus with an increase in oxy-hemoglobin at the site of neural activity, the distortion of the magnetic field is reduced, resulting in an increase in fMRI signal (1-5%). The spatial resolution of BOLD fMRI can be as high as $1mm^3$ depending on characteristics of the scanner like magnetic field strength[60]. Although whole brain images can be obtained in less than 2 seconds, the overall temporal resolution is limited by the much slower hemodynamic response (12-18 sec)[49]. The non-invasiveness, high spatial and temporal resolution of fMRI makes it an enticing method for assessing neural activity surrounding Ingestive behavior.

Use in research

Both PET and fMRI have been routinely used in this line of research, with the less invasive BOLD fMRI becoming increasingly prominent. Experimental designs assessing food reward typically use a 'block design' in which blocks (periods) of stimuli are presented, generally for 30 seconds each. Stimulus blocks are presented and alternated with a control condition and/or comparison stimuli, each of which is repeated several times. By using stimuli related averaging, this design serves to increase the statistical power of the experiment, and is used to investigate visual, olfactory, and gustatory stimuli.

Identifying Brain Regions Involved with Hunger, Satiety, and Food Reward (Table 1)

Brain Regions Associated with Hunger & Satiety

Only one study to date specifically identified the neural responses associated with hunger and satiety by varying energy status (fed vs. fasted) and controlling for sensory stimulation. In a study by Tataranni et al. [13], 11 normal weight adult men, on 2 separate days, were scanned using positron emission tomography (PET) following a 36-hr fast or after the consumption of a large meal (i.e., 50% of daily energy needs) through a tube placed in subjects mouths. During the scans, the subjects remained in a resting state with no stimulus (i.e., no sensory input, visual pictures, etc.). Using this paradigm, any differences in brain activation can be attributed to the acute fasted vs. fed states. Fasting led to an increase in activation in the following homeostatic, and 'limbic brain' brain regions: hypothalamus, insula, medial orbital frontal cortex, anterior cingulate, parahippocampal gyrus, hippocampus, and ventral striatum (all regions, fasted vs. fed states, $p<0.005$ uncorrected). Alternatively, the fed (satiated) state led to an increase in the ventromedial and dorsolateral prefrontal cortex (all regions, fed vs. fasted states, $p<0.005$ uncorrected).

Two subsequent studies show support for these findings, indicating positive correlations between hunger and brain activation. Specifically, increased hunger was associated with increased activation in the hypothalamus, ventral striatum, anterior cingulate, insula, and medial prefrontal cortex

($p<0.05$) [23,61]. Taken together, the findings from these studies establish the foundation of brain regions implicated with hunger and satiety.

Brain Activity Surrounding Food Reward

The consumption of food involves not only the consumption of the energy content of the food but the taste, smell, and sight of food. The later involves the reward (hedonic) properties of food, not necessarily homeostatic effects of food consumption. While the neural activation surrounding the homeostatic control of appetite can be determined through the manipulation of varying energy states in the absence of any external stimuli, the activation in response to reward/hedonic aspects of food can only truly be accessed through alterations in sensory stimuli (i.e., gustatory, olfactory, visual).

Gustatory Stimuli

For centuries, chocolate has been known for its high hedonic properties, eliciting strong desire and pleasure similar to that seen with other addictive behaviors such as alcohol, drugs and sexual behaviors [62,63]. As a result, the use of chocolate as a gustatory reward has provided insight into our understanding of food reward and motivation to eat. Several studies have been completed which examine the brain activation patterns in response to tasting chocolate. To date experiments have compared the neural activity of receiving chocolate to both neutral control solutions and slightly aversive (saline) solutions; and feeding chocolate under differing states of desire (i.e. following a fast in which subjects 'want' the food (deemed a highly pleasurable experience) and following chocolate induced satiety in which subjects no longer 'want' the food (deemed an aversive experience). Using small quantities of chocolate in conjunction with subjects desire, the taste of chocolate has been found to activate several brain regions including the insula, cingulate gyrus, and the lateral orbital frontal cortex [64,65]. On the other hand, the slightly aversive saline condition was found to activate the amygdala, medial orbital frontal cortex, cingulate gyrus, and hippocampus[65]. Also of interest is that the brain regions encoding the pleasurable taste of chocolate were found to be modulated depending on

whether the subject experiences chocolate satiety (i.e., when over-exposed to the chocolate stimuli). Collectively, these results provide supporting evidence that the 'limbic brain' encodes feelings/perceptions of rewarding, aversive gustatory stimuli and potentially the degree of reward experienced.

Olfactory Stimuli & Combined Gustatory Effects

Undeniably, the sense of smell is a part of the pleasurable experience of eating, both as an independent factor and as a component of food flavor, which incorporates taste and smell. To identify the neural activity underlying both aspects of this response, Araujo et al. [14] scanned healthy subjects receiving pleasurable food odors alone, or in combination with the taste of the respective foods. The pleasurable food odors resulted in increased activation in the caudolateral orbital frontal cortex and amygdala ($p<0.005$, uncorrected). Additionally, the combined smell and taste of the pleasurable food led to heightened activation in the anterior orbital frontal cortex above the individual responses to these stimuli ($p<0.005$). Based on these findings, the 'limbic brain' brain appears to activate alone or in combination with gustatory stimuli and food odors. In fact, the combined experience of flavor elicits a synergistic effect on activation in frontal regions of the 'limbic brain' brain, above the effects of taste and smell alone [14].

Order of Brain Activation

Using the rewarding (i.e., flavor) properties of food, it is now possible to partition the neural activity responsible for the two underlying aspects of reward: hedonic 'liking' and appetitive 'wanting'. The rationale behind this line of research involves the following concept. If there are separate brain regions encoding 'liking' and 'wanting', then those regions that continuously respond to pleasurable food stimuli, regardless of whether the drive to eat the respective food is still present or not, would be implicated in 'liking'. On the other hand, those regions that are initially activated in response to a

pleasurable food stimulus but become inactivated with repetitive exposure to the respective food would be involved with 'wanting.'

To support this concept, Gottfried et al. [15] designed a study in which subjects smelled pleasurable odors (i.e., vanilla or peanut butter) before and after eating foods associated with these odors (i.e., vanilla ice cream or peanut butter sandwiches). The quantity of a food consumed was designed to lead to a decreased motivation to consume that food (i.e. satiety). The subjects randomly completed all combinations of odors and foods. When the subjects were presented with the food odor which was NOT associated with the food consumed to satiety, there was an increased activation in the ventral striatum, insula, and cingulate gyrus ($p<0.001$, uncorrected). However, these responses were decreased when presented with the food odor which was associated with the food consumed to satiety. These data suggest that there are specific limbic structures underlying the degree of 'liking' [15]. Unlike the other previously mentioned regions, the medial orbital frontal cortex and amygdala ($p<0.001$, uncorrected) were only found to decrease when presented with the food odor associated with the food consumed to satiety and are thereby implicated in processing the 'wanting' aspect of reward. This higher order processing in the orbital frontal cortex, combining subject desire and underlying 'liking', is supported by correlations between neural activity in this region and subjective pleasantness of ratings of food flavor [14]. Moreover, these results are supported by both studies of similar designs [56] and studies using solely taste stimuli [66]. Taken together, these data support that drive/motivation to eat incorporates several 'limbic brain' regions but is ultimately controlled by the higher order, processing of the orbital frontal cortex and amygdala.

Collectively, the use of neuroimaging methodologies incorporating gustatory and olfactory stimuli has provided substantial evidence for the role of the 'limbic brain' brain in the many aspects surrounding motivation to eat and reward; including the partitioning of the degree of hedonic (liking) and appetitive drive (wanting) toward rewarding stimuli. However, recent evidence suggests that the

rewarding properties of gustatory and olfactory stimuli involved with food flavor is significantly impacted by visual food cues as well [50]. Thus, current research is focusing on the visual component of human ingestive behavior.

Visual Stimulation and Reward

Recent evidence has emphasized that vision is another extremely important component of this process. To illustrate this point, one study found that when participants were blindfolded during the consumption of a standardized meal, intake at that meal was reduced by 24% (blindfolded vs. un-blinded; $p<0.05$)[53]. On a daily basis, Americans are exposed to two main types of visual food cues: 1) stimuli occurring during the act of eating and 2) stimuli in which immediate consumption is not possible (i.e. vending machines, food-related billboards, TV commercials, etc). The later places an increased emphasis on the memories/past perceptions of the reward value of the visual food cues. Data have begun to accumulate showing that increased exposure to visual food cues may be the main driving force to chronic overconsumption[67]. This effect is especially evident in children and adolescents where substantial increases in energy intake occur following exposure to food advertisements and other food cues[12,68], with the largest increase occurring in obese subjects[12,68]. Thus, increased attention has been placed on assessing the brain regions associated with the rewarding properties of visual food stimuli.

Visual Food Cues and Brain Activity Surrounding Reward

To identify the regions of the brain that are specifically responsive to visual food stimuli, neuroimaging studies have been performed which compare visual food stimuli (i.e., food pictures) to non-food visual stimuli (blurred images, landscapes, people, animals, etc). These types of comparisons adjust for the brain activation that occurs in response to simply viewing pictures (i.e., blurred images) as well as viewing highly pleasurable, but non-appetizing pictures (i.e., animal, people, etc.). It should also be noted that viewing food pictures, instead of being exposed to actual food, places a larger emphasis

on past memories/experiences (i.e. individual perceptions) of food instead of the stimuli experienced when food in consumed.

One of the first studies incorporating this approach identified brain activation patterns when viewing food vs. non-food pictures [19]. Specifically, nine adolescents viewed food pictures (deemed highly pleasurable and appetizing) compared to non-food, animal pictures (deemed highly pleasurable but non-appetitizing) and baseline, blurred pictures (deemed non-pleasurable and non-appetizing) during fMRI. Overall, viewing food pictures elicited greater activation in the insula, frontal and prefrontal cortices, parahippocampal gyrus, and fusiform when compared to non-food pictures (food vs. blurred images & non-food animals; $p< 0.05$, 3 contiguous voxels). In a similar study, 12 adults viewed food and non-food (i.e., landscaping, people etc) pictures during fMRI[21]. Increased brain activation in the insula and lateral orbital frontal cortex were observed when viewing food vs. non-food pictures ($P<0.05$, corrected).

To extend these findings, Beaver et al[17] identified brain activation patterns in response to different categories of pictures ranging in appetitive appeal. In this study, 14 adults viewed pictures of 1) highly pleasurable, dessert foods (e.g. chocolate cake, ice cream); 2) bland foods (e.g. uncooked rice, potatoes); 3) non-food items (e.g. video tapes, iron); and 4) aversive food (e.g. moldy bread, rotten meat). When compared to the bland and non-food items, the appetizing foods led to increased activation in the lateral orbital frontal cortex and the ventral striatum ($p > 0.001$, uncorrected). Similar findings were also observed in other studies comparing 1) food pictures (appetizing and appealing) vs. non-food pictures (e.g. tools)[18] and 2) high calorie foods (burgers, cakes, and chocolate) vs. low-calorie food (salads, fruit, and vegetables)[20]. In the former study, increased brain activation was observed in the prefrontal cortex, insula, and striatum regions in response to viewing appetizing vs. non-food pictures (all comparisons, $P< 0.001$; 8 contiguous voxels); the later study found greater activation in the

orbital frontal cortex, insula, amygdala, and ventral striatum (all comparisons, $p<0.001$) when viewing higher vs. lower calorie food pictures[20].

Taken together, these studies identify specific regions of the 'limbic brain' which are involved with food motivation and reward. However, the relationship between reward-driven brain activation and visual food stimuli can be strengthened by assessment across acute energy states. This approach will provide information regarding the effects of motivation state (i.e., fed/fasted) on brain activity during exposure to appetizing and pleasurable visual food stimuli.

Visual Stimulation When Varying Acute Energy States

In any given day, people are exposed to visual food stimuli both prior to and following the consumption of meals. However, it was previously unknown if the nutritional state of an individual effects the neural activation of the perceived reward to visual food stimuli. Fuhrer et al[18] conducted an experiment to determine if this effect does indeed exist. Specifically, 12 men were scanned through fMRI in two conditions: 1) following a 14-h fast and 2) one hour after ad-libitum feeding. During the scan, the subjects viewed food and non-food pictures (i.e., household tools)[18]. When compared to the fed (satiated) state, fasting led to increased brain activation in the amygdale, anterior cingulate and medial orbital frontal cortex (food vs. non-food in fed vs. fasting state; $P<0.001$; 8 contiguous voxels). These data show that activation of brain regions implicated in encoding reward value is heightened during exposure to food pictures in accordance with subject's motivational state.

These findings were extended in a study by Goldstone et al.[22], which incorporated both altered energy states and visual food stimuli differing in nutritional content[22]. In this study, 20 adults were scanned using fMRI in two conditions: 1) following an overnight fast with no morning meal and 2) following an overnight fast with a satiating morning meal of ~700 kcal. During the scans, the subjects viewed pictures of 1) high calorie foods; 2) low-calorie foods; 3) non-food stimuli (i.e., household products); and, 4) baseline non-food stimuli (i.e. Gaussian blurred pictures). During fasting, the high

calorie food stimuli led to increased activation in the striatum, amygdala, insula, and orbital frontal cortex compared to the low calorie food (high vs. low calorie foods; P<0.001). In contrast, no differences in brain activation were observed when viewing the high vs. low calorie foods in the fed condition. When comparing motivational states, the fasting condition led to increased activation in the striatum, amygdala, insula, and orbital frontal cortex (high vs. low calorie foods in fasting vs. fed states; P<0.001) whereas the fed condition led to increased activation in the hippocampus, cingulate gyrus, and prefrontal cortex (high vs. low calorie foods in fed vs. fasting states; P<0.001). These findings support that of Fuhrer et al.[18] and indicate that activity in the 'limbic brain' is increased in response to both the nutrition content of food pictures, which is an indication of the degree of food reward, and motivational state.

Given that there could be an increased risk in children and adolescents for environmental stimuli to trigger overconsumption of food/energy, it is imperative to determine if similar brain activation patterns are also observed in children and adolescents. As previously described, Holsen et al.[19] performed fMRI scans in nine adolescents. In this study, the subjects were scanned in a pre-meal (fasted) condition and immediately after consuming a 500 kcal meal [19]. Again, during the scans, the subjects viewed food pictures (deemed highly pleasurable and appetizing) and non-food, animal pictures (deemed highly pleasurable but non-appetitizing), and baseline, blurred pictures (deemed non-pleasurable and non-appetizing). The pre-meal condition led to increased activation in the amygdala, orbital frontal cortex, medial frontal cortex, insula, parahippocampal gyrus, cingulate gyrus, and fusiform gyrus vs. post-meal (food vs. baseline; pre vs. post-meal; $p< 0.05$, 3 contiguous voxels). When comparing the pre vs. post-meal responses using the food vs. non-food (animal stimuli) contrast, increased activation was observed in the superior frontal and fusiform gyrus ($p< 0.05$, 3 contiguous voxels). These results indicate that children and adolescents exhibit stronger responses to visual food stimuli as supported by the additional brain activation in several regions of the 'limbic brain' which were

not observed in adults. These findings suggest a heightened susceptibility to environmental food stimuli in children and adolescents.

Visual Food Stimuli: Obese vs. Lean

The importance of heightened neural activity relating to food reward has become paramount in neuroimaging studies surrounding obesity. Many have sought to identify the relationship between chronic overconsumption and perceived reward experienced from visual food stimuli. According to Stoeckel et al.[24] viewing pictures of high calorie foods led to heightened activity in various regions of the 'limbic brain', including the orbital frontal cortex, prefrontal cortex, amygdala, ventral striatum, cingulate gyrus, and hippocampus in obese subjects compared to leans subject ($p<0.01$, 7 contiguous voxels).

These findings were extended by Martin et al.[23] using the same experimental design previously discussed in Holsen et al.[19]. In the pre-meal scan, the obese subjects exhibited greater activation in the anterior cingulate the medial prefrontal cortices, and the medial, middle, and inferior frontal cortex compared to the lean subjects (food vs. non-food; obese vs. lean; $p<0.001$, 3 contiguous voxels). In the post meal condition, the obese subjects exhibited greater responses in the medial prefrontal cortex, superior frontal cortex, and hippocampus vs. lean subjects (food vs. non-food; obese vs. lean; $p<0.001$, 3 contiguous voxels). in concert, these findings indicate that exposure to external visual food stimuli, which is commonplace in our society, leads to heightened activity both prior to and following the consumption of meals in obese vs. lean individuals.

Gaps in the Literature

Neuroimaging has led to the identification of the specific brain regions within the 'limbic brain' involved with appetite, satiety, and food motivation. We can now use this approach to assess whether the reward-driven regions of the brain are responsive to specific dietary interventions developed to

combat obesity and subsequently delay the initiatiation of eating and/or reduce the quantity of energy consumed.

Two of the more common dietary strategies which have been suggested to improve appetite control and body weight management are higher protein intake and breakfast consumption [27,69]. The data is extensive and conclusive regarding the beneficial effects of higher protein intake but limited with respect to the daily addition of breakfast. However, while the majority of these studies have assessed the homeostatic control of appetite and energy intake in response to these interventions, no studies to date have identified whether the non-homeostatic, reward-driven control is modulated by dietary protein and breakfast.

Chapter 3: Research Methods and Procedures

Participants

Adolescent girls were recruited from the Kansas City, KS area through advertisements, flyers, and email listserves to participate in the study. Eligibility was determined through the following inclusion criteria: 1) age range 13-18 y; 2) overweight to obese (BMI: 25-34.9 kg/m^2; 85-99th percentile for BMI for age); 3) no metabolic or neurological diseases or other health complications; 4) not been clinically diagnosed with 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) frequently eats lunch (i.e., ≥ 5 eating occasions/wk); 7) infrequently eats breakfast (i.e., ≤ 2 breakfast occasions/wk); and 8) is right-handed.

Fifty-one volunteers were interested in participating in the study. Twelve met the screening criteria and began the study, and 10 completed all study procedures. The two participants who did not complete the study procedures were unable to stay awake during the fMRI brain scan and were thus excluded from all analyses. Subject characteristics of those who completed the study are presented in Table 1.

Table 1: Subject characteristics of the 10 'breakfast skipping' adolescent girls who completed all study procedures

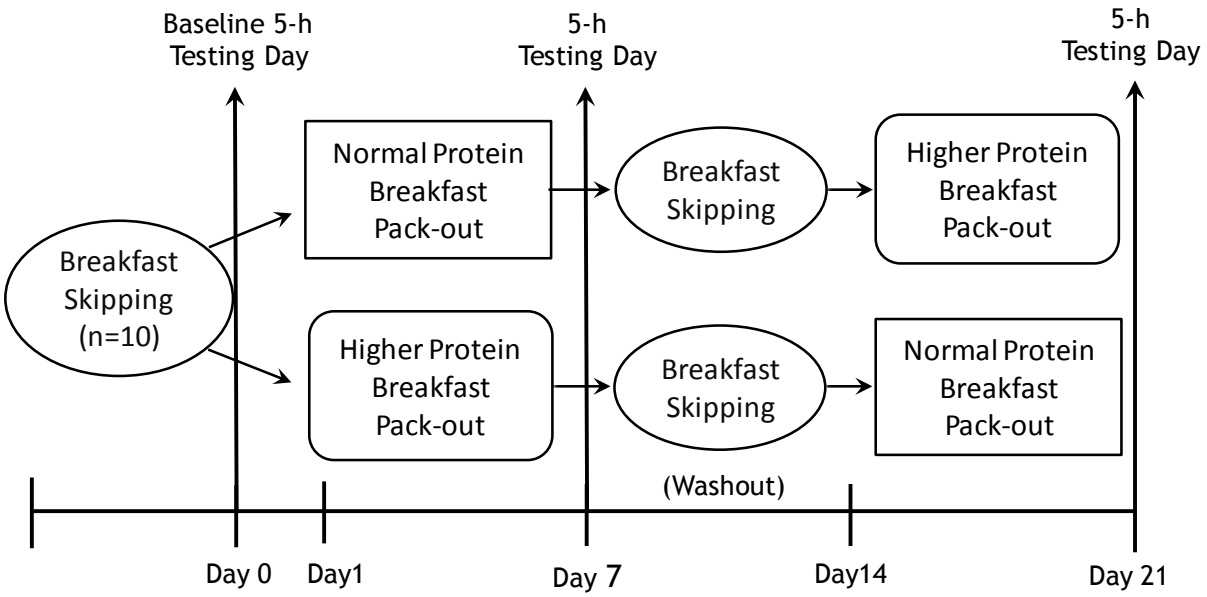
Subject Characteristics	Mean ± SEM
Age (y)	15 ± 1
Height (cm)	165 ± 2
Weight (kg)	79.1 ± 3.3
BMI	
Percentile for age and gender (%ile)	93.1 ± 1.4
Actual (kg/m^2)	29.0 ± 1.0
Breakfast Skipping (#/week)	5 ± 1

All participants and their parents (or legal guardians) were informed of the study purpose, procedures, and risks and signed the consent/assent forms. The study procedures were approved by the University of Kansas Medical Center Human Subjects Committee and the General Clinical Research Center Advisory Committee. The subjects received $120 for completing all study procedures.

Experimental Design (Figure 1)

This study was performed in conjunction with another study examining the effects of increased dietary protein at breakfast on appetite control and the regulation of food intake with respect to the physiological, energy regulatory hormonal signals. The current study utilized a semi-randomized, crossover design consisting of 3 separate testing days. Following the baseline 'breakfast skipping' (BS) testing day, the participants were randomly provided with a normal protein breakfast (NP; 18 ± 1 g protein/meal) or a higher protein breakfast (HP; 50 ± 1 g protein/meal) to consume at home for six days. On the seventh day of each pattern, the participants completed the respective testing day. Each participant performed both breakfast patterns, with a one week washout (i.e., breakfast-skipping) period between treatments.

Figure 1: Study design



During each testing day, the participants arrived at the General Clinical Research Center (GCRC) following an overnight fast. At time +0 min, they were provided with the respective breakfast meal (i.e., NP or HP) or continued to skip breakfast (i.e., BS). Perceived appetite, satiety, and brain activation responses were measured 3 hour post-breakfast.

Breakfast Patterns and Respective Testing Day Meals

Following the baseline, BS testing day, the participants randomly completed the NP and HP breakfast patterns. For each pattern, the participants were provided with the respective breakfast meals and asked to consume these at home between 7-9 am for 6 consecutive days. On day 7 of each pattern, the participants returned to the GCRC to complete the respective testing day. Each breakfast pattern consisted of 2 types of meals which were provided throughout the 7 days in an alternating style. The NP meals included two types of breakfast cereals (i.e., crispy squares or crispy rice) and milk, whereas the HP meals consisted of baked items (i.e., oatmeal bars or waffles with syrup) and yogurt. Each of the breakfast meals within each treatment were matched for protein, carbohydrates, fat, sugar, fiber, energy content, and palatability.

The dietary characteristics and palatability of the testing day breakfast meals are shown in Table 2. The meals contained approximately 24% of estimated energy needs (490 ± 10 kcal) for normal to overweight adolescents[70]. The NP meal was comprised of 15% protein (18 ± 1 g protein), 65% carbohydrates and 20% fat, whereas, the HP meal included 40% protein (50 ± 1.1 g protein), 40% carbohydrates, and 20% fat. Both meals were served with 237 ml of water. Total energy content, fat, sugar, fiber, energy density, and palatability were similar between the breakfast meals. During the baseline, BS day, the participants were only provided with the 237 ml of water. This day served as the subjects' normal eating pattern and was used to identify the normal (baseline) responses.

Table 2: Dietary characteristics of the testing day breakfast meals

Dietary Characteristics*	Normal-protein (NP) Breakfast	Higher-protein (HP) Breakfast
Energy Content (kcal)	490 ± 10	490 ± 10
PRO (g)	18.3 ± 0.3	49.9 ± 0.1
CHO (g)	77.2 ± 1.6	51 ± 1.4
Sugar (g)	12.6 ± 0.3	12.8 ± 0.2
Fiber (g)	6.8 ± 0.1	7.1 ± 0.1
Fat (g)	10.7 ± 0.2	10.9 ± 0.2
Testing Day menu:	<u>Crispy Square Cereal with Milk:</u> Toasted rice cereal 32 g Toasted wheat cereal 62 g Whole milk 32 g Reduced sugar 2% milk 235 g	<u>Waffle with Syrup:</u> 1%fat cottage cheese 85 g Liquid egg substitute 80 g Margarine 10.8 g Unbleached flour 32.2 g Lavash bread 47 g Maple syrup 3.2 g Sugar free maple syrup 44 g <u>Yogurt:</u> Whipped 1% cottage cheese 85 g Raspberries 20 g
Palatability (mm)[†]	64 ± 9	58 ± 7

*Data Presented as Mean ± SEM

[†]Palatability assessed from a questionnaire completed during breakfast (+15 min) (scale: 1-100 mm)

fMRI

Brain activation responses were assessed prior to lunch (i.e., 180 min after the start of the testing day) in each of the 3 testing days. During the fMRI procedure, the participants laid down in a supine position on the sliding MRI table and focused on the photographs which were projected onto a screen and easily viewed through a mirror.

The fMRI paradigm has been previously published and incorporated stimuli from three categories of pictures including food, animals, and blurred baseline images[19,23] . The pictures from each category were presented in blocks of images. Ten photographs (of the same type of stimuli) were presented per block. The scan involved three repetitions of each block of stimulus-producing images(i.e., food, animal), alternated with blocks of randomized blurred images. Each photograph was projected for 2.5 seconds, with an interstimulus interval of 0.5 seconds. There was a total of 13 blocks of stimuli presented. Animal pictures were used to control for visual richness and general interest (i.e., appealing but not appetizing). Each functional scan lasted approxamtely seven minutes and was performed in duplicate. Scanning was performed at the Hoglund Brain Imaging Center at the University of Kansas Medical Center on a 3 Tesla Allegra scanner (Siemens Medical Solutions, Erlangen, Germany).

Recognition memory for the presented images was examined following the scanning session to identify whether the participants were attentive throughout the scans (See reference [23] for further detail). Functional scans were excluded if the participants scored less than chance (i.e., <50%). Removal of duplicate scans on any one testing day led to the exclusion of the subject.

Questionnaires

Perceived fullness, hunger, desire to eat, and prospective food consumption were assessed at the beginning of each testing day (+0 min) and prior to fMRI (+180 min) on each the testing day. The questionnaires contained validated visual analog scales incorporating a 100mm horizontal line rating

scale for each response[71]. The questions are worded in the following manner: 'how strong is your feeling of' with the anchors of 'not at all' to 'extremely'.

Data and Statistical Analyses

The brain activation responses were analyzed using the BrainVoyager QX statistical package and random effects (Brain Innovation, Maastricht, Netherlands, 2004). Preprocessing steps included trilinear three-dimensional motion correction, sinc-intrerpolated slice scan time correction, two-dimensional spatial smoothing with 4-mm Guassian filter, and high-pass filter temporal smoothing[23]. Functional images were realigned to the anatomic images obtained within each session and standardized using BrainVoyager Talairach transformation, which conforms to the space defined by the Talairach and Tournoux's stereotaxic atlas[72]. Functional scans were discarded if head movement > 3mm along any axis (x,y or z).

To determine the effects of breakfast on the neural encoding of reward-driven eating, a repeated measures ANOVA was performed on the brain activation maps within the Brain Voyager software using Stimulus (i.e., appetizing and appealing Food vs. non-appetizing but appealing Non-food, Animal) x Breakfast (BS vs. NP) comparisons. To identify whether the macronutrient composition of the breakfast meal would differentially effect the neural encoding of reward-driven eating, a repeated measures ANOVA was again performed using Stimulus (i.e., Food vs. Non-food) x Breakfast (NP vs. HP).

Variables representing the experimental conditions were modeled with a hemodynamic response filter and entered into the model using random effects. Contrast between conditions was assessed with *t*-statistics using random effects. A-priori regions of interest (ROIs) included the amygdala, hippocampal formation (hippocampus and parahippocampal cortex), cingulate, insula, oribitofrontal cortex, and pre-frontal cortex. Activations were considered significant at a statsistical threshold of $p < 0.001$ (uncorrected) and minimum cluster size of six contiguous voxels. Other areas

were considered significant if they exceeded a threshold of $p < 0.0001$ and a minimal cluster size of six contiguous voxels.

Regarding the ROI data analysis, follow-up analyses of a-priori regions of interest were conducted in regions noted above that achieved statistical significance in the breakfast pattern analyses. Mean percent signal change (Food vs. Baseline) in the maximum voxel within each region for each individual was exported to the latest version of the Statistical Package for the Social Sciences (SPSS; 17.0; SPSS Inc.; Chicago, IL).

Pearson's correlations were computed between the percent signal change in the a-priori regions reaching significance and perceived appetite and satiety. Data are expressed as mean ± SEM. A $p<0.05$ was considered statistically significant. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS; 17.0; SPSS Inc.; Chicago, IL).

Results

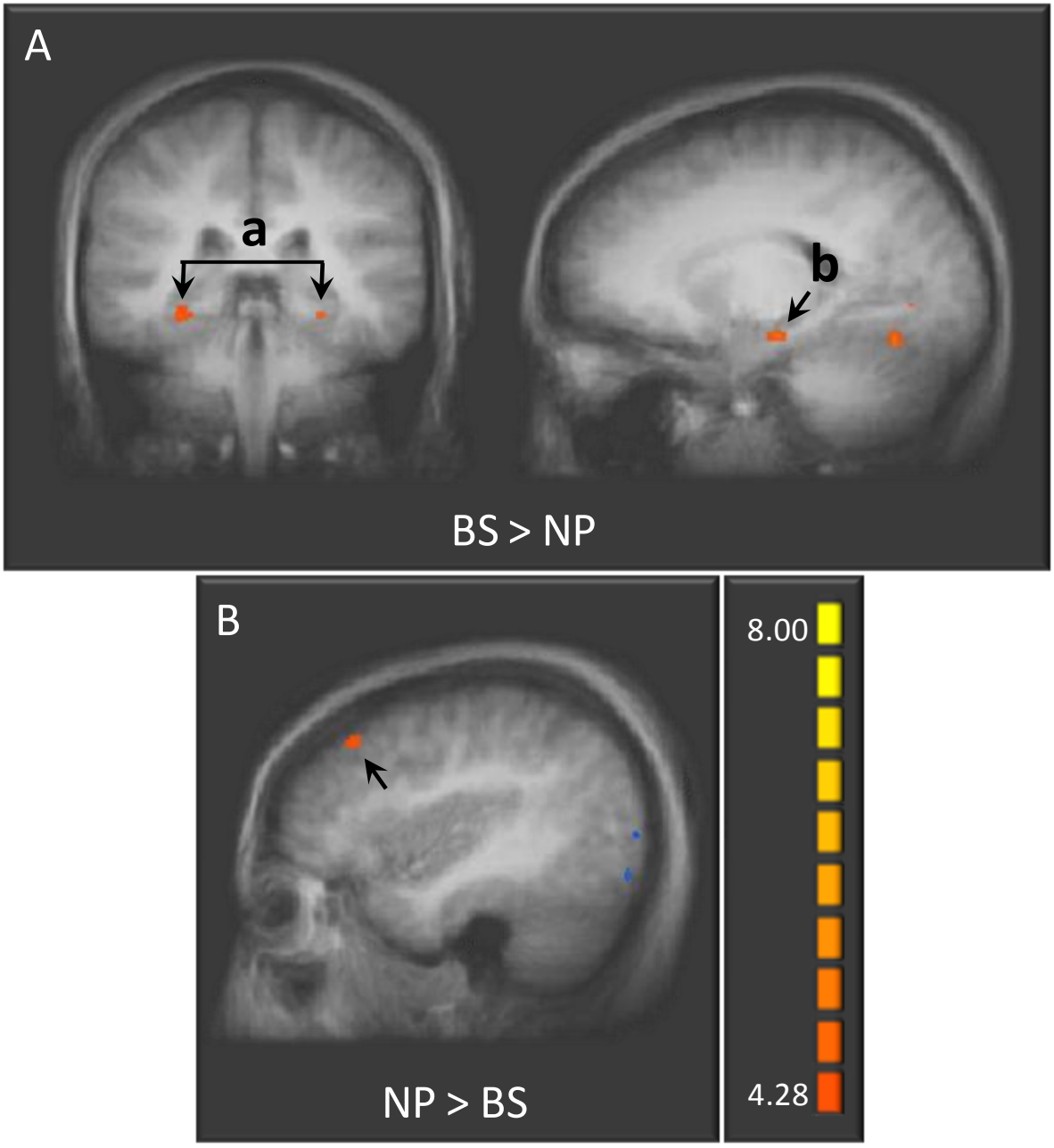
Breakfast Skipping vs. Breakfast

All brain regions significantly affected by breakfast consumption are presented in Table 3. Pre-lunch neural activity in response to visual food cues (i.e., Food>Non-food) was greater in the parahippocampus (x, y, z = 30, -34, -11; -27, -34, -11) and hippocampus (-18, -19, -14) when breakfast was skipped (i.e., BS) compared to consuming the NP breakfast ($p < 0.001$; 4 contiguous voxels; Table 3; Figure 2A). Conversely, the middle frontal gyrus (-39, 26, 40) showed increased activity during the NP vs. BS testing day ($p < 0.001$; 4 contiguous voxels; Table 3; Figure 2B).

Table 3: Brain regions reaching significance (Food > Non-food) at +180 min (i.e., Pre-lunch) during the Breakfast Skipping (BS), Normal Protein (NP), and Higher Protein (HP) testing days

Contrast and region	Coordinates			*t*	No. of voxels
	X	Y	Z		
BS > NP					
Parahippocampal gyrus	30	-34	-11	7.57	238
	-27	-34	-11	6.25	30
Hippocampus	-18	-19	-14	10.32	51
Occipital gyrus	12	-76	-8	7.28	7
	-9	-73	-8	7.78	10
	-12	-82	-8	8.42	10
Cerebellum	-12	-73	-17	8.42	7
	-15	-67	-17	11.42	44
NP > BS					
Middle frontal gyus	-39	26	40	5.56	24
NP > HP					
Insula	33	17	13	5.79	12
HP > NP					
No regions found					

Figure 2: Pre-lunch *a priori** brain activation patterns following the addition of breakfast; all comparisons Food > Non-food (Animal)



*P<0.001; 4 contiguous voxels; BS, Breakfast Skipping; NP, Normal Protein

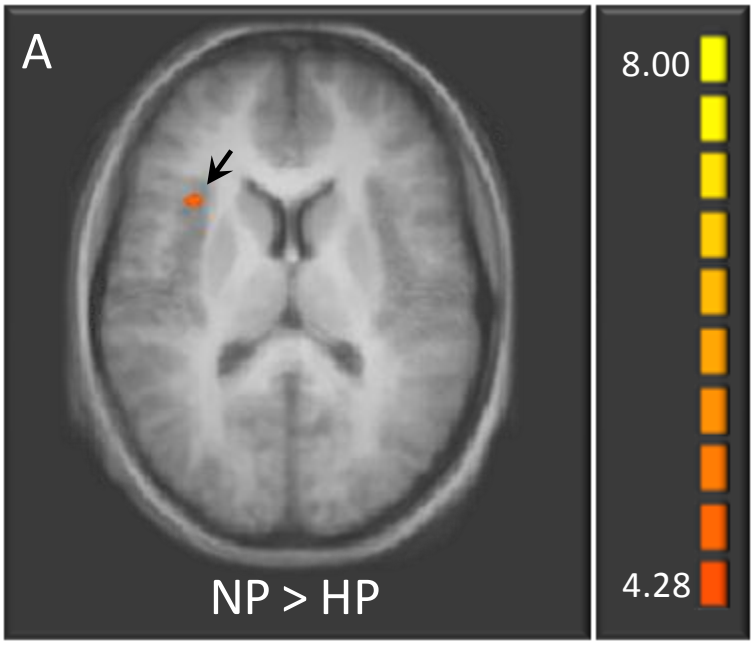
A: Greater activation following BS vs. NP; a) bilateral parahippocampus; b) hippocampus

B: Greater activation following NP vs. BS; in the middle frontal gyrus;

NP vs. HP Breakfast Meals

The brain regions significantly influenced by increased dietary protein at breakfast (i.e., HP vs. NP) are shown in Table 3. In comparing breakfast type, consumption of the NP breakfast resulted in increased activation in the insula (x, y, z = 33, 17, 13; p<0.001; 4 contiguous voxels, food>nonfood) vs. the HP breakfast (Table 3; Figure 3A).

Figure 3: Pre-lunch *a priori** brain activation patterns following the Normal Protein (NP) vs. Higher Protein (HP) Breakfast Treatments; all comparisons Food > Non-food (Animal)



*P<0.001; 4 contiguous voxels

A: Greater activation following NP vs. HPI in the insula

Correlations between the Brain Activation Patterns and Perceived Appetite and Satiety

As shown in Table 4, percent signal change in the parahippocampal gyrus and insula were positively correlated with perceived sensations of hunger, desire to eat, and prospective food consumption (all correlations, $p < 0.05$). In addition, percent signal change in the insula was negatively associated with feelings of fullness (i.e., satiety; $p < 0.05$; Table 4).

Table 4: Correlations between pre-lunch neural activity and perceived appetite and satiety

	Parahippocampal gyrus		Insula	
Variable	R	p-value	R	P-value
Perceived Appetite				
Hunger	0.506	0.012	0.303	0.029
Desire to eat	0.458	0.024	0.334	0.014
Prospective food consumption	0.505	0.012	0.270	0.048
Perceived Satiety				
Fullness	NS	NS	-0.329	0.015

Chapter 4: Discussion

The present study was conducted to examine the effects of breakfast on neural activity surrounding reward driven eating in overweight/obese adolescents who habitually skip breakfast. The addition of breakfast resulted in attenuation of brain activation responses to appetizing food cues in a-priori regions including the hippocampus and parahippocampus and increased activation in the 'higher order' medial frontal gyrus. With respect to whether additional alterations were observed with increased dietary protein, we found that the higher protein breakfast meal led to decreased activation in the anterior insula. Further, reduced parahippocampus and/or insula activations were associated with reduced perceived appetite and inversely associated with increased satiety. Taken together, these data indicate

brain regions encoding reward-driven eating and suggest that dietary recommendations which include increased protein at breakfast might lead to reduced motivation and desire to eat, and thus prevent overeating in the modern environment.

Until recently, it was difficult to assess the effectiveness of dietary interventions to reduce non-homeostatic, reward driven eating behavior. Although the cortico-limbic system has been implicated with this type of eating, it wasn't until the advancements in neuroimaging, specifically incorporating visual food stimuli during fMRI, that has led to the systematic identification of the key brain regions involved with food motivation and reward.

Previous studies have used this methodology to assess the neural activation in response to 1) examining visual food cues that vary in nutritional value (high calorie vs. low calorie)[21,22]; 2) varying the energy state of the participants when viewing visual food stimuli (fasted vs. fed; obese vs. lean)[22-24,46], and 3) combining both aspects[22]. By design, the use of food images places emphasis on past experiences and memories of the reward value of food. Recent evidence has shown that the expectation of reward may be a major underlying factor contributing to overeating especially in

adolescents[68], further supporting the need to develop dietary interventions which reduce the responses to the current, reward-centered, food environment.

The hippocampus and parahippocampus are regions known for their involvement with the development of short and long term memories, respectively[73,74]. Previous neuroimaging studies have shown increased activation of these regions in adults and adolescents in response to food cues during acute energy deficiency (i.e. fasting) when hunger is elevated [19,61]. Alternately, the hippocampus has been implicated in the development of food cravings[45]and is activated more in obese subjects during exposure to food cues both in the fed and fasted energy states in comparison to healthy weight controls[23]. In the present study, brain activation in these regions in response to appetizing food images was attenuated prior to lunch following the addition of breakfast in overweight/obese 'breakfast skipping' adolescent girls. These data indicate that the daily addition of breakfast appears to reduce the neural encoding of food reward/memories, potentially leading to reduced desire/motivation to eat and perhaps reduced overeating.

Along with the activations previously mentioned, we also observed increased activation in the medial frontal gyrus following breakfast. Prior studies examining the effects of satiation (i.e., fed state) in lean and obese individuals have indicated that obese individuals exhibit heightened activity in frontal regions of the brain[75]. This response may be due to the role of the frontal regions in inhibiting the reward centers[75]. In regard to the specific role of the medial frontal region, activation in this region has been implicated not only in increased cognitive control[76] but also the integration of physiologic and hedonic/reward signals[77]. This is supported by the findings of increased activation in the medial frontal cortex following the infusion of the physiologic satiety hormone PYY[39]. Previous research from our lab has shown that PYY is significantly elevated following breakfast as compared to skipping breakfast[78]. Collectively, the increased activation in the medial frontal gyrus following breakfast consumption may

reflect control over the limbic reward/memory centers (namely, the hippocampus and parahippocampus) and/or the effects of the resulting physiologic signals in the region.

The macronutrient composition, specifically dietary protein, of meals has been shown to alter the physiologic signals involved with appetite control and the regulation of energy intake in adolescents[78]. Our current study extends these findings to examine whether increased dietary protein alters the brain activation surrounding non-homeostatic, reward-driven eating behavior. When compared to the normal protein breakfast meal, the higher protein breakfast led to reduced activation in the anterior insula. The insula is typically considered to include the primary gustatory cortex, responding to the taste of food[79]. However, recent evidence also demonstrates its responsiveness to the degree of reward experienced[64], food cravings[45] and visual food cues [80]. The anterior insula is also hyper- responsive during the anticipation of reward in obese vs. lean adults[46] and adolescents girls[81]. Additional, practical implications were observed in the previously mentioned study[81] such that the adolescent girls who exhibited heightened activity in the anterior insula also showed increased weight gain during a one year follow-up as compared to those displaying less activation[81]. In the present study activity in this region correlated with perceived appetite and was negatively associated with satiety. This finding is supported by previous experiments using visual food cues[21]. Further, PYY, which is a key physiologic, satiety signal has been shown to be secreted in response to dietary protein[82] and has also been found to decrease insula activation[40]. Taken together, these data suggest that the incorporation of a higher protein breakfast has the ability to reduce insula activation which may decrease perceptions of reward in response to food-related environmental stimuli which would otherwise lead to increased energy intake.

Strengths & Limitations

This study is novel in its use of a fMRI food motivation paradigm to assess the effects of two common dietary interventions on the mechanisms involved with reward-driven eating in

overweight/obese adolescents. Further, we assessed the effects of these interventions three hours post-consumption when the next eating occasion would typically occur. Although we were unable to examine the immediate effects of breakfast consumption, this approach allowed for the identification of whether breakfast consumption had a sustained impact on motivation and desire to eat prior to the next eating occasion. Another strength of the study includes the one-week acclimation period prior to each breakfast testing. This allowed the breakfast skipping adolescents to habitualize to the breakfast eating pattern, and therefore provided a more accurate assessment of the effects of incorporating breakfast on a daily basis.

Some of the study limitations are described below. The baseline, breakfast skipping testing day was performed without alteration of the participant's habitual dietary habit of skipping the morning meal. Although this served as the most accurate assessment of baseline responses, the authors are aware of the confounding factors attributable to the order of the testing days. An example of which is the effects of expectation and novelty on the orbital frontal cortex[83] and the habitualization effect seen in the amygdala[19]. Other limitations include the unknown physiologic responses to the breakfast interventions. As previously mentioned, this study was performed along with another study assessing the physiologic signals involved with appetite control and the regulation of energy intake. In addition, the current sample size was small with only 10 subjects in a repeated measure design, and the analyses did not correct for multiple comparisons across the whole brain. This was a first, but vital step to identify whether brain activation differences exist with breakfast and dietary protein. We are currently in the process of completing a follow-up study, including a larger sample size, that will examine the effects of breakfast and increased dietary protein on brain activation patterns prior to dinner to assess reward-driven eating behavior later in the day and evening hours which is the time in which adolescents tend to overeat.

Adolescents in the United States are exposed to limitless food stimuli, on a daily basis, that lead to overeating and[68,84,85] breakfast skipping is a common dietary practice that is strongly associated with overweight/obesity in adolescents and could be adversely contributing to the susceptibility to these detrimental environmental stimuli. Dietary recommendations to combat reward-driven eating are lacking, especially in adolescents. In the current study, we demonstrate that the addition of breakfast in overweight/obese 'breakfast skipping' adolescents leads to reductions in brain activation in specific regions involved with reward-driven eating. Additionally, a breakfast higher in dietary protein leads to additional improvements.

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Appendix A:

SCREENING CONSENT FORM

Project Title: The Body's Response to Food Intake in Adolescents

Sub-study Title: The Brain's Response to Food Intake in Adolescent Young Women

GCRC Protocol # 102

INTRODUCTION:

Your daughter is being invited to participate in a research study about how the brain responds to breakfast. This research study will be conducted at the University of Kansas Medical Center with Heather J. Leidy, PhD as the principal investigator. Approximately 15 subjects will be enrolled.

Your daughter does not have to participate in this research study. Before you and your daughter make a decision to participate, you should read the rest of this form, and your daughter should read the assent form. The main purpose of research is to benefit individuals and society at large. Your daughter might personally benefit from participating in this study, but you and your daughter should understand that the primary purpose of research is to create new knowledge.

BACKGROUND:

Over 17 million young people in the US are currently overweight or obese and are already experiencing serious health problems. More effort is needed to identify successful treatment and prevention strategies to eliminate this problem. One main contributor to obesity is the increase in unhealthy eating habits. The most common, yet unhealthy eating habit is skipping breakfast, which is strongly connected to over-eating (especially in the evening), weight gain, and obesity. Skipping breakfast has also been shown to reduce attention and memory in young people.

Several ways to determine the benefits of eating breakfast are to identify the differences in appetite, motivation to eat, and food intake when a person does and does not eat breakfast. We will take blood samples to measure several appetite hormones (which are chemicals in your body that respond to eating) and will identify how your brain patterns change when looking at pictures of food.

PURPOSE:

The purpose of this study is to determine how the brain responds to the daily consumption of different breakfast meals in 'breakfast skipping' adolescent women.

SCREENING PROCEDURES: TOTAL TIME: 30 min

The following screening procedure will be completed at one of the information sessions:

1.) Body Weight and Body Height Measurements: (5 min)

Your daughter's body weight will be measured to the nearest 0.1 kg using a research scale. Also at this time, her height will be measured using a wall-mounted ruler.

The following screening procedures will be completed *prior* to the first information session:

1.) Medical History Questionnaire: (15 min)

The purpose of this questionnaire is to provide us with information concerning your daughter's medical history. 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 your daughter has any food allergies, food intolerances, or if she is latex-intolerant. You will provide, to the best of your knowledge, a complete history of all of your child'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).

2.) Dietary Questionnaire: (10 min)

The purpose of this questionnaire is to provide us with information concerning the day-to-day dietary practices, habits, and foods eaten and avoided by your daughter.

RISKS:

There are no known risks when completing medical history or dietary questionnaires nor are there any known risks with having body weight and height measurements taken.

BENEFITS:

Your daughter may benefit from participation in this screening by gaining information about her body weight status as well as being informed of the negative effects of skipping breakfast along with the benefits of eating breakfast on a daily basis for better appetite control, food intake, body weight management, and cognitive function.

ALTERNATIVES:

The only alternative to the methods proposed in this screening will be to decline to participate. Your daughter's participation in this screening is voluntary. Deciding not to participate will have no effect on the care or services she will receive at University of Kansas Medical Center.

COSTS:

Except for the cost of traveling to and from the information meeting, there is no cost to you and no cost to your daughter related to her participation in the screening.

PAYMENT TO SUBJECTS:

There is no payment for completing any of the screening procedures.

IN THE EVENT OF INJURY:

In the event that your daughter experiences a serious side effect during this screening, you or your daughter should immediately contact the principal investigator, Dr. Heather Leidy at 913-588-7650. If it is after 5:00 p.m., a holiday or a weekend, you should call 765-714-7928 or email hleidy@kumc.edu.

If your daughter has any bodily injury as a result of participating in this screening, care will be provided for her at the usual charge. Claims will be submitted to your health insurance policy, your government program, or other third party, but you will be billed for the costs of that care to the extent insurance does not cover them. Payment for lost wages, disability, or discomfort is not routinely available. You do not give up any of your legal rights by signing this form.

INSTITUTIONAL DISCLAIMER STATEMENT:
If you think your child have been harmed as a result of participating in research at the University of Kansas Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Under certain conditions, Kansas state law or the Kansas Tort Claims Act may allow payment to persons who are injured in research at KUMC.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION:
Efforts will be made to keep your daughter's personal information confidential. Researchers cannot guarantee absolute confidentiality. The privacy of your daughter's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). By signing this consent form, you are giving permission ("authorization") for KUMC to use and share your daughter's health information for the purposes of this research study. If you decide not to sign the form, your daughter cannot be in the study.

To do this screening, we need to collect health information that identifies your child. We will collect information from activities described in the Procedures section of this form.

Your daughter's screening-related health information will be used at KU Medical Center by Dr. Heather Leidy, members of the research team, and the KUMC Human Subjects Committee and other committees and offices that review and monitor research studies. Screening records might be reviewed by government officials who oversee research, if a regulatory review takes place.

All screening information that is sent outside of KU Medical Center will have your daughter's name and other identifying characteristics removed, so that your daughter's identity will not be known. Because identifiers will be removed, your daughter's health information will not be re-disclosed by outside persons or groups and will not lose its federal privacy protection.

Your permission to use and share your daughter's health information will not expire unless you or your daughter cancels it.

QUESTIONS:
You have read the information in this form. Dr. Leidy has answered your question(s) to your satisfaction. If you have any more questions, concerns or complaints after signing this, you may contact Dr. Leidy at (913) 588-7650. If you have any questions about your daughter's rights as a research subject, you may call (913) 588-1240 or write the Human Subjects Committee, Mail Stop #1032, University of Kansas

Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE SCREENING:

Your daughter's participation in this screening is voluntary, and the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care or treatment your daughter receives now or in the future at the University of Kansas Medical Center. The screening may be discontinued for any reason without your daughter's consent by the investigator conducting the study. Your daughter has a right to change her mind about allowing the research team to have access to her health information. If you or your daughter wants to cancel permission to use her health information, you or your daughter should send a written request to Dr. Heather Leidy. The mailing address is Heather Leidy, University of Kansas Medical Center; Mail Stop #4013, 3901 Rainbow Boulevard, Kansas City, KS 66160. If you or your daughter cancels permission to use her health information, your daughter will be withdrawn from the screening. The research team will stop collecting any additional information about her. The research team may use and share information that was gathered before they received you or your daughter's cancellation.

SCREENING CONSENT

Dr. Heather Leidy has given you and your daughter information about this screening. She has explained what will be done and how long it will take. She explained any inconvenience, discomfort, or risks that may be experienced during this screening process.

I freely and voluntarily consent to allow my daughter to participate in this screening. I also agree to allow the use of my daughter's data during this screening process as part of the baseline study information (should she be approved to participate in the study and decides to participate). I have read and understand the information in this form and have had an opportunity to ask questions and have them answered. **I will be given a signed copy of the consent form to keep for my records.**

_____

Type/Print Subject's Name

_____

Type/Print Parent's Name Giving Consent

_____ _____ _____

Signature of Parent of Subject Time Date

_____

Type/Print Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

SCREENING ASSENT FORM

Project Title: The Body's Response to Food Intake in Adolescents

Sub-study Title: The Brain's Response to Food Intake in Adolescent Young Women

GCRC Protocol # 102

You being asked to participate in a research study that looks at how the brain responds to different breakfast foods. If you want to be part of the study, you must first complete the following screening procedures:

1.) Your body weight and height will be measured.

2.) You will help one of your parents complete the Medical History Questionnaire which asks questions about your past health conditions, diseases, and medications. It also asks questions about any food allergies or foods that you cannot eat.

3.) You will complete a Dietary Questionnaire that asks questions about how many times a day you eat, when you eat, and foods you like or dislike.

None of the screening procedures have any known risks.

I have read this "parental permission" form and understand that I am agreeing to participate in this screening. I have been given the opportunity to ask any questions that I might have about my participation. Also, I understand that by signing on the line below I am indicating my willingness to participate. I understand that I may withdraw my assent and stop participating at any time.

_____
Name of Adolescent Subject

_____ _____
Signature of Adolescent Subject Date

Appendix B

STUDY CONSENT FORM

Project Title: The Body's Response to Food Intake in Adolescents

Sub-study Title: The Brain's Response to Food Intake in Adolescent Young Women

GCRC Protocol # 102

INTRODUCTION:
Your daughter is being invited to participate in a research study about how the brain responds to breakfast. This research study will be conducted at the University of Kansas Medical Center with Heather J. Leidy, PhD as the principal investigator. Approximately 15 subjects will be enrolled.

Your daughter does not have to participate in this research study. Before you and your daughter make a decision to participate, you should read the rest of this form and your daughter should read the assent form. The main purpose of research is to benefit future patients and society in general. Your daughter might get personal benefit from participating in this study, but you and your daughter should understand that the purpose of research is to create new knowledge.

BACKGROUND:
Over 17 million young people in the US are currently overweight or obese and already experience serious health problems. More effort is needed to identify successful treatment and prevention strategies to eliminate this problem. One main contributor to obesity is the increase in unhealthy eating habits. And, the most common, yet unhealthy habit includes skipping breakfast which is strongly connected to over-eating (especially in the evening), weight gain, and obesity. Skipping breakfast has also been shown to reduce attention and memory in young people.

Several ways to determine the benefits of eating breakfast is to identify the differences in appetite, motivation to eat, and food intake with and without eating breakfast. We will take blood samples to measure several appetite-hormones (which are chemicals in your body that respond to eating) and will identify how your brain patterns change when looking at pictures of food.

PURPOSE:
The purpose of this study is to determine how the brain responds to the daily consumption of different breakfast meals in 'breakfast skipping' adolescent young women.

PROCEDURES: TOTAL TIME: 4 WEEKS

During study WEEK 1, your daughter will complete the following BASELINE TESTING procedures:

1.) <u>3-Day Measurement of Daily Food Intake</u>: TOTAL TIME: 1 ½ hours
Your daughter will complete a 3-day food log in which she will simply write down all the foods and beverages consumed on 2 week days and 1 weekend day. She will be given instructions on how to complete these.

2.) <u>Physical Activity Questionnaire</u>: TOTAL TIME: 15 min
The purpose of this questionnaire is to provide us with information concerning the day-to-day activities, sports, clubs, and overall physical activity level that your daughter is involved with. Your daughter will be asked to maintain this physical activity for the duration of the study.

3.) <u>'Breakfast Skipping' Testing Day</u>: TOTAL TIME: 6 hrs
After an overnight fast, your daughter will arrive at the General Clinical Research Center (GCMC) at KUMC. She will arrive approximately 30 minutes before the testing period begins (between 7-9 am). Upon arriving, your daughter will be asked to collect a urine sample to test for pregnancy. She will then sit in a reclining chair and numbing cream may be placed in both elbow joints to prep for the catheter insertion. All of the testing procedures will be explained one more time. After approximately 30 minutes, a catheter, which is a thin flexible, plastic tube, will be inserted into a vein in the front of one of her elbows by a trained GCRC registered nurse.

When your daughter is ready to begin, a small blood sample (~ ½ teaspoon) will be drawn from the catheter. There should be no pain experienced with any of the blood collections. The blood sample will be used to measure various hormones, which are chemical messengers in the body, that respond to food intake. Also at that time, your daughter will be given a study palm-pilot in order to complete questionnaire asking about her feelings, thoughts, and mood.

Following these procedures, she will be given a glass of water but will not be given a breakfast meal. Throughout the remaining 4 hours, blood samples and palm-pilot questionnaires will be collected every 20 minutes and several types of mental, memory, motor, and hand-eye coordination tasks will be performed every hour.

After 3 hours, your daughter will be taken to the Hoglund Brain Imaging Center (HBIC) on the KUMC campus to have a brain scan 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 and can provide information on blood flow, metabolism, and brain function. This is a non-radioactive (i.e., no x-rays), non-invasive technique. Upon arriving at HBIC, your daughter will lie on a table that 'slides' into the scanner. Her head will be set in a specific testing position-making it difficult for her to move her head. During the scan, she will view numerous pictures of food and animals. She will be asked to remember the pictures that she saw during the scanning. This procedure lasts approximately 1 hour. At the end of the scan, your daughter will return to the GCRC for several final study procedures.

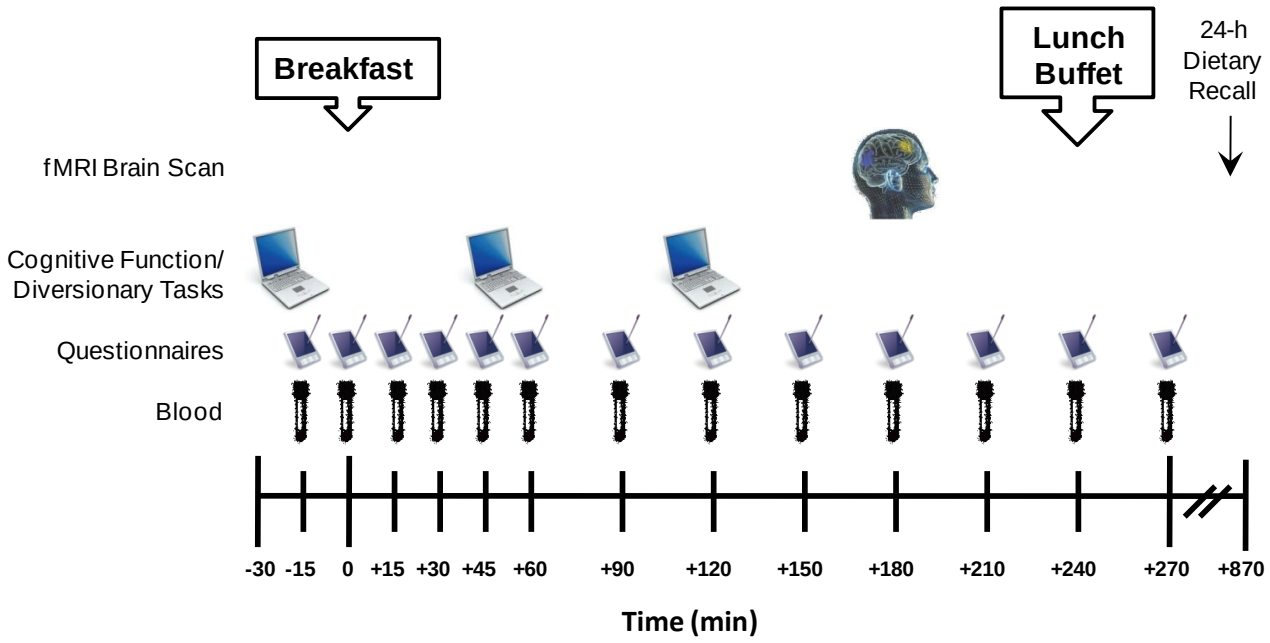
Back at the GCRC, she will first be provided with a lunch buffet. Over the next 30 minutes, your daughter will be able to eat as much or as little as she would like until feeling 'comfortably full.' After that time, the catheter will be removed from her arm and she will be free to leave the laboratory.

Over the remaining 24 hours, your will complete a 24-h food record followed by a 24-h d dietary recall. The dietary recall will occur through a phone interview. Using this approach, one of the members of the

research team will contact your daughter the following morning and ask her what she ate and drank the following day.

Here is a diagram of the 5-hour testing day:

Study Testing Day



During study WEEK 2, your daughter will be randomly provided with 1 of 2 breakfast diets: 'Rise' or 'Shine' and will complete the following procedures:

1.) <u>Daily Consumption of the Provided Breakfast Meals</u>: TOTAL TIME: 2 hrs

Your daughter will be given 6 days of either the 'Rise' or the 'Shine' Breakfast. Each breakfast meal will be prepared in a separate container and marked as Breakfast Day 1-6. Every morning (for 6 days), she will eat her assigned breakfast between 7-9:30 am. At breakfast, she will be permitted to only eat the foods provided to her. However, after breakfast is completed, she can eat anything else she chooses 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. Below is a brief description of the breakfast meals.

Shine Breakfast Menu

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt

Rise Breakfast Menu

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk

5-h Testing Day Meals

2.) <u>Daily Breakfast Meal Instructions & Procedures</u>: TOTAL TIME: 20 min

Each morning, 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 your daughter's feelings, thoughts, and mood towards the breakfast meal.

3.) <u>'Rise' or 'Shine Testing Day</u>: TOTAL TIME: 6 hrs

Your daughter will complete the same procedures as previously described for the 'Breakfast Skipping' testing day; however, during this testing day, she will be given the breakfast pattern that she was previously following during the past 6 days.

During study WEEK 3, your daughter will go back to her typical morning habit of skipping breakfast and will complete the following:

1.) <u>Daily Breakfast Skipping Log</u> : TOTAL TIME: 10 min

Your daughter will be asked to skip breakfast for the next 7 days. She will complete a Breakfast Skipping Log that asks her whether or not she ate breakfast that day and when her first eating occasion occurred.

During study WEEK 4, your daughter will be provided with the other breakfast diet that she was not given during WEEK 2. She will then complete the following procedures:

1.) <u>Daily Consumption of the Provided Breakfast Meals</u>: TOTAL TIME: 2 hrs

2.) Daily Breakfast Meal Instructions & Procedures: TOTAL TIME: 20 min

3.) 'Rise' or 'Shine' Testing Day: TOTAL TIME: 6 hrs

For each of these, your daughter will complete the same procedures as previously described for WEEK 2.

Following this week, the study will be completed.

RISKS

There are no known risks when completing the study questionnaires, food intake logs or recalls, or mental, memory, motor, and hand-eye coordination tasks.

There may be some risks when having a catheter inserted into the arm. During the insertion, your daughter may feel some pain which feels like a small pinch. However, the GCRC nurses may apply a numbing cream prior to the catheter insertion which will reduce the pain. There is a risk of a small bruise and/or infection developing. However, the catheters are inserted by a highly trained GCRC nurse using sterile techniques. Lastly, your daughter may also feel lightheaded and there is a slight risk of fainting due to the sight of blood. The total amount of blood to be drawn in this study is a total of ½ of a cup (approximately 4 oz) over the 3 study visits and is small enough not to present any hazard to your daughter's physical well being. This amount of is approximately ¼ of the amount taken during a routine blood donation. However, if your daughter is 17 yrs of age, she must agree not to donate blood for at least one month prior to, during, and for one month after the study.

The risk involved with the MRI measurements is very small. Again, there are no X-rays or radiation involved. Even though there are no known adverse effects for MRI, this procedure does release a small amount of energy to the body. As a precaution, a pregnancy test is performed during the morning of each testing day to screen out pregnancy. If a positive pregnancy test occurs, your daughter will discontinue all study procedures. MRI scanning is not associated with any health risks, but it is important that your daughter complete the metal screening form accurately prior to each testing day. If your daughter has a pacemaker, blood vessel clips, or other internal metal, she may not be allowed to participate in this study. If your daughter has a pacemaker or vascular clip and accidentally enter the MRI suite, a life-threatening situation can develop. If between visits she has any devices surgically implanted, or if she had an accident that resulted in metal being implanted in her body, the investigators will need to know. If that happens, she may not be able to continue the study.

Your daughter may become uncomfortable in the scanner due to the small space and annoying sounds. To minimize the discomfort with the small space, your daughter will be able to look through a mirror to see the outside of the scanner. Additionally, your daughter will be given earplugs and earphones to block out the nose from the scanner. Further, at any time, your daughter can request to stop the test. Your daughter may feel lightheaded immediately following the fMRI scan. However, this will subside within 3-5 minutes after leaving the fMRI room.

There may be some risks of your daughter's stomach or bowels becoming upset due to any changes in his/her usual food and beverage intake.

There may be other risks that have not yet been identified, and unexpected side effects that have not been previously observed may occur.

NEW FINDINGS STATEMENT

You and your daughter will be informed if any significant new findings develop during the course of the study that may affect her willingness to participate in this study.

BENEFITS

Your daughter may benefit from participation in this study by gaining a better understanding of how her brain and body responds to her typical eating patterns and what happens when other, more healthy dietary habits are practiced. Safe and effective ways to control food intake and body weight are important to prevent or delay the onset of complications from obesity, such as diabetes, and heart disease. It is hoped that additional information gained in this research study may be useful in the treatment of other adolescents with poor eating habits.

ALTERNATIVES

The only alternative to the methods proposed in this work will be to decline to participate. Your daughter's participation in this study is voluntary. Deciding not to participate will have no effect on the care or services your daughter will receive at University of Kansas Medical Center.

COSTS

Except for the cost in traveling to and from KUMC, there is no cost to you and no cost to your daughter related to her participation in the study.

PAYMENT TO SUBJECTS

Your daughter will be paid a total of $150 for completing all study visits. Specifically, your daughter will receive $40 for each 5-hour testing day. She will also receive $15 for completing each of the 6 day patterns of eating the provided breakfast (at home). Your daughter will receive these payments at the end of each study week. If your daughter quits before completion of all the study weeks, then she will be paid for each completed week.

It is up to you and your daughter to decide who will receive payment for the participation in this study. The KUMC Research Institute will be given either your name, address, and social security number or your daughter's name, address, and social security number, and the title of this study. This will allow them to write checks for your daughter's study payments. These payments will be sent to your home address after each completed study week. If your daughter chooses to quit the study, the KUMC Research Institute will also be immediately contacted to write a check for the number of study weeks completed. Study payments are taxable income. A Form 1099 will be sent to you and to the Internal Revenue Service if your payments are $600 or more in a calendar year.

IN THE EVENT OF INJURY

In the event that your daughter experiences a serious side effect during this study, you or your daughter should immediately contact the principal investigator, Dr. Heather Leidy at 913-588-7650. If it is after 5:00 p.m., a holiday or a weekend, you should call 765-714-7928 or email hleidy@kumc.edu.

If your daughter has any bodily injury as a result of participating in this study, care will be provided for her at the usual charge. Claims will be submitted to your health insurance policy, your government program, or other third party, but you will be billed for the costs of that care to the extent insurance does not cover them. Payment for lost wages, disability or discomfort is not routinely available. You do not give up any of your legal rights by signing this form.

INSTITUTIONAL DISCLAIMER STATEMENT

If you think your child have been harmed as a result of participating in research at the University of Kansas Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Under certain conditions, Kansas state law or the Kansas Tort Claims Act may allow payment to persons who are injured in research at KUMC.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Efforts will be made to keep your daughter's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies your daughter will be removed.

The privacy of your daughter's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). By signing this consent form, you are giving permission ("authorization") for KUMC to use and share your daughter's health information for the purposes of this research study. If you decide not to sign the form, your daughter cannot be in the study.

To do this research, we need to collect health information that identifies your daughter. We will collect information from activities described in the Procedures section of this form.

Your daughter's study-related health information will be used at KUMC by Dr. Heather Leidy, members of the research team, and the KUMC Human Subjects Committee and other committees and offices that review and monitor research studies. Study records might be reviewed by government officials who oversee research, if a regulatory review takes place.

All study information that is sent outside KUMC will have your daughter's name and other identifying characteristics removed, so that her identity will not be known. Because identifiers will be removed, her health information will not be re-disclosed by outside persons or groups and will not lose its federal privacy protection.

Your permission to use and share your daughter's health information will not expire unless you or your daughter cancels it.

QUESTIONS

You have read the information in this form. Dr. Leidy has answered your question(s) to your satisfaction. If you have any more questions, concerns or complaints after signing this you may contact Dr. Leidy at (913) 588-7650. If you have any questions about your daughter's rights as a research subject, you may call (913) 588-1240 or write the Human Subjects Committee, Mail Stop #1032, University of Kansas Medical Center, and 3901 Rainbow Blvd., Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

Your daughter's participation in this study is voluntary and the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care or treatment your daughter receives now or in the future at KUMC. The entire study may be discontinued for any reason without your daughter's consent by the investigator conducting the study. She has a right to change her mind about allowing the research team to have access to her health information. If you or your daughter wants to cancel permission to use her health information, you or your daughter should send a written request to Dr. Heather Leidy. The mailing address is Heather Leidy, University of Kansas Medical Center; MS #4013; 3901 Rainbow Boulevard, Kansas City, KS 66160. If you or your daughter cancels permission to use his health information, she will be withdrawn from the study. The research team will stop collecting any additional information about her. The research team may use and share information that was gathered before they received you or your daughter's cancellation.

CONSENT

Dr. Heather Leidy has given you and your daughter information about this research study. She has explained what will be done and how long it will take. She explained any inconvenience, discomfort or risks that may be experienced during this study.

I freely and voluntarily consent to allow my daughter to participate in this research study. I have read and understand the information in this form and have had an opportunity to ask questions and have them answered. **I will be given a signed copy of the consent form to keep for my records.**

_____

Type/Print Subject's Name

_____

Type/Print Parent's Name giving Consent

_____ _____ _____

Signature of Parent of Subject Time Date

_____

Type/Print Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

Use of Data and/or Blood Samples for Future Research

Please check only **one** of the two boxes below

☐ I agree to allow the use of my daughter's data and/or blood samples collected during this study to be used for future research that is unrelated to this study. Specifically, the data will be kept for 25 years and will be included in a large data base. All data will be de-identified to prevent any identification back to your daughter. 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 data and/or blood samples. However, at any time, I (or my daughter) can request that the data and/or blood samples be destroyed if I (or my daughter) change our mind. If this occurs, I will provide a written request to Dr. Leidy at the address listed below. Lastly, I understand that Dr. Leidy can use and share information that was gathered before this request was received.

Heather Leidy, PhD
Dept. of Dietetics & Nutrition
The University of Kansas Medical Center
Mail Stop: #4013
3901 Rainbow Blvd
Kansas City, KS 66160

_____ _____
Parent's Signature Date

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

_____ _____
Parent's Signature Date

STUDY ASSENT FORM
The Benefits of Breakfast in Adolescent Young Women
GCRC Protocol #102

You are being asked to participate in a research study that looks at how the brain responds to different breakfast foods. If you want to be part of the study, you must complete the following procedures:

During study WEEK 1,

1.) You will write down all of the foods and drinks that you consumed during 2 week days and 1 weekend day.

2.) You will write down the activities, sports, and exercises that you generally do. Throughout the study, you will try to not change your activity habits.

3.) The night before the 1st testing day, you will not eat or drink anything but water after 9 pm. On the day of each testing, you will arrive at the GCRC between 7-9 am and will collect a urine sample in a cup so the GCRC can determine whether you are pregnant. If you would like to reduce the pain or discomfort which may occur when the catheter is inserted into your arm, the GCRC nurse will put some numbing cream on a small spot on both of my arms. You will then recline in a comfortable chair and will be told about all of the study procedures you will complete. After 30 minutes, a trained nurse will insert a catheter (a flexible, plastic tube) into your arm vein. You may feel a small amount of pain (like a pinch) when the catheter is being inserted. However, this pain will quickly go away and will not be felt throughout the rest of day.

Afterwards, you will complete some study questionnaires on a palm-pilot that asks questions about how you are feeling and what you are thinking about. A blood sample will be taken from the catheter in your arm. You will not experience any pain when this occurs. During this day, you will not be given a breakfast meal. Over the remaining 4 hours, blood samples will be taken and you will complete the study questionnaires. You will also participate in several games and exercises that test my hand-eye coordination, memory, and mental and motor skills.
After 3 hours, you will be taken down to the Hoglund Brain Imaging Center where you will have a brain scan. During this time, you will lie on a sliding table that will be placed inside the scanner (which looks like a big tube). During the scanning procedure, food and animal pictures will be shown. You will be asked to remember these pictures at the end of the procedure. During the testing procedure, the scanner will make numerous noises. You will wear ear plugs to reduce the sound. If you feel uncomfortable, you can request to leave the scanner at any time.

Afterwards, you will be taken back to the GCRC and given lunch. You will be able to eat as much or as little as you would like over the next 30 minutes. After you are finished, the catheter will be taken out of your arm and you will be free to leave the laboratory.

Over the remaining 24 hours, you will record all of the food and beverage you consume. You will also be called by one of the research staff members and asked about what you ate over the last 24 hours.

The amount of blood taken from your arm vein over the entire day is so small that you should not

feel any physical symptoms from this. You may get a small bruise on your arm where the catheter was inserted. This will go away in several days. You may also feel a little lightheaded immediately after the fMRI procedure. This will stop shortly after you leave the fMRI room.

During study WEEK 2, you will be given either the 'Rise' or the 'Shine' diet and will complete the following procedures:

1.) Every morning for 6 days, you will eat the breakfast meals that are given to you. You must eat these between 7-9:30 am every morning for 6 days. At breakfast, you are only permitted to eat the foods provided. However, after breakfast is completed, you can eat anything else you choose to eat throughout the remainder of the day.

The next page shows the foods that you will eat during these diets

Shine Breakfast Menu

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt	Home Baked Vanilla Oatmeal Bars Blueberry Yogurt	Belgium Waffles with Maple Syrup Raspberry Yogurt

Rise Breakfast Menu

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk	Crispy Rice Cereal with Milk	Square Crisp Cereal with Milk

5-h Testing Day Meals

2.) Each day, you will read and follow the breakfast instruction sheet. This contains the directions on how to prepare your breakfast meals, a check-off sheet that lists all of the foods to consume, and several short questionnaires asking about your feelings, thoughts, and mood about the breakfast meal.

3.) At the end of WEEK 2 (Day 7), you will complete the 2nd testing day which is very similar to the 1st testing day. However, during this day, you will be given either the 'Rise' or the 'Shine' meal to consume.

During study WEEK 3, you will go back to your typical morning habit of skipping breakfast and will complete the following:

1.) You will skip breakfast for the next 7 days and will complete a Breakfast Skipping Log that asks whether or not you ate breakfast and when your first eating occasion was that day.

During study WEEK 4, you will be given the other breakfast diet and will complete the same procedures as described during WEEK 2. Then, you will be finished with the study.

I have read this "parental permission" form and understand that I am agreeing to participate in this research study. I have been given the opportunity to ask any questions that I might have about my participation. Also, I understand that by signing on the line below I am indicating my willingness to participate. I understand that I may withdraw my assent and stop participating at any time.

_____
Name of Adolescent Subject

_____ _____
Signature of Adolescent Subject Date