

## ABSTRACT

Title of Document: APPETITE SIGNALS IN THE BRAIN: HIGH FRUCTOSE CORN SYRUP SWEETENED COLA EFFECTS HYPOTHALAMIC ACTIVITY AS MEASURED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Functional Magnetic Resonance Imaging has been used for over a decade to measure the effects of sugar on the hypothalamus, the appetite-regulating center of the brain. Hypothalamic activity decreases in a dose-dependent manner in response to glucose solution consumption. Fructose elicits an increase in hypothalamic activity. This study aimed to understand the effects of high fructose corn syrup, a combination of glucose and fructose on hypothalamic activity. Fasting blood samples were collected from 8 (4M /4F) healthy adult volunteers who were then fed a standard breakfast and transported to the Maryland Neuroimaging Center. Participants underwent two successive scans. Prior to the first scan participants drank 12 oz. of either cola or water. After the first scan participants received 6 oz. of either cola or water immediately prior to the beginning of the second scan. Treatments were assigned in a Latin square crossover design. Each scan included ~17mins of T2 weighted functional scanning of the hypothalamus (12 mm mid-sagittal slice; echo time: 5ms; repetition time 40ms; flip angle: 40°; FOV: 210 mm; in-plane resolution: 1.6mm X 1.6mm). MANOVA revealed a

statistically significant three-way interaction between time, volume and treatment when the model was weighted with either fasting insulin ( $p < 0.0001$ ) or fasting triglycerides ( $p = 0.023$ ). Gender also significantly interacted with time, volume and treatment ( $p = 0.008$ ). Further, age was negatively correlated with overall average hypothalamic signal intensity with volume as well with treatments. Both demographics and metabolites strongly influence hypothalamic response, making it difficult to determine the specific effects of high fructose corn syrup sweetened cola on appetite signals in the hypothalamus.

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COLA EFFECTS HYPOTHALAMIC ACTIVITY AS MEASURE BY FUNCTIONAL  
MAGNETIC RESONANCE IMAGING**

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Thank you to my family for all of your support. In dedication to my father, whose unconditional love and support has helped me remember who I really am and to strive to be that person without wavering. And to my beautiful daughter Blair, without her I would be lost.

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## List of Abbreviations

11 $\beta$ HSD1	11 $\beta$ -hydroxysteroid dehydrogenase 1
AGRP	Agouti-related Protein
ANACOVA	Analysis of Co-Variance
BHNRC	Beltsville Human Nutrition Research Center
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
CBF	Cerebral Blood Flow
DA	Dopamine
FMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
G3P	Glyceraldehyde-3-phosphate
G6PDH	Glucose 6 phosphate dehydrogenase
GI	Gastrointestinal
GLP-1	Glucagon like Protein 1
GLU	Glucose
HFSC	High Fructose Corn Syrup
HPA	Hypothalamic Pituitary Adrenal
IV	Intravenous
LAH	Lateral Anterior Hypothalamus
LPH	Lateral Posterior Hypothalamus
MD	Medial Dorsal Nucleus
MNC	Maryland Neuroimaging Center

NAcc .....	Nucleus Accumbens
NPY .....	Neuropeptide-Y
PCR .....	Polymerase Chain Reaction
PFC .....	Prefrontal Cortex
PYY.....	Peptide Tyrosine Tyrosine
ROI .....	Region of Interest
T1R .....	Taste Receptor Type 1
T2R .....	Taste Receptor Type 2
TE.....	Echo Time
TG .....	Triacylglycerol
TR.....	Repetition Time
UAH .....	Upper Anterior Hypothalamus
UPH .....	Upper Posterior Hypothalamus
VTA .....	Ventral Tegmental Area

## Chapter 1: Literature Review

## Introduction

The objective of this review is to introduce the reader to a new approach to the study of food intake and its control. Functional magnetic resonance imaging (fMRI) is now being used to study the neural mechanisms controlling hunger and appetite. Rather than attempt to summarize the entire field (that is growing exponentially) in what follows we hope to summarize a subset of that literature by focusing on the effects of sugars on brain activity as measured by fMRI. We start this brief overview with an attempt to place this work in context. The control of food intake has a long and rich history. Contemporary students of the neural controls of hunger and appetite recognize that multiple systems within the brain act in concert with one another to a common end. Hypothalamic controls, hind brain integration of peripheral signals and taste/reward systems all contribute to our ability to monitor and control caloric intake.

The scientific study of the control of food intake has a history that spans the last 100 years. The pioneering works of C.P. Richter, Jean Mayer, Gordon Kennedy, Eliot Stellar, Jacques LeMagnen and dozens of others have all contributed to our understanding of the biological bases of hunger and satiety. Rather than attempt to review the entire area here, we offer the reader two excellent recent reviews that summarize contemporary hypotheses about hunger and satiety. Guyenet and Schwartz<sup>5</sup> and Choi<sup>6</sup> have clearly outlined not only the role of the hypothalamus in the regulation of energy balance, but also noted that a segment of that region (specifically the ventromedial nucleus) plays several interactive roles in maintaining intake. They also note that energy regulation results from an interaction not only of hypothalamic neurons, but includes input from brain reward as well as brainstem – sensory systems (Reviewed in 7).

We have chosen to focus on sugar solutions because of their relative simplicity from a nutrition perspective and because sweetened beverages constitute a major source of calories in the American diet. By studying the mechanisms involved with sugar solution intake we hope to develop a better understanding of how and why our usual appetite mechanisms fail to adequately protect us from overeating when we are presented with sweetened beverages. Such an understanding could be instrumental in reversing the obesity epidemic facing the country.

### ***Obesity epidemic***

Recent statistics from the Center for Disease Control reveal that the obesity epidemic continues to spread throughout the US, with obesity rates in most states greater than 30% of adults. Even more troubling is that in some segments of the American population an even greater percentage of people are affected. For example more than 4 of 5 adult African American women are currently either overweight or obese. Perhaps most discouragingly of all the recent statistics is that no single factor, or combination of factors has been definitively identified as the cause or causes of the recent epidemic. Failure in determining these root causes only leads to the persistence of the condition, and has resulted in the prediction that, for the first time, today's US children will not be expected to live as long as their parents.

### ***Recent controversy about fructose and high fructose corn syrup***

Researchers have an ample number of hypotheses to consider guiding their efforts in determining the causes of obesity. One such hypothesis is that the composition of the American diet has shifted from a diet low in sugars and high in complex

carbohydrates to one that is high in sugar. Bray and colleagues<sup>1</sup> have noted that the typical American now consumes 1000%+ more sugar than only one generation ago (Figure 1). Several lines of evidence have linked high sugar diets with insulin resistance and subsequent obesity and several researchers have identified specific sugars that are suspected of being effective in promoting obesity. In particular, high fructose corn syrup has been linked to a myriad of metabolic changes conducive to promoting obesity.

### ***Effects of single nutrients on gene expression***

Mindful of these developments, we examined the short-term effects of sugar consumption on liver and adipose tissue 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$  HSD1) and glucose 6 phosphate dehydrogenase (G6PDH) message and expression<sup>2</sup>. These two enzymes have been linked to obesity (and in particular omental obesity) in both human and animal models. The question posed was: “Could any one sugar affect the regulation and/or expression of these enzymes?” We reported that rats given 24h access to dilute fructose solutions dramatically increased hepatic 11 $\beta$  HSD1 and G6PDH message and protein, whereas controls given access to the same concentration of glucose had no effect on either enzyme. We went on to show that 7-day access to sucrose had a comparable effect on hepatic 11 $\beta$  HSD1. Again, 7-day access to glucose failed to induce any significant effect on either enzyme, despite comparable sugar intake to either sucrose or fructose fed rats. Clearly, different sugars could influence these critical components of sugar-induced obesity.

### ***Effect of fructose in particular***

More recently we repeated one of London et al.’s experiments by giving rats 24h access to one of 4 different dilute sugar solutions in addition to unrestricted access to standard diet<sup>3</sup>. Hypothalamic samples taken from the brains of these animals were then probed using PCR arrays (BioScience Inc., Gaithersburg, MD). Several differences in appetite-regulating genes between sugar fed and control rats

were noted, including a dramatic suppression in cholecystokinin that was specific to fructose-fed rats. Differences in gene expression could be attributed to several factors, such as over-eating, novelty, and differences in sensory and reward factors that accompany sugar intake. However fructose (and not high fructose corn syrup or any other sugar tested) promoted several unique changes in hypothalamic controls and was the only sugar tested that additionally produced a pronounced increase in circulating triglycerides. Although Storlien<sup>4</sup> and others have reported that long-term exposure to fructose can induce hypertriglyceridemia, to our knowledge we were the first to demonstrate that this effect could be produced with as little as overnight access. These studies provide convincing evidence that fructose is uniquely suited to promote both regulatory and metabolic conditions that cause obesity.

### **Glucose affects hypothalamic signaling**

The main function of carbohydrate metabolism is to modulate postprandial blood glucose to avoid high or low glucose concentrations – both of which cause cellular damage. Postprandial carbohydrates, disaccharides and polysaccharides, are broken down by enzymes on intestinal villi into one unit sugars called monosaccharides. Glucose, a monosaccharide, is considered the physiologically preferred sugar for energy use and storage. From the intestine, glucose is absorbed into the blood stream. The brain uses about 25% of blood glucose as a primary fuel source. The glucose rich blood travels through the hepatic portal vein, which stimulates a biphasic insulin secretion from pancreatic beta cells proportionate to the concentration of blood glucose. Insulin then travels through the blood stream facilitating glucose uptake into cells for storage or energy use. Within the hypothalamus insulin can bind and inhibit the action of Neuropeptide-Y/ Agouti-related Protein (NPY/AGRP) neurons, known modulators of food intake. Additionally, glucose-sensing neurons of the hypothalamus are inhibited by a

rise in blood glucose. As a consequence, glucose ingestion and subsequent insulin secretion reduce neuronal activity within the hypothalamus.

### ***FMRI***

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive method of measuring changes in the brain. Prior to the inception of functional imaging, MRI had been used almost exclusively for clinical imaging of abnormal or damaged tissues (Figure 2). True to its namesake, MRI uses magnetic resonance to image particular tissues when set to a particular frequency. A proton spins about itself on an axis but when a magnetic field is introduced that axis will align with the field. The alignment of the protons with the magnet creates a “resting” energy state, which can be disrupted by radiofrequencies. The application of the radiofrequency wave increases the energy state of the protons. When the radiofrequency wave is removed the protons return to their magnetic alignment, doing so releases energy frequencies, which are integrated through the radiofrequency coils to create an image. A series of multiple images that reveal moment-to-moment changes in the brain is called functional magnetic resonance imaging (fMRI). Blood oxygen level dependent (BOLD) fMRI takes measuring changes within the brain a step further. BOLD fMRI is based on the imaging property of blood in its oxygenated or deoxygenated state (Figure 3). When imaged at a particular frequency oxygenated blood appears almost black on a gray scale, while deoxygenated blood appears light gray. When a neuron becomes active oxygen is required; therefore, changes in blood flow occur to supply the neuron with oxygen. This translates into BOLD fMRI, which measures blood oxygenation levels within the brain to determine differences in neuronal activity.

The development of BOLD fMRI technology has promoted its use in research. The most common application is to measure cognitive functions such as learning and memory. Imaging of the hypothalamus could reveal differences in substrate metabolism as well as demographic variations in



responsiveness to nutrient challenges. However, the location of the hypothalamus makes imaging challenging. The presence of a major sinus immediately adjacent to the hypothalamus makes imaging the region particularly difficult, as echoes from the empty sinus interfere with imaging.

### *Applying fMRI to the study of the effects of sugars on the hypothalamus*

Using BOLD fMRI, a decrease in hypothalamic activity in response to both oral and intravenous glucose administration has been reported. Smeets<sup>8</sup> observed oral consumption of a 25g or 75g glucose solution elicited a 1-2.5% signal decrease in the hypothalamus shortly after consumption. Furthermore, the 75g dose induced a greater reduction in hypothalamic activity compared to the 25g dose; the 75g dose was also significantly different from control for at least 5 minutes longer than the 25g dose. When analyzed regionally, only the upper anterior hypothalamus (UAH) and upper posterior hypothalamus (UPH) observed a reduction in signal due to glucose administration; only the upper anterior hypothalamus responded to glucose in a dose dependent manner. Smeets<sup>9</sup> was able to repeat his previous results of a 1.5-2% hypothalamic signal decrease in response to a 75g glucose solution administered orally. When they compared oral and intravenous glucose administration, an equivalent glucose infusion resulted in a transient 1% hypothalamic signal decrease. Furthermore, the signal reduction extended from an onset time of 9 minutes to at least 20 minutes when glucose was administered orally versus an onset time of 4 minutes to 17 minutes for IV glucose administration. The difference observed in magnitude is likely a result of GI regulatory mechanisms stimulated when glucose is orally administered. Smeets<sup>8</sup> sought to determine if hypothalamic signal decreases were a function of sweet taste or caloric density. A non-nutritive sweetened solution of aspartame was created with equal sweetness of a 75-g glucose solution in addition to an equicaloric solution of maltodextrin. Neither solution decreased hypothalamic activity nor did they differ from control. Regional analysis of the UPH

showed inconsistent but significant decreases in response to the maltodextrin solution. These results suggest that hypothalamic signal decrease is dependent on both calories and sweet taste.

Extrahypothalamic signal changes also occur in response to glucose administration. Liu<sup>10</sup> analyzed the whole brain response after oral glucose administration. This study looked at clusters of activity change over a time period and noted two peaks in activity. The first peak was observed in the supplementary motor area, somatosensory cortex, cerebellum, anterior cingulate and orbitofrontal cortex. This peak is most likely the result of sensory and visceral signaling but is not considered a result of “eating” because control did not produce the same peak. The second peak of activity was observed as a decrease in signal within the hypothalamus around 7.7 – 12.8 minutes post ingestion. These results are consistent with previously discussed effects of glucose on hypothalamic activity and serves as validation for research in hypothalamic brain activity changes in response to sugar solutions.

Changes in circulating glucose and circulating insulin levels in response to a glucose solution are believed to be the predominating factor for changes in hypothalamic activity when a glucose solution is consumed. However, several signaling pathways respond to the consumption of both glucose solutions or an equal volume of water. Matsuda<sup>11</sup> was among the first to observe a decrease in hypothalamic activity in response to the water control. By using obese and lean subjects they sought to determine differences in hypothalamic signal changes in response to a glucose solution. To measure controls 8 of the 20 subjects were randomly chosen to consume an equal volume of water as the glucose treatment solution. When analyzed regionally the consumption of the water elicited a signal decrease in hypothalamic activity that was reportedly “virtually identical to that of glucose”. This result suggests that gastric motility may attenuate hypothalamic signals. It is important to note that this is the only study that observed a response to water consumption.

Increases in hypothalamic activity can be observed when subjects undergo hyperinsulinemic clamp sessions. Page<sup>12</sup> demonstrated that a decrease in blood glucose results in a two-fold increase in hypothalamic activity dependent on counter regulatory hormones like glucagon, epinephrine and growth hormone. Subjects observed an increase in hypothalamic activity when plasma glucose was around 77.2 +/- 2 mg/dL. Musen<sup>13</sup> demonstrated that non-diabetic subjects had hypothalamic activation when blood glucose dropped to 68 +/- 9mg/dL, the same results were seen in insulin dependent diabetic subjects at 76 +/- 8mg/dL. Note that these numbers are not significantly different.

### ***Specificity: Glucose vs. Fructose***

Glucose is not the only monosaccharide that is metabolized by the body. Fructose is another monosaccharide that the body metabolizes - but through an unregulated pathway. Almost all absorbed fructose upon entering the blood stream is quickly taken up by the liver. Fructose, unlike glucose, becomes metabolized to 2 glyceraldehyde-3-phosphate (G3P) molecules immediately with minimal regulatory mechanisms. This is where the term “instant triose” originates. The metabolic processes that take place after this are highly debated. Many believe that the influx of G3Ps are eventually metabolized into Acetyl-CoA, which in turn increases *de novo* lipogenesis. Others believe that fructose is simply incorporated in to the metabolic pathway of glucose. Page<sup>12</sup> performed a study to determine if fructose could cross the blood brain barrier and be metabolized within the brain. Using rats, a 20% fructose infusion raised plasma fructose levels more than 20-fold. As a result, Page observed an increase in the amount of fructose found in dialysis fluid taken from the ventral medial hypothalamic region. Additionally, GLUT5 and ketohexokinase mRNA was expressed in the hypothalamus, which indicates that fructose could be metabolized within the hypothalamus. These results suggest that fructose could be used as an energy source within the brain. It is important to note that the fructose was infused and not orally administered, and in that way bypassed hepatic uptake of fructose. We are left to conclude that

fructose may be used in the brain as an energy source, but the amount of fructose that reaches the brain is likely negligible.

Other appetite regulatory mechanisms can be altered differentially by glucose and fructose. For example, Page<sup>12</sup> demonstrated that a fructose solution affects the hypothalamus differently than a glucose solution. Consistent with several other previously reported studies, they found a decrease in hypothalamic activity in response to the consumption of a glucose solution. They also reported that 15 minutes after subjects consumed a glucose solution a reduction in hypothalamic cerebral blood flow of -6.84 mL/g was observed. Hypothalamic cerebral blood flow (CBF) increased 5.10 mL/g in response to an equal concentration fructose solution. An overall decrease in hypothalamic cerebral blood flow averaged -5.45 mL/g in response to a glucose solution and 2.84 mL/g in response to a fructose solution. Several other differences between glucose and fructose treatments were reported. Glucose administration increased plasma glucose, insulin and GLP-1 whereas fructose administration increased circulating lactate and PYY. There were no differences in circulating leptin or ghrelin between glucose and fructose treated subjects. In an earlier study, Purnell<sup>14</sup> attempted to find a difference between glucose and fructose when delivered intravenously. However, their results were inconclusive and showed fructose decreasing hypothalamic activity and glucose increasing hypothalamic activity in the control region. Smeets<sup>9</sup> demonstrated that hypothalamic signal changes are difficult to measure when glucose is administered intravenously rather than orally. Additionally, the hypothalamus is difficult to image using fMRI technology due to size and placement.

The hypothalamus is not the sole regulator of appetite in the brain. There are many pathways the hypothalamus communicates with to respond to changes in appetite. Page<sup>12</sup> measured the functional connectivity between the hypothalamus and the rest of the brain when subjects were given either a glucose or fructose solution. After glucose administration subjects' functional connectivity increased in

the thalamus, caudate and putamen, whereas connectivity was only increased to the thalamus after fructose consumption. In addition to a reduction in CBF in the hypothalamus reductions were seen in the thalamus, insula, anterior cingulate and striatum after glucose consumption. In response to the consumption of the fructose solution CBF decreased in the thalamus, hippocampus, posterior cingulate cortex, fusiform gyrus, and visual cortex. The changes in activity observed when a glucose solution was administered are conducive to a satiety response for both homeostatic and reward centers. The response to fructose consumption suggests an inability to properly regulate appetite for both homeostatic and reward centers.

## I. Taste And Reward

**Reward Pathway** The reward pathway is a brain circuit that plays a critical role in motivating reward-based learning, development of goal-directed behaviors, responses to food intake, and monetary rewards<sup>15-17</sup>. This network is part of the cortico-basal ganglia that includes the prefrontal cortex (PFC), nucleus accumbens (NAcc), ventral tegmental area (VTA), and the medial dorsal nucleus (MD) of the thalamus<sup>15</sup>. The reward pathway uses dopamine (DA) as one of its main neurotransmitters to mediate signals in response to rewards<sup>18</sup>. This pathway also responds to drugs of abuse such as cocaine or heroin<sup>19</sup>. Recently sugar has been suggested to stimulate the reward pathway as well and possibly bring about sugar “addiction” or addiction related behaviors<sup>20,21</sup>.

**Gustatory (Taste) Pathway** The gustatory (taste) pathway involves signals that originate from taste receptors in the mouth and throat<sup>22</sup>. The five main taste sensations are sweet, salty, bitter, sour, and umami<sup>23</sup>. These signals travel to the brain via nerves, including the chorda tympani and glossopharyngeal nerves, through the nucleus of the solitary tract in the medulla, the thalamus and primary taste cortex to the amygdala and hypothalamus<sup>22,24</sup>. The taste pathway is important in discriminating not only taste but also post-ingestive consequences regarding caloric content to the

brain<sup>25</sup>. Additionally, sweet and bitter taste receptors (T1R and T2R respectively) have been identified in the gastrointestinal tract (GI) indicating taste signals are not limited to the mouth and throat region<sup>26,27</sup>. Taste perception can be disrupted in obese people resulting in positive reward system activation in response to any, not just palatable, taste cues<sup>28</sup>. Furthermore, artificial sweeteners can also play a role in disrupting the natural homeostatic taste mechanisms responsible for regulating taste and food ingestion<sup>29</sup>.

### **Reward and Calorie Content**

Two of the recurring questions asked by researchers over the years have been “How does the reward value of a food item relate to its calorie content?” and “What is the connection between a reward received and the ability of the brain to discern its caloric content?” In a study focusing on the caloric content of food, Killgore<sup>30</sup> and associates reported that amygdalar activation as measured by fMRI was high following presentation of food pictures when compared to non-food pictures. High-calorie foods produced increased activation of the hypothalamus and frontal cortex areas<sup>30</sup>, thereby contributing to the brain’s ability to evaluate the reward value of higher-calorie foods.

This natural mechanism can be complicated with the use of artificial sweeteners such as saccharin, a non-nutritive sweetener. Green<sup>29</sup> has reported that there are differences in brain activity between subjects who commonly consume diet soda when compared to subjects who regularly consume soda sweetened with caloric sweeteners. In diet soda drinkers, there was a large response in the dopaminergic midbrain as measured by fMRI when consuming either saccharin or sucrose<sup>29</sup>. These results indicate the reward system has modified itself within the diet soda drinker to respond to both caloric and non-caloric sweet taste, and does not discriminate based on caloric content. Green speculated that this change may in fact lead to changes in body weight as a person may not be able to evaluate caloric content and may overeat. These findings are consistent with those of Davidson and Swithers<sup>31</sup>

who reported that rats given intermittent sweet taste and calories were unable to compensate for a sweet and caloric pre-meal compared to rats given consistent sweet taste and calories. While rats are much better at tightly regulating their caloric intake, there may still be similar changes seen in human populations.

Rudenga<sup>32</sup> has reported that fMRI-measured activation of the amygdala was affected differently between people who drink artificially sweetened drinks and those who drink sugar-sweetened beverages. They reported that there was a negative correlation in people who regularly used artificial sweetener and their amygdala response to sucrose. The amygdala has been shown to be involved in flavor-nutrient conditioning, which allows for preference of flavor previously paired with sucrose over saline<sup>33</sup>. Reduced amygdalar activation as measured by fMRI resulted from treatment with a  $\mu$ -opioid receptor inverse agonist in subjects consuming palatable food<sup>34</sup>. As already noted above, disruptions in flavor sensing and the ability to judge caloric content have the potential to impact regulation of caloric intake.

### **Taste and Reward in Obese vs. Non-Obese**

The brain activity of obese people is significantly different from that of normal weight controls. Some of these differences include the reward pathway. In an fMRI study, people with high body mass index measurements showed activation in their reward pathway in response to high-calorie taste cues<sup>35</sup>. Furthermore, the people showing high reward pathway activity also had a greater number of binge-eating symptoms. These two observations make it tempting to suspect that reward pathway activation links reward processing with binge eating<sup>35</sup>. In another fMRI study, obese subjects had high levels of activation in the NAcc for all samples that provided a taste (sweet, fatty, or quinone) whereas controls varied their response depending on taste cues<sup>28</sup>. This study suggests the obese subjects did not discriminate among tastes, but rather discriminated against the lack of taste. Leptin deficient obese

subjects treated with leptin had lower activation in the striatum in response to food images<sup>36</sup>.

Additionally, in obese women there is wide spread activation of the reward system in response to high-calorie food pictures<sup>37</sup>. These results indicated that changes might occur within the reward system either as a result of or as a correlate to obesity, which represses the natural taste and reward systems.

### ***Obesity and Type II Diabetes***

Obesity and type II diabetes are polygenic diseases for which a cause is still unknown. However, type II diabetes is often preceded by obesity though a genetic component which can be exacerbated by environmental factors. One environmental factor is diet and as previously mentioned sugar intake has been shown to be a potential contributor to these diseases. Obesity in general is the inability of the body to properly maintain energy homeostasis. Matsuda<sup>11</sup> sought to determine any differences in hypothalamic activity in response to a glucose solution between lean and obese subjects. They observed an attenuated and delayed response in the obese group. Both groups still experienced a decrease in hypothalamic activity but the obese group decreased by 4.8% whereas the lean group decreased by 7.9%. Additionally, obese subjects did not observe a significant reduction in hypothalamic activity until 9.4 minutes post ingestion whereas the lean group responded sooner, at 6.4 minutes post ingestion. This delay was observed in two different regions of the hypothalamus, the lateral posterior hypothalamus (LPH) as well as the UAH. Plasma glucose concentrations were not different between groups during the fMRI session. However, fasting plasma insulin and mean plasma insulin were significantly higher in this same obese group. Additionally, fasting plasma leptin was 21  $\mu\text{g/L}$  higher in the obese group versus the lean group.

Smeets<sup>9</sup> compared hypothalamic activity in response to consumption of an oral glucose solution between type 2 diabetic subjects and healthy controls. The control group observed a reduction in



hypothalamic activity congruent with previous studies. By contrast, the type II diabetic group's BOLD signal in any hypothalamic area remained unaffected. Additionally, when the type II diabetic group consumed water the hypothalamic activity was increased significantly in the lateral anterior hypothalamus (LAH) and LPH. These results suggest that subjects with type II diabetes may lack a regulatory mechanism required for energy homeostasis within the hypothalamus. The results do not give any indication as to what regulatory mechanism is missing and whether this response contributed to or was caused by the type II diabetes.

## **Conclusions**

Functional magnetic resonance imaging is providing new perspectives into how the brain controls food intake and energy metabolism. This review offers only a limited insight into the extent to which this new technology is contributing to a better understanding of how and why we eat what we eat. Here we have focused on the effects of sugars on brain regions that are known to participate in hunger and appetite. The early reports of this work make it clear that sugar affects several neural systems that can be monitored by fMRI. Hypothalamic, sensory and reward pathways are all influenced by sugar. It remains to be determined how different sugars influence the activities of each pathway. Early evidence supports the speculation that not all sugars act alike. The application of this technology also serves to test in humans hypotheses about regulatory mechanisms that originated in animal models. For example, can the suppressive effect of fructose on hypothalamic cholecystokinin be observed using fMRI? Future applications of this technology will undoubtedly advance our understanding of these effects and in that way support the need for better approaches to the treatment and prevention of diet-induced obesity.

Chapter 2: Appetite Signals in the Brain: High Fructose Corn Syrup Sweetened  
Cola Effects Hypothalamic Activity as Measured by fMRI

## Introduction

Contemporary students of the neural controls of hunger and appetite recognize that multiple systems within the brain act in concert with one another to a common end. Guyenet and Schwartz<sup>5</sup> and Choi<sup>6</sup> have clearly outlined not only the role of the hypothalamus in the regulation of energy balance, but also noted that a segment of that region (specifically the ventromedial nucleus) plays several interactive roles in maintaining intake.

Recent statistics from the Center for Disease Control reveal that the obesity epidemic continues to spread throughout the US, with obesity rates in most states greater than 30% of adults. Even more troubling is that in some segments of the American population an even greater percentage of people are affected. For example, more than 4 of 5 adult African American women are currently either overweight or obese. Perhaps most discouragingly of all the recent statistics is that no single factor, or combination of factors has been definitively identified as the cause or causes of the recent epidemic.

Sweetened beverages constitute a major source of calories in the American diet. Bray and colleagues<sup>1</sup> have noted that the typical American now consumes significantly more sugar than only one generation ago. Several lines of evidence have linked high sugar diets with insulin resistance and subsequent obesity and several researchers have identified specific sugars that are suspected of being effective in promoting obesity. In particular, high fructose corn syrup has been linked to a myriad of metabolic changes conducive to promoting obesity. Rats given 24h access to one of 4 different dilute sugar solutions in addition to unrestricted access to standard diet showed several differences in appetite-regulating genes between sugar fed and control rats when hypothalamic tissue was sampled<sup>3</sup>.

A series of multiple images that reveal moment-to-moment changes in the brain is called functional magnetic resonance imaging (fMRI). Blood oxygen level dependent (BOLD) fMRI takes measuring changes within the brain a step further. BOLD fMRI is based on the imaging property of

blood in its oxygenated or deoxygenated state. When a neuron becomes active oxygen is required; as a consequence changes in blood flow occur to supply the neuron with oxygenated blood. This translates into BOLD fMRI, which measures blood oxygenation levels within the brain to determine differences in neuronal activity.

fMRI is an excellent non-invasive method for imaging the hypothalamus, although the location of the hypothalamus makes imaging challenging. The presence of a major sinus immediately adjacent to the hypothalamus makes imaging the region particularly difficult, as echoes from the empty sinus interfere with imaging. Several researchers have successfully imaged the hypothalamus. Smeets<sup>8</sup>, observed oral consumption of a 25g or 75g glucose solution elicited a 1-2.5% signal decrease in the hypothalamus shortly after consumption. Furthermore, the 75g dose induced a greater reduction in hypothalamic activity compared to the 25g dose; the 75g dose was also significantly different from control for at least 5 minutes longer than the 25g dose. Smeets<sup>9</sup> also demonstrated that glucose infusion does not decrease hypothalamic activity to the same magnitude as oral glucose, thus the change in hypothalamic activity is only partially attributed to blood glucose concentration. When Page<sup>12</sup> compared glucose to fructose, hypothalamic response to both solutions was quite different. Fructose increased hypothalamic activity while glucose decreased hypothalamic activity by the same magnitude from baseline. Fructose is taken up by the liver after digestion, leaving almost none available to travel to the brain. Therefore, the effects of fructose are most likely a secondary response to fructose metabolism. Americans are consuming a combination of glucose and fructose rather than one or the other, which makes these results poorly suited to identify possible mechanisms in obesity.

This study aims to identify the effects of high fructose corn syrup on hypothalamic activity via cola. High fructose corn syrup (HFCS) comes in many glucose to fructose ratios. The most common is 45:55 glucose to fructose. Given the opposing effects of glucose and fructose, hypothalamic activity

could remain unchanged when administered simultaneously. However, glucose acts directly on the brain in conjunction with insulin, whereas fructose does not. Therefore, we predict hypothalamic response will reflect the affects of the glucose rather than the opposing of both sugars By studying the mechanisms involved with sugar solution intake we hope to develop a better understanding of how and why our usual appetite mechanisms fail to adequately protect us from overeating when we are presented with sweetened beverages.

## **Materials and Methods**

### *Subjects*

Eight healthy adult men (n=4) and women (n=4), between 25 and 70 years of age with a body mass index (BMI) between 19 and 38 kg/m<sup>2</sup>, were recruited from the Baltimore-Washington area. Participants were screened at the Beltsville Human Nutrition Research Center (BHNRC). Eligibility criteria included: participants were non-smokers; free of active cardiovascular disease, diabetes, and metabolic or malabsorption syndromes; and were not taking lipid-lowering medications. In addition, participants were required to have blood pressure  $\leq$  180/100 mm Hg, fasting triacylglycerol  $\leq$  300 mg/dl and fasting glucose  $\leq$  126 mg/dl. Written informed consent was obtained from all subjects. This study's protocol was approved by University of Maryland's Institutional Review Board.

### *Experimental Procedure*

Each testing day participants came into the BHNRC, following a 12-hour overnight fast. After a fasted blood draw each participant was fed a standardized breakfast meal. The standardized test meal consisted of a breakfast sandwich (English muffin, egg, sausage, cheese) and a hash brown (Table 1). Participants

were required to refrain from consuming caffeine, as well as any other foods or beverages prior to testing.

### *Blood Sampling and Processing*

Serum and plasma samples were collected at each blood draw. Plasma samples were gently inverted and placed on ice. Blood collected for serum was gently inverted and held at room temperature for 30 minutes after collection prior to centrifugation. Plasma and serum samples were portioned into cryogenic vials and stored at -80° C for subsequent analyses.

### *Plasma and Serum Assays*

Plasma insulin concentrations were measured by ELISA (LINCOplex; LINCO Research). Triacylglycerol (TG) and glucose (GLU) were both measured using an automated clinical chemistry analyzer (Vitros 5,1 FS) (Orthoclinical Diagnostics, Rochester, NY). TG were measured in serum and GLU was measured in sodium fluoride/EDTA plasma using colorimetric/rate microslide technology.

### *fMRI Scanning*

Following breakfast, participants proceeded to the University of Maryland Neuroimaging Center (MNC). Participants underwent magnetic resonance imaging in a 3T Siemens MAGNETOM Trio scanner. Each subject was positioned supine on the patient table, equipped with ear plugs and headphones to reduce noise. Participants were then allowed to sit up and consume 12 oz. of treatment 1. No time requirement was given, but participants were urged to consume their treatment quickly but not too fast as to cause discomfort. Participants were then returned to the supine position, where a Siemens 12 channel head matrix coil was placed over the participant's head. Foam cushions were supplied

between the head coil and the head to reduce movement. Functional scans began promptly using conventional gradient echo sequence (T2-weighted) to scan a 12 mm midsagittal slice, manually positioned to capture the hypothalamus (TR= 40 ms, TE=5, flip angle=40°, FOV of 210 mm, FOV phase 100%, Phase resolution 100%, In-plane resolution of 1.6×1.6 mm). The functional imaging run took approximately ~17 minutes during which a total of consecutive 50 scans were performed. Upon completion of the functional scans, the participant was removed from scanner and allowed to take short break (~5 min) if desired. Scanning procedures were then repeated with the participant receiving 6 oz. of treatment 2. Upon completion of the second functional run, a T1-weighted anatomical scan (Sagittal MPRAGE, TR=1900ms, TE=2.52ms, flip angle 9°, FOV=250 mm, resolution= 1.0 x 1.0 x 1.0 mm, 176 slices with 18.2% slice oversampling) was taken.

#### *Beverage testing*

To measure the effects of Coca-Cola on the hypothalamus, subjects reported to the MNC on 4 testing days, separated by no less than a week. Each testing day participants underwent two successive imaging sessions. The experimental condition prior to the first session was either 12oz of Coca-Cola or 12oz of bottled water. Upon completion of the first session participants received either 6oz of Coca-Cola or 6oz of bottled water immediately prior to the beginning of the second scan. Treatments were assigned in a crossover design using Latin square order to ensure each order of treatments was accounted for over the 4-week period.

#### *Data Preprocessing*

For each imaging session, the T1-weighted MPRAGE image was used to manually trace the region-of-interest (ROI) of the hypothalamus on the middle sagittal plane of the data. The 50 functional images

were processed in a consecutive order, and then an image registration program (<http://afni.nimh.nih.gov/afni/>) was used to correct the head motions in a 2-dimensional setting, and aligned the functional images with the MPRAGE image in the middle sagittal plane. The hypothalamus functional signal at each time point was then estimated by averaging the image intensities of all the pixels within the hypothalamus ROI in the aligned functional data.

### *Data Analysis*

Average signal intensity of the region of interest (ROI), which traced the hypothalamus, were obtained from each slice and then entered into a spreadsheet. Overall average signal intensity is the average of all 50 average signal intensities. The signal intensities of the ROI were used for statistical analyses. Analysis of Co-Variance (ANACOVA) adjusted for repeated measures was used to test for effects of treatment and volume over the 17 minutes of functional scanning. Adjustments for the effect of metabolic factors like fasting triglycerides were weighted in ANACOVA analyses. Correlational analyses were also ran using overall average signal intensity against co-variants like fasting insulin and age.

## **Results**

### *Subjects*

All of the subjects who began the study were able to complete the entire study (Table 2). The original scanning parameters, which imaged two functional slices, did not produce a reliable signal from the hypothalamus. Therefore, the scanning parameters were changed and all scans using the original scanning parameter could not be used. The data used for analysis is missing 6 imaging sessions of the 32



in total. Of the 6 imaging sessions that were lost, the first two imaging sessions for subjects 1 and 2 as well as the first imaging session for subjects 3 and 4 were lost.

#### *Hypothalamic activity responds to dose*

Fifty functional slices were obtained during the 17-minute functional imaging session. Therefore, the average signal intensity within the hypothalamus was recorded 50 times approximately 17 minutes post ingestion. Preliminary analyses revealed that volume is most likely affecting the hypothalamus, perhaps via stomach distension or fullness signals sent from the vagus nerve to the hypothalamus (Figure 4). Multivariate analysis revealed no significant effect of volume or treatment in hypothalamic signal intensity. However, the signal intensities are significantly affected by time ( $p=0.005$ ). When the signal intensities across the 50 time points are fitted with a line, there is a slight positive slope. These results suggests dose, the combination of treatment and volume, does affect neuronal activity in the hypothalamus but differential effects of the treatments (Cola and water) and volumes (12oz and 6oz) were not found. In previous studies, the hypothalamus has been shown to respond to treatment rapidly then slowly return to baseline activity. Because there is a delay between delivering the treatment and the initiation of the functional scanning, the initial response to treatment may have been missed. The slope of the response can infer the return to baseline. The first imaging session, 12oz treatment, had no statistically significant effect on the second imaging session, 6oz treatment. Therefore, subjects' hypothalamic activity was returned to baseline before the second treatment was administered. It is also important to note that there was no effect of the testing day. Subjects underwent the entire experimental procedure over 4 separate testing days; which could theoretically alter response due to variables like anticipation. However, the order of treatment was structured in a Latin square design to help counter any of these possible effects. It is important to note that the effect of the testing day was calculated using

subjects 5-8 because some data was lost for subjects 1-4. Therefore, only subjects 5-8 completed a Latin square.

### *Insulin and Triglycerides Affect Hypothalamic Response*

Further multivariate analyses that adjusted the model for fasting insulin revealed a strong significant three-way interaction between time, volume and treatment ( $p < 0.0001$ ). Furthermore, including fasting triglycerides data, but not fasting glucose data, produced a significant three-way interaction between time, volume and treatment ( $p = 0.023$  and  $p = 0.23$  respectively). These results suggest that circulating triglycerides and circulating insulin in some way influence hypothalamic responses. Insulin binds to many neurons within the hypothalamus as a modulator of intake including NPY/AGRP neurons.

Triglycerides are metabolized into free fatty acids, which might indirectly modulate hypothalamic activity by regulating leptin activity. Glucose acts on the glucose sensing neurons of the hypothalamus. However, the exact function of these neurons has not been determined. The locations of these neurons are most dense in the lateral hypothalamus. Unfortunately the lateral hypothalamus may be poorly represented due to scanning limitations.

Correlational analyses were used to further analyze the relationship between hypothalamic response and circulating metabolites. Hypothalamic response as measured by overall average hypothalamic signal intensity is positively correlated with fasting triglycerides as well as fasting insulin but not fasting glucose (Table 3). Higher fasting triglycerides and insulin were associated with higher hypothalamic signal intensity, thus the hypothalamus was less responsive to treatment. High fasting triglycerides and insulin are indicators of metabolic dysfunction, thus hypothalamic response is partially inhibited by errors of metabolism. As previously demonstrated by Matsuda<sup>11</sup>, hypothalamic responses to a glucose solution are blunted in obese subjects when compared with lean controls. These results support

previous reports of a relationship between metabolic dysfunction and decreased responsiveness in the hypothalamus<sup>11</sup>. Interestingly, Body Mass Index (BMI) does not correlate with hypothalamic signal intensity. This result may be due to BMI's inability to reliably measure metabolic dysfunction. While a BMI greater than 30 is the definition of obesity, BMI is a ratio of height-to-weight but does not reliably measure body composition or unhealthy fat depots like abdominal obesity which are better indicators of metabolic dysfunction.

#### *Age and Gender predict hypothalamic response*

Demographics such as age and gender appear to impact hypothalamic response to treatment. When gender is included into the mixed effects model, a significant 4-way interaction between gender, time, volume and treatment is revealed (Figure 5). Men and women are inherently different metabolically due to differences in body composition. Therefore, the same dose given to a woman would potentially be diluted metabolically by the increase in lean body mass and total body mass in men. Age was negatively correlated to the hypothalamic signal response to both treatment and volume (Table 4). These results indicate that as an individual ages their hypothalamus is either more responsive to dose or their baseline hypothalamic activity is lower. BOLD fMRI is dependent on blood flow and the ability to oxygenate blood, which the normal degradation of these systems could result in lower baseline activity. Without baseline data it is difficult to determine the most likely cause of decreased hypothalamic response with age (Figure 6).

## **Discussion**

Appetitive and metabolic signals from various systems are received and processed in the hypothalamus. The hypothalamus then sends its own signals to various systems in the periphery to

orchestrate metabolic processes. Therefore, the hypothalamus is the center for appetite and metabolism. Dysfunction of this regulatory system results in the inability to properly regulate body weight, caloric intake as well as other metabolic processes. These data demonstrate the ability to elicit change in hypothalamic activity by administering sugar solutions of different volumes. However, differential effects of high fructose corn syrup sweetened cola compared to water were not evident. One factor that may have hindered our ability to observe statistically significant effects of treatment could be a lack of statistical power. Factors, such as cost, limited the size of the sample population. Furthermore, these data show that the sensitivity of hypothalamic responsiveness is dependent on circulating metabolites like insulin and triglycerides but not glucose. Other factors that influence metabolism such as age and gender were also strong predictors of hypothalamic response. Clearly, metabolic status influences how the brain responds and reacts to HFCS-sweetened cola.

Our results show the hypothalamus responded to both treatments of cola and water. Matsuda<sup>11</sup> has reported hypothalamic response to water. He reported that the response to water was identical to the glucose response but only within a segmented region of the hypothalamus, as opposed to the entire hypothalamic area. Others observed no change from baseline<sup>8,12</sup>. However, all previous reports are based on fasted subjects whereas our study fed all subjects a standardized meal. It may be that stomach distention was a greater influence in this study. The effect of being fed rather than fasted could be partially responsible for why differences were not evident between cola and water. Fasting and feeding are both methods used to ensure subjects are on similar metabolic planes and will respond similarly. Other studies used fasted subjects which may have lead the hypothalamus to be hyper responsive to the sugar solutions<sup>8-12</sup>. Conversely, our subjects may have been less responsive to our treatment because their systems had recently been metabolizing the standardized meal. In the case of measuring the effects of high fructose corn syrup, hypothalamic response may be minimized due to the opposing effects of

fructose and glucose. Therefore, hypothalamic response to high fructose corn syrup may be best measured in fasted subjects, who are likely to be more responsive to treatment.

The influence of metabolites like insulin and triglycerides on hypothalamic activity has been implicated in previous studies<sup>8,12</sup>. High levels of insulin and triglycerides are often indicative of the body's inability to properly metabolize substrate. These errors of metabolism were apparent in our subjects' reduced hypothalamic responses when fasting triglycerides or fasting insulin were elevated. Previous research has shown the hypothalamus of obese subjects is less responsive to a glucose solution compared to that of lean subjects. Interestingly, plasma insulin was higher in obese subjects but plasma glucose was the same between obese and lean.

Aging is the slowing and breakdown of metabolic processes, which often leads to lower basal activity. Our data shows lower hypothalamic activity in older subjects; a response most likely due to the result of lower baseline activity. An alternative hypothesis would be that the hypothalamus is hyper reactive with increasing age due to an inability to finely modulate response. Apart from age biases, our results showed differential responses between men and women. Apart from differences in body composition, the hypothalamus is sexually dimorphic. For example, the estrogen receptors are differentially expressed in neurons between men and women. Previous studies have not reported age or gender biases in hypothalamic responses. However, previously reported sample populations were not appropriately diverse to determine age or gender effects<sup>9,12</sup>.

Understanding the impact of hypothalamic response on obesity will continue to be studied using fMRI. While the method is not perfected and the use of the scanner is expensive, the benefits of observing time-lapsed responses in the human brain are invaluable. Future studies on hypothalamic response to sugar solutions should focus on finding new parameters for measuring hypothalamic activity. Single slice fMRI imaging is not ideal, partly because motion correction software cannot

process the 2D slices without the entire brain in 3D slices. The current method of imaging will be sufficient for measuring simple hypothalamic responses but analysis of extra-hypothalamic interactions, like reward pathways, are extremely limited in two dimensions.

The hypothalamus processes many peripheral signals. Here we demonstrated the impact of triglycerides and insulin on hypothalamic response. Other peripheral systems like the GI and liver have long known interactions with the hypothalamus. Studying these systems in conjunction with hypothalamic response would help with further understanding of where obesity originates. For example, how does the modern diet impact liver metabolism and does that impact then dampen hypothalamic responses? The effect of other nutrients, such as fat, on hypothalamic response would be the next logical nutrient given the extensive effects of fat on the HPA axis.

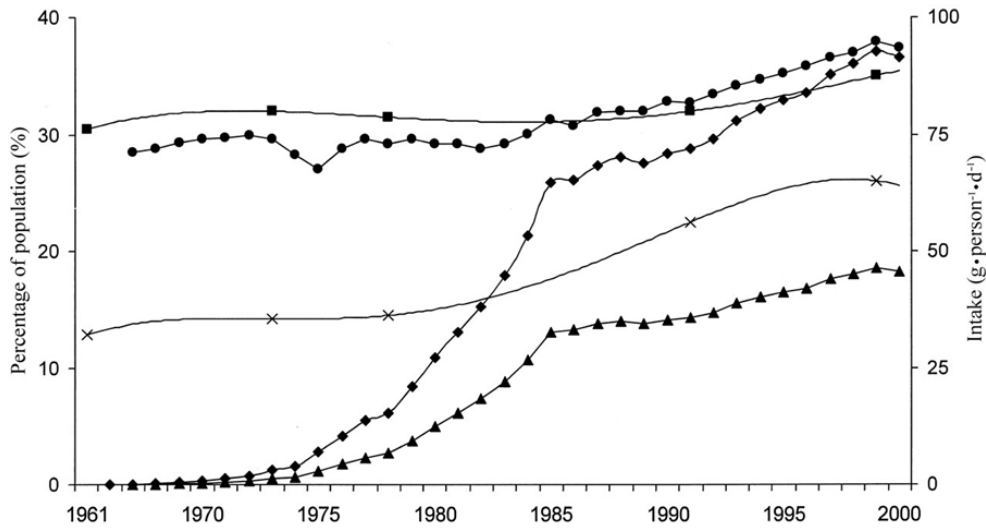
Our immediate next steps are two fold. We must first focus on the effects of high fructose corn syrup on hypothalamic activity using baseline measurements and increasing our number of treatments to include glucose, fructose and sucrose. Because caffeine is a vasoconstrictor and is present in many types of cola, the effect of caffeine on hypothalamic response and fMRI imaging must be delineated. Furthermore, increasing the sample sizes would be beneficial to not only analysis of hypothalamic response to treatment but to the co-variables noted in this study, like age. Common sample sizes in fMRI research are at least 30 subjects due to the variability in functional imaging. Additionally, a larger sample size that includes a wider range in the age of adults would allow the impact of age to be better defined.

The hypothalamus is a dense region of appetite and metabolic processing. Hypothalamic dysfunction has been shown to impact health and obesity. Understanding the many intricate systems within the hypothalamus will enable researchers to add another piece to the puzzle of obesity in the United States and will support the development of new therapies to prevent and treat this disease.



Estimated intakes of total fructose (●), free fructose (▲), and high-fructose corn syrup (HFCS, ◆) in relation to trends in the prevalence of overweight (■) and obesity (x) in the United States.

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Bray G A et al. Am J Clin Nutr 2004;79:537-543

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Figure 1. Fructose Consumption 1961-2000. Graphical representation of estimated total fructose (●), free fructose (▲), and high-fructose corn syrup (HFCS, ◆) intake in relation to increasing prevalence of overweight (■) and obesity (x) in the United States from 1961 - 2000. Adapted from Bray GA, et al. Am J Clin Nutr 2004;79:537-543.





Figure 2. fMRI 3-Tesla Magnet. Image of the fMRI 3-Tesla Magnet used at the University of Maryland, Neuroimaging Center.

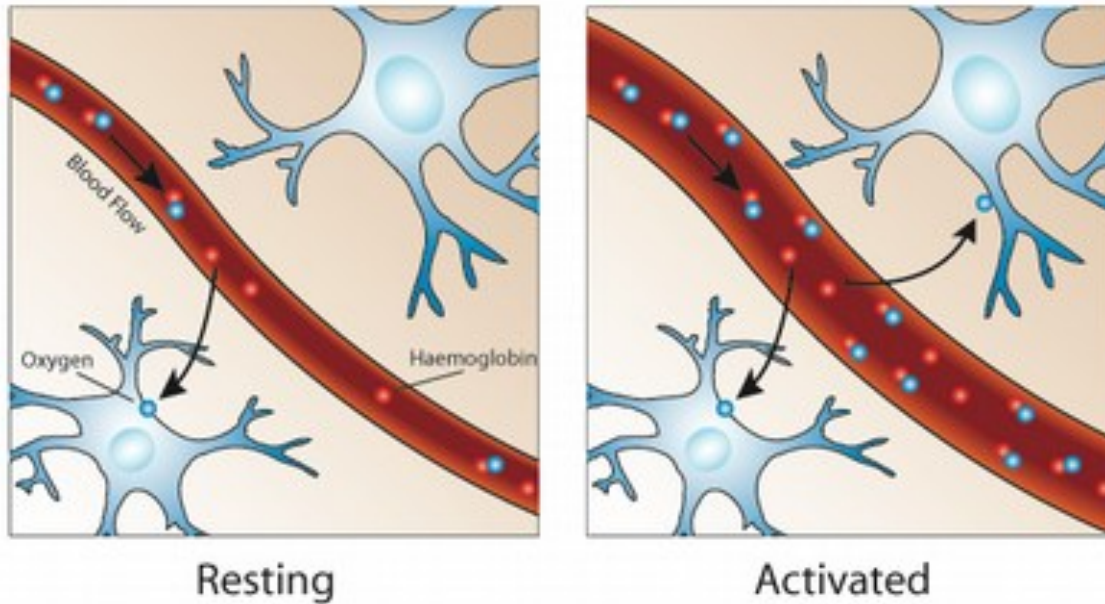


Figure 3. BOLD Signal. When blood hemoglobin is oxygenated it contains no unpaired electrons and thus is not attracted to a magnetic field. However it becomes paramagnetic (has unpaired electrons) when deoxygenated. This difference in magnetic properties makes it possible to detect small differences in the MR signal of blood. Thus, the changes in oxygenation reflect levels of neural activity. This form of MRI is known as blood oxygenation level dependent (BOLD) imaging. Adapted from Huettel, S., Song, A., & McCarthy, G. (2009). *Functional magnetic resonance imaging*. (2nd ed.). Sunderland, MA: Sinauer Associates, Inc.

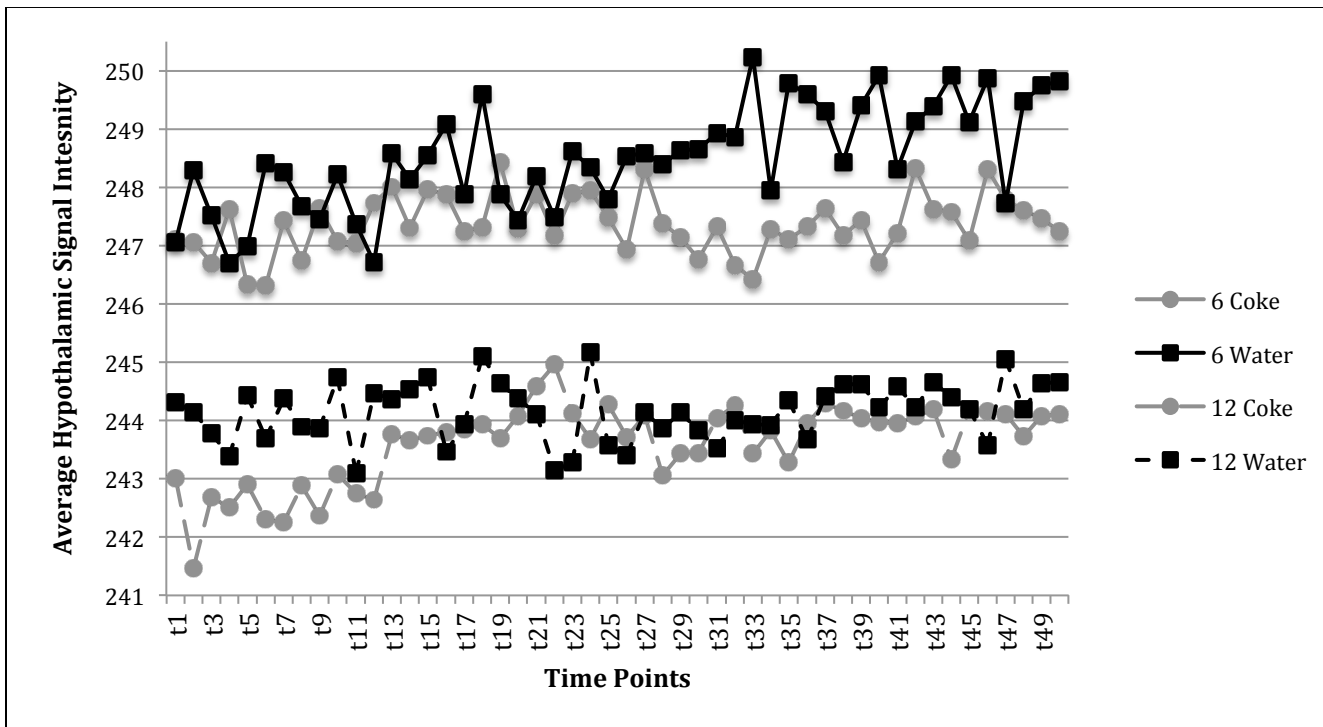


Figure 4. Dose Response in Hypothalamus. Illustrated above is the average hypothalamic signal intensity at each of the 50 time points, separated by dose (Treatment and Volume). These data show differential response of the hypothalamus to volume, hypothalamic activity appears to be lower when either 12oz treatment is administered compared to the 6oz treatments. Differential effects of Coke vs. Water in hypothalamic response cannot be inferred.

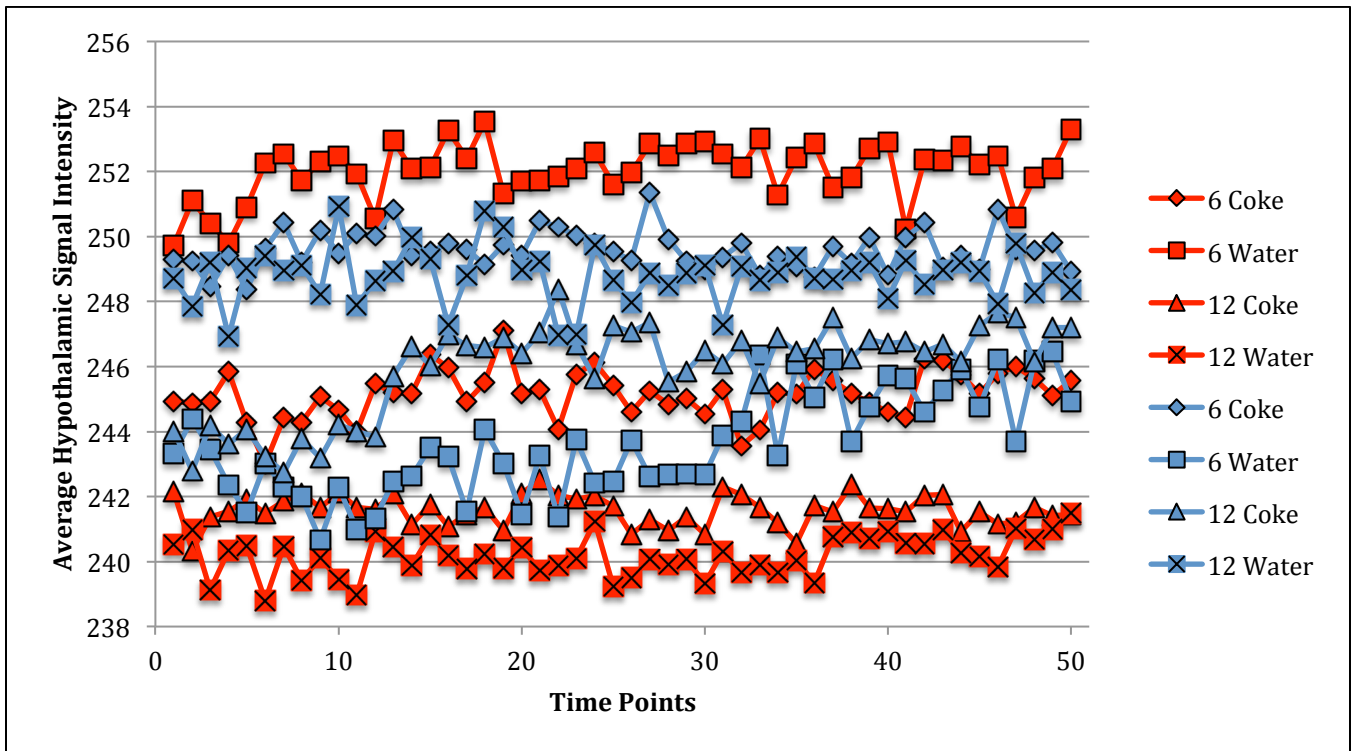


Figure 5. Four-Way Interaction Gender\*Time\*Treatment\*Volume. Illustrated above is the average hypothalamic signal intensity across each of the 50 time points separated by gender and dose. (Red data=Women, Blue data=Men) These data show differences in response to dose, dependent on gender. The women's responses were relatively constant and showed greater differentiation to dose. The men's responses were sporadic and show almost no differentiation to volume or treatment. It is important to note that some data from the male subjects is omitted (p=0.008).

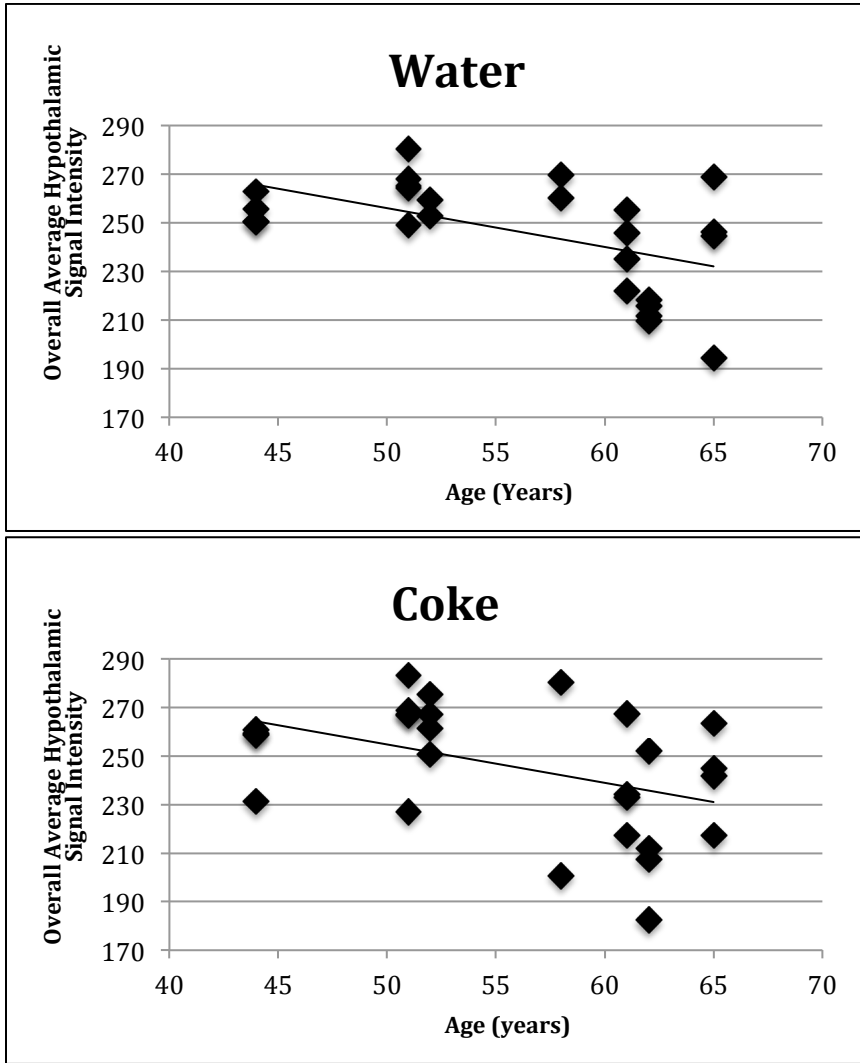


Figure 6. Increased Age Associated with Increased Hypothalamic Response. Illustrated above are the overall average hypothalamic signal intensity compared to age. These data show a significant negative correlation between increased age and decreased hypothalamic activity when data is separated by treatments (Water  $p=0.023$ , Cola  $p=0.005$ ).

Weight (g)	193
Calories (kcal)	460
Total fat (g)	27
Saturated fat (g)	8
Trans fat (g)	0
Protein (g)	15
Carbohydrate (g)	41
Dietary fiber (g)	3
Sodium (mg)	1100
Potassium (mg)	170
Cholesterol (mg)	125

Table 1. Nutrient Analysis of Standardized Breakfast. This table shows the nutrient profile of the standardized breakfast the subjects were fed the morning of each imaging session after the fasted blood draw. Nutrient analysis was calculated using The Food Processor: Version 10.11.0 (ESHA Research, Salem, OR). Standardized meal consisted of: egg, cheese, sausage, English muffin, and a hash brown.

	Women n=4 Data are means $\pm$ SEM	Men n=4 Data are means $\pm$ SEM
	Mean	Mean
Age (years)	55.3 $\pm$ 4.8	55.8 $\pm$ 2.6
BMI (kg/m <sup>2</sup> )	27.7 $\pm$ 1.0	28.5 $\pm$ 1.0
Fasting plasma glucose (mg/dL)	98.5 $\pm$ 1.5	101.4 $\pm$ 0.9
Fasting serum insulin (mU/l)	7.4 $\pm$ 0.7	10.1 $\pm$ 1.1
Triglycerides (mg/dL)	122.1 $\pm$ 9.8	137.8 $\pm$ 60.3

Table 2. Subject Data. This table shows subject data, separated by gender, from the screening process.

<b>Table 3. Metabolite Correlations</b>				
		<b>Fasting Metabolite</b>	<b>Correlation Coefficient</b>	<b>P- value</b>
<b>Volume</b>	12 oz.	Triglyceride	0.567	0.003
		Insulin	0.518	0.007
		Glucose	-0.160	0.435
	6 oz.	Triglyceride	0.575	0.002
		Insulin	0.445	0.023
		Glucose	-0.039	0.851
<b>Treatment</b>	Cola	Triglyceride	0.577	0.002
		Insulin	0.468	0.014
		Glucose	-0.133	0.507
	Water	Triglyceride	0.555	0.004
		Insulin	0.503	0.010
		Glucose	-0.079	0.710

Table 3. Fasting Metabolites are Predictive of Hypothalamic Response. This table shows the correlation between overall average hypothalamic signal intensity and each of the three fasting metabolites, triglycerides, insulin and glucose. These data are separated by volume and treatment.



<b>Table 4. Age Correlations</b>			
		<b>Correlation Coefficient</b>	<b>P-value</b>
<b>Volume</b>	12oz	-0.552	0.003
	6oz	-0.423	0.032
<b>Treatment</b>	Cola	-0.522	0.005
	Water	-0.454	0.023

Table 4. Hypothalamic Response Increases with Age. This table shows the negative correlation between overall average hypothalamic signal intensity and age. Older subjects had the lowest hypothalamic activity compared to the younger subjects, when data is separated by volume and treatment.

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