Title
Differences in Brain Responses Between Lean and Obese Women to a Sweetened Drink

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Differences in Brain Responses Between
Lean and Obese Women to a Sweetened Drink

A thesis submitted in partial satisfaction
of the requirements for the degree Master of Science
in Clinical Research

by

Lynn Shapiro Connolly

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ABSTRACT OF THE THESIS

Differences in Brain Responses Between
Lean and Obese Women to a Sweetened Drink

by

Lynn Shapiro Connolly

Master of Science in Clinical Research
University of California, Los Angeles, 2012
Professor Robert Elashoff, Chair

Background: Ingestion of sweet food is driven by central reward circuits and restrained by interoceptive satiety signals. Obesity is in part related to an upregulation of these reward mechanisms in association with a downregulation of vagally mediated satiety mechanisms. The specific influence of sucrose intake on central affective and reward circuitry, and differences in these influences in the obese population is incompletely understood.

Hypotheses: 1) Similar brain regions are engaged by the stimulation of sweet taste receptors by sucrose and by non-nutrient sweeteners. 2) During visual food related cues, obese subjects show greater brain responses specifically to sucrose compared to lean controls.
Methods: In a two-day, double blind, crossover design, 10 obese and 10 lean healthy females received either a sucrose or a non-nutrient sweetened beverage prior to viewing agreeable food or neutral images. BOLD signal was measured using a 1.5Tesla MRI scanner.

Results: Viewing food images after ingestion of either the sucrose or the non-nutrient drink was associated with engagement of similar brain regions, including the amygdala, hippocampus, thalamus and dorsolateral prefrontal cortex, Obese differed from lean subjects in both behavioral and brain responses: While obese subjects rated both beverages as less tasteful and satisfying, they showed greater brain responses. Obese subjects also showed engagement of an additional brain network (including anterior insular and anterior cingulate cortices, hippocampus, amygdala), and this was only seen after sucrose ingestion.

Conclusion: Obese subjects had a reduced behavioral hedonic response yet a greater engagement of affective brain networks in response to food images, particularly after sucrose ingestion. These findings suggests that in obese subjects lingual and gut derived signaling generate less central hedonic effects than recalling memories of food experiences in response to visual cues, analogous to response patterns implicated in food addiction.
The thesis of Lynn Shapiro Connolly is approved.

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2012
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Chapter two is a version of “Differences in brain responses between lean and obese women to a sweetened drink” a manuscript in preparation for submission. The authors include:

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Chapter 1: Background

Thirty four percent of the population is obese and an additional 34% is considered overweight, with minority women showing the highest percentage of affected individuals.\(^1\) Obesity-attributable medical expenditures related to the metabolic syndrome and other associated diseases reached $75 billion in the US in 2003.\(^2\) Even with the billion-dollar diet industry, long-term weight loss results with dieting have been disappointing. And despite extensive research into possible targets for drug development aimed at the obesity epidemic, only surgical interventions including gastric bypass surgeries have shown persistent benefits.\(^3\)

Food intake is subject to intense regulation by homeostatic and hedonic systems. Homeostatic feeding is driven by nutritional or caloric deficiency and involves humoral and neuronal signaling from the gastrointestinal (GI) tract to the hypothalamus and posterior insula (pINS), known as the interoceptive cortex. Hedonic feeding occurs when one is not deprived of nutrients and is instead driven by central reward circuitry and regions of the brain involved in evaluating the motivational value of a food cue (including the nucleus accumbens and the orbitofrontal and anterior insular cortices). Distinct, but overlapping brain networks are thought to regulate the interplay between these two mechanisms. In lean individuals, the two systems are known to interact in a self-regulatory manner in order to produce optimal behavior that results in adequate food intake to keep body weight stable.

It is thought that overeating may be the result of an imbalance between satiety mechanisms triggered by nutrients in the intestine that reach the brain via endocrine and vagal afferent signals, neuronal circuits relating to motivation and reward, and prefrontal circuits that are involved in a more top-down executive control of ingestive behavior. A similar alteration
between interoceptive, reward, and executive control mechanisms is seen in drug addiction where an altered interaction of these networks results in an enhanced reinforcing value of the drug and an altered reward threshold. Over the last decade, significant progress has been made in understanding obesity related disparities in peripheral encoding mechanisms, and altered hypothalamic control mechanisms related to homeostatic control of ingestive behavior in rodents. However, it has only been recently recognized that alterations in cognitive and reward related control mechanisms have been implicated in the pathophysiology of obesity.

A number of investigators have looked into the role of food related memories in obesity, by studying the impact of viewing food images on brain activation. Comparing lean and obese subjects have established differences in brain response to images of high calorie versus low calorie foods. It has also been demonstrated that brain activity changes in response to these images when a subject has been given glucose versus water, and that this interaction between visual food cues and ingested nutrients differs in obese compared to lean groups. A limitation of these studies is that subjects can taste the difference between high and low calorie foods and between glucose and water ingestion; thereby possibly confounding the results by the subject’s preconceived expectations based on what they are seeing, smelling and tasting. Non-caloric sweeteners stimulate the same sweet taste receptor as glucose and sucrose, thereby theoretically providing the same hedonic value without the calories. For this reason, they are an ideal means to study the specific effect of sucrose absorption on brain activation in obese and lean subjects.

Sweet taste perception of both non-caloric (artificial) sweeteners and sucrose is peripherally mediated by tongue heteromic T1R2/T1R3 sweet taste receptors. The sensory information is then transmitted by cranial nerves VII, IX and X to the nucleus tractus solitarius
and to the anterior INS (aINS). The aINS together with the operculum is part of the human gustatory cortex; it receives input from homeostatic, prefrontal, and affective brain regions, and has close connections to reward networks.

The subjective perception of sweet taste is multidimensional, including the assessment of taste quality, hedonic “liking” and the incentive motivational component “wanting”. Reduced satisfaction to actual food ingestion (“liking”) combined with greater engagement of hedonic circuits during expectation of food intake (“wanting”), has been proposed as a mechanism underlying food addiction. This pattern is similar to one reported in drug addiction where craving for the drug is amplified, while satisfaction after drug intake is reduced. This model might explain why some obese individuals over respond to food cues and can eat compulsively even when it is not perceived as pleasurable.

During the last decade, great progress has been made in understanding homeostatic mechanisms of feeding behavior in rodents (the ability to maintain internal equilibrium or balance). Despite this gain in knowledge, translational efforts to develop effective treatments for human obesity have been disappointing. While the relative importance of alterations in hedonic (pleasure) versus homeostatic (internal equilibrium) mechanisms responsible for driving the human obesity epidemic is not known, the lack of understanding the role of hedonic mechanisms in humans may have contributed to the translational disappointments. Thus, studying the effect of nutrient intake on these two mechanisms in obese and lean individuals, could provide important insights into the etiology of human obesity.

In the current study, we aimed to test the following hypotheses: (1) Brain regions involved in reward circuitry will be differently engaged by the stimulation of sweet taste receptors by non-nutrient sweetener versus sucrose ingestion. (2) Brain regions activated by oral
liquid non-nutrient sweetener versus sucrose will differ between lean and obese subjects. To test these hypotheses, we assessed brain responses using functional magnetic resonance imaging (fMRI) in lean and obese healthy female subjects during two drink conditions (sucrose and a non-nutrient sweetener) that both stimulate sweet taste receptors. Our findings suggest that although similar brain regions were engaged after consumption of both beverages, obese subjects had an increased response to the viewing of food images and to the ingestion of the sucrose drink, as well as differential satisfaction after beverage consumption.
Differences in brain responses between lean and obese women to a sweetened drink

Background:
Ingestion of sweet food is driven by central reward circuits and restrained by interoceptive satiety signals. Obesity is in part related to an upregulation of these reward mechanisms in association with a downregulation of vagally mediated satiety mechanisms. The specific influence of sucrose intake on central affective and reward circuitry, and differences in these influences in the obese population is incompletely understood.

Hypotheses:
1) Similar brain regions are engaged by the stimulation of sweet taste receptors by sucrose and by non-nutrient sweeteners. 2) During visual food related cues, obese subjects show greater brain responses specifically to sucrose compared to lean controls.

Methods:
In a two-day, double blind, crossover design, 10 obese and 10 lean healthy females received either a sucrose or a non-nutrient sweetened beverage prior to viewing agreeable food or neutral images. BOLD signal was measured using a 1.5 Tesla MRI scanner.

Results:
Viewing food images after ingestion of either the sucrose or the non-nutrient drink was associated with engagement of similar brain regions, including the amygdala, hippocampus, thalamus and dorsolateral prefrontal cortex, Obese differed from lean subjects in both behavioral
and brain responses: While obese subjects rated both beverages as less tasteful and satisfying, they showed greater brain responses. Obese subjects also showed engagement of an additional brain network (including anterior insular and anterior cingulate cortices, hippocampus, amygdala), and this was only seen after sucrose ingestion.

Conclusion:
Obese subjects had a reduced behavioral hedonic response yet a greater engagement of affective brain networks in response to food images, particularly after sucrose ingestion. These findings suggest that in obese subjects lingual and gut derived signaling generate less central hedonic effects than recalling memories of food experiences in response to visual cues, analogous to response patterns implicated in food addiction.

Keywords: obesity, fMRI, and artificial sweeteners
INTRODUCTION

In many parts of the industrialized world, obesity has reached epidemic proportions. In the United States, thirty four percent of the population is obese and an additional 34% is considered overweight, with minority women showing the highest percentage of affected individuals.\textsuperscript{1} Obesity-attributable medical expenditures related to the metabolic syndrome and other associated diseases reached $75 billion in the US in 2003.\textsuperscript{2} Even with the billion-dollar diet industry, long-term weight loss results with dieting have been disappointing. Despite extensive research into possible targets for drug development aimed at the obesity epidemic, only surgical interventions including gastric bypass surgeries have shown persistent benefits.\textsuperscript{3}

While tremendous progress has been made in the characterization of peripheral and hypothalamic mechanisms related to food intake and satiety in rodents\textsuperscript{4-9}, it has only been recently that affective, reward and cognitive processes related to ingestive behavior and obesity have been studied in human subjects.\textsuperscript{10,11} Based on these human studies, it has been suggested that overeating may be the result of an imbalance between vagally and endocrine mediated satiety mechanisms from the intestine on the one side, and central reward circuits, and prefrontal circuits involved in executive control of ingestive behavior on the other side. A similar shift between interoceptive, reward, and executive control mechanisms is seen in drug addiction where an altered interaction of these networks results in an enhanced reinforcing value of the drug and a distorted reward threshold.\textsuperscript{10,12}

A number of investigators have looked into the role of food related memories in obesity, by studying the impact of viewing food images on brain activation.\textsuperscript{13-15} Studies comparing lean and obese subjects have established differences in brain response to images of high calorie
versus low calorie foods.\textsuperscript{13, 14} It has also been demonstrated that brain activity changes in response to these food images after a subject consumes glucose versus water, and that this interaction between visual food cues and ingested nutrients differs in obese compared to lean groups.\textsuperscript{15} A limitation to all these studies is the fact that subjects can taste the difference between high and low calorie foods and between glucose and water ingestion; thereby confounding the results by the subject’s preconceived expectations based on what they are seeing, smelling and tasting. Non-caloric sweeteners stimulate the same sweet taste receptor as glucose and sucrose, thereby theoretically providing the same hedonic value without the calories\textsuperscript{16, 17}. For this reason, they are an ideal means to study the specific effect of sucrose absorption on brain activation in obese and lean subjects.

In the current study, we aimed to test the following main hypotheses: i) Brain responses to stimulation of sweet taste receptors by non-nutrient sweetener versus sucrose ingestion will be similar. ii) During visual food related cues, obese subjects will show greater affective and/or hedonic brain and behavioral responses to sucrose compared to lean controls. To test these hypotheses, we assessed brain responses using functional magnetic resonance imaging (fMRI) in lean and obese healthy female subjects during two drink conditions (a 300-calorie sucrose drink and an under 10-calorie non-nutrient sweetened beverage) that both stimulate sweet taste receptors.

\textbf{MATERIALS AND METHODS}

\textit{Participant Selection}
Twenty healthy lean and obese female participants were recruited from the clinical research unit of the UCLA Center for Neurobiology of Stress, from the UCLA Center for Human Nutrition, and from community advertisements. Subjects were between the ages of 18-40 years and were age-matched. The ten lean subjects had a body mass index (BMI) between 19-25 kg/m\(^2\) and the ten obese subjects had a BMI between 30-37 kg/m\(^2\). All subjects were right-handed, were regularly menstruating and were studied during the follicular phase (4-12 days after the first day of last menstrual period) given brain reward mechanisms vary with reproductive phase.\(^\text{18}\)

Exclusion criteria were as follows: i) a history of any gastrointestinal surgery, psychiatric and neurologic disorders, or head trauma with loss of consciousness; ii) a past or current history of an eating disorder; iii) a current history of chronic pain; iv) being pregnant or breast-feeding; v) tobacco use of more than 5 cigarettes per month; vi) a history of excessive exercise; vii) postmenopausal status; viii) use of any medications/drugs that affect the central nervous system, gastrointestinal motility, autonomic activity or pain sensation within 4 weeks of enrollment; ix) a history of serious psychiatric, neurologic, cardiovascular, respiratory, or renal illnesses.

UCLA Office of Protection of Research Subjects (OPRS) approved the study protocol. All subjects provided written informed consent before participation.

Psychosocial Evaluations

Because psychological processes may influence gastrointestinal sensory and motor function as well as brain responses,\(^\text{19}\) all subjects were evaluated using the MINI+5.0, the Hospital Anxiety and Depression scale (HAD), and the Spielberger State and Trait Anxiety Inventory (STAI). The MINI+5.0 is a brief structured interview for the major Axis I psychiatric
disorders in DSM-IV and ICD-10. The HAD scale is a measure of current anxiety and depression symptoms validated for non-psychiatric samples. The STAI is a 40-item self-report assessment that differentiates between state anxiety and trait anxiety.²⁰

**Appetite Assessments**

Taste, hunger, satiation, and satisfaction were assessed with two visual analog scales (VAS). The 10-point VAS for appetite examines taste and desire for specific food and has been shown to be reproducible and not influenced by prior diet standardization.²¹ The 10-point Fullness Questionnaire (FQ) is used to measure hunger and satisfaction and has been shown to correlate positively with the weight and caloric composition of foods, and negatively with palatability.²²

**Functional MRI Paradigm**

This study was part of a larger study that used both resting state images and task functional MRI to evaluate differences in obese and lean women after a sucrose drink versus a non-nutrient sweetened beverage. In this paper we are reporting on the evoked brain responses to visual food cues after nutrient ingestion in obese and lean subjects.

A double-blind randomized crossover design was utilized over two separate days of functional MRI (fMRI) scanning, no less than 2 days apart. Subjects fasted 6 hours prior to scan appointments, which occurred between 9:00 am and 11:00 am. The two scanning days were identical except the drink order was counterbalanced. At the start of each scan day, the subjects were given a synopsis of the study tests and placed in the scanner for a brief structural scan followed by two additional scans: a resting scan and a functional food images scan. The
objective of the resting scan was to determine whether or not the networks that are activated during resting state of the brain differ between lean and obese. This was a separate aim from the current study and the results will be reported elsewhere.

The 15-minute functional food images scan had three 5 min functional RUNS (RUNS 1–3). During each RUN, 36 images (18 food, 18 neutral images of brick walls) were presented in random order. Subjects were instructed to focus their attention on the stimuli. Three images were shown for 4 s each followed by a 12 s dark screen with a central fixation cross. A total of 108 images were displayed (54 food, 54 scenery).

Ten minutes before both the resting scan and the functional food images scan, subjects consumed a 10 oz beverage consisting of either a non-nutrient sweetened beverage (Diet Ocean Spray Cranberry Juice with 10 tsp of Truvia; <10 calories) or a sucrose beverage (Ocean Spray Cranberry Juice with 10 tsp of sugar; 300 calories). The beverages were designed to be similar in taste and sweetness. Pilot testing in 5 healthy individuals confirmed that the drinks could not be differentiated on the basis of taste. Subjects consumed both beverages on each scan day but the drink order was counterbalanced. Randomization was performed using Excel random number generator function. Subjects and investigators were blinded to the drink order. The beverage was presented in a non-descriptive container. Nose clips were applied to minimize olfactory influences and subjects were instructed to drink through a straw.

Subjects completed four FQs: a baseline FQ, a second FQ 10-minutes after the first beverage, a third FQ immediately prior to the second beverage, and a fourth FQ ten-minutes after the second beverage. A VAS for appetite questionnaires was completed ten-minutes after each beverage.
**fMRI Acquisition and Preprocessing**

MRI scanning was performed using a 1.5T MRI scanner (Siemens Sonata; Siemens, Erlangen, Germany). A high resolution structural image was acquired from each subject with a magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, repetition time (TR) = 2200 ms, echo time (TE) = 4.38 ms, slice thickness = 1 mm, 176 slices, 256 x 256 voxel matrices, and 1\(^3\) mm voxel size.

Food-related image stimuli were presented using E-Prime 2.0 Professional through MR compatible goggles. Functional blood oxygen-level dependent (BOLD) images were acquired with an echo-planar T2*-weighted imaging (EPI) sequence, TR = 2000 ms, TE = 45 ms, flip angle = 77°, slice thickness = 5 mm, 220 x 220 voxel matrices, and 3.4 x 3.4 x 5 mm voxel size.

Using SPM5 software (Welcome Department of Cognitive Neurology, London, UK), data were slice-time and motion corrected, spatially normalized to the MNI template using the structural images, and spatially smoothed at both 3 mm\(^3\) and 8 mm\(^3\) Gaussian kernel. The first two volumes were discarded to allow for stabilization of the magnetic field.

**Data Analysis**

**Behavioral Analyses.** Behavioral analyses were performed in PASW v17.0 (Chicago, IL). Group differences in STAI and HAD anxiety and depression ratings were evaluated by independent samples t-tests. Taste ratings were evaluated in a Group (lean; obese) x Condition (sucrose; non-nutrient sweetener) analyses of variance (ANOVA). Appetite ratings for satisfaction and hunger were evaluated in Group (lean; obese) x Condition (sucrose; non-nutrient sweetener) x Time (before ingestion; after ingestion) repeated measure ANOVAs. All data are given as mean ± standard error.
fMRI Analyses. Two complementary analyses were performed to test our hypotheses: general linear model (GLM) and partial least squares (PLS) analyses. GLM is the standard, univariate approach that assesses each voxel or region for differences in activity while PLS is a multivariate analysis that assesses differences in network activity. The two methods complement each other as one is more focused on individual regions while the other is more holistic and focused on large networks within the brain.

General Linear Model (GLM) Analyses. A GLM was applied in SPM5 to analyze the fMRI time series of the data smoothed at 8mm³. The flexible factorial model option was utilized to calculate a random effects general linear model specifying subjects, groups (lean vs. obese), and conditions (sucrose, non-nutrient sweetener, food images, and neutral images) as main effects along with the interaction of group and condition. We also examined the effect of order and there was no order effect so it was not included in the final model. Conjunction analyses were performed. Region of interest (ROI) analyses were conducted by applying a small volume correction (SVC) for the ROIs and significance was defined at a probability value less than 0.05 corrected using the family-wise error (FWE) algorithm. Anatomically based ROIs were selected a priori based on areas known to be involved in both hedonic and homeostatic networks (amygdala, hippocampus, hypothalamus, anterior and posterior cingulate cortices, anterior and posterior insula [INS]). ROIs were created using the Wake Forest University PickAtlas toolbox in SPM5 and were applied to the contrasts maps. Only the significant values from ROI analyses are reported in the paper; all ROI results will be reported in a supplemental table.

Multiple regression and the region of interest described above were used to determine group (lean non-nutrient sweetener, lean sucrose, obese non-nutrient sweetener, obese sucrose) differences in the association between hunger scores and brain activity during the viewing of
food images and viewing neutral images. Hunger scores were collected after the consumption of the second drink.

*Partial Least Squares (PLS) Analyses.* PLS is a multivariate statistical technique considered to be more sensitive than standard univariate analyses of neuroimaging data such as SPM.\textsuperscript{23, 24} PLS is analogous to principal components analysis (PCA), but the solutions can be restricted to the part of the covariance structure that is attributable to conditions or groups in an experimental design. A task PLS analysis was employed to identify distributed patterns of regions associated with viewing pleasant images of food in lean and obese women. Task PLS will identify experimental contrasts accounting for the maximum amount of variance in the data and the brain regions whose activity relates, as a whole, to these contrasts. In addition, a non-rotated PLS analysis was employed to examine group by condition interactions. The difference between a non-rotated and a task PLS analysis is that a priori contrasts of interest are used in the non-rotated but not the task PLS. Contrasts representing group differences in response to food images in the high and low calorie conditions were entered into the analysis. PLS was implemented with freely available code ([http://www.rotman-baycrest.on.ca](http://www.rotman-baycrest.on.ca)) and performed on data spatially smoothed at 3mm\(^3\). Voxel reliability was determined using bootstrap estimation (500 samples). The ratio of the observed weight to the bootstrap standard error was calculated and voxels were considered reliable if the absolute value of the bootstrap ratio (BSR) exceeded 2.81 and clusters greater than 20 voxels are reported.

**RESULTS**

*Patient Population*
**Clinical Variables.** A total of 22 subjects were enrolled in the study (Table 1). The first two subjects were excluded from the GLM and PLS analyses due to suboptimal quality of our initial food and neutral images. Therefore, 20 subjects were used for the GLM and PLS analyses. The mean BMI of the lean group was 22.4 kg/m$^2$ (SE 0.5), and 32.9 kg/m$^2$ (SE 0.7) for the obese group (p<0.05). All obese and lean subjects’ had similar STAI and HAD depression and anxiety ratings that were within the normal range. No subject had current depression or anxiety based on the MINI+ interview.

**Appetite Measures.** There was no difference between the sucrose and the non-nutrient sweetened drinks, in how their taste was rated by the two groups (Fig 1). Beverage consumption significantly reduced hunger ratings (p=0.005), increased satisfaction ratings (p<0.001) and reduced desire for sweetness (p<0.001) across all subjects, without any differences between the two drinks. However, obese subjects rated the taste of both beverages significantly lower than lean subjects (p=0.005), and obese women reported less satisfaction compared to lean subjects after both drinks, consistent with reduced subjective hedonic responses in the obese (p=0.016) (Table 2).

**Correlation of subjective sensations with brain responses**

In both obese and lean subjects, a greater subjective hunger score on the FQ correlated with a greater engagement of the left posterior INS using ROI analyses (z= 3.70; p=0.045) when viewing food images after beverage consumption. Compared to lean subjects, obese subjects had a trend for greater correlation of the right anterior INS with a subjective feeling of hunger (z = 3.29; p= 0.074). These findings confirm a central role of the insula in the perception of interoceptive feelings related to food intake.
**Brain responses related to test drinks in both lean and obese**

ROI analyses indicate that similar brain regions were engaged after ingestion of the two test drinks in both groups, when simultaneously viewing food images. After both drinks, there was significant engagement of bilateral amygdala, bilateral hippocampus, and bilateral thalamus, substantia nigra, right dorsolateral prefrontal cortex, and a trend towards engagement of the right anterior INS, consistent with engagement of a network of brain regions related to affect, memory, interoception and executive control (Table 3).

**Brain responses to food cues and differences between obese and lean subjects**

Several analysis approaches were used to characterize the brain’s responses to visual food cues, and to identify differences between lean and obese subjects.

We first looked at the brain response to viewing images of food (without contrasting them to neutral images). After the high-calorie sucrose beverage, the obese subjects had greater engagement of the right anterior INS when compared to the lean subjects (z=3.82; p=0.016). In contrast, there were no differences between obese and lean subjects when viewing food images after drinking the non-nutrient sweetener beverage.

In order to identify a network of interactive regions that were engaged differently with the task of viewing food versus neutral images, we first combined responses obtained during both test meals, and used a multivariate analysis approach (PLS). In both subject groups, the viewing of food images was associated with activation of a network that included the left INS (35 -12 20, boot strap ratio [BS] ratio= 4.02, p<.001), bilateral pregenual cingulate (x=12, y=46, z=2, BS ratio= 3.91, p<.001; x=-2, y=48, z =8, BS ratio= 3.87, p<.001), bilateral amygdala
(x=28, y=-2, z=-14, BS ratio= -5.05, p<.001; x=-22, y=-4, z=-14, BS ratio= -3.70, p<.001), and left hippocampus (x=-30, y= -18, z= -12, BS ratio= -3.4, p<.001). This network accounted for 41% of the crossblock variance (p<0.001), and was engaged to a greater extent in the obese compared to lean subjects (Fig. 2).

Using a multivariate analysis approach, we found an additional network that was more engaged by obese subjects only after the high calorie sucrose beverage compared to all other group/conditions. This network included bilateral anterior INS (44 10 -6, BS ratio=6.51, p<.001; -40 8 2, BS ratio= 5.63, p<.001), right ACC (10 28 32, BS ratio= 5.31, p<.001), the right lateral amygdala (26 -4 -18, BS ratio= 3.72, p<.001), the right hippocampus (38 -20 -16, BS ratio= 4.88, p<.001), and the visual cortex (30 -80 24, BS ratio= 10.56; -34 -88 0, BS ratio= 7.26, p<.001). This network accounted for 27% of the crossblock variance (p=0.028) (Fig. 3).

**DISCUSSION**

The main findings of the study are: 1) Similar brain regions were engaged after ingestion of either sucrose or a non-nutrient sweetener while viewing hedonic food images. 2) In both groups, regardless of drink type, the viewing of pleasant food images was associated with brain networks involving interoceptive, affective and cognitive brain regions. 3) While all subjects rated the taste and the satisfaction of the two drinks similarly, obese women rated both drinks as less tasteful and satisfying than lean women, consistent with a reduced hedonic response to the drinks. 4) This reduced behavioral hedonic response in the obese was associated with greater responses in affective and memory related brain regions to both viewing of food images, and to the ingestion of the sucrose drink. These results suggest that in obese subjects, gut derived
sucrose related signaling generates less hedonic effects than recalling memories of pleasant food in response to visual cues. As the latter response appears to be reinforced by sucrose ingestion, it may be a mechanism whereby sucrose ingestion perpetuates the craving for more sweets.

**Similarities in brain responses to sucrose and non-nutrient sweetener**

In the combined sample, similar brain regions were engaged after ingestion of the two test drinks while viewing hedonic food images. The regions included the bilateral amygdala, bilateral hippocampus, bilateral thalamus, substantia nigra, right dorsolateral prefrontal cortex and a trend towards engagement of the right anterior INS. These findings are consistent with the multidimensional encoding of interoceptive, affective, memory related and cognitive aspects of the experience of a sweet test meal, as previously reported. Non-nutrient sweeteners bind to lingual taste receptors with equal if not greater affinity as sucrose. The similarity of the brain response between the two drinks suggests that the interoceptive input to the brain results primarily from lingual and possibly intestinal sweet taste receptor activation, and does not require other encoding mechanisms, which require glucose absorption and interaction with glucose sensing mechanisms in the portal vein, the pancreas and the brain (hypothalamus, NTS).

In a previous study by Frank et al comparing brain responses to lingual application of sucrose and sucralose, results indicated that both sucrose and sucralose activate functionally connected primary taste pathways and related brain regions. However, in their study, sucrose tasting led to greater engagement of the anterior INS, striatum, ACC and prefrontal cortex compared to sucralose. There are several possible explanations for why we did not find this difference. Frank et al’s study had a smaller sample size and the sweeteners were only tasted...
orally, limiting the stimuli to lingual and intestinal sweet taste receptors. In addition, subjects in Frank et al’s study did not view food images, thereby limiting the overall hedonic food-related experience.

Differences in subjective ratings of the test meal by obese and lean subjects

Despite the difference in caloric content between the two drinks, we found that subjects rated the taste of the sucrose and the non-nutrient sweetened beverage similarly, suggesting similar activation of lingual sweet taste receptors by the two drinks. Sweet taste perception of both non-nutrient sweeteners and sucrose is peripherally mediated by tongue heteromic T1R2/T1R3 sweet taste receptors. The sensory information is then transmitted by cranial nerves VII, IX and X to the nucleus tract solitarius (NTS), and to the human gustatory cortex within the anterior INS. Previous studies have shown artificial sweeteners bind to these taste receptors with equal if not greater affinity compared to sucrose. 16, 17, 28

Obese subjects rated the taste of both beverages lower than lean subjects, and reported less satisfaction after consuming the beverages compared to lean subjects, consistent with a reduction in the hedonic aspect associated with ingestion of a sweet drink. The subjective perception of sweet taste is a multidimensional experience and reflects the modulation of anterior INS activity by inputs from interoceptive, affective, reward, and prefrontal/orbitofrontal inputs. 21 Taste perception includes the assessment of taste quality, hedonic “liking” and the incentive motivational component “wanting”. 27, 29 Reduced satisfaction to actual food ingestion (“liking”) despite greater engagement of hedonic circuits during expectation of food intake (“wanting”), has been proposed as a mechanism underlying food addiction. 30 This pattern is similar to drug
addiction where craving for the drug is amplified, while actual satisfaction after drug use is reduced. In our study, the subjects were shown palatable images of food after beverage consumption and in between behavioral measure assessment. Altogether, these findings imply that in obese subjects, gut derived signaling generates less hedonic effects than recalling memories of food in response to visual cues.

**Recall of food-related memories by visual stimuli and interaction with interoceptive stimuli**

It has long been known that amnesic patients readily eat a second meal offered immediately after a full meal suggesting that memory recall of food related experiences are as equally significant as caloric need in the decision to eat. Representations of food-related experiences are generated in networks involving the prefrontal and orbitofrontal cortices, anterior INS, amygdale, hippocampus and reward pathways. Consistent with previous reports, we found that in both lean and obese subjects, regardless of drink type, the viewing of pleasant food images was associated with activation of interoceptive, affective and memory related brain regions. Similarly, obese and lean subjects showed the expected positive correlation between the subjective feeling of hunger with the engagement of insular cortex when viewing food images postprandially.

In order to maintain homeostasis, the interoceptive input from the gut at some point must override the memory recall attenuating the motivation to eat. This hypothesis is supported by a report by Veit et al who demonstrated that oral glucose but not water ingestion decreased the BOLD response in the hypothalamus when subjects were shown images of high calorie foods. This suggests that glucose, presumably acting via glucose sensors in the intestine, portal vein or centrally, modifies hypothalamic signaling to high calorie food stimuli to maintain caloric
equilibrium. Interestingly, we did not see a difference in BOLD response to the two drinks in the hypothalamus in either group or condition comparison. One possible explanation is that our sample size limited our ability to detect a difference in this brain region. Alternatively, the homeostatic network might be linked to sweet taste receptor activation (as opposed to caloric consumption), in which case one would not expect to see a difference in homeostatic response to these two beverages, both of which activate the sweet taste receptors.\textsuperscript{16, 17, 28}

**Obese subjects have an exaggerated response to food images after a sweetened drink**

While similar brain regions were engaged with both the sucrose and the non-nutrient sweetened beverage in the combined lean and obese groups, there were differences in brain responses when the obese women were compared to the lean group. When obese subjects viewed food images after the sucrose beverage (but not the non-nutrient sweetened drink), a network including the anterior INS, ACC, right lateral amygdala, right hippocampus, and the visual cortex was more engaged compared to lean subjects. Similarly, Rothemund et al\textsuperscript{36} found that high-calorie food images yielded BMI-dependent activations in regions associated with taste information processing (anterior INS and lateral OFC), motivation (OFC), and emotion and memory functions (posterior cingulate). These findings are consistent with the concept that viewing food images produces a brain response involving recall of previous food experiences, and that this response is exaggerated in obese subjects.

While both obese and lean subjects showed the expected positive correlation between the subjective feeling of hunger with the engagement of insular cortex when viewing food images postprandially,\textsuperscript{33, 34} obese subjects had a greater correlation of the right anterior INS with the subjective feeling of hunger. This suggests a greater modulation in the obese subjects of this
brain region by emotional and reward pathways when craving for food. Anterior INS activation has also been demonstrated in association with the conscious feeling of urge in drug addiction.\textsuperscript{12}

Our findings suggest that in obesity, additional interoceptive inputs other than that generated by lingual/intestinal sweet taste receptor activation plays a role in the associated brain response to a sweetened beverage in the context of viewing food cues. In addition to sweet taste receptor activation on intestinal enteroendocrine cells, glucose-sensing mechanisms have been described in the pancreas, portal vein, hypothalamus and NTS\textsuperscript{25,26,37-39}. Likewise, group differences between lean and obese subjects in peripheral glucose, insulin, or incretin levels after sucrose intake may play a role.

As an alternative to peripheral differences in glucose sensing, central differences between obese and lean populations might also explain our findings. In the drug addiction model, as addiction increases, stimuli within the environment that are associated with drug use (cigarettes, bottles of alcohol, drug paraphernalia) become powerful reinforcing incentives to drive ongoing drug use. This suggests the interoceptive cortex has a central role in conscious cue-induced urges by encoding a representation of the salient effects of drug use that become activated when an addicted person is exposed to drug cues. It is believed that the amygdala and hippocampus are also involved in conditioning to addictive substances and relative cues in addiction.\textsuperscript{40}. Similarly, in obesity an addiction to sucrose might prompt increased engagement of this salience network when viewing images of palatable food after ingesting sucrose.

**Limitations**

A potential limitation to our study is the fact that the volumes of the food were the same for obese and lean, possibly contributing to the finding that obese subjects were less satiated –
obese women might require a larger volume to feel full. However, the absence of a statistical
differences in hunger ratings pre and post beverage consumption, suggest that volume did not
play a significant role in the satiety measures. Similarly, group differences in gastric emptying
may have contributed to the observed differences. The findings in rodent studies\textsuperscript{41} that no
difference in gastric emptying after infusion of sucrose or an artificially sweetener is observed,
argues against a group difference.

**Summary and possible clinical implications**

In summary, we found several obesity-related differences in behavioral and brain
responses to sucrose versus a non-nutrient sweetened beverage. Obese women verbally reported
a reduced hedonic behavioral response to either sweetened beverage, yet they demonstrated a
greater hedonic brain response particularly after sucrose ingestion. This increased brain response
is driven more by recalling memories of food experiences in response to visual cues than by
lingual and gut derived signaling. Despite the extensive literature on obesity related changes in
peripheral satiety mechanisms,\textsuperscript{6,7} these findings are most consistent with a difference in central
modulation of ingestive behavior in the obese, and are suggestive of food addiction.

Artificial sweeteners were developed with the goal of reducing caloric intake without
sacrificing sweet taste. Ideally reducing caloric intake should promote weight loss. It is unclear,
however, if this is the case. At first glance, the similar taste perception and brain activation with
both drinks would suggest that artificial sweeteners are an ideal substitute for sugar. However,
the smoking cessation literature suggests that tobacco substitutes such as nicotine-free inhalers,
still play a peripheral and central role in the ritual of smoking and are not always effective at
reducing cravings.\textsuperscript{42,43} Similarly, it might be that in at risk populations, these artificial
sweeteners might promote the same salience and addictive pathways as sugar, eventually leading to even more caloric ingestion.
**Figure 1.** Comparing taste ratings of sucrose and non-nutrient sweetened beverages between obese and lean subjects (*p*>0.05). Subjective taste ratings were quantified using a 10-point VAS, from “bad = 0” to “good = 10”.

**Figure 2.** Obese show greater engagement of brain network including posterior INS (left panel) and bilateral amygdala (right panel) to viewing food images, using a multivariate fMRI analysis approach, as described in Methods. A blue circle marks respective brain regions.
Figure 3. Obese subjects had greater engagement of a network including the anterior INS (blue circle) following the high calorie sucrose beverage, using multivariate fMRI analysis approach, as described in Methods. (p<.001)

<table>
<thead>
<tr>
<th></th>
<th>Lean Mean (SE) n=10</th>
<th>Obese Mean (SE) n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24.91 (1.24)</td>
<td>27.64 (1.87)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.39 (0.45)</td>
<td>32.82 (0.68)</td>
</tr>
<tr>
<td>HAD Depression</td>
<td>1.09 (0.37)</td>
<td>2.36 (0.43)</td>
</tr>
<tr>
<td>HAD Anxiety</td>
<td>5.45 (1.15)</td>
<td>4.76 (0.98)</td>
</tr>
<tr>
<td>STAI</td>
<td>48.45 (3.31)</td>
<td>47.1 (2.56)</td>
</tr>
</tbody>
</table>

Table 1. Demographics and Baseline Characteristics
Table 2. Taste and appetite ratings at baseline and after the second beverage consumption.

Subjective ratings were quantified using a 10-point VAS, as described in the methods.

<table>
<thead>
<tr>
<th>Taste</th>
<th>Baseline</th>
<th>After 2nd Drink</th>
<th>Baseline</th>
<th>After 2nd Drink</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.18(.39)</td>
<td>7.73(.50)</td>
<td>6.86(.61)</td>
<td>5.73(.71)*</td>
</tr>
<tr>
<td>Hunger:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.40(.61)</td>
<td>5.35(.85)</td>
<td>5.8(.49)</td>
<td>5.50(.44)</td>
</tr>
<tr>
<td>After 2nd Drink</td>
<td>3.28(.98)</td>
<td>3.55(.76)</td>
<td>4.75(.60)</td>
<td>4.20(.61)</td>
</tr>
<tr>
<td>Satisfaction:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.50(.40)</td>
<td>1.60(.31)</td>
<td>2.30(.55)</td>
<td>2.20(.52)</td>
</tr>
<tr>
<td>After 2nd Drink</td>
<td>4.44(.85)</td>
<td>4.80(.84)</td>
<td>3.70(.44)</td>
<td>3.95(.48)</td>
</tr>
<tr>
<td>Desire for Sweetness:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.85(.71)</td>
<td>3.45(1.02)</td>
<td>5.35(.65)</td>
<td>4.40(.73)</td>
</tr>
<tr>
<td>After 2nd Drink</td>
<td>6.94(.85)</td>
<td>7.95(.50)</td>
<td>7.25(.59)</td>
<td>7.10(.53)</td>
</tr>
</tbody>
</table>

*p<0.05 between group comparisons

Table 3. Similar brain regions were engaged when looking at images of food after ingestion of both sucrose and the non-nutrient sweetener beverages in lean and obese subjects, using ROI conjunction analysis, as described in Methods.
References

5. de Lartigue G, de La Serre CB, Raybould HE. Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. Physiol Behav 2011;105:100-5.
42. Darredeau C, Barrett SP. The role of nicotine content information in smokers' subjective responses to nicotine and placebo inhalers. Hum Psychopharmacol 2010;25:577-81.
Chapter 3: Appendix

This study was a double-blind, placebo-controlled, randomized clinical trial, aimed to test the following two hypotheses: (1) Brain responses to stimulation of sweet taste receptors by non-nutrient sweetener versus sucrose ingestion will be similar. (2) During visual food related cues, obese subjects will show greater affective and/or hedonic brain and behavioral responses to sucrose compared to lean controls.

Compared with behavioral measures, which show high inter-individual variation, the reliability of functional brain measures is generally much higher, due to the fact that hundreds of repeated measures of brain function can be obtained within a given subject for a given condition. Simulation studies have been conducted to assist investigators with the estimation of power and determination of sample sizes for within-group fMRI region-of-interest analysis based upon expected percent signal change, and estimates of intra- and inter-subject variability in the signal change\(^1\). Results indicate that a minimum of 10 subjects are required to achieve adequate statistical power (80\%) to detect a 75\% signal change with spatial smoothing at FWHM of 5mm in an activation analysis at an alpha=0.002 and the number of subjects increases to 25 after correcting for multiple comparisons, e.g., alpha = .000002. For a more conservative signal change of 50\% with a similar smoothing kernel, 12 subjects are required to achieve adequate statistical power (80\%) to detect a 50\% signal change with spatial smoothing at FWHM of 5mm in an activation analysis. Based on the simulation studies described and the between-group ROI analysis (p<=0.05 (one-tailed), uncorrected for multiple tests), a minimum of 10 subjects are required to adequately power the between-group comparisons for each experiment. We conducted a fMRI pilot and feasibility study, and therefore recruited 20 subjects to meet the study objectives (10 subjects in each group).
Behavioral Analyses

Behavioral analyses were performed in PASW v17.0 (Chicago, IL). Group differences in HAD anxiety and depression ratings were evaluated by independent samples t-tests. An independent samples Student t-test was performed instead of a paired student t-test because for the behavioral data there were no repeated measures; the anxiety and depression ratings were evaluated at one time at the start of the study.

Taste ratings were evaluated with analyses of variance (ANOVA). The two groups were the lean and obese subjects and the two conditions were sucrose ingestion and the non-nutrient sweetener ingestion. ANOVA was performed instead of multiple two-sample student t-tests in order to decrease the chance of having a type I error. A fixed-effects model was used because the treatment (type of beverage) was set.

Appetite ratings for satisfaction and hunger were evaluated with repeated measure ANOVAs. The two groups were the lean and obese subjects and the two conditions were sucrose ingestion and the non-nutrient sweetener ingestion. A repeated measure ANOVA was performed because appetite ratings were measured both before beverage consumption and after beverage consumption. Again a fixed-effects model was used.

General Linear Model (GLM) Analyses

Statistical parametric maps are obtained using whole brain image subtraction routines utilizing spatially registered functional images warped to stereotactic coordinates using the T1 to define the transformation. To minimize the severity of the correction for multiple tests inherent in the whole-brain approach (where every voxel of the brain is analysed), the primary analysis
was a hypothesis driven region of interest (ROI) analysis. Anatomically based ROIs were selected a priori based on areas known to be involved in both hedonic and homeostatic networks (amygdala, hippocampus, hypothalamus, anterior and posterior cingulate cortices, anterior and posterior insula [INS]). ROIs were created using the Wake Forest University PickAtlas toolbox in SPM5 and were applied to the contrasts maps.

A GLM was applied in SPM5 to analyze the fMRI time series of the data. A flexible factorial model was specified with groups (lean vs. obese), conditions (sucrose, non-nutrient sweetener, food images, and neutral images) and their interaction as factors. Conjunction analyses were performed when indicated because of concern for overestimation of variances due to small samples (random-effects). Region of interest (ROI) analyses were conducted by applying a small volume correction (SVC) for the ROIs and significance was defined at a probability value less than 0.05 corrected using the family-wise error (FWE) algorithm. SPM allows for two corrective options: FWE and the false discovery rate (FDR). FWE was used as it is the most stringent correction.

The ROI conjunction analysis uses the flexible factorial design in SPM5. SPM5 flexible factorial design allows for no more than three factors when building a matrix. Our three factors were subject, group (lean versus obese), and task (non-nutrient sweetener viewing food images, non-nutrient sweetener viewing neutral images, sucrose viewing food images, and sucrose viewing neutral images). “Independence” and “Variance” are configured for each factor separately. Independence refers to whether the errors are independent between the different factor levels, while variance refers to whether the error variances are equal or unequal between the factor levels. For subject independence was set as yes and variance was set as equal (give the between-subjects effect). For group, independence was set as yes and variance set as unequal
(given the between-group effect). For task independence was set as no and variance set as equal. In the subject section, the input images for each subject were specified along with the factor matrix that maps the images onto the different factor levels. Finally, we looked at both main effects and interactions. We added a covariate for order given this was a crossover design study (with a washout) and this was not significant so it was not included in the final model.

In neuroimaging, a conjunction analysis looks for brain areas activated by task A and by task B, or a conjunction of tasks\(^3\). A positive conjunction test implies that the region is commonly activated across the tasks. In our study we looked at areas of the brain activated by both ingestion of the sucrose beverage and the non-nutrient sweetened beverage. The conjunction null hypothesis is the state of no conjunction effects: (not M\(_A\)) OR (not M\(_B\)); whereas the conjunction hypothesis is M\(_A\) AND M\(_B\). We used the contrasts created from the flexible factorial model to test this conjunction null hypothesis.

Hunger covariate analyses were run using a GLM covariate model in SPM5. Hunger scores were collected after the consumption of the second drink and compared to four groups based on weight (lean vs. obese) and caloric intake (non-nutrient sweetener vs. sucrose). Models were run to compare the impact of the hunger scores of these four groups in the two image conditions (food vs. neutral). The same ROIs were run for each of the models as in the flexible factorial analysis.

**Multivariate analysis. Partial Least Squares (PLS) analysis**

PLS is a multivariate statistical technique considered to be more sensitive than standard univariate analyses of neuroimaging data such as SPM.\(^4,\,5\) PLS is analogous to principal components analysis (PCA), but the solutions can be restricted to the part of the covariance
structure that is attributable to conditions or groups in an experimental design.\textsuperscript{6} As used in neuroimaging, partial least squares can be used to describe the relation between a set of measures (such as design contrasts, behavioral scores, or seed activity) and a set of functional brain images. PLS refers to two related methods: (1) symmetric PLS or Partial Least Squares Correlation (PLSC), and (2) asymmetric PLS or Partial Least Squares Regression (PLSR).\textsuperscript{7} While PLSR is used to predict behavior from brain activity, PLSC is used to analyze associates between behavior and brain activity.\textsuperscript{7} PLSC is the more popular version of PLS for neuroimaging and is what we used in our analysis.

There are three main types of PLSC used in neuroimaging based on the nature of the set of measures used in the analysis.\textsuperscript{6} Task-PLS is an analysis of brain activity related to elements of the experimental design (expressed by design contrasts) and identifies patterns of brain areas related to changing task demands and/or group differences. Behavioral-PLS is an analysis of brain-behavior correlation patterns that are either common among tasks/groups or task/group-dependent. Seed-PLS is an analysis of the functional connectivity of a theoretically important brain region (the seed). Seed-PLS can identify functional connectivity patterns that are either common among tasks/groups or task/group-dependent.

In this study, a task PLS analysis was employed to identify distributed patterns of regions associated with viewing pleasant images of food in lean and obese women. Task PLS will identify experimental contrasts accounting for the maximum amount of variance in the data and the brain regions whose activity relates, as a whole, to these contrasts. In addition, a non-rotated PLS analysis was employed to examine group*condition interactions. The difference between a non-rotated and a task PLS analysis is that a priori contrasts of interest are used in the non-
rotated but not the task PLS. Contrasts representing group differences in response to food images in the high and low calorie conditions were entered into the analysis.

PLS was implemented with freely available code (http://www.rotman-baycrest.on.ca). As with other multivariate approaches, PLS requires the data to be in matrix form so that the entire data structure can be analyzed at once. PLS analyzes the relationship between the matrices $X$ (brain activity matrix) and $Y$ (behavior/design matrix). The columns of $X$ and $Y$ are stored in a cross-product matrix, denoted $R$, which is computed as $R=Y^TX$. The relationship between the $j$th column of $X$ and the $k$th column of $Y$ is measured by the scalar (dot) product between these two columns. The dot product gives the covariance between these two columns when they are centered. When these two columns are normalized (e.g. expressed as Z scores), the dot product expresses the correlation between these two columns. Covariance and correlation are not directional so the analysis focuses on shared information.

Voxel reliability was determined using bootstrap estimation (500 samples). The ratio of the observed weight to the bootstrap standard error was calculated and voxels were considered reliable if the absolute value of the bootstrap ratio (BSR) exceeded 2.81 and clusters greater than 20 voxels are reported.
Bibliography


42. Darredeau C, Barrett SP. The role of nicotine content information in smokers' subjective responses to nicotine and placebo inhalers. *Hum Psychopharmacol* 2010;25:577-81.
