

Neural Correlates of Obesity:
Disgust, Inflammation, and Brain Function

By

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CHAPTER I: INTRODUCTION

Overview

Over one billion adults have a body mass index (BMI) of 25-30 kg/m² (overweight) worldwide and more than 300 million have a BMI greater than 30 kg/m² (obese). (Flegal et al., 2010)

Within the last decade, the prevalence of obesity and obesity-related diseases has increased markedly within the US adult population. (Flegal et al., 2010) Simply stated, body weight is controlled by the homeostatic feeding system that tightly controls the balance between energy intake and energy expenditure. (Schwartz et al., 2000) When this homeostatic feeding system breaks down, the balance between energy intake and energy expenditure shifts toward energy intake. There are several factors known to contribute to excess weight gain and obesity. (1) Individuals struggling with obesity commonly consume excess calories by choosing calorie dense foods rich in saturated fats and sugar over healthier options. (Schwartz and Porte, 2005; García-García et al., 2012) (2) Even with a consistent diet, decreases in exercise or metabolic rate may lead to an overweight or obese phenotype. (3) Individual variations in endocrine hormone levels may contribute to and further cement obesity. (Schwartz and Porte, 2005) (4) Psychiatric disorders such as depression and anxiety or altered emotions such as reduced disgust proneness are also associated with weight gain and obesity. (Houben and Havermans, 2012; Watkins et al., 2016)

Homeostatic feeding is coordinated by neuroendocrine feedback loops involving nutrient and hormonal signals indicating energy store levels to the hypothalamus. Robust and redundant

biological systems have developed to defend energy supply. (Berthoud et al., 2008) Non-homeostatic feeding can be initiated via complex neural networks, including the ventral striatum (reward and motivated behavior), dorsal striatum (habit learning), the prefrontal cortex (executive function and inhibition) and the insula (gustatory and disgust). (Berthoud et al., 2012). Given this central role of the brain in homeostatic and non-homeostatic feeding, it is likely that obesity is associated with changes in brain structure and function, as well as changes in eating habits and physical activity.

The obesity phenotype is relatively easy to create in the research setting. Researchers typically use the diet-induced obesity (DIO) model to study obesity and its comorbidities. In this model, researchers manipulate the food available to the organism studied (typically rodents, but sometimes dogs or non-human primates). Food is typically supplied *ad libitum* and consists of at least a 45% high fat diet. A diet of at least 45% fat can lead to obesity and its associated comorbidities, including chronic low-grade inflammation. (Xu et al., 2003b; De Souza et al., 2005b; Buettner et al., 2006) The DIO model allows researchers to examine the short-term effects of high fat diet before the onset of obesity and the effects of long-term obesity, including within the context of systemic inflammation and neuroinflammation.

Effects of Obesity

Hormonal

Any shift towards energy intake in excess of body energy needs leads to increased adipose tissue deposition, obesity, and increased risk for associated metabolic disorders including,

dyslipidemia, cardiovascular disease, stroke, insulin resistance, and type 2 diabetes. (Kopelman, 2000) There are three primary hormones that regulate energy intake and expenditure: insulin, leptin, and ghrelin. Insulin is synthesized and secreted by β cells in the pancreas and regulates metabolic function by acting in the liver, muscle, adipose tissue, and the brain. Insulin is rapidly released in response to increased blood glucose levels. Systemically, insulin facilitates glucose transfer into the cell, glycogen synthesis, and glycolysis. (Basu et al., 2004; Rivera et al., 2010) In the brain, elevated levels of circulating insulin augment counter-regulatory responses to hypoglycemia and alter feeding behavior by acting on anorexigenic insulin receptors distributed throughout the hypothalamus. (Rodin et al., 1985; Fruehwald-Schultes et al., 1999)

Ghrelin is a primary orexigenic hormone peptide secreted from the stomach, gastrointestinal tract, pancreatic α cells, adrenal cortex, and the hypothalamus. (Kojima et al., 1999; Korbonits et al., 2001; Nakazato et al., 2001; Date et al., 2002) Ghrelin secretion is largely dependent upon nutritional state, showing preprandial increases and postprandial decreases. (Ariyasu et al., 2001; Cummings et al., 2001; Morton et al., 2006)

Adipose tissue, once considered to be primarily involved in energy storage, is now understood to function also as an endocrine organ that secretes various bioactive substances, including leptin. (Ouchi et al., 2003; Berg and Scherer, 2005) Leptin synthesis and secretion by adipose tissue is dependent upon the total amount of adipose tissue. (Schwartz et al., 2000; Berg and Scherer, 2005; Zhou and Rui, 2013) Several rodent studies demonstrate that leptin functions as an anorexigenic feedback mechanism to inhibit food intake and regulate body weight by acting on leptin receptors in the hypothalamus. (Halaas et al., 1995; Pelleymounter et al., 1995; Schwartz

et al., 1996; Sahu, 2003) These three primary hormones (insulin, ghrelin, and leptin) influence systemic and hypothalamic energy regulation in a highly complex manner.

Systemic Inflammation

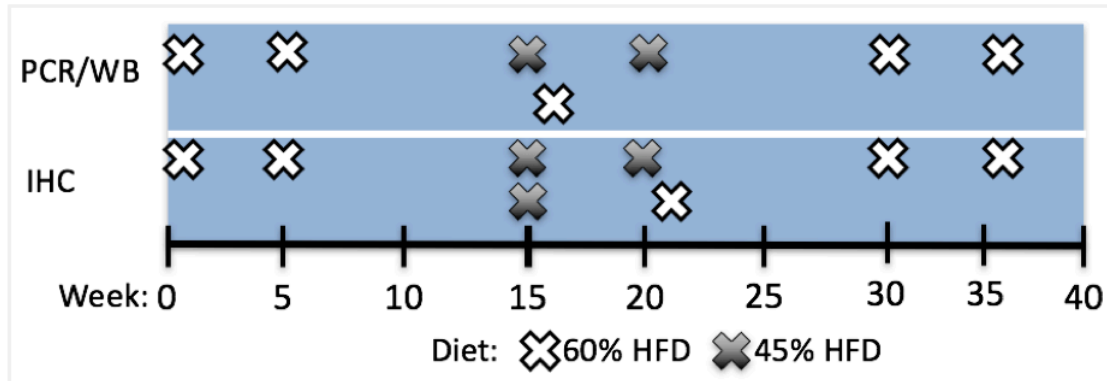
Accumulating evidence suggests that chronic inflammation plays a major role in the pathogenesis of obesity-related metabolic and neural dysfunction. (Cai, 2009; Cai and Liu, 2012; McNay et al., 2012) Beyond functioning as a long-term energy storage organ, adipose tissue plays a key role in the integration of systemic energy metabolism via secretion of various adipokines. (Berg and Scherer, 2005; Ouchi et al., 2011; Kwon and Pessin, 2013) The secretion of these adipokines is tightly controlled during normal body weight conditions, resulting in a balance of pro- and anti-inflammatory factors. (Ouchi et al., 2011) However, in rodents and humans, excess energy storage leads to an increase in pro-inflammatory adipokines (e.g. CRP, IL-6, IL-1 β , TNF α) and a decrease in anti-inflammatory adipokines (e.g. adiponectin, SFRP50) after short- and long-term periods of high fat diet intake. (Lam et al., 2005; Milanski et al., 2009; Velloso, 2009; Olefsky and Glass, 2010; Zhou and Rui, 2013) This imbalance leads to chronic low-grade neural inflammation, insulin and leptin resistance in the body and the brain, and increased recruitment of both microglia and astrocytes, specifically to the hypothalamus. (Kaiyala et al., 2000; Weisberg et al., 2003; Xu et al., 2003a; Kim et al., 2008; Mori et al., 2010) It has also been shown that the consumption of a high fat diet similarly affects the balance of pro- and anti-inflammatory factors, even before the development of an obese phenotype. (Chatterjee et al., 2009; Thaler et al., 2012) It remains unclear whether this shift towards a pro-inflammatory environment is caused by increased adipose tissue, a high fat diet, or both. There

is, however, a clear understanding of which signaling cascades lead to an increase in pro-inflammatory adipokine expression.

Brain Inflammation

There are a few primary signaling cascades that are consistently implicated in the neural inflammatory process. A number of studies have observed an increase in inflammatory markers in the brain as early as 3 days after high fat diet and up to 40 weeks of high fat diet and obesity. **(Figure 1)** A 20-week high fat diet feeding study found increase reactive oxygen species (ROS), increased prostaglandin E2 production, and upregulation in NF- κ B signaling in the rat cortex. (Zhang et al., 2005) In the hypothalamus, investigators reported increased activation of both Jnk and IKK β /NF- κ B pathways, as well as induction of endoplasmic reticulum [ER] stress. (De Souza et al., 2005a; Zhang et al., 2008; Milanski et al., 2009; Ozcan et al., 2009; Posey et al., 2009) Not only does activation of these pathways increase expression of pro-inflammatory adipokines, IL-1 β , TNF α , and IL-6, the increased activation of these pathways has a timecourse similar to that of the development of hypothalamic insulin resistance. (Münzberg et al., 2004; Fam et al., 2007) Targeting these signaling cascades, specifically IKK β /NF- κ B, may be an effective strategy to reduce the chronic, low-grade neural inflammation and the insulin/leptin resistance associated with high fat diet (HFD) and diet-induced obesity (DIO). Chronic, low-grade neural inflammation (inflammation not resulting from serious infection or injury) can lead to cellular-level structural changes within the brain.

Figure 1: Timeline of studies reporting increases in inflammatory markers and hypothalamic parenchymal change.



Note: PCR/WB represents inflammatory markers studies. IHC represents hypothalamic parenchymal changes studies. (PCR) Polymerase Chain Reaction (WB) Western Blot (IHC) Immunohistochemistry. Figure adapted from (Zhang et al., 2005; De Souza et al., 2005a; Zhang et al., 2008; Milanski et al., 2009; Ozcan et al., 2009; Posey et al., 2009; Thaler et al., 2012)

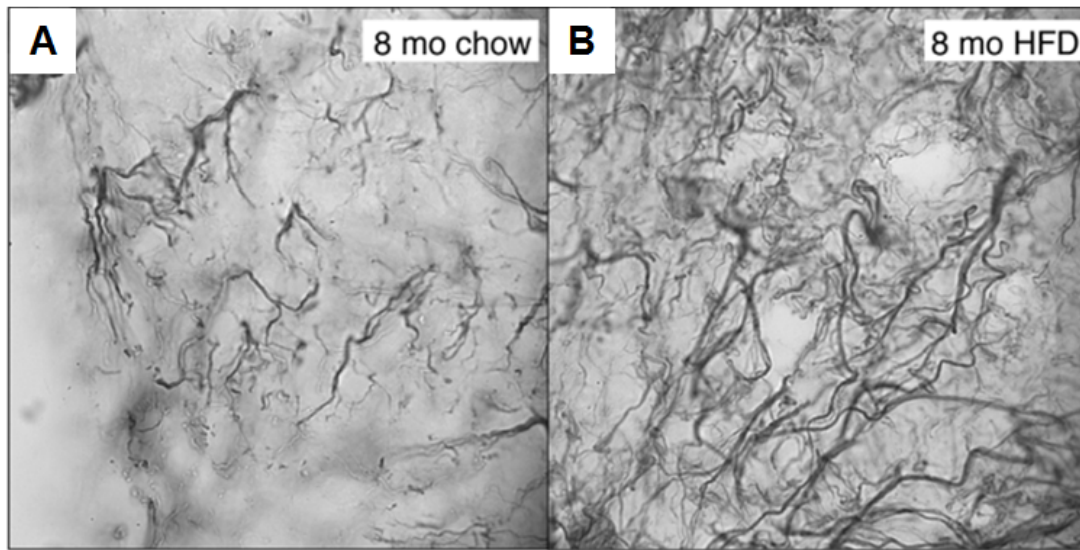
Gliosis

Brain function, ranging from cellular to systems level functioning, is largely dependent upon healthy parenchyma. Brain parenchyma is comprised of neurons, astrocytes, and microglia that operate via tightly controlled electrochemical mechanisms. Slight changes in parenchymal makeup lead to local changes in function and possibly major changes in neural systems, homeostatic functions, and cognition (Zhou et al., 2005; Esposito et al., 2008; Alafuzoff et al., 2009; Posey et al., 2009) It is well documented that chronic low-grade inflammation causes damage to brain parenchyma, or gliosis (as characterized by morphological changes to and excessive recruitment of glia, neuronal damage, and altered cell-circuit function). (Schwartz et al., 2000; Thaler and Schwartz, 2010; Cai and Liu, 2011)

Brain regions with gliosis have an overall increase in glia density and the glia within that region exhibit changes in size and shape. (Namavar et al., 2012) With gliosis, astrocyte projections

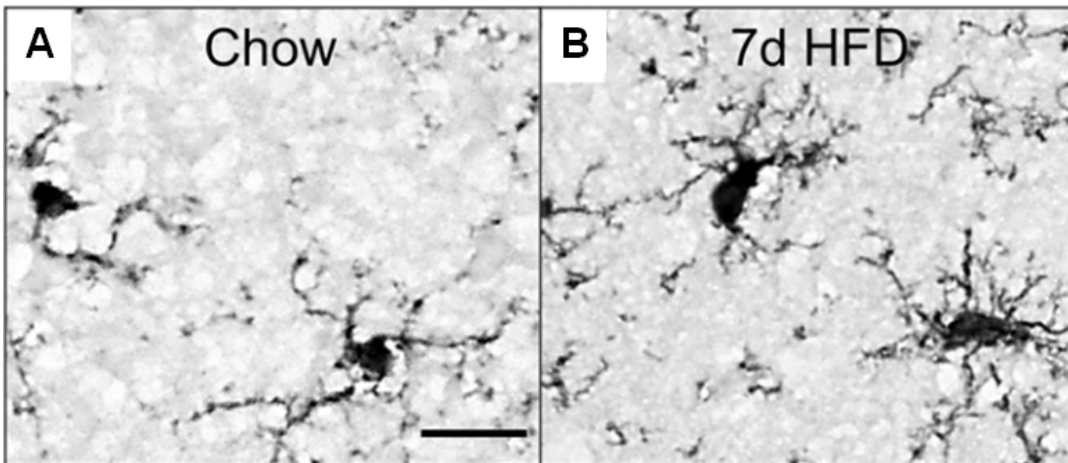
become asymmetrical and overlap or tangle with nearby astrocytes. **(Figure 2)** while microglia cell bodies become elongated and their projections become shorter and stockier, resembling an M1 macrophage. (Thaler et al., 2012) **(Figure 3)** Pro-opiomelanocortin neurons cell types associated with anorexigenic action are also affected by gliosis -- and they degenerate and are engulfed by surrounding microglia. (Thaler et al., 2012) Although the entire brain may be affected by neuroinflammation and gliosis, the hypothalamus is a well-documented example of how neuroinflammation may lead to local parenchymal damage and homeostatic dysfunction. (Thaler et al., 2012; Buckman et al., 2013; Lee et al., 2013; Berkseth et al., 2014)

Figure 2: Effect of HFD and obesity on astrocyte morphology in rat arcuate nucleus



Note: Figure from (Thaler et al., 2012)

Figure 3: Effect of HFD and obesity on microglia morphology in rat arcuate nucleus



Note: figure from (Thaler et al., 2012)

Hypothalamus Structure

The hypothalamus has long been implicated as a primary region for controlling food intake and energy expenditure. (Anand and Brobeck, 1951) Lesion studies in rats first suggested the hypothalamus as a satiety center and further suggest the hypothalamus also functions as a hunger center. (Brobeck et al., 1943; Anand and Brobeck, 1951) It was later posited that individual nuclei within the hypothalamus performed unique tasks. (Stellar, 1994) Lesion studies in the ventromedial hypothalamic nucleus (VMN) resulted in hyperphagia while a lesion in the lateral hypothalamic area (LHA) resulted in hypophagia. (Stellar, 1994) Current opinion, however, does not designate individual hypothalamic nuclei as independent centers controlling food intake. Instead, the hypothalamus is viewed as a region consisting of discrete pathways responsible for generating integrated responses to afferent input related to changes in bodily energy storage. This intricate system is highly coordinated by the arcuate nucleus (ARC).

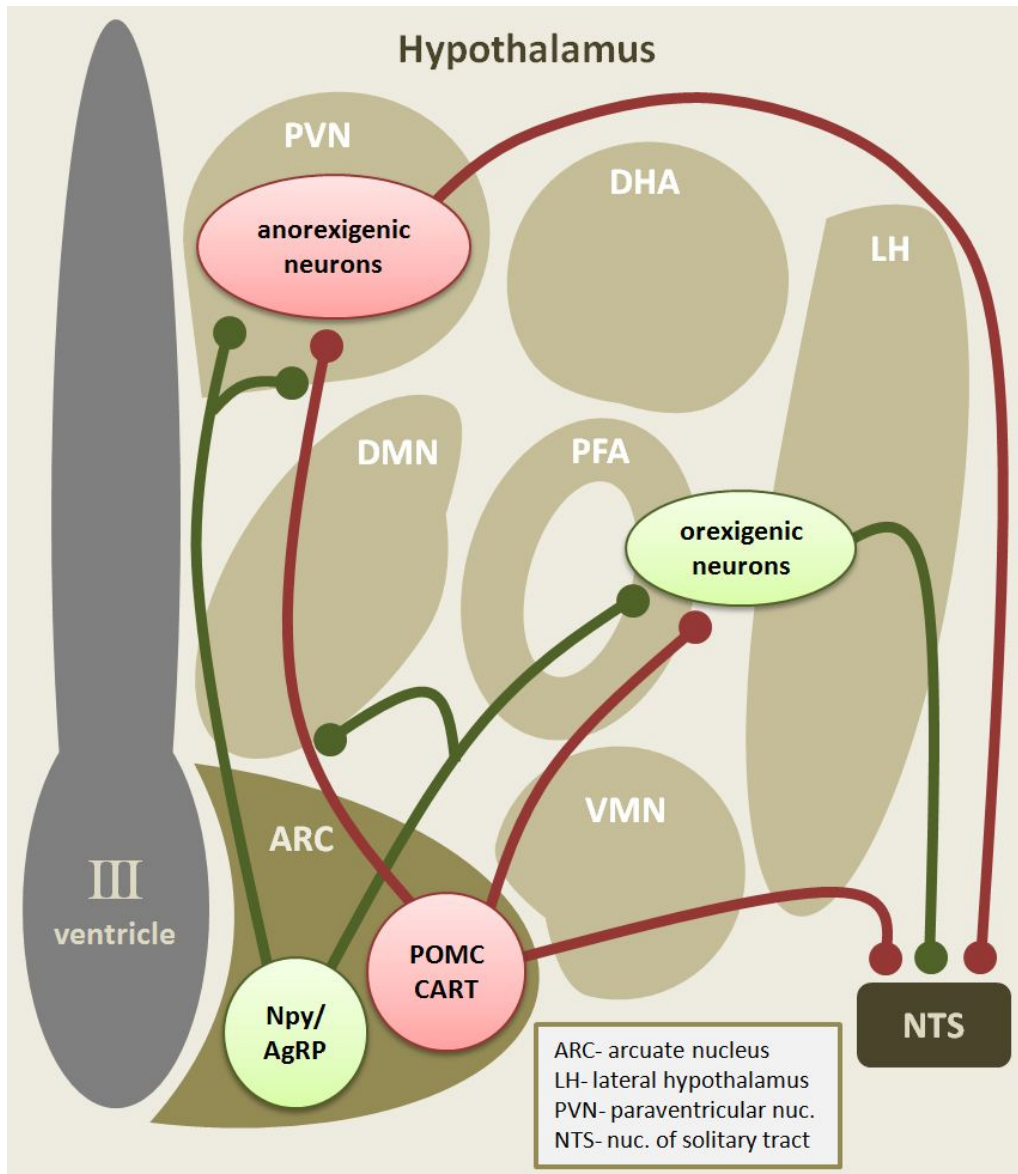
Arcuate Nucleus Cell Populations

The ARC contains orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related protein (AGRP) and anorexigenic neurons expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcripts (CART) that act as sensors for bodily energy stores and subsequently coordinate a complex network of neurons that ultimately control hunger and satiety signals. (Flier and Maratos-Flier, 1998) These neurons are capable of detecting both immediate and chronic changes in levels of hormones or nutrients in the blood stream (e.g. insulin, leptin, ghrelin), and function as a major aspect of homeostatic feeding. (Flier and Maratos-Flier, 1998) NPY/AGRP neurons stimulate food intake when activated and are inhibited by insulin and leptin. (Farooqi et al., 1999; Doyon et al., 2001) NPY/AGRP neurons also project

to POMC/CART neurons, inhibiting their action via release of GABA. (Horvath, 2005)

POMC/CART neurons inhibit food intake and are activated by insulin and leptin. (Schwartz et al., 2000; Wren et al., 2001) Ghrelin has the reverse effect of leptin and insulin on both cell types, as it activated NPY/AGRP to initiate feeding and inhibits POMC/CART neurons to reduce feeding. (Cummings et al., 2001; Nakazato et al., 2001; Wren et al., 2001) Both cell subpopulations project to adjacent areas, paraventricular nucleus (PVN) and LHA. (Flier and Maratos-Flier, 1998) The PVN is comprised of neurons that reduce food intake (anorexigenic), whereas the neurons within the LHA increase food intake (orexigenic). (Horvath, 2005; Horvath et al., 2010) Signal propagated from the PVN to LHA is directed downstream to the nucleus of the solitary tracts (NTS), an area implicated in satiety signaling. (Schwartz et al., 2000) The colocalization of these distinct cell types among various nuclei suggests that the hypothalamus plays a highly specialized role in energy homeostasis. **(Figure 4)**

Figure 4: Organization of hypothalamic nuclei associated with energy intake and expenditure and their projections



Note: POMC/CART are anorexigenic (red represents decrease feeding) and Npy/AgRP are orexigenic (green represents increase feeding). (*AgRP*) *agouti-related peptide* (*ARC*) *arcuate nucleus*, (*CART*) *cocaine- and amphetamine-related transcript* (*DHA*) *dorsal hypothalamic area*, (*DMN*) *dorsomedial nucleus*, (*NPY*) *neuropeptide Y*, (*NTS*) *nucleus of solitary tract*, (*LH*) *lateral hypothalamus*, (*PFA*) *paraforminular nucleus*, (*POMC*) *pro opiomelanocortin*, (*PVN*) *paraventricular nucleus*, (*VMN*) *ventromedial nucleus*

Cognitive

Obesity is also associated with reduced cognitive performance in adolescents and adults, including changes in memory, attention, and spatial ability. (Elias et al., 2003; Cohen et al., 2011; Yau et al., 2012) Obese adolescents showed significantly lower arithmetic, spelling, attention, and mental flexibility, and a trend for lower overall intelligence. These findings were strengthened when the number of symptoms of metabolic syndrome were used as a predictor of cognitive impairment instead of BMI. (Yau et al., 2012) In adults, similar cognitive deficits are observed with current and persisting obesity. Obese adult men performed worse than their lean counterparts on tests of learning, memory, executive functioning, and abstract reading during an initial testing period and a 4 to 6-year follow-up study. (Elias et al., 2003)

Measures

There are many tools and methods available to measure changes in obesity, behavior, inflammation, and neural changes.

Body Weight

Obesity is characterized by excess body weight, specifically excess adipose tissue. There are several ways to calculate obesity that are dependent upon the measurements taken. The most widely utilized, primarily due to its simplicity, is the body mass index (BMI). BMI is calculated by dividing weight (kilograms) by height (meters) for the formula of $BMI = \text{kg/m}^2$. BMI ranges are separated into four main categories: underweight ($x < 18.5$), healthy ($x = 18.5-24.9$), overweight ($x = 25-29.9$), and obese ($x > 30$). Individuals with a BMI > 35 are considered

severely obese, and individuals with a BMI > 40 are considered morbidly obese. (Flegal et al., 2010) Although not as common, some researchers rely on waist-hip ratio measures as a measure of obesity. Waist-hip ratio is calculated by dividing the measurement of the waist (W) by the hips (H) for a formula of $r = W/H$. The waist-hip ratio is a better indicator of abdominal visceral adiposity storage, which is more strongly correlated with obesity comorbidities than BMI. (Price et al., 2006) However, BMI and waist-hip ratio measurements yield similar results when used in MRI research.

Eating Behavior Questionnaires

Overeating is a prominent behavioral aspect of obesity. Several behavioral questionnaires examine various behaviors associated with overeating and obesity. The Three Factor Eating Questionnaire (TFEQ) examines three main factors associated with overall food intake. (Stunkard and Messick, 1985) These factors include 1) cognitive restraint of eating, 2) disinhibition, and 3) general hunger. The TFEQ is effective at discerning eating behaviors by examining the amount of cognitive restraint needed to restrain eating and when that restraint fails, the threshold at which an individual's disinhibition leads to food intake. Lastly, the TEFQ quantifies the overall hunger levels of an individual. A similar questionnaire, the Dutch Eating Behavior Questionnaire (DEBQ), examines an individual's structure of eating behavior. (van Strien et al., 1986) The factors include external eating, restrained eating, and emotional eating and describe the drive behind an individual's decision to eat, whether it be due to external stimuli, a lack of restraint, or an emotional response.

The Grand Hunger Scale specifically examines an individual's hunger pertaining to the recency of past meals and anticipation of future meals. (Grand, 1968) Additionally, the Grand Hunger Scale is critical in assessing current hunger, an important covariate for experimental studies involving brain responses to food or hunger. The Night Eating Questionnaire examines night eating patterns, a behavior associated with obesity. (Allison et al., 2006) Some scales like the Pittsburgh Sleep Quality Index (PSQI) are used to examine the indirect effects of overeating and obesity, like sleep disturbances and sleep apnea, which are common comorbidities with obesity and may even contribute to sustained obesity. (Buysse et al., 1989) Finally, one visual analog scale uses drawings of men and women with low and high BMIs to examine the relationship between actual BMI and self-perception of body size. (Kakeshita and de Sousa Almeida, 2006) Interestingly, many obese individuals underestimate their own body size.

Cognitive Assessments

A battery of cognitive assessments is regularly administered to obese subjects due to the reported associations of obesity and cognitive deficits. (Cazettes et al., 2011; Cohen et al., 2011; Yau et al., 2012) The focus of these assessments range from general reading and memory to strategic planning and goal-directed behavior. Simpler tests like the Wide Range Assessment of Memory and Learning test examine immediate and delayed memory as well as learning ability. (Gioia, 1998) Verbal fluency and attentional capacities are measured using the Controlled Oral Word Association Test and Psychomotor Vigilance Task, respectively. (Basner et al., 2011; Patterson, 2011) More complicated assessments like the Tower of London Test and Wisconsin Card Sorting Test utilize tightly-controlled games to measure executive function, planning, and goal-directed behavior. (Riccio et al., 2004; Nyhus and Barceló, 2009) Lastly, some assessments, like

the Wide Range Achievement Test, are multifaceted and include tests on reading, sentence comprehension, and mathematics. (Miller, 1979)

Immunohistochemistry

Direct, invasive methods exist to examine the effects of obesity and inflammation on the brain. Stereological techniques have been used to examine changes in hypothalamic volume and neuronal density in obese mice after exposure to high fat diet. Mice on high fat diet for 8 weeks have larger hypothalamic volumes, a decrease in hypothalamic neuron density, and increased glia density. (Namavar et al., 2012) These results suggest that high fat diet alters the neuronal structure of the hypothalamus. However, this study failed to examine astrocyte and microglia changes. Due to the heterogeneous population of cells within the hypothalamus (neurons, astrocytes, microglia), immunohistochemical techniques are used to examine which cell populations are affected by inflammation and obesity.

Fluoro-Jade C effectively labels degenerating neuronal populations, but does not label degenerating or healthy glia. (Gu et al., 2012) Using glial fibrillary acidic protein (GFAP) to examine the differential distributions of astrocytes within the hypothalamus, researchers found that obesity is associated with astrogliosis (increased astrocyte population and density) within the mediobasal hypothalamus. (Buckman et al., 2013) Utilizing GFAP and the histochemical microglial marker, ionized calcium-binding adapter molecule 1 (IBA1), researchers consistently observe an increase in glia cell density and morphological changes in the mediobasal hypothalamus of rats and mice fed high fat diet. Importantly, these changes were observed within 1 to 3 days of high fat diet intake, persisted for up to 8 months, and are further supported by an

increase in inflammatory markers in serum. (Cai, 2009; Thaler et al., 2012; Purkayastha and Cai, 2013) Pertinent inflammatory markers found in the serum include increased levels of pro-inflammatory adipokines (CRP, IL-16 IL-1 β , TNF α , S100B) and a decrease in anti-inflammatory adipokines (adiponectin, SFRP5). (Cummings et al., 2001; Morton et al., 2006; Cazettes et al., 2011; Mueller et al., 2012) Other markers of inflammation include elevated serum levels of reactive oxygen species (ROS) production, increases prostaglandin E2 (pgE2), and increased markers of cyclooxygenase-2 (COX-2). (Farooqi et al., 1999; Wren et al., 2001; Horvath, 2005; Cai, 2009, 2013; Cai and Liu, 2012; McNay et al., 2012)

Neuroimaging

Gliosis

Investigators have developed novel MRI techniques to address the limitations to exploring the neural changes associated with obesity and inflammation in humans. This technique -- building from studies correlating T₂-weighted MRI signal with post-mortem tissue gliosis in patients with neurodegenerative disease -- quantifies subtle changes in T₁- or T₂-weighted signal as a marker for neural changes associated with obesity and inflammation. (Marshall et al., 1988; Coulthard et al., 1999; Briellmann et al., 2002) Simply stated, an increase in signal on a T₂-weighted scan or a decrease in signal on a T₁-weighted scan suggests parenchymal change or gliosis. A landmark study using MRI and immunohistochemical techniques in tandem reported a positive correlation of T₂-weighted signal with mean fluorescent intensity of GFAP staining in mouse mediobasal hypothalamus. (Lee et al., 2013) These results strongly support the hypothesis that neural changes associated with obesity and inflammation can be measured with MRI. A similar study in humans extracted the signal from *a priori* regions of interest from T₂-weighted MRI scans and

showed a positive correlation of BMI with signal change in the mediobasal hypothalamus, suggesting that obesity is associated with gliosis in the mediobasal hypothalamus. (Thaler et al., 2012)

The above techniques, however, are being replaced with more sophisticated imaging parameters to address the limitations of single echo MRI scans. Single echo structural scans are susceptible to multiple types of contrast mechanisms that influence the acquired signal, potentially confounding the interpretation of such data. Daily differences in the scanner environment, and individual differences within the same subject require single echo structural scans to be internally normalized for every scan for each subject. In serial scans, data are normalized by sampling the baseline signal from a control region presumed to be unaffected by neuroinflammation or parenchymal change. (Briellmann et al., 2002; Thaler et al., 2012) The major caveat to this method is that it is impossible to ensure the control region is, in fact, unaffected by neuroinflammation. Thus, quantitative mapping of T_1 and T_2 is the emerging method of preference for exploring neuroinflammation and parenchymal changes.

Quantitative mapping of T_1 and T_2 enables objective determination of whether abnormalities are present within a particular patient, group, or longitudinally. (Bernasconi et al., 2000; Wendel et al., 2001; Briellmann et al., 2004; Berkseth et al., 2014) Quantitative maps do not need to be normalized to a control region. Instead, the T_2 signal intensity value is normalized within the values of the multiple echoes acquired. (Briellmann et al., 2004; Berkseth et al., 2014; Schur et al., 2015) The fundamental drawback to this approach is the increased time, limiting its use in the clinical setting. In research however, quantitative mapping has been used to successfully

identify increased T_2 values within the hypothalamus of obese rodents and humans. (Berkseth et al., 2014; Schur et al., 2015) Furthermore, T_2 changes are strongly correlated with direct immunohistological measures of neuroinflammation and gliosis. Taken together, these results strongly suggest that quantitative mapping is effective at identifying brain regions affected by neuroinflammation and gliosis and can be applied to obese populations.

fMRI

The aim of most functional neuroimaging methods is to assess brain activity relating to cognition, affect, and behavior. A common paradigm in fMRI studies is to examine the brain's response to visual, olfactory, or gustatory food cues. One emerging paradigm focuses on how the brain responds to images of food that differ in categories such as palatability or caloric content. The majority of these studies compare lean and obese subjects, although a few groups perform longitudinal studies examining brain changes after weight change. (Murdaugh et al., 2012) Visual food cue paradigms consistently show that obese individuals have greater levels of activation in brain regions associated with the reward system (insula, caudate, putamen, hippocampus, nucleus accumbens, orbital frontal cortex, prefrontal cortex, and anterior cingulate cortex) when individuals view images of high-calorie, palatable foods compared to images of low-calorie foods or non-foods. (Rothmund et al., 2007; Stoeckel et al., 2008) Like visual cues, olfactory cues differentially affect BOLD activation in lean versus obese individuals. Obese individuals show greater BOLD activation in the hippocampus to odors of sweet and fat-related foods than lean individuals. (Bragulat et al., 2010)

Differences in BOLD activation between lean and obese subjects predict behavioral, physiological, and neural changes. Less BOLD activation in executive function areas (e.g. prefrontal cortex) predicts greater weight gain in women over a 1.3 – 2.9 year period. (Kishinevsky et al., 2012) In overweight children, prefrontal cortex activity during an executive function task increases after a 13-week exercise program. (Davis et al., 2011) Future weight loss is predicted by BOLD activation in regions associated with reward and motivation. Murdaugh et al (2011) found that greater activation in brain regions mediating motivational and attentional salience of food cues in obese individuals at the start of a weight-loss program was predictive of less weight loss in the program and poorer weight control over a 9-month follow-up period. (Murdaugh et al., 2012) Functional MRI allows researchers to assess the efficacy of various interventions on neural and behavioral changes in obesity. Together, the results of these cross-sectional and longitudinal studies suggest that obesity is associated with heightened responses to visual and olfactory food cues in a distributed network of brain regions involved in reward, motivation, executive function, memory, and emotion.

One important covariate includes individual hunger level or time since last meal. During a visual task examining attentional bias for food cue images, obese and lean individuals equally favored food cue images during a fasting state. After a meal, however, only obese individuals continued to favor food cue images. (Castellanos et al., 2009) In addition to changes in food preference, the pattern of BOLD activation while viewing high-calorie versus low-calorie food images is influenced by hunger. After a 500kcal meal, activation shifts from the ACC and mPFC (pre-meal) to the caudate, hippocampus, mPFC, and superior frontal gyrus (post-meal). (Martin et al.,

2010) Therefore, it is now standard to administer a hunger scale, such as the Grand Hunger Scale, before studies on food cues and brain activation. (Grand, 1968)

Volumetric Based Morphometry

Structural neuroimaging methods allow for the quantification of volumetric differences in the white and grey matter of subject brains. Differences in brain volume between lean and obese individuals may provide insight into the neurological changes associated with obesity. One study comparing lean and obese groups found smaller grey matter densities in the post-central gyrus, frontal operculum, putamen, and middle frontal gyrus. (Pannacciulli et al., 2006) MRI structural scans demonstrate a negative correlation of BMI with overall brain volume, and specifically grey matter volume. (Ward et al., 2005; Gunstad et al., 2008) In men, BMI is negatively correlated with grey matter volume in the medial temporal lobes, occipital lobe, frontal lobe, and precuneus. (Taki et al., 2008)

Waist circumference is another strong indicator of grey matter volume. Waist circumference is negatively correlated with grey matter volume in frontal and temporal lobes, pre- and post-central gyri, insula, cingulate cortex, parahippocampus, hippocampus, amygdala, putamen, precuneus, and thalamus. (Janowitz et al., 2015) Waist circumference and BMI are similarly negatively correlated with grey matter volume in the hypothalamus, prefrontal cortex, insula, and inferior parietal lobe. (Kurth et al., 2013) Few longitudinal studies have been conducted but evidence suggests that an increase in BMI causes a decrease in grey matter volume over a period of 1 and 5 years. (Yokum et al., 2012; Bobb et al., 2014)

Disgust

One factor that may contribute to an increased BMI and obesity is individual variations in the emotion of disgust.

The Emotion of Disgust

Disgust is a human emotion that primarily functions to protect us from disease or contamination. (Oaten et al., 2009) However, an individual may experience moral disgust if societal norms or morals are broken. (Rozin and Haidt, 2013) Therefore, disgust may be broken into two general categories including physical disgust and moral disgust. (Tybur et al., 2013) Physical disgust (commonly referred to as core disgust) is associated with the motivation to avoid body products (e.g. feces, blood, vomit), specific animals (e.g. roaches, flies), and contaminated food. (Rozin and Fallon, 1987) The physical disgust response (e.g. closing of the nostrils, nausea, and gagging) functions to prevent ingestion of toxic or unpleasant tasting substances by ultimately terminating eating. (Rozin and Fallon, 1987) Moral disgust is elicited by deviating from social and moral societal norms. (Borg et al., 2008; Tybur et al., 2013) Moral disgust is further divided into two categories including sexual disgust (e.g. pedophilia, incest, prostitution) and non-sexual disgust (e.g. lying, cheating, murder). (Borg et al., 2008; Tybur et al., 2013) Moral disgust both adheres to and further cements societal norms. Although both physical and moral elicitors induce disgust, physical elicitors mainly evoke a feeling of dirtiness whereas moral elicitors of disgust induce feelings of indignation and contempt. (Ottaviani et al., 2013) Despite differences in feelings elicited by each subset of disgust, the emotion of disgust serves to prevent actions that may lead to either physical or moral contamination. Taken in the context of obesity, physical

and moral disgust may contribute to an individual's decision to abstain from activities likely to cause excessive weight gain.

Disgust and Obesity

Moral disgust and physical disgust may both contribute to maintaining a healthy weight. Lean individuals sometimes view obese individuals with moral disgust (e.g. gluttonous, sloth). (Crandall et al., 1994; Townend, 2009) Increased levels of disgust are associated with negative feelings towards overweight compared to thin people. (Vartanian, 2010) The physical appearance of an obese individual may elicit moral disgust because it conflicts with societal norms on slimness and beauty. (O'Brien et al., 2009) Additionally, obesity may conflict with unconscious drives and biases related to evolutionary fitness. (O'Brien et al., 2009) Overall, some individuals may view obesity as a breach of social- and morality-based norms. (Lieberman et al., 2012)

Deficits in the experience of physical disgust, specifically in relation to food, may be one factor that contributes to obesity. (Watkins et al., 2016) Increased levels of food-related disgust have been associated with the eating disorders bulimia and anorexia nervosa. (Davey et al., 1998; Troop et al., 2002) For example, one study found higher levels of disgust to various stimuli, especially in response to foods, in individuals with anorexia nervosa. (Aharoni and Hertz, 2012) Because anorexia nervosa is characterized by a decreased BMI, it is possible that increased disgust proneness that prevents adaptive eating behavior contributes to reduced BMI.

Conversely, a recent study found significantly lower disgust proneness scores in obese compared to lean individuals. (Houben and Havermans, 2012) **(Figure 5)** These findings suggest that

increased disgust proneness among individuals with anorexia nervosa may partially account for lower BMI, whereas decreased disgust proneness among obese individuals may be associated with higher BMI.

Insula and Disgust

The insula is the primary brain region associated with the disgust response. Insula activation has been associated with individual differences in disgust proneness when viewing disgusting stimuli. (Phillips et al., 1997; Mataix-Cols et al., 2008) There is greater insula activation to disgusting images compared to neutral images in lean individuals. (Wicker et al., 2003; Wright et al., 2004; Baumann and Mattingley, 2012) Not only visual, but disgusting olfactory stimuli results in insula BOLD activation. (Wicker et al., 2003) Viewing images of disgusting foods, in contrast with images of non-food items or appetizing foods, elicits insula activation. (Calder et al., 2007) Combined, the data strongly implicate the insula as a critical region in the disgust response.

Disgust Propensity and Sensitivity Scale - Revised

Recent developments in the psychometric assessment of individual differences in disgust proneness may allow for a more precise delineation of the role of disgust in obesity. Indeed, the development of the Disgust Propensity and Sensitivity Scale - Revised (DPSS-R) allows investigators to distinguish between two characteristics of disgust proneness: how easily people are disgusted (propensity) and how unpleasant the experience of disgust is appraised (sensitivity). (van Overveld et al., 2006; Olatunji et al., 2007) The DPSS-R serves as a useful alternative to the Disgust Scale (Haidt et al., 1994) and the Disgust and Contamination

Sensitivity Questionnaire (Rozin and Fallon, 1987) in that it does not measure disgust for specific elicitors. This is of particular importance for examining food-related disgust because it limits the possibility that between-group differences in disgust are due to irrelevant elicitors such as spiders or blood. (van Overveld et al., 2006; Olatunji et al., 2007; Watkins et al., 2016)

Summary

There are many factors that contribute to obesity and a continually evolving set of research tools available to examine these factors. Emerging data sheds light on the association of disgust proneness with obesity. Obese individuals, when compared to their lean counterparts, have less behavioral disgust proneness. Interestingly, there is no evidence of altered insula activation or grey matter volume between lean and obese individuals. An in depth study examining changes in insula activation and grey matter volume in the context disgust proneness and obesity would greatly contribute to the literature. These results would be novel within the field of disgust proneness and identifying regions with structural and functional would strongly suggest that the insula is a major brain region associated with disgust.

In addition to disgust proneness, chronic neuroinflammation and gliosis is associated with obesity. Immunohistochemistry studies consistently report gliosis in obese rodents. Although immunohistochemistry is the gold standard for identifying regions of gliosis and identifying specific cell populations contributing to gliosis, the technique is invasive and not suitable for use in humans. In order to further study gliosis in humans, existing MRI methods must be modified to detect parenchymal changes consistent with gliosis in human populations. Initial structural

MRI methods rely on T_2 -weighted imaging. Determining whether clinical T_1 -weighted is sensitive enough to detect changes in gliosis would greatly benefit the field. If T_1 is appropriately sensitive, researchers could utilize existing MRI repositories to explore various association with gliosis in any existing patient MRI dataset.

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CHAPTER II: STUDY 1 -- DISGUST PRONENESS AND ASSOCIATED NEURAL SUBSTRATES IN OBESITY

Abstract

Defects in experiencing disgust may contribute to obesity by allowing for the overconsumption of food. However, the relationship of disgust proneness and its associated neural locus has yet to be explored in the context of obesity. Thirty-three participants (17 obese; 16 lean) completed the Disgust Propensity and Sensitivity Scale-Revised (DPSS-R) and an fMRI paradigm where images from four categories (food, contaminants, contaminated food, or fixation) were randomly presented. Independent two-sample t-tests revealed significantly lower levels of Disgust Sensitivity for the obese group (mean score=14.7) compared to the lean group (mean score=17.6), $p=0.026$. The obese group had less activation in the right insula than the lean group when viewing contaminated food images. Multiple regression with interaction analysis revealed one left insula region where the association of Disgust Sensitivity scores with activation differed by group when viewing contaminated food images. These interaction effects were driven by the negative correlation of Disgust Sensitivity scores with beta values extracted from the left insula in the obese group ($r=-0.59$) compared to a positive correlation in the lean group ($r=0.65$). Given these BMI-dependent differences in Disgust Sensitivity and neural responsiveness to disgusting food images it is likely that altered Disgust Sensitivity may contribute to obesity.

Introduction

Obesity is a growing public health concern with many contributing factors (Cohen, 2008; Caballero, 2007). Numerous biological, social, and learned factors contribute to feeding behavior, including initiation and termination of food intake (Berthoud, Woods, Cowley, Levin, & Kelley, 2002; Berthoud, 2012). Darwin initially proposed that experiencing disgust facilitates food rejection (Darwin, 2009). This idea has been corroborated by Rozin and Fallon; who assert that even the physical disgust response (e.g. closing of the nostrils, nausea, and gagging) functions to terminate eating (Rozin & Fallon, 1987). Therefore, deficits in the experience of disgust specifically in relation to food may be one factor that contributes to obesity.

Increased levels of food-related disgust have been associated with the eating disorders bulimia and anorexia nervosa (Troop, Treasure, & Serpell, 2002; Davey, Buckland, Tantow, & Dallos, 1998). For example, one study found higher levels of disgust to various stimuli, especially in response to foods, in anorexia nervosa (Aharoni & Hertz, 2012). Because anorexia nervosa is characterized by a decreased body mass index (BMI), it is possible that increased disgust proneness that prevents adaptive eating behavior contributes to reduced body mass index (BMI). Conversely, a recent study found significantly lower disgust proneness scores in obese compared to lean individuals (Houben & Havermans, 2012). These findings may suggest that increased disgust proneness among individuals with anorexia nervosa may partially account for lower BMI whereas decreased disgust proneness among obese individuals may be associated with higher BMI.

Recent developments in the psychometric assessment of individual differences in disgust proneness may allow for a more precise delineation of the role of disgust in obesity. Indeed, the development of the Disgust Propensity and Sensitivity Scale – Revised (DPSS-R) allows investigators to distinguish between two characteristics of disgust proneness: how easily people are disgusted (propensity) and how unpleasant the experience of disgust is appraised (sensitivity). The DPSS-R serves as a useful alternative to the Disgust Scale (Haidt, McCauley, & Rozin, 1994) and the Disgust and Contamination Sensitivity Questionnaire (Rozin, Fallon, & Mandell, 1984) in that it does not measure disgust for specific elicitors. This is of particular importance for a study examining differences in food-related disgust because it limits the possibility that between-group differences are due to irrelevant elicitors such as spiders or blood (van Overveld, de Jong, Peters, Cavanagh, & Davey, 2006). Given that the neural basis for potentially altered disgust response in obesity is unclear, utilizing the DPSS-R in conjunction with a food-related disgust fMRI task is a first step in the exploration of the neurobehavioral disgust response.

Delineation of brain regions associated with disgust responding may offer important insights into eating behaviors that contribute to obesity. Insula activation has been associated with individual differences in disgust proneness when viewing disgust stimuli (Mataix-Cols et al., 2008; Phillips et al., 1997). Baumann and colleagues showed greater insula activation to disgusting images compared to neutral images in normal weight individuals (Baumann & Mattingley, 2012; Wright, He, Shapira, Goodman, & Liu, 2004; Wicker et al., 2003). The insula is thought to regulate interoceptive awareness and may play a role in satiety (Craig, 2003; Craig, 2009).

Viewing images of disgusting foods, in contrast with images of non-food items or appetizing foods, elicits insula activation (Calder et al., 2007). Therefore, it is likely that the insula actively regulates the disgust response. This pattern of finding raises the possibility that brain regions associated with disgust may be underactive among obese individuals, especially in response to food cues.

Although imaging studies have identified the insula as a primary region implicated in the disgust response, much remains unknown about the neural correlates of the disgust response in obesity. To our knowledge, no studies have examined differences in brain activation in response to disgusting food-related images between lean and obese individuals. Thus, we paired disgusting food-related images paired with the administration of the DPSS-R to examine the neurobehavioral correlates of the disgust response specifically in the context of food. We hypothesized that obese individuals compared to lean individuals, 1) will have decreased disgust proneness scores and 2) exhibit decreased insula activation during a disgust-probing food-specific fMRI task.

Methods

Participants

Thirty-three participants (17 obese; 16 lean) who met the following eligibility criteria were recruited via email and poster advertisement: no current medical illness, no past brain trauma, no use of psychotropic medications, and no current or past drug or alcohol abuse. Height and weight were measured on the initial screening day and BMI was calculated [BMI = weight(kg) /

height²(m)]. Participants with a BMI > 30 were classified as obese and participants with a BMI < 25 were classified as lean. Individuals with a BMI of 25-30 were classified as overweight and excluded from the study.

Study Procedures

Each participant completed the Disgust Propensity and Sensitivity Scale-Revised (DPSS-R) before an fMRI task (van Overveld et al., 2006). The DPSS-R distinguishes between two characteristics of disgust proneness: how easily people are disgusted (propensity) and how unpleasant the experience of disgust is appraised (sensitivity). Participants rated how often a statement is true based on a five point Likert ranging from "never" to "always". Example statements include "I avoid disgusting things" (propensity) and "When I feel disgusted, I worry that I might pass out" (sensitivity). All questionnaires were completed in the afternoon after a 4-hour fast. The study protocol was approved by the Vanderbilt University Institutional Review Board and the procedures were in accordance with the guidelines of the Helsinki Declaration on human experimentation.

fMRI Task

Participants completed a randomized jittered rapid-event-related fMRI paradigm where images (14 per category) from four categories: food (e.g. crackers, cookies), contaminants (e.g. mold, toilet), contaminated food (e.g. moldy cracker, cookies on a toilet seat), or fixation (white crosshairs on black background) were randomly presented. The fixation cross was used as the baseline condition. Food, contaminants, and contaminated food images were generally matched for color, intensity, and brightness but not for additional visual parameters or complexity. The

order of images was randomized across all trials. The order of category of image was also randomized to prevent possible order effects. Participants saw each image only once throughout the study. Images from the food category consisted of high and low energy dense foods. These images were not selected to examine differences in caloric content, but instead selected to represent the full spectrum of food choices available across several diets. The images in the contaminated food category were created by combining the images from the food category with the images from the contaminates category. Some of these images were obtained with a digital camera (e.g. pizza left in a dumpster) and the other images were created in Photoshop (e.g. bugs crawling over a filet of salmon). Although the level of disgust elicited by each image was not quantified, the disgust stimuli were modeled after examples listed in various disgust questionnaires and International Affective Picture System (IAPS) images.

The images were presented for 4 seconds with an interstimulus interval presentation of a fixation cross jittered between 2-8 seconds. To enhance the ecological validity of the task, participants were instructed to think about whether they would eat what was presented in each image. All fMRI scans were conducted in the afternoon after a 4-hour fast in order to induce a hunger state in participants. Participant hunger was assessed using a self-report hunger scale that provided a composite hunger index that included individual questions on current hunger and time since last meal (Grand, 1968).

fMRI Data Acquisition

Participants underwent functional magnetic resonance imaging (fMRI) on a 3T Intera Achieva MRI scanner (Phillips Medical Systems, Andover, MA). In each 270 second functional run, 28

field echo EPI (128 dynamics, 4.50mm slice thickness with 0.45mm gap, 2s TR, 34ms TE, 79° flip angle, FOV = 240, matrix = 80 X 78) scans were acquired.

Analysis of Normality

Normality was determined by calculating the Fisher Z score (skewness / standard error of skewness). The data were normally distributed if $|\text{Fisher Z}| < 2.58$ ($p = 0.01$). Pearson's correlations were used for normally distributed data and Spearman's Rho correlations were used for non-normally distributed data. One subject was labeled as an outlier and excluded from the dataset because extracted beta values were greater than five standard deviations from the mean (mean = -2.45, s.d. = 9.75, outlier beta value = -56.6).

Self-Reported Data Analysis

Scale score differences between the two groups were conducted using independent *t*-tests. Statistical significance of the between groups differences was determined at a $p \leq 0.05$.

fMRI Data Analysis

Data were analyzed using SPM8 (Wellcome Department of Cognitive Neuroscience, London) utilizing the General Linear Model (GLM) and a random effects analysis. The functional data were slice-time corrected using the first slice as the reference slice then motion-corrected by being spatially aligned to their mean functional image. Images were stereotactically normalized to the SPM EPI template (Montreal Neurological Institute) then smoothed with a full-width half maximum (FWHM) 8mm Gaussian Kernel.

All conditions (i.e. food, contaminants, contaminated foods) were modeled by group (i.e. obese, lean) in a full-factorial model in SPM8. This method allows for the calculation of all possible contrasts of interests (e.g. Lean > Obese: Food, Lean > Obese: Contaminates, Lean > Obese: Contaminated Food, Obese > Lean: Food, Obese > Lean: Contaminates, Obese > Lean: Contaminated Foods, Lean > Obese: Contaminated Food > Contaminates + Food, Obese > Lean: Contaminated Food > Contaminates + Food). All contrasts displaying significant results were calculated, reported, and discussed in future sections of this manuscript.

Regions of interest analysis was restricted to bilateral insula. The insula mask was created using WFUpickatlas. We used the AFNI based Alphasim program to run a Monte Carlo simulation to determine the extent threshold and voxel cluster size for uncorrected p values to generate a family wise error (FWE) corrected $p \leq 0.05$ for our bilateral insula mask. The extent threshold cluster size for voxel level p values of $p \leq 0.05$ and $p \leq 0.01$ were 74 voxels and 35 voxels, respectively.

fMRI Regression Analysis

Our primary fMRI regression analysis was used to examine the association of the behavioral measure of disgust with the disgust-based fMRI task. We used the multiple regression module in SPM8 to examine the association of Disgust Sensitivity scores with BOLD activation elicited from viewing each image condition (food, contaminate, contaminated food). In order to control for the effect of group (lean v. obese), group was modeled as a condition of no effect in our primary regression model. A subsequent regression model -- with the addition of an interaction term of Disgust Sensitivity scores with group -- was created to determine where the association

of Disgust Sensitivity scores with BOLD activation elicited from viewing each image condition differed between the lean and obese groups. A corrected p value of 0.05 was achieved at a voxel level $p \leq 0.01$ and extent threshold voxel cluster size of 35. Beta values were extracted from ROIs utilizing REX. SPSS was used to examine group level correlations of Disgust Sensitivity scores with extracted beta values.

Results

Demographics

Mean (min, max) BMIs of the lean and obese groups were 21.5 (19.1, 23.7) and 36.4 (30.0, 45.6), respectively. Groups were equivalent on sex (lean males = 7; females = 9, obese males = 7; females = 10) and age (lean = 25.2, obese = 27.7). **(Table 1)** BMI and Age were normally distributed ($|Fisher Z| < 2.58$).

Table 1: Subject demographics

	Group		<i>p</i> -value
	Lean (<i>n</i> =16)	Obese (<i>n</i> =17)	
Male/Female (<i>n</i>)	7/9	7/10	0.384 (χ^2)
	Mean (Min, Max)	Mean (Min, Max)	
Age	25.2 (20, 32)	27.7 (21, 35)	0.077
BMI	21.5 (19.1, 23.7)	36.4 (30.0, 45.6)	< 0.001

Behavioral

Hunger scores, Disgust Sensitivity scores, and Disgust Propensity scores were normally distributed ($|Fisher Z| < 2.58$). Groups did not differ on the hunger index ($p = 0.26$; mean lean score = 19.33, 25 percentile = 16.56, 75 percentile = 22.69; mean obese score = 18.28, 25 percentile = 14.56, 75 percentile = 20.19). The obese groups demonstrated statistically significantly lower levels of Disgust Sensitivity (mean score = 14.7, SD = 3.7, min = 8, max = 22) than the lean group (mean score = 17.6, SD = 3.7, min = 12, max = 26), $p = 0.026$. However, there was no statistically significant difference between the groups in Disgust Propensity (mean obese score = 20.9, SD = 4.9, min = 12, max = 32; mean lean score = 21.0, SD = 3.1, min = 16, max = 26; $p = 0.906$). (**Table 2**)

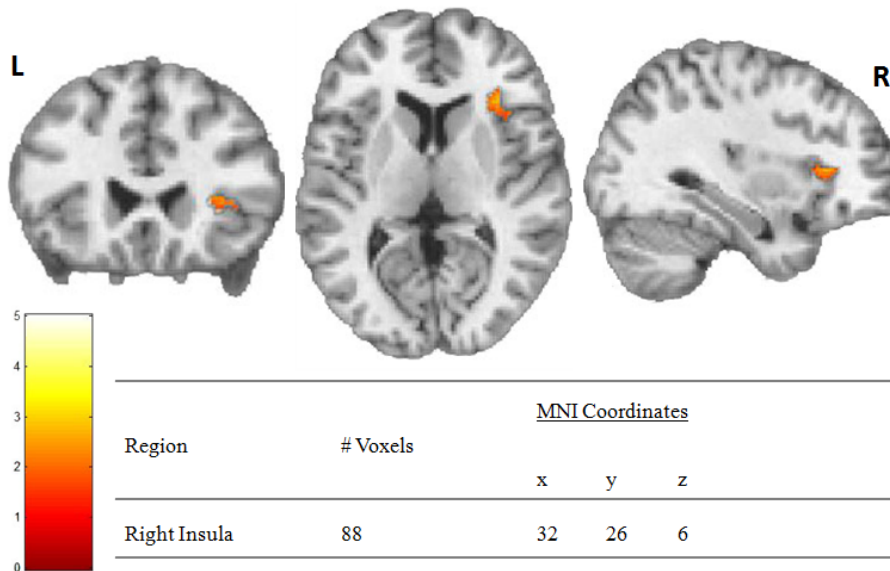
Table 2: Disgust Propensity and Sensitivity Scale - Revised Scores

	Group		<i>p</i> -value
	Lean (<i>n</i> =16)	Obese (<i>n</i> =17)	
	Mean (Min, Max)	Mean (Min, Max)	
Disgust Sensitivity Score	17.6 (12, 26)	14.7 (8, 22)	0.026
Disgust Propensity Score	21.0 (16, 26)	20.9 (12, 32)	0.906

Neural Activation

Between groups *a priori* bilateral insula analysis in SPM8 revealed one cluster in the right anterior insula where obese individuals had less BOLD activation than lean individuals when viewing contaminated food images (lean > obese: contaminated food > baseline). **(Figure 1)** No significant between groups differences were observed for any other contrast (e.g. obese > lean: food > baseline, lean > obese: contaminate > baseline, lean > obese: food > baseline).

Figure 1: Insula activation is lower in obese subjects when viewing contaminated foods.



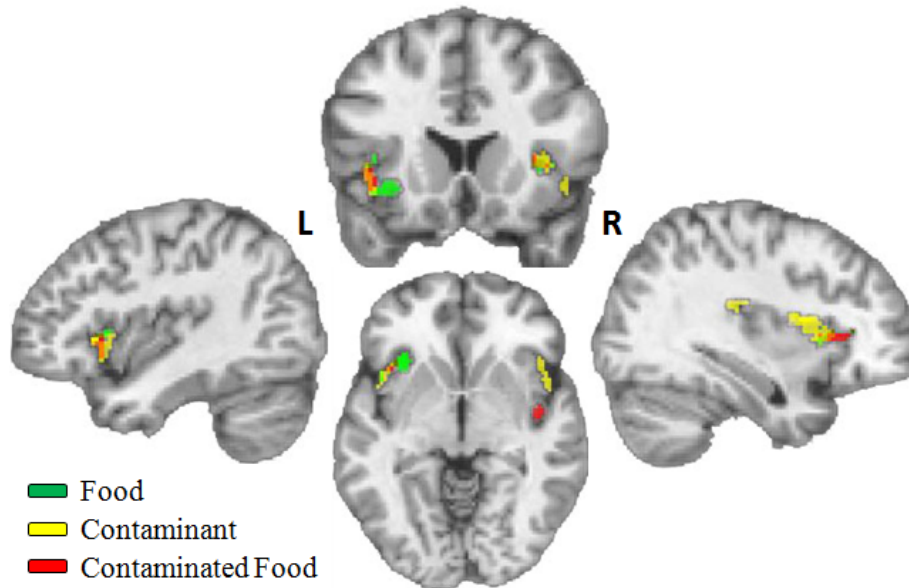
Note: Color bar represents T values for activated voxel group at statistical threshold of $p < 0.05$ and extent threshold = 74 voxels for corrected FWE $p < 0.05$

We next examined the association of Disgust Sensitivity scores with BOLD activation while viewing images from each condition (i.e. food, contaminate, and contaminated food). Multiple regression analysis revealed several bilateral insula regions where disgust sensitivity scores were positively correlated with BOLD activation for each condition. **(Table 3)** There was considerable overlap in BOLD activation across all three conditions. Notably, all right posterior BOLD activation was restricted to the contaminant and contaminated food conditions. All regions with positive associations are displayed on a single subject brain and color-coded by condition. **(Figure 2)** There were no negative associations of Disgust Sensitivity scores with BOLD activation in any condition.

Table 3: Positive association of Disgust Sensitivity scores with activation while viewing food, contaminate, and contaminant food images.

Region	# Voxels	MNI Coordinates		
		x	y	z
Food				
Left Insula	144	-34	24	0
Right Insula	36	32	16	4
Contaminate				
Left Insula	73	-44	16	0
Right Insula	135	38	6	14
Right Insula	39	32	-20	22
Right Insula	62	46	22	-6
Contaminated Food				
Left Insula	48	-38	22	0
Right Insula	45	42	-10	-10
Right Insula	71	38	28	6

Figure 2: Positive association of disgust sensitivity scores with activation while viewing food, contaminant, and contaminated food images.



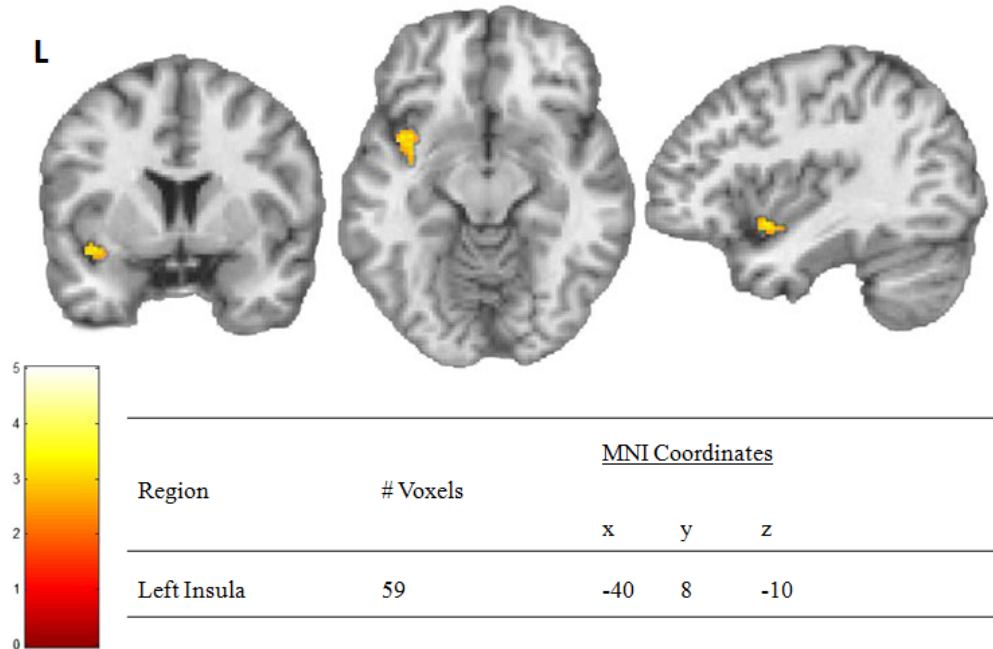
Note: Activated voxel group at statistical threshold of $p < 0.01$ and extent threshold = 35 voxels for corrected FWE $p < 0.01$

Finally, we determined where the association of Disgust Sensitivity scores with BOLD activation elicited from viewing each image condition differed between the lean and obese groups.

Multiple regression with interaction analysis revealed one left insula region where the association of Disgust Sensitivity scores with BOLD activation differed by group when viewing contaminated food images. **(Figure 3)** This effect was not present for the food or contaminant condition. To determine if the level of Disgust Sensitivity scores were associated with insula activation, we examined the group-level correlations of Disgust Sensitivity scores with beta values extracted from the significant left insula region. This analysis revealed a negative correlation of Disgust Sensitivity scores with beta values in the obese group ($r = -0.59$) and a positive correlation of Disgust Sensitivity scores with beta values in the lean group ($r = 0.65$,

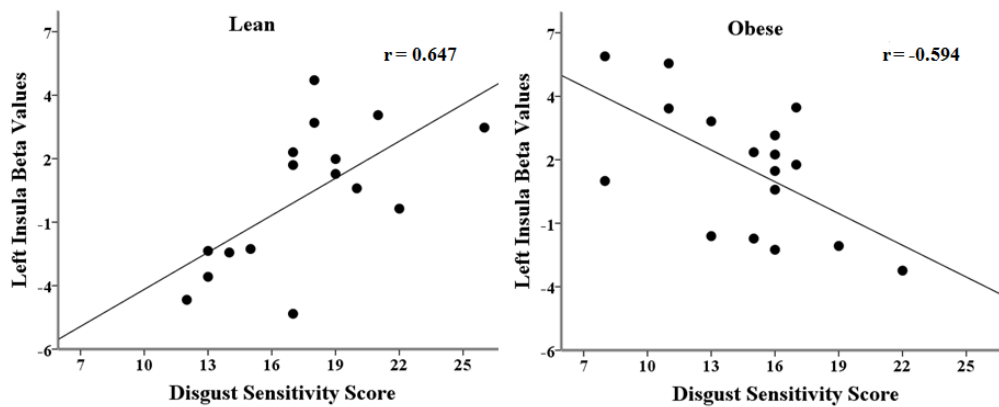
difference: $Z = 3.77, p < 0.001$). **(Figure 4)** BMI was not correlated with Disgust Sensitivity scores or extracted beta values, and therefore no mediation analysis was necessary.

Figure 3: Activation for the interaction of group (lean v. obese) and Disgust Sensitivity scores when viewing contaminated foods



Note: Color bar represents T values for activated voxel group at statistical threshold of $p < 0.01$ and extent threshold = 35 voxels for corrected FWE $p < 0.01$

Figure 4: Inverse association of Disgust Sensitivity scores and activation in obese individuals.



Discussion

The present study identified differences in disgust proneness and associated neural substrates between obese and lean individuals. Although lean and obese individuals did not significantly differ in how easily they are prone to experiencing disgust (propensity), obese individuals were less likely to appraise the experience of disgust as negative (sensitivity). The finding that obese individuals have lower Disgust Sensitivity scores further supports the possibility that a diminished disgust response may be one factor associated with obesity. Our findings are consistent with the notion that the experience of the disgust response and an associated negative appraisal are factors necessary to inhibit the drive for overconsumption. Whereas individuals with higher levels of disgust (e.g. individuals with anorexia nervosa) over-inhibit their drive to eat, it is possible that individuals with lower levels of disgust (e.g. obese individuals) may have a reduced ability to inhibit their drive to eat. In other words, obese and lean individuals may recognize they are consuming excess calories (Disgust Propensity) but obese individuals

experience diminished negative appraisal (Disgust Sensitivity), which may contribute to their failure to reduce caloric consumption.

As hypothesized, the insula had decreased activation in the obese group as compared to the lean group when viewing contaminated food images. This is, to our knowledge, the first identification of reduced insula activation to disgust stimuli in obese relative to lean individuals. This difference in insula activation was restricted to the right hemisphere, suggesting that the left insula may be less sensitive to contaminated food stimuli. Alternatively, if the observed findings are a consequence of obesity rather than a risk factor for it, the left insula may be resistant to the effects of obesity. Decreased insula activation was only observed when viewing contaminated foods, and not when viewing food or contaminant alone, suggesting that differences in disgust responses for lean versus obese individuals are specific to the interaction of food and contaminant. These findings raise the possibility that lower Disgust Sensitivity and reduced insula activation may contribute to the tendency to overeat among obese individuals.

The regression analysis revealed several overlapping areas within the insula with a positive association of Disgust Sensitivity scores with BOLD activation while viewing images from each category (food, contaminant, contaminated food). The majority of overlap was found in bilateral anterior insula, a region commonly associated with object valence (Britton et al., 2006; Viinikainen et al., 2010). Therefore, it is possible that the positive association of Disgust Sensitivity scores with BOLD activation within this subregion of the anterior insula is driven by the need to assign a positive or negative valence to a viewed image. This is especially likely considering the rapid-event-related design of the experiment. When rapidly viewing and

appraising whether one would eat various images of food, contaminant, and contaminated foods, one must quickly discern whether a food item is edible or contaminated. This concept may be translated to animal foraging behavior, where an animal needs to identify fresh and edible items from rotting or poisonous items.

The regression analysis also revealed some distinct areas within the insula with a positive association of Disgust Sensitivity scores with BOLD activation while viewing images from the contaminant and contaminated food categories. While one area of activation associated with contaminated food images was only slightly posterior to the primary anterior insula region, an area of activation associated with contaminant images was located in the far posterior insula. This suggests that the insula may be organized as a gradient, where the valence of food related items is processed in the anterior insula and the valence of pure contaminants is processed in the posterior insula. Further studies are necessary to fully understand the anterior-posterior organization of the insula in terms of valence processing.

The interaction analysis revealed one area of activation within the anterior left insula where the association of Disgust Sensitivity scores with BOLD activation while viewing contaminated food images differed by group. Within this area of activation, there was a positive association of Disgust Sensitivity scores with BOLD activation in the lean group, whereas there was a negative association of Disgust Sensitivity scores with BOLD activation within the obese group. This finding suggests that there is a functional dissociation between self-report of disgust sensitivity (Disgust Sensitivity scores) and neural activation (extracted beta values) in obese individuals. In a typical excitatory neurobehavioral system, neural activation should increase as a measurable

behavior increases. In this context, this system remains intact in the lean group, but is disrupted in the obese group. This observed uncoupling of disgust-related behavior and neural activation among obese individuals may reflect a fundamental dysregulation in a disgust system that may help mediate appropriate eating behavior.

The behavioral result of dysregulation in the disgust system has been demonstrated in a previous study showing that lower measures for core and contamination disgust predict a greater likelihood of eating high calorie food (Houben & Havermans, 2012). Via this mechanism, lower disgust in obesity might lead to a greater probability of ingesting higher calorie foods, which are associated with obesity. Lower behavioral disgust, paired with a negative association of disgust scores with neural activation in disgust regions, may lead to increased food intake and subsequent weight gain. A reduced disgust response may slightly extend the extent to which obese individuals will consume food. This may present as an increase in the total calories consumed in one meal or as the frequency an individual will consume a high calorie food over a low calorie food (Houben & Havermans, 2012). This can be directly contrasted with individuals with anorexia nervosa -- with higher levels of disgust -- who are less likely to consume excess calories over an extended timeframe (Hadigan et al., 2000).

Barrett and Simmons (2015) propose that the anterior insula is one region within an overall interoceptive network responsible for a unified homeostatic and allostatic response (Barrett & Simmons, 2015). This network uses expectations about the world based on past experiences to estimate the body's upcoming metabolic needs. In this context, any disruption in this system -- such as an underactive disgust response -- might potentially lead to an imbalance of the predicted

need of metabolic resources and slightly bias the individual to choose higher calorie foods. Over the long-term, this chronic imbalance may lead to continual intake of excess calories and associated increase in BMI.

There were several limitations to the study. Although stratifying the groups into lean (BMI < 25) and obese (BMI > 30) allows for stronger between group comparisons, the addition of an overweight group (BMI = 25-30) may yield insight into the differences in disgust response during the transition from a lean BMI to an obese BMI. Because correlative findings do not reveal causative factors, a prospective study would be critical to determine if these differences were the cause or the consequence of obesity. Next, we did not quantify the level of disgust elicited by each image. However, the disgust stimuli were modeled after examples listed in various disgust questionnaires and IAPS images. Furthermore, it seems unlikely that individuals become obese by eating contaminated foods. Instead, the stimuli used in the study were designed to elicit a general disgust response and a food-specific disgust response. Lastly, we did not screen individuals for specific dietary restrictions or guidelines (e.g. gluten-intolerance, veganism), allowing for the potential for individual brain changes inconsistent with those of a typical omnivore.

Our results are inconsistent with those reported by Houben et. al., showing that obese individuals have lower Disgust Proneness, as measured by the Disgust Scale – Revised (Houben & Havermans, 2012). We observed that obese individuals have Disgust Propensity scores equivalent to lean individuals, but have decreased Disgust Sensitivity scores when compared to lean individuals. We believe that methodological differences may have accounted for the

divergent findings – specifically the use of different disgust scales and differences in the BMI of the lean and obese groups. The Disgust Scale – Revised measures only disgust proneness and does not differentiate between two components of disgust proneness (propensity and sensitivity). In addition, Houben et. al. divided their sample into low BMI (defined as 1 standard deviation below the mean BMI) and high BMI (defined as 1 standard deviation above the mean BMI). Their low BMI group consisted of individuals with a BMI range of 13.86-18.73 and their high BMI group consisted of individuals with a BMI range of 28.79-38.96. Therefore, their low BMI group was comprised of a mix of underweight individuals (as defined by BMI < 18) or individuals with BMIs that fall in the low range of healthy weight (BMI = 18-24.9). It is not possible to distinguish whether the reported between group differences for disgust proneness are influenced by the underweight nature of the low BMI group, or if they reflect a difference between lean and obese individuals.

Our results do not exhibit the commonly observed increase in neural activation in obese individuals when viewing food cues (Davids et al., 2009; Martens et al., 2013; Dimitropoulos, Tkach, Ho, & Kennedy, 2012). This is likely due to our unique task designed to examine the effects of altered disgust rather than altered activation in response to food cues. We did not attempt to stratify the food cues into images of high- and low-calorie foods, and this potentially accounts for the failure to elicit significant differences in food-related activation between the lean and obese groups. Furthermore, the disgust cues and food-related disgust cues were interleaved with the food cues. Because the task was rapid event-related, it is possible that the intermixed disgust cues had a lingering effect that may have impacted any group differences in

food-related neural activation. A future study using both a rapid event-related design with disgust cues and a block design with only food cues could allow us to address this limitation.

Examination of differences in Disgust Sensitivity and associated neural substrates in the present study supports the notion that a reduced disgust response may contribute to obesity. The present study offers a dimensional complement to previous studies showing that individuals with anorexia nervosa have significantly elevated disgust response, specifically to food related items, as compared to controls (Aharoni & Hertz, 2012; Troop et al., 2002). That is, whereas individuals with anorexia nervosa have a heightened food disgust response, obese individuals have a diminished food disgust response (Anorexic > Lean > Obese). Because this diminished disgust response was observed at both the level of personality and at the level of neural responses, it might be possible that a decreased disgust response could encourage overeating. In the context of obesity, the disgust response may be a potential target for intervention.

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CHAPTER III: STUDY 2 -- THE ASSOCIATION OF INSULA GREY MATTER VOLUME WITH DISGUST PRONENESS

Abstract

Recent studies provide evidence that Disgust Proneness is associated with the obesity phenotype, and reduced Disgust Proneness may contribute to overeating and a higher body mass index (BMI). Although Disgust Proneness is commonly associated with insula BOLD activation, our understanding of the association of brain grey matter volume (GMV) with Disgust Proneness is limited. This study aims to identify the associations of insula subregions GMV with two primary subtypes of Disgust Proneness: Disgust Sensitivity and Disgust Propensity. Additionally, the study explores the association of BMI with insula subregion GMV. Thirty-three participants (16 obese, 17 lean) completed the Disgust Propensity and Sensitivity Scale - Revised and underwent a structural MRI session. The obese group had significantly lower Disgust Sensitivity Scores than the lean group ($p = 0.05$). Grey matter volumes in bilateral subregions of the insula were positively correlated with Disgust Sensitivity and Disgust Propensity scores. This study, in conjunction with previous publications, provides evidence for a structural and functional link within the insula responsible for Disgust Proneness behavior. Additionally, there is currently no evidence that obesity is associated with abnormal alterations of insula GMV, suggesting that behavioral differences in Disgust Proneness are primarily driven by functional, and not structural, changes in the brain.

Introduction

Disgust is a basic emotion that functions to protect the body from disease, infection, and poison and is triggered by inedible, unclean, and infectious stimuli. (Oaten et al., 2009) The disgust response likely evolved from gustatory mechanisms that protect organisms from ingesting unsafe food. (Woolley et al., 2015) The disgust response, or Disgust Proneness, can be broken into two categories: Disgust Propensity (how easily people are disgusted) and Disgust Sensitivity (how unpleasant the experience of disgust is appraised). (van Overveld et al., 2006; Olatunji et al., 2007) Recent studies provide evidence that Disgust Proneness is also associated with the obesity phenotype, and reduced Disgust Proneness may contribute to overeating and a higher body mass index (BMI). (Watkins et al., 2016) Although Disgust Proneness is commonly associated with insula BOLD activation, our understanding of the association of brain grey matter volume (GMV) with Disgust Proneness is limited. This study aims to identify the associations of insula subregions GMV with two primary subtypes of Disgust Proneness: Disgust Sensitivity and Disgust Propensity. Additionally, the study explores the association of BMI with insula subregion GMV.

Identifying insula subregions that are structurally associated with Disgust Proneness will develop a better understanding of the neural origination of Disgust Proneness. While several studies have been published on the association of Disgust Proneness and functional BOLD activation in the brain, specifically the insula, few studies have examined the structural association of Disgust Proneness. Identifying the association of insula subregion grey matter volume with behavioral Disgust Proneness will further clarify the neural underpinnings of the emotion of disgust.

Anterior insula activation has been associated with individual differences in Disgust Proneness when viewing disgusting stimuli. (Wicker et al., 2003; Wright et al., 2004; Stark et al., 2007; Mataix-Cols et al., 2008; Baumann and Mattingley, 2012) Not only visual, but disgusting olfactory stimuli results in BOLD activation within the anterior insula. (Wicker et al., 2003) Anterior insula BOLD activation also correlates with self-reported measures of Disgust Proneness while subjects viewed disgusting stimuli. (Calder et al., 2007; Mataix-Cols et al., 2008) Even presenting vocal (Phillips et al., 1998) or facial expressions of disgust result in anterior insula BOLD activation in healthy adults. (Phillips et al., 1997; Sprengelmeyer et al., 1998; Krolak-Salmon et al., 2003) Direct electrode implantation studies on pre-surgical epilepsy patients revealed ventral anterior insula activation in response to observed facial expressions of disgust. (Krolak-Salmon et al., 2003) Finally, presenting images of disgusting foods elicited anterior insula BOLD activation in lean adults (Calder et al., 2007) and reduced anterior insula BOLD activation in obese compared to lean adults. (Watkins et al., 2016)

Initial studies suggest an inverse association of Disgust Proneness with BMI. Increased levels of disgust have been associated with clinically low BMIs in individuals with eating disorders such as anorexia nervosa. (Davey et al., 1998; Troop et al., 2002) Food-related disgust is also higher in similar samples of individuals with anorexia nervosa and clinically low BMIs. (Aharoni and Hertz, 2012) Conversely, a recent study found significantly lower Disgust Proneness scores in high-BMI individuals compared to low-BMI individuals. (Houben and Havermans, 2012) Extending this finding, Watkins et. al (2016) reported that obese compared to lean individuals had lower levels of Disgust Sensitivity and reduced insula BOLD activation while viewing

images of food-related disgust. (Watkins et al., 2016) The current study seeks to determine if there are structural GMV differences similar to the functional differences observed in obese individuals.

Although functional imaging studies have identified the anterior insula as a primary region implicated in Disgust Proneness, much remains unknown about the association of structural GMV with Disgust Proneness. Additionally, preliminary research identifies differences in insula BOLD activation between lean and obese individuals but there is no data on insula GMV differences between lean and obese individuals. Therefore, this study seeks to provide evidence for a structural and functional link within the anterior insula responsible for Disgust Proneness and to determine if any observed structurally (GMV) differences are present between lean and obese individuals. We hypothesized that (i) insula subregions with an association of GMV with Disgust Proneness would be located within the anterior insula, similar to those found in functional studies and (ii) obese individuals would have lower GMV within those insula subregions.

Methods

Participants

Thirty-three participants (16 obese, 17 lean) were recruited via poster advertisements and email. Eligibility requirements included: no current medical illness, no use of psychotropic medications, no past brain trauma, and no current or past drug or alcohol abuse. Height and weight were measured during the initial screening day for BMI calculations [BMI = weight (kg) / height²

(m)]. Participants with a BMI greater than 30 were classified as obese and participants with a BMI less than 25 were classified as lean. Overweight individuals, classified as a BMI of 25-30, were excluded from the study.

Study Procedures

Each participant completed the Disgust Propensity and Sensitivity Scale – Revised (DPSS-R) before the MRI portion of the experiment. (van Overveld et al., 2006; Olatunji et al., 2007) The DPSS-R measures two independent characteristics of disgust proneness: how easily people are disgusted (Propensity) and how unpleasant a disgusting event is appraised (Sensitivity). The scale requires individuals to rate how often a statement is true based on a five point Likert rating scale capped with the options "never" and "always". Example statements include "I avoid disgusting things" (Propensity) and "When I feel disgusted, I worry that I may pass out" (Sensitivity). Study participants completed all questionnaires in the afternoon after a 4-hour fast. The study protocol was approved by the Vanderbilt University Institutional Review Board and the procedures were in accordance with the guidelines of the Helsinki Declaration on human experimentation. Participants also completed the Grand Hunger Scale, a 4-item questionnaire designed to assess hunger and appetite. (Grand, 1968)

MRI Data Acquisition

For each subject, 128 T₁-weighted volumetric 3D SPGR whole-brain sagittal images were acquired in a Phillips 3 Tesla scanner with SENSE head coil. The following parameters were used: Flip = 20 degrees, echo time = 4.2 ms, slice thickness = 1.2 mm, no skip, field of view = 24x24 cm, matrix size = 256x256 pixels for an in-plane resolution of 0.88 mm.

Analysis of Normality

Normality was determined by calculating the Fisher Z score (skewness / standard error of skewness). The data were normally distributed ($p = 0.01$) if $|\text{Fisher } Z| < 2.58$. Pearson's correlations were used for normally distributed data and Spearman's Rho correlations were used for non-normally distributed data. Two subjects were removed from the original dataset due to artifact and signal dropout located in the right parietal-occipital lobe.

Self-Reported Data Analysis

Questionnaire scale score differences between the two groups were conducted using independent t -tests. Statistical significance of the between-group differences was determined by a $p \leq 0.05$.

VBM Data Analysis

Preprocessing

Individual T_1 -weighted structural images were processed using the VBM8 toolbox implemented in the Statistical Parametric Mapping (SPM8) packages (Wellcome Department of Cognitive Neuroscience, London). Images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using default settings (i.e. high-dimensional DARTEL normalization algorithms and modulation for non-linear components). The resulting segmented images were then normalized to a study-specific mean template created in MNI space from the average of all brains in the cohort. The GM data was then smoothed with an 8 mm full-width-half-maximum isotropic Gaussian kernel. Finally, an absolute threshold value of > 0.1 was applied to remove spurious voxels. A quality check was performed using tools from the SPM

Toolbox, which revealed artifacts in the right parietal-occipital lobe of two subjects. The two subjects with artifacts were removed from the dataset.

Analysis

The primary hypothesis of the study specifically examines changes in the insula ROI. Therefore, a bilateral insula mask was created using WFUpickAtlas and dilated by 1 then applied as an explicit mask for all image analyses. Dilating the mask by 1 allows for the detection of insula grey matter volume changes without improperly constraining the area examined. We used the AFNI-based program, AlphaSim, (Cox, 1996) to run a Monte Carlo style simulation to determine the extent threshold and voxel cluster size for uncorrected P values to generate a family wise error corrected $P \leq 0.05$ for our bilateral insula mask. The extent threshold cluster size for voxel-level P values of $P \leq 0.05$ and $P \leq 0.01$ were 74 and 35 voxels, respectively. Between group differences in insula grey matter volume were examined using the SPM8 T-test module. This model allowed for the calculation of lean > obese and obese > lean contrasts.

VBM Regression Analysis

Our primary VBM regression analysis was used to examine the association of the behavioral measures of Disgust Proneness (DPSS-R scores) with GMV within the insula. We used the multiple regression module in SPM8 to examine the association of Disgust Sensitivity or Disgust Propensity scores with insula GMV. To control for the effect of group (lean vs. obese), group was modeled as a condition of no effect in our initial regression model.

A subsequent regression model, which included an interaction term of Disgust Sensitivity or Disgust Propensity scores with group (lean vs. obese), was created to determine if the association of Disgust Sensitivity or Disgust Propensity scores with insula GMV differed between lean and obese groups. A family wise error corrected P value of 0.05 was achieved at a voxel level $P \leq 0.01$ and extent threshold voxel cluster size of 35. REX was used to extract beta values from statistically significant clusters. SPSS was used to examine group-level correlations of Disgust Sensitivity and Disgust Propensity scores with extracted beta values.

Results

Demographics

Mean (min, max) BMIs of the lean and obese groups were 21.4 (19.1, 23.6) and 36.7 (30.5, 45.6), respectively. Groups were equivalent on sex (lean men = 7, women = 10; obese men = 7, women = 9) and age (lean = 25.2, obese = 27.3) (**Table 1**) BMI and age were normally distributed ($|\text{Fisher } Z| < 2.58$).

Table 1: Subject Demographics

	Group		<i>p</i> -value
	Lean (<i>n</i> =17)	Obese (<i>n</i> =16)	
Male/Female (<i>n</i>)	7/10	7/9	0.88 (χ^2)
	Mean (Min, Max)	Mean (Min, Max)	
Age	25.2 (20, 32)	27.3 (21, 35)	0.08
BMI	21.4 (19.1, 23.6)	36.7 (30.5, 45.6)	< 0.001

Behavioral

Hunger scores, Disgust Sensitivity scores, and Disgust Propensity scores were normally distributed ($|Fisher Z| < 2.58$). Groups did not differ on hunger index ($P = 0.26$, mean lean score = 19.33, 25 percentile = 16.56, 75 percentile = 22.69; mean obese score = 18.31, 25 percentile = 14.56, 75 percentile = 20.19). The obese group demonstrated statistically significant lower levels of Disgust Sensitivity (mean score = 14.5, s.d. = 3.74, min = 8, max = 22; $P = 0.05$) than the lean group (mean score = 17.2, s.d. = 3.76, min = 12, max = 26). However, there was no statistically significant difference between the groups in Disgust Propensity (mean obese score = 20.6, s.d. = 5.01, min = 12, max = 32; mean lean score = 20.7, s.d. = 3.18, min = 16, max = 26).

(Table 2)

Table 2: Disgust Propensity and Sensitivity Scale – Revised (DPSS-R) Scores

	Group		<i>p</i> -value
	Lean (<i>n</i> =16)	Obese (<i>n</i> =17)	
	Mean (Min, Max)	Mean (Min, Max)	
Disgust Sensitivity Score	17.2 (12, 26)	14.5 (8, 22)	0.05
Disgust Propensity Score	20.7 (16, 26)	20.6 (12, 32)	0.95

Between-Group Volumetric Differences

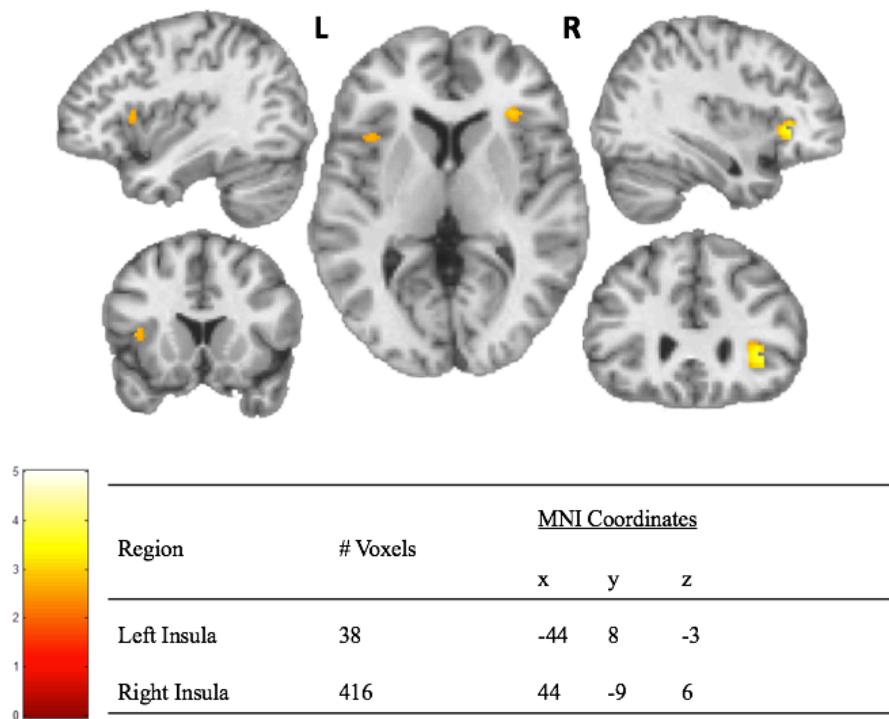
We first examined if the insula GMV differed between lean and obese groups. No between group volumetric differences were observed using a $p \leq 0.01$ with cluster size $k = 35$ for corrected family wise error ($P \leq 0.05$).

Regression Analysis

We examined whether Disgust Proneness was associated with insula GMV. Across the entire sample, Disgust Sensitivity and Disgust Propensity scores were positively correlated with insula GMV. The regression analysis examining the association of Disgust Sensitivity scores with insula GMV revealed ROIs within the left and right insula (38 voxels and 416 voxels, respectively). **(Figure 1)** The regression analysis examining the association of Disgust Propensity scores with insula GMV revealed ROIs within the left and right insula (41 voxels and 268 voxels, respectively). **(Figure 2)** The left insula clusters for Disgust Sensitivity and Disgust

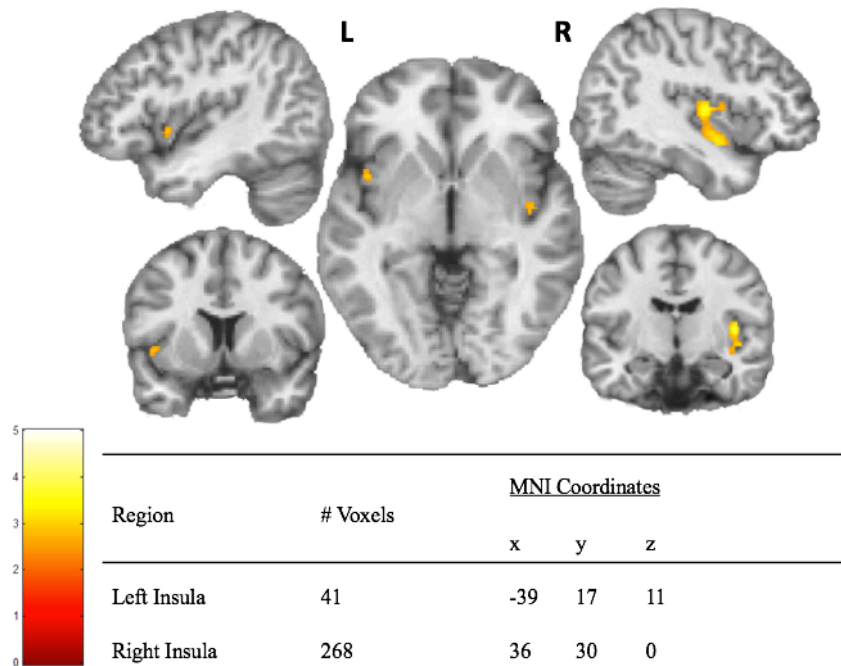
Propensity were both located in the anterior insula. However, the right insula clusters for Disgust Sensitivity and Disgust Propensity were located in the anterior insula and posterior insula, respectively.

Figure 1: Disgust Sensitivity scores positively correlate with insula grey matter volume



Note: Color bar represent T values for grey matter volume change at statistical threshold of $p < 0.01$ and extent threshold = 35 voxels for corrected FWE $p < 0.05$

Figure 2: Disgust Propensity scores positively correlate with insula grey matter volume



Note: Color bar represent T values for grey matter volume change at statistical threshold of $p < 0.01$ and extent threshold = 35 voxels for corrected FWE $p < 0.05$

The beta values were extracted from all four ROIs. Regression analyses revealed a positive correlation of Disgust Sensitivity scores with the left insula beta values ($r = 0.42$) and right insula beta values ($r = 0.45$) across the entire sample. **(Figure 3)** Regression analyses revealed a positive correlation of Disgust Propensity scores with the left insula beta values ($r = 0.39$) and right insula beta values ($r = 0.46$). **(Figure 4)**

Figure 3: Disgust Sensitivity scores positively correlate with insula grey matter volume

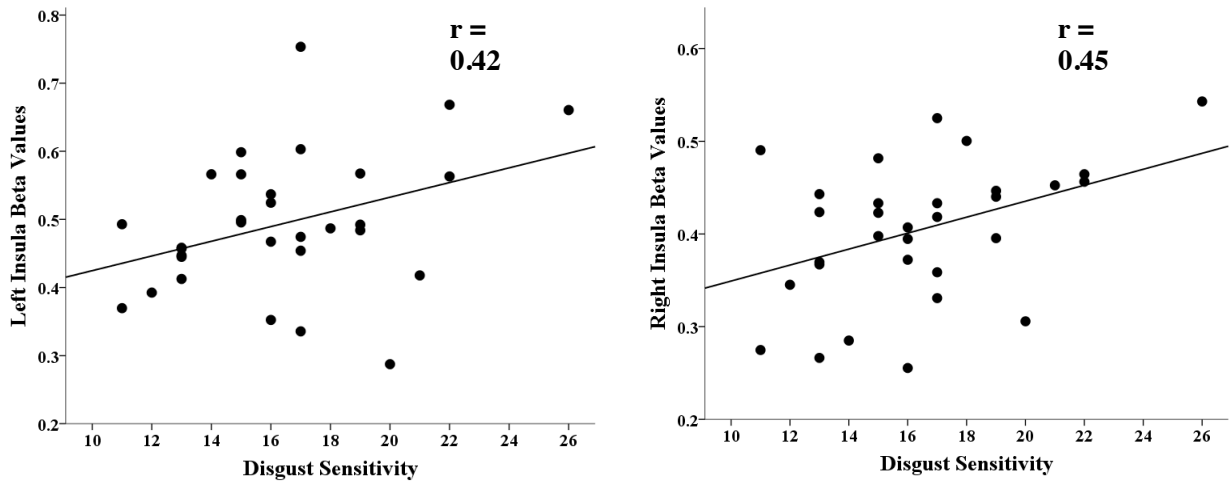
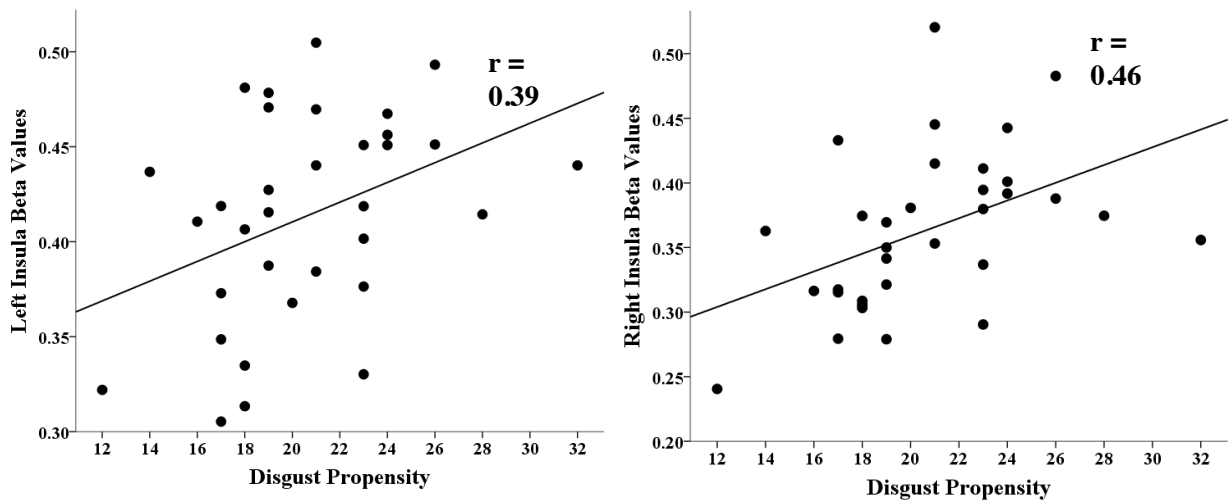


Figure 4: Disgust Propensity scores positively correlate with insula grey matter volume



We next examined whether the associations of Disgust Sensitivity scores with insula GMV differed between the lean and obese groups. Disgust Sensitivity scores were not positively correlated with extracted beta values from the left insula in the lean group but were positively correlated in the obese group (lean $p = 0.189$, $r = 0.225$; obese $p = 0.004$, $r = 0.680$). Comparison of regression coefficients revealed no statistically significant difference by group ($p = 0.848$, $t =$

0.194). Disgust Sensitivity scores were positively correlated with extracted beta values from the right insula in both groups, however the obese group was a trend level association (lean $p = 0.024$ $r = 0.545$; obese $p = 0.055$ $r = 0.488$). Comparison of regression coefficients revealed no statistically significant difference by group ($p = 0.392$, $t = -0.869$).

Lastly, we examined whether the associations of Disgust Propensity scores with insula GMV differed between the lean and obese groups. Disgust Propensity scores were positively correlated with extracted beta values from the left insula in the lean group but not the obese group (lean $p = 0.036$, $r = 0.510$; obese $p = 0.210$ $r = 0.331$). Comparison of regression coefficients revealed no statistically significant difference by group ($p = 0.176$, $t = -1.386$).

Disgust Propensity scores were positively correlated with extracted beta values from the right insula in the lean group but not the obese group (lean $p = 0.012$ $r = 0.594$; obese $p = 0.131$ $r = 0.394$). Comparison of regression coefficients revealed no statistically significant difference by group ($p = 0.109$, $t = -1.651$).

Discussion

This study examined the association of self-reported Disgust Proneness scores with insula GMV. Additionally, the study examined differences in Disgust Proneness and insula GMV between lean and obese individuals. The results indicate that obese individuals have reduced Disgust Sensitivity than their lean counterparts and that both groups have similar measures of Disgust Propensity. Therefore, obese individuals are equally as likely to experience disgust (Proneness) as compared to their lean counterparts, but were less likely to identify the experience as negative

(Sensitivity). The sample used in this analysis heavily overlaps with the sample used in the Watkins (2016) paper. (Watkins et al., 2016) Two subjects from each group were excluded for artifacts found in their structural MRI scans but replaced by subjects excluded from the prior study for artifacts found in their functional MRI scans. Though not entirely novel, these behavioral findings reaffirm that obese individuals experience reduced Disgust Sensitivity as compared to controls.

The regression analysis identified a positive association of Disgust Sensitivity and Disgust Propensity with GMV of insula subregions. The subregions observed in the left hemisphere are similarly located in the anterior insula for both Disgust Sensitivity and Disgust Propensity. However, the subregions observed in the right insula are in the anterior insula for Disgust Sensitivity and the posterior insula for Disgust Propensity. These findings identify subregions where insula GMV may be driving, or driven by, the level of behavioral Disgust Sensitivity or Disgust Propensity experienced. These structural findings overlap with published findings on the association of Disgust Sensitivity and Disgust Propensity with functional BOLD activation. (Watkins et al. 2016)

The insula subregions representing a positive association of Disgust Sensitivity with GMV lend further insight into the neural underpinnings of the behavioral Disgust Sensitivity response. A prior study revealed a positive association of Disgust Sensitivity scores with functional BOLD activation in anterior insula subregions spatially similar to those reported in the current study. (Watkins et al., 2016) Taken together, we have identified an insula subregion that is both structurally and functionally related to Disgust Sensitivity. Although the insula is commonly

considered to be a primary region for Disgust Proneness processing, we believe Disgust Sensitivity in particular is mediated by the reported subregions within the anterior insula.

The insula subregions representing a positive association of Disgust Propensity with GMV lend further insight into the neural underpinnings of the behavioral Disgust Propensity response. Prior studies reveal a positive association of Disgust Propensity scores with functional BOLD activation in insula subregions similar to those reported in the current study (Schienle et al., 2008; Schäfer et al., 2009) The left insula subregion associated with Disgust Propensity is located in the anterior insula whereas the right insula subregion is located in the posterior insula. Our results, combined with those reported by Schienle *et al* and Schäfer *et al* (2008, 2009) strongly support the notion that Disgust Propensity is associated with both structure and function within the reported subregions of the insula. (Schienle et al., 2008; Schäfer et al., 2009)

The identification of insula subregions associated with Disgust Proneness provides specific ROIs to examine before and after behavioral interventions. Abnormal Disgust Proneness has been identified as one factor contributing to eating disorders including anorexia nervosa and obesity. (Davey et al., 1998; Troop et al., 2002; Aharoni and Hertz, 2012; Houben and Havermans, 2012; Watkins et al., 2016) Psychiatric interventions, such as Cognitive Behavioral Therapy (CBT), reduce behavioral Disgust Proneness and therefore may be useful in treating individuals with anorexia nervosa. (Taboas et al., 2014; Ludvik et al., 2015) Future research endeavors should conduct longitudinal functional and structural imaging studies to examine the neurological effect of disgust-specific CBT therapy on subregions observed in the present study. Conversely, a disgust-based implicit priming task is effective at increasing Disgust Proneness in obese

individuals and subsequently decreasing preference for high-calorie foods. (Legget et al., 2015) The logical extension of such studies would be to examine the longitudinal effects on structure and function within the subregions reported in the present study. The proposed studies would shed light on whether the behavioral change precedes the neural change or the neural change precedes the behavioral change.

We found no evidence to support our hypothesis that insula GMV is associated with BMI. There were no significant insula GMV differences between the lean and obese group and the correlation of measures of Disgust Sensitivity and Disgust Proneness with insula GMV did not differ by group. The lack of between group differences in insula GMV may be due to a relatively small sample size of $n = 33$. Alternatively, it is possible that behavioral changes in Disgust Proneness are more strongly associated with functional brain changes independent of structural changes. So, although the reported insula subregions are associated with disgust proneness across all BMIs, higher BMI is only associated with altered functional BOLD activation.

The disconnect between BMI-related reductions in insula structure and insula function may be explained by differences in brain metabolism and glucose uptake between lean and obese individuals. Insulin facilitates systemic and neural glucose uptake. (Jauch-Chara et al., 2012) Thus, impaired insulin action due to insulin resistance, as observed with obesity, leads to a decrease in neural glucose uptake. (Kahn and Flier, 2000; Schwartz et al., 2000; Carvalheira et al., 2003; De Souza et al., 2005; Schwartz and Porte, 2005) Because neural glucose uptake is strongly correlated with functional BOLD activation, an impairment in neural glucose uptake, as

observed in obese individuals, may lead to a reduction in functional BOLD activation without reducing GMV within the same region. (Rothman et al., 1999; Hyder et al., 2001) This concept should be further studied and characterized by utilizing either Magnetic Resonance Spectroscopy or Chemical Shift Imaging to measure cerebral adenosine triphosphate (ATP) and phosphocreatine levels during a Disgust Proneness task.

This study, in conjunction with previous publications, provides evidence for a structural and functional link within the insula responsible for Disgust Proneness behavior. Additionally, there is currently no evidence that obesity is associated with abnormal alterations of insula GMV, suggesting that behavioral differences in Disgust Proneness are primarily driven by functional, and not structural, changes in the brain. The identification of specific insula subregions responsible for Disgust Proneness should be used to determine the neural efficacy of future disgust-related interventions.

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CHAPTER IV: STUDY 3 -- DETECTING PARENCHYMAL DENSITY CHANGES WITH T₁-WEIGHTED MRI

Abstract

Brain function is dependent upon a healthy parenchyma (structural organization of glia and neurons). Obesity, the related increase in pro-inflammatory cytokines, and development of insulin and leptin resistance, lead to neuroinflammation and parenchymal changes.

Investigators have developed novel techniques utilizing MRI in order to address the methodological limitations of exploring the parenchymal changes associated with obesity in humans. This study seeks to explore the viability of using T₁-weighted MRI scans to identify parenchymal density changes (density of all cells) in human subjects before and after weight loss and insulin detemir treatment. The study was designed to expand upon data suggesting that single echo T₂-weighted MRI is sensitive enough to quantify signal intensity changes that are positively correlated with BMI. We examined the mediobasal hypothalamus because of its known function in feeding and a subregion of the insula for its known role in the disgust response. Both regions may have cellular level structural underpinnings responsible for the functional differences reported in obese groups. Our null results suggest that T₁-weighted MRI is not a suitable alternative to single echo T₂-weighted MRI or quantitative T₂ mapping.

Introduction

Brain function is dependent upon a healthy parenchyma (structural organization of glia and neurons). Accumulating evidence suggests that chronic inflammation, as a function of both nutrient excess and excess adipose tissue, plays a major role in the pathogenesis of parenchymal-related neural dysfunction. (Kaiyala et al., 2000; Cai, 2009; Thaler and Schwartz, 2010; Cai and Liu, 2012; McNay et al., 2012) Beyond functioning as a long-term energy storage organ, adipose tissue plays a key role in the balanced secretion of pro- and anti-inflammatory adipokines. (Thaler and Schwartz, 2010; Ouchi et al., 2011) When excess adipose tissue is stored, there is greater release of pro-inflammatory adipokines (e.g. CRP, IL-6, IL-1 β , TNF α) than anti-inflammatory adipokines (e.g. adiponectin, SFRP5) (Lam et al., 2005; Milanski et al., 2009; Olefsky and Glass, 2010; Zhou and Rui, 2013), which may contribute to neural insulin and leptin resistance. Both peripheral and neural insulin and leptin resistance, as well as an increase in pro-inflammatory cytokine production, are associated with neuroinflammation in areas abundant in insulin and leptin receptors. (Huang et al., 1996; Bjørbaek et al., 1998; Münzberg et al., 2004; Lam et al., 2005; Fam et al., 2007; Zhou and Rui, 2013; Schur et al., 2015) Thus, obesity, the related increase in pro-inflammatory cytokines, and development of insulin and leptin resistance, lead to neuroinflammation and parenchymal changes.

The alterations in brain parenchyma associated with obesity have yet to be fully characterized, especially in humans. Direct, yet invasive, techniques are commonly used in rodent studies. Stereological techniques have been used to identify regional brain volume and neural density changes in the hypothalamus of obese mice. (Namavar et al., 2012) Immunohistochemical

techniques provide greater specificity for cell-type specific changes in the brain parenchyma. Immunohistological techniques have been used to identify morphological changes and increases in the density of astrocytes and microglia within the hypothalamus of rodents fed high fat diet. (Thaler and Schwartz, 2010; Cai and Liu, 2011; Thaler et al., 2012; Buckman et al., 2013; Cai, 2013) These cellular changes occur on a bimodal timeline. Effects are observed after two days of *ad libitum* access to high fat diet, disappear after one week, re-emerge within two weeks, and persist with continued high fat diet intake. (Thaler et al., 2012) Although these invasive techniques allow for direct measurement of parenchymal alterations, they are not suitable for human studies.

Investigators have developed novel techniques utilizing MRI in order to address the methodological limitations of exploring the parenchymal changes associated with obesity in humans. This technique – building from rodent and human studies correlating T₂-weighted MRI signal with post-mortem tissue gliosis in patients with neurodegenerative disease – quantifies subtle changes in T₁- or T₂-weighted signal as a marker of parenchymal density change. (Marshall et al., 1988; Coulthard et al., 1999; Briellmann et al., 2002) Simply stated, an increase in signal intensity on a T₂-weighted scan or a decrease in signal intensity on a T₁-weighted scan suggests a general increase in parenchymal density (defined as an increase in neural or glial density). Using this method, Thaler and colleagues (2012) report a positive correlation of T₂-weighted signal in human mediobasal hypothalamus (MBH) with body mass index (BMI). (Thaler et al., 2012) Their findings suggest that an increase in T₂-weighted signal is associated with obesity and parenchymal changes in the MBH. Because the MBH is the primary feeding center in the brain (Anand and Brobeck, 1951; Schwartz et al., 2000), it is plausible that the

observed parenchymal changes in the MBH result in functional changes in feeding behavior and overconsumption.

The initial reports of the Thaler et al paper (2012) led us to examine existing longitudinal data using the same methodology. One important note about the clinical imaging parameters used by Thaler and our group – these clinical MRIs only use a single echo to acquire the structural image. Single echo scans are susceptible to multiple types of variability that influence the baseline values critical to studies of neuroinflammation. Daily differences in scanner environment, and individual differences within the same subject require single echo structural scans to be internally normalized for every scan for each subject. Data are normalized by sampling the baseline signal from a control region presumed to be unaffected by neuroinflammation and parenchymal change. (Briellmann et al., 2002, 2004; Thaler et al., 2012) The major caveat to this method is that it is impossible to ensure the control region is not affected by neuroinflammation. Despite this caveat, single echo structural images may be useful because: (1) they are quicker to acquire, (2) they are commonly used in the clinical and general research setting, and (3) major data repositories are likely to only have T_1 or T_2 -weighted scans, and not quantitative T_2 maps. These reasons provide a strong rationale for exploring the viability of using single echo structural scans to explore parenchymal changes in clinical populations such as obesity – even when a more sensitive and specific imaging method exists (detailed in the discussion section).

This study seeks to explore the viability of using T_1 -weighted MRI scans to identify parenchymal density changes in human subjects before and after weight loss and insulin detemir treatment. The longitudinal design allows us to conduct a within-subject analysis of parenchymal change.

Additionally, the inclusion of a healthy lean group allows us to conduct a between-group analysis of baseline parenchymal density. The entire cohort allows us to examine: (1) the association of BMI with parenchymal density (2) the combined effects of insulin detemir and weight loss on parenchymal density and (3) if T₁ MRI is sensitive enough to detect initial differences or longitudinal changes in parenchymal density.

The two regions of interest are the mediobasal hypothalamus (MBH) and insula. The inclusion of both regions is based on evidence of structural and functional deficits associated with obesity. The MBH is the major feeding center in the brain and contains both orexigenic and anorexigenic cell populations. (Schwartz et al., 2000; McNay et al., 2012) In lean individuals, these opposing cell populations are balanced and function to sustain a homeostatic energy balance. In obese individuals, these opposing cell populations are imbalanced, which results in greater drive of the orexigenic cell population, increased feeding, and subsequent obesity. (Morton et al., 2006; McNay et al., 2012) In this model, cellular level structural changes lead to changes in neural function and ultimately feeding behavior. These structural changes are caused by chronic neuroinflammation and increased parenchymal density (gliosis) within the MBH.

The insula is responsible for many functions, including the disgust response. Recent studies show that obese individuals have reduced Disgust Proneness and reduced activation within the insula during a disgust task. (Watkins 2016, *submitted*) Although insula grey matter volume is associated with behavioral disgust proneness, lean and obese subjects have equivalent insula grey matter volume. (Watkins 2016, *submitted*) Interestingly, hypothalamic grey matter volume does not differ between lean and obese groups either. (Carnell et al., 2012; Yokum et al., 2012;

Shefer et al., 2013) It is possible that the underlying cause of the observed functional and behavioral changes associated with the insula are driven by cellular level structural changes similar to those observed in the hypothalamus. Therefore, we will use the T₁-weighted imaging method to examine between-group differences and longitudinal changes within the insula.

If we can successfully use T₁ MRI to identify changes in parenchymal density in humans, then we can use this noninvasive tool to test the efficacy of future weight loss interventions. Weight loss is typically only temporary, but monitoring parenchymal density change in obesity may be the key to successful, long-term weight loss.

We hypothesize that: (1) the obese diabetic group, compared to the lean group, will have lower normalized mean signal intensities extracted from the MBH and insula, (2) in the obese group only, mean signal intensity extracted from the MBH and insula will correlate with measures of adiposity and insulin function, and (3) in obese diabetics, mean signal intensity will increase as a function of the combinatory factors of weight loss and insulin detemir administration.

Methods

Participants

Demographics

Lean and obese diabetic participants were recruited via the Vanderbilt Eskin Diabetes Clinic and primary care clinic and by advertisements. Participants completed an online survey to

determine eligibility. During multiple visits, participant height, weight, BMI, adiposity, insulin levels, and glucose levels were recorded.

Eligibility Requirements

Eligibility requirements included: (1) between 31-50 years of age, (2) body mass index (BMI) between 18-25 kg/m² or 30-59 kg/m², (3) body weight < 350 lbs, (4) (5) stable body weight during the previous 3 months with less than 5 lbs self-reported weight change, (6) type 2 diabetes, insulin naive (except for use during gestational diabetes), on either metformin, sitagliptin, dipeptidyl-4 inhibitor, or thiazolidines, (7) HbA1c levels between ~6-9%, (8) abstain from alcohol and exercise 48 hours before visits, (9) able and willing to follow prescribed meal plans.

Exclusion Criteria

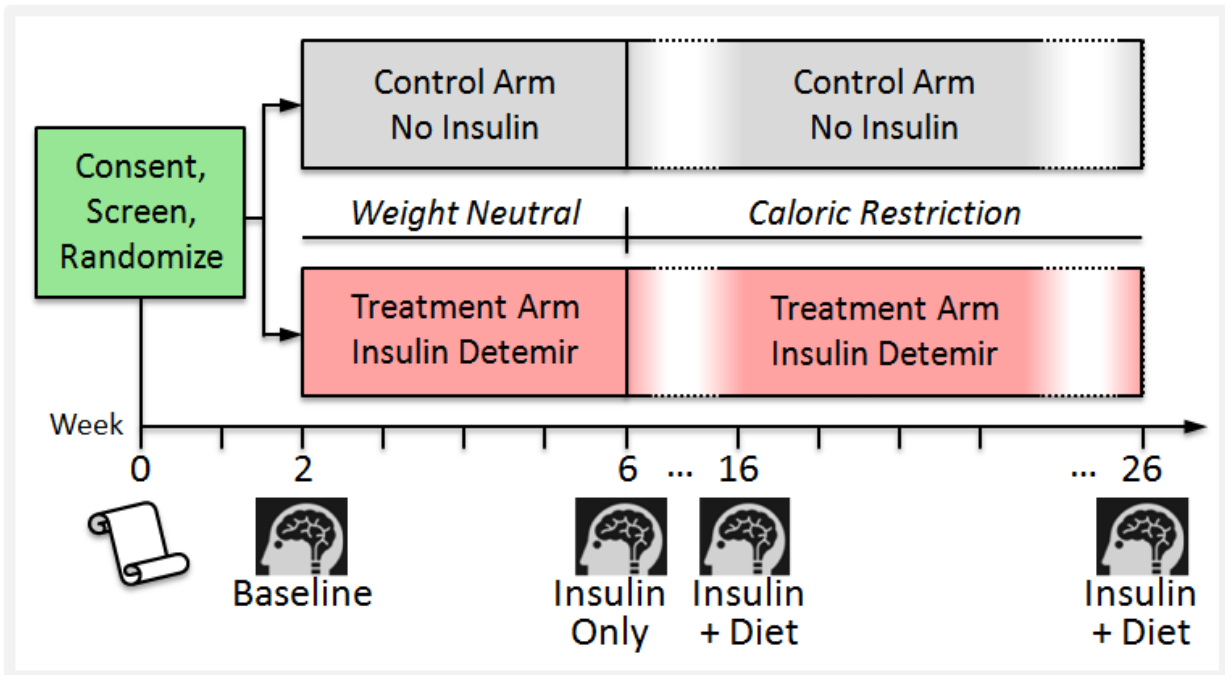
Exclusion criteria include: (1) known or suspected hypersensitivity to insulin detemir, (2) significant comorbidities (cardiovascular disease, metabolic disease, liver or renal insufficiency), (3) anemia, (4) hypertension requiring more than 2 medications to control or uncontrolled hypertension (resting BP >140/90), (5) history of substance abuse, (6) tobacco use, (7) history of psychiatric disorder, (8) use of centrally acting, psychotropic medications, (9) and contradiction which would interfere with MRI imaging, (10) females who are pregnant or breast-feeding, (11) obesity induced by other endocrine disorders (Cushing Syndrome, Polycystic Ovarian Syndrome), (12) postmenopausal female, (13) previous surgery for weight loss, (14) appetite suppressing supplement use within the last 6 months, (15) dietary supplements such as EPA, DHA, or omega-3 fatty acids.

Study procedures

The study lasted 26 weeks with 2 weeks of run-in and then 24 weeks of intervention.

Participants were first given informed consent and screened to determine eligibility based on inclusion and exclusion criteria (week 0). Participants visited with a registered dietician and registered nurse weekly from week 0-6, then biweekly until the study ended. Week 2 was the first of four study visits that occurred at weeks 2, 6, 16, and 26. Study visits measured the main study outcomes including body weight, adipose tissue, and MRI scans. Randomization and drug intervention (insulin detemir) occurred at completion of the first study visit (week 2). From week 0-6, participants were asked to maintain body weight. Therefore, week 2-6 of the study was the *Weight Neutral* phase of the intervention. After the second study visit (week 6), all obese participants started the *Weight Loss* phase, which included a hypocaloric diet intervention from week 6-26. Lean subject only completed the first study visit (week 2), including measures of body weight, and MRI scans. Ultimately, there were 3 total groups: (1) lean, (2) obese with weight loss intervention, and (3) obese with weight loss intervention and insulin detemir treatment. **(Figure 1)**

Figure 1: Study design



Note: Control group (n = 18) was scanned at baseline (week 2) only

Insulin Detemir Treatment (1/2 of obese subjects; weeks 2-26)

Insulin detemir was administered subcutaneously, once daily, in doses ranging from 0.1 U/kg to 0.6 U/kg. The dosing regimen strategy was similar to the "303" algorithm, where bedtime insulin doses were titrated up by 3 units until AM fasting sugars within the prescribed protocol range to achieve (90-110 mg/dl). The goal of the insulin therapy was to achieve near normoglycemia with a low rate of hypoglycemic episodes. The insulin detemir-naïve group did not receive a placebo injection. Homeostatic model assessment of insulin resistance (HOMA-IR) scores were calculated by using the following formula: $([\text{Glucose mg/dL} \times \text{Insulin mU/L}] / 405)$

Dietary Intervention (all obese subjects; weeks 6-26)

Both groups were exposed to a modest caloric restriction dietary intervention of 15% from calculated needs. Study dietitians calculated the caloric prescription for weight loss by subtracting 15% kcal from measured total energy expenditure via doubly labeled water or measured resting energy expenditure multiplied by a factor of 1.3 to adjust for total energy expenditure.

MRI data acquisition

Fat-Water Imaging (FWI)

Lean and adipose tissue volumes/masses were measured for every obese participant at every study visit. Participants were in a supine position with arms extended about the head, positioned to enter the scanner feet-first. A multi station protocol with multiple (~17) table positions were used to acquire whole-body data. Each position consisted of a multi-slice, multi-echo gradient echo (fast field echo, FFE) acquisition with 12 slices, slice thickness 8 mm, zero slice gap. Other acquisition details include: TR/TE1/TE2/TE3 [ms] = 75/1.34/2.87/4.40; FA=20° (QBC for transmit and receive); water-fat shift (WFS) = 0.325 pixels (BW=1335.5 Hz/pixel); field of view (FOV) = 500mm x 390 mm, acquired matrix size = 252 x 195; acquired voxel size = 2mm x 2mm x 8mm. FWI data was stripped of identifiers and reconstructed and analyzed by collaborators Dr. Joel Kullberg and Lars Johansson under a sub-contract to the University of Uppsala. After post-processing, values for total lean tissue (TLT), total adipose tissue (TAT),

subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT), were reported to Dr. Kevin Niswender of Vanderbilt University.

Structural Brain MRI

Multiple brain images were acquired on each study day. However, only pertinent brain image parameters are discussed. High resolution T1-weighted anatomical images were acquired using a 3T Intera Achieva (Phillips Medical Systems, Andover, MA). 3D T₁-weighted inversion recovery (IR) - turbo fast echo (TFE) scans were acquired with isotropic 1mm³ resolution (voxel size = 1x1x1mm), TI/TR/TE = 1000/9.9/4.6 ms, flip angle 8°, scan time = 6.5min. T₁-weighted images were examined for artifacts. No pre- or post-processing was done on the images in order to preserve the raw signal intensity values. Images were never combined into a group analysis, and therefore were not coregistered for group analysis.

Analysis of normality

Normality was determined by calculating the Fisher Z score (skewness / standard error of skewness). The data were normally distributed ($p = 0.01$) if $|\text{Fisher Z}| < 2.58$. Pearson's correlations were used for normally distributed data and Spearman's Rho correlations were used for non-normally distributed data. T-tests and Paired T-tests were performed for normally distributed data. The Mann-Whitney U test was used to compare group means of non-normally distributed data. Non-normally distributed data were log transformed (LG10 function in SPSS) prior to running Paired T-tests to satisfy the normal-theory-based assumption required to perform Paired T-test.

Region of interest signal extraction protocols

Signal intensity was extracted from T₁-weighted images across two adjacent slices using the program, MIPAV. All ROIs were visually inspected for abnormalities or artifact. Mean signal intensity was calculated for each lateral region by averaging the values extracting from two adjacent slices. The regions of interest (ROIs) included the: mediobasal hypothalamus (MBH), insula subregion, amygdala, and putamen. ROIs were located using the following protocols:

MBH: *Coronal*: locate mammillary bodies in medial position; move 2-3 slices posterior; MBH is situated in most ventral portion and anterior to the descending white matter tracts (located 4-5 slices posterior from the mammillary bodies). *Sagittal*: Crosshairs fall slightly anterior to the pituitary gland. Sample 6 voxels (2 horizontal X 3 vertical) per slice, for a total of 12 voxels per sample. There must be at least a 1 voxel buffer between the ventricles and the sampled region. This buffer reduces the chance of partial voluming, where the signal intensity of that voxel is the mean of signal from the high intensity ventricle and the lower intensity grey matter of the MBH. Partial volume effects are particularly likely in smaller structures that border different tissue types, like the MBH.

Insula: *Coronal*: third ventricle opening to mammillary bodies; symmetrical "wing" shaped ventricles across the y axis; ventral most portion of the y-shaped insula region. *Sagittal*: 3-4 slices lateral of the most medial portion of the insula; eye sockets become visible; clear view of pre-, post-, and central-sulci. *Axial*: slightly dorsal to visible eye sockets; clear view of the dorsal brain stem; posterior of the y-shaped insula region. Sample 9 voxels per slice (3 x 3) for a total of 18 voxels per sample.

Amygdala: *Coronal*: located in the same slices as MBH; large grey matter area ventral and lateral to the MBH. *Axial*: anterior and medial to the hippocampus. Sample 12 voxels per slice (4 horizontal X 3 vertical) for a total of 24 voxels per sample.

Putamen: *Coronal*: located in the same slice as MBH and amygdala; large grey matter area resembling "pie crust"; medial to insular sulcus. *Axial*: Oblong, horn-shaped grey matter structure lateral to the third ventricle and anterolateral to the lateral ventricles. Sample 12 voxels per slice (3 horizontal X 4 vertical) for a total of 24 voxels per sample.

Normalizing ROI signal intensities

We followed the signal intensity protocol established by Thaler et al (2012). (Thaler et al., 2012) Ratios were calculated by comparing the mean extracted signal intensity of the MBH with the mean extracted signal intensity of the ipsilateral amygdala. The same control region (ipsilateral amygdala) was used for the mean extracted signal intensity of the insula. A control ratio compared the mean extracted signal intensity of the amygdala with the mean extracted signal intensity of the ipsilateral putamen. This control ratio provided a mathematical rationale for using the ipsilateral amygdala ROI as a control for our experimental ROIs.

Between-group analysis

Data were analyzed using SPSS with either Students T-tests or Mann-Whitney U tests to determine if extracted signal intensity values differed between lean and obese groups at baseline. Tests were chosen based on whether the data were normally distributed. Signal intensities were extracted with the MRI software, MIPAV. Signal intensity from the MBH and insula ROIs was normalized to signal intensity extracted from the amygdala ROI. If the signal intensity extracted

from an ROI significantly differed by group ($p = 0.05$), the signal from that ROI was examined in the next phase (regression analysis). If the signal intensity extracted from an ROI did not significantly differ by group, there was no rationale for examining the association of adiposity measures, BMI, or HOMA-IR, because those ROI signal intensities were considered to be within the normal range (as compared to the lean, healthy controls).

Regression analysis

To test the association of obesity and insulin function with our measure of parenchymal density (gliosis), extracted signal intensities from ROIs that differed significantly from the lean control group were correlated with measures of adiposity (visceral, subcutaneous, and total), BMI, and HOMA-IR scores. Regression analyses only included data from the obese group to avoid presenting results with statistical overlap with the between-group analyses.

Longitudinal analysis

To examine changes in parenchymal density after insulin detemir treatment, we used Paired T-tests to compare within-subject mean ROI signal intensities at week 2 (baseline) and week 26 (study end). HOMA-IR scores, however, were sampled at week 6 and week 26. This was due to the nature of how HOMA-IR scores are calculated. Insulin-naive diabetic patients have low insulin levels by definition and introducing insulin detemir artificially increases measured insulin levels. Week 6 was the earliest point where insulin and glucose levels were measured after insulin detemir treatment began, and therefore is the earliest HOMA-IR value that can be appropriately compared to the week 26 values.

Results

Demographics

Forty-nine participants (31 obese, 18 lean) in total were included in the analyses. For the between-groups analysis, 37 participants (19 obese, 18 lean) were included. The mean (min, max, standard deviation) BMI was 22.8 (19.0, 25.1, 1.95) for the lean group and 36.3 (29.7, 49.8, 5.32) for the obese group. **(Table 1)** For the within-subjects analysis, 20 obese subjects receiving insulin detemir were included. The mean (min, max, standard deviation) BMI was 36.2 (29.9, 41.0, 3.18). **(Table 2)** A total of eight obese subjects were included in both analyses. Tables 1 and 2 include biological measures, including BMI, adiposity, and HOMA-IR scores throughout applicable timepoints.

Table 1: Demographics for between-group participants

	Group		<i>p</i> -value
	Lean	Obese	
Male/Female (<i>n</i>)	9/9	10/9	
	Mean (Min, Max) S.D.	Mean (Min, Max) S.D.	
BMI	22.83 (19.0, 25.1) 1.95	36.29 (29.7, 49.8) 5.32	< 0.001
Adipose Tissue		53.47 (38.6, 74.6) 11.57	
Visceral AT		7.06 (3.3, 13.5) 2.95	
Subcutaneous AT		12.02 (6.3, 19.2) 3.67	
Lean Tissue		44.26 (31.3, 62.9) 9.10	
HOMA-IR		9.20 (4.43, 19.01) 4.19	

Table 2: Demographics of within-group participants (obese diabetics only)

	Timepoint		<i>p</i> -value
	Week 2	Week 26	
Male/Female (<i>n</i>)	10/10	10/10	
	Mean (Min, Max) S.D.	Mean (Min, Max) S.D.	
BMI	36.27 (29.9, 41.0) 3.18	33.14 (26.8, 37.8) 3.16	< 0.001
Weight (kg)	107.48 (77.1, 129.7) 14.31	98.2 (68.2, 123.6) 14.48	< 0.001
Adipose Tissue	52.42 (38.6, 68.2) 8.40	44.81 (31.1, 61.5) 7.97	< 0.001
Visceral AT	7.25 (3.3, 12.5) 2.77	6.15 (2.7, 11.5) 2.46	< 0.001
Subcutaneous AT	11.55 (6.3, 15.6) 2.74	9.57 (4.9, 13.4) 2.52	< 0.001
Lean Tissue	44.98 (32.7, 61.4) 8.78	42.63 (30.1, 58.8) 8.63	< 0.001
HOMA-IR*	16.83 (3.67, 65.91) 16.07	12.00 (1.78, 30.48) 8.90	0.11

* baseline measurement taken from week 6

Normality

Fisher Z tests revealed that the extracted signal intensity from two ROIs was not normally distributed. For both the between-group and within-group datasets, the signal intensity extracted from the Left MBH and Left Insula ROIs were not normally distributed. The appropriate statistical tests were used for the non-normal data. All other ROIs and biological measures were normally distributed ($|\text{Fisher } Z| < 2.58; p = 0.01$).

Between-group signal intensity

There were no significant group differences for signal intensity extracted from the right or left MBH or left insula ROIs ($p = 0.45$, $p = 0.15$, $p = 0.39$; respectively). There was a statistically significant group difference for signal intensity extracted from the right insula ROI ($p = 0.05$).

(Table 3) Therefore, the obese group right insula ROI was further analyzed in the regression analysis phase of the study. Of note, the significant between-group difference may be influenced by abnormally low signal intensity in the right insula of the lean group, rather than an abnormally high signal intensity in the right insula of the obese group. When statistically examining the values within each group, we found that the right insula signal intensities for the lean group were statistically lower than the left insula signal intensities for the lean group ($p = 0.01$). The left and right insula signal intensities did not differ within the obese group ($p = 0.07$). Therefore, the lean group right insula ROI was further analyzed in the regression analysis phase as well. The control ROI, signal intensity extracted from the amygdala and normalized to putamen, did not differ by group for the left of right hemisphere ($p = 0.62$, $p = 0.71$; respectively).

Table 3: Between-group differences in baseline signal intensity

	Group		<i>p</i> -value
	Lean	Obese	
	Mean (Min, Max) S.D.	Mean (Min, Max) S.D.	
Right MBH	1.040 (.81, 1.41) 0.14	1.007 (0.84, 1.23) 0.11	0.45
Left MBH	1.090 (.89, 1.41) 0.14	1.022 (0.89, 1.35) 0.10	0.15
Right Insula	0.750 (0.48, 0.89) 0.10	0.823 (0.56, 0.99) 0.11	0.05
Left Insula	0.839 (0.75, 1.02) 0.08	0.877 (0.72, 1.13) 0.13	0.39

* values normalized to Amygdala

Regression analysis

The signal intensity extracted from the right insula was significantly higher in the obese group than the lean control group. Therefore, the signal intensity extracted from the right insula of obese diabetics was examined for associations with markers of insulin sensitivity (HOMA-IR), and body composition (BMI, adipose tissue, visceral AT, subcutaneous AT, and lean tissue). For the obese diabetic group, right insula signal intensity was not associated with any markers of insulin sensitivity or body composition. **(Table 4)** Only BMI was measured for the lean group. However, there was no significant association of right insula signal intensities with BMI for the lean group ($p = 0.51$, $r = 0.16$).

Table 4: Right insula signal intensity is not associated with biological markers in obese diabetics

Right Insula Signal Intensity		
	<i>p</i> -value	<i>r</i> *
HOMA-IR	0.93	-0.02
BMI	0.58	-0.15
Adipose Tissue	0.27	-0.27
Visceral AT	0.34	-0.24
Subcutaneous AT	0.59	-0.14
Lean Tissue	0.21	-0.31

* Pearson correlation coefficient

Longitudinal analysis

There were no statistically significant changes in mean signal intensity after 24 weeks of insulin detemir administration and 18 weeks of weight loss intervention. Paired T-tests revealed slight decreases in mean signal intensity for bilateral MBH and insula, but no changes were statistically significant. **(Table 5)** The insulin detemir administration and calorie restriction weight loss intervention was successful at reducing BMI, weight, adipose tissue, visceral adipose tissue, subcutaneous adipose tissue, lean tissue, and HOMA-IR scores. **(Table 2)** It is important to note that the HOMA-IR baseline measurements were recorded at week 6, instead of week 2. This is due to the effect of exogenous insulin detemir administration on the HOMA-IR formula. Patients with type 2 diabetes have low basal insulin levels, and therefore have low baseline HOMA-IR scores. Administering insulin detemir to these patients artificially increases their HOMA-IR scores, simply by introducing exogenous insulin. Week 6 was the first available timepoint where

HOMA-IR was measured and subjects were receiving insulin detemir. Thus, we used the HOMA-IR scores from week 6 as the baseline.

Table 5: Insulin detemir and weight loss intervention does not lead to changes in signal intensity

	Timepoint		<i>p</i> -value
	Week 2	Week 26	
	Mean (Min, Max, S.D.)	Mean (Min, Max, S.D.)	
Right MBH	1.041 (0.88, 1.19, 0.09)	1.036 (0.89, 1.24, 0.09)	0.7
Left MBH	1.052 (0.92, 1.41, 0.12)	1.046 (0.88, 1.35, 0.11)	0.62
Right Insula	0.852 (0.60, 1.05, 0.13)	0.847 (0.61, 1.03, 0.12)	0.57
Left Insula	0.837 (0.67, 1.17, 0.12)	0.833 (0.66, 1.31, 0.11)	0.63

* values normalized to Amygdala

Discussion

In this study, we investigated if T₁-weighted MRI is sensitive enough to quantify signal intensity differences between lean and obese diabetic groups. Additionally, we investigated if signal intensity changed from baseline after weight loss and insulin detemir administration. The study was designed to expand upon data suggesting that single echo T₂-weighted MRI is sensitive enough to quantify signal intensity changes that are positively correlated with BMI. (Thaler et al., 2012) Our results do not support any of our original hypotheses: (1) the obese diabetic group, compared to the lean group, will have lower mean signal intensities extracted from the MBH and insula, (2) in the obese group only, mean signal intensity extracted from the MBH and insula will

correlate with measures of adiposity and insulin function, and (3) in obese diabetics, normalized mean signal intensity will increase as a function of the combinatory factors of weight loss and insulin detemir administration. Together, our results suggest that T_1 -weighted MRI is likely not a suitable alternative to single echo T_2 -weighted MRI or quantitative T_2 mapping. The absence of a positive control marker of parenchymal density change prevents us from fully asserting that T_1 -weighted MRI is insensitive to changes in parenchyma in humans.

This study utilized a mixed factorial design, combining both between-group and within-group design. The mixed factorial design allowed us to analyze baseline signal intensity differences between lean and obese diabetic groups and examine the long-term effects of weight loss and insulin detemir intervention on signal intensity within the obese diabetic group. Although the study was designed appropriately to correctly test our hypotheses, the MRI methodology was not potentially sensitive enough to quantify subtle changes in signal intensity. This is likely due to using T_1 - instead of T_2 -weighted images or quantitative T_2 maps.

T_1 - and T_2 -weighted MRI both rely on measuring the changes in interactions between water molecules and tissue macromolecules to assess the spatial distribution between grey and white matter. (Crooks et al., 1987; Tofts and du Boulay, 1990; Miot-Noirault et al., 1997) Small changes in the interactions of molecules can be measured to detect lesions within the grey and white matter of the brain. However, T_1 - and T_2 -weighted images are differentially sensitive to changes in molecule interactions by tissue type. In particular, T_1 is better at detecting the spatial distribution of myelin-bound cholesterol within brain white matter. (Koenig, 1991) Thus, T_1 is most commonly used in clinical populations suffering from white matter lesions or atrophy, such

as Multiple Sclerosis. (Neema et al., 2007; Polman et al., 2011) Conversely, T_2 relates to proton transfers, molecular exchange, and diffusion of water, making it most suitable for imaging changes in cellular density changes in grey matter, which is low in cholesterol and fat. (Miot-Noirault et al., 1997; Barkovich, 2000) Our null results, in combination with the positive results reported by Thaler *et al* (2012), support the notion that T_2 -weighted imaging is the preferred imaging sequence for the quantification of small changes in grey matter cellular density.

It is important to discuss the method of using the ratio of T_1 - and T_2 -weighted images acquired from the same subject. This ratio eliminates the MR-related intensity bias and enhances the contrast to noise ratio for myelin. (Glasser and Van Essen, 2011; Ganzetti et al., 2014)

Unfortunately, this method further decreases sensitivity to signal changes in grey matter.

Not only is T_1 -weighted imaging relatively insensitive to grey matter signal intensity, the use of single echo imaging presents an issue of signal normalization. Single echo scans are susceptible to multiple types of variability that influence the baseline values of the acquired signal intensity. Therefore, each scan must be internally normalized in order to compare the data between groups or within subjects. For this study, we followed the normalization protocol described by Thaler et al (2012). Signal intensity from the ipsilateral amygdala was used to normalize the signal extracted from the MBH and insula ROIs. Although this normalization approach has a mathematical rationale, there is no evidence to support its biologic rationale. Almost all of the studies on diet- and obesity-induced parenchymal changes (gliosis) look at the MBH. There are no IHC studies that examine the amygdala, and therefore no evidence to suggest the amygdala is unchanged by diet and obesity. This issue extends to the majority of brain regions, with the

exception of the hippocampus, which is associated with changes in neural density. (Namavar et al., 2012) Quantitative T₂ mapping addresses this issue.

The advantage of quantitative T₂, compared to single echo imaging, is the unbiased quantitative nature of the values obtained. Quantitative T₂ can reliably detect subtle differences in T₂ relaxation times, which allow researchers to identify subregion-level changes in parenchyma. (Bernasconi et al., 2000; Wendel et al., 2001; Briellmann et al., 2004; Lee et al., 2013; Berkseth et al., 2014) Multiple echoes allows the researcher to extrapolate a single T₂ signal intensity value that does not need to be normalized to a control region. A T₂ map can be created by calculating T₂ values using an exponential fit function, signal intensity at echo time t , $S(t) = S_0 \times e^{-t/T_2}$. Thus, the T₂ signal intensity value is normalized within the values of the multiple echoes acquired. (Briellmann et al., 2004; Lee et al., 2013; Berkseth et al., 2014; Schur et al., 2015) The fundamental drawback to using quantitative T₂ is the increased time required to acquire each scan, limiting its use in the clinical setting. In research however, quantitative T₂ has been used to successfully identify increases in T₂ signal intensity within the hypothalamus of obese rodents and humans. (Lee et al., 2013; Berkseth et al., 2014; Schur et al., 2015)

Using quantitative T₂ and immunohistochemistry (IHC), an initial study by Lee et al (2013) reports that longer T₂ relaxation times are a marker of increased parenchymal density (gliosis). (Lee et al., 2013) Longer T₂ relaxation times within the MBH are reported in mice fed a 60% high fat diet for 21 weeks, when compared to a control group fed a 12% fat chow diet. IHC shows that the same mice on high fat diet have increased astrocyte and microglia density, compared to controls. For the mice fed high fat diet, astrocyte density is positively correlated with T₂ relaxation times within the MBH. (Lee et al., 2013) A follow up study in mice also

detected diet- and obesity-induced increases in parenchymal density that were measured with quantitative T_2 and confirmed with IHC. (Berkseth et al., 2014) Combined quantitative T_2 MRI and IHC analysis on post-mortem human brains yielded similar results. (Schur et al., 2015) IHC measures of parenchymal density correlated with T_2 relaxation times, suggesting that quantitative T_2 is the best current method available for detecting parenchymal density in human brains.

There was one statistically significant result in our between-group baseline analysis. The obese group had significantly higher signal intensity values in the right insula when compared to controls. Unfortunately, this effect likely reflects a lower mean signal in the lean group, rather than a higher signal in the obese group. The mean signals extracted from the bilateral insula ROIs of the lean group are significantly different ($p = 0.01$), whereas the mean signals extracted from the bilateral insula ROIs of the obese group are statistically similar ($p = 0.07$). Thus, we examined both lean and obese group mean signals in the subsequent regression analysis.

The regression analysis did not reveal any significant findings to support the hypothesis that T_1 -weighted MRI is sensitive enough to measure differences in signal intensity associated with measures of body composition or insulin sensitivity. This may be due to MRI limitations, as discussed above. Alternatively, there is the possibility that parenchymal density in this specific cohort is unaffected by body composition and insulin sensitivity. Given the foundation of animal literature, combined with emerging human data, it is most likely that T_1 -weighted MRI is not sensitive enough to detect such subtle changes.

The longitudinal analysis does not provide any significant results to support the hypothesis that T₁-weighted MRI is sensitive enough to measure changes in parenchymal density as a result of decreased adiposity and improved insulin sensitivity. This may be due to the limitations of T₁-weighted MRI or may be due to a lack of parenchymal density change associated with decreased adiposity and improved insulin sensitivity. The body composition and endocrine measures provide evidence that the interventions were successful. BMI, weight, adipose tissue, visceral adipose tissue, subcutaneous adipose tissue, lean tissue, and HOMA-IR scores all decreased from baseline. Because better insulin sensitivity and lower BMI have both been associated with lower parenchymal density, it is likely that our lack of significant findings can be attributed to MRI limitations. (Berkseth et al., 2014; Schur et al., 2015)

Overall, T₁-weighted MRI is not recommended to detect changes in parenchymal density. Single echo T₂-weighted MRI may be sensitive enough for parenchymal density studies, but more research must be done to determine an appropriate control region for signal normalization. Single echo T₂ is an attractive method because of it is commonly used in the clinical environment and is a common image type found in research MRI repositories. Quantitative T₂ mapping is the leading method for quantifying changes in parenchymal density, even with the major drawback of increased acquisition times.

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CHAPTER V: DISCUSSION

Body weight is tightly controlled by the homeostatic feeding system, which is primarily reliant upon communication between the endocrine system and the brain. The endocrine system and brain are highly complex systems with a myriad of components that can present with altered function. Changes in neural insulin or leptin sensitivity and the neural underpinnings of disgust are associated with obesity. As such, it is important to explore new factors that are associated with obesity and to continually expand upon existing methodologies to increase our understanding of this multi-faceted problem.

Summary of Results

Our results across two studies reveal the structural and functional underpinnings of Disgust Proneness. Identifying insula subregions that are structurally associated with Disgust Proneness (Disgust Sensitivity and Disgust Propensity) will develop a better understanding of the neural origination of Disgust Proneness. Using self-reported data from the Disgust Propensity and Sensitivity Scale – Revised (DPSS-R), VBM regression analysis revealed a positive association of bilateral insula grey matter volume (GMV) with Disgust Proneness scores. These findings identify subregions where insula GMV may be driving, or is driven by, the level of behavioral Disgust Proneness experienced.

The significant subregions identified in the GMV study spatially overlap with significant subregions identified in the fMRI study. Using a task designed to evoke disgust and food-related

disgust, we identified insula subregions where BOLD activation was positively correlated with Disgust Proneness scores. The significant insula subregions found in our fMRI study spatially overlap with the insula subregions identified in our VBM study. Combined, these studies reveal the insula subregions where structure and function are associated with Disgust Proneness. We can use our understanding of the neural underpinnings of Disgust Proneness to explore BMI-dependent neurobehavioral differences in disgust.

Emerging data sheds light on the association of disgust proneness with obesity. Disgust Proneness is negatively associated with BMI, where individuals with anorexia nervosa have the highest levels of Disgust Proneness, and obese individuals have the lowest levels of Disgust Proneness. Obese individuals have lower levels of Disgust Sensitivity, as measured by the Disgust Propensity and Sensitivity Scale – Revised (DPSS-R). Using an fMRI-optimized task designed to elicit disgust and food-related disgust, we found that obese individuals have less insula BOLD activation than the lean group. This is the first identification of altered levels of BOLD activation in obese individuals within the insula, the primary region implicated in the disgust response. Furthermore, the self-reported measures of Disgust Sensitivity were positively correlated with insula activation extracted from the lean group, but negatively correlated with insula activation extracted from the obese group. This finding suggests that there is a functional dissociation between self-report of Disgust Sensitivity and neural activation in obese individuals. This observed uncoupling of disgust-related behavior and neural activation among obese individuals may reflect a fundamental dysregulation in a disgust system that may help mediate appropriate eating behavior.

As the logical extension to our findings of altered insula functional activity, we next explored insula grey matter volume differences between obese and lean individuals. The cohort used in this study was the same as that used in our fMRI analysis, but included 4 new subjects that were excluded from the fMRI study due to imaging artifacts. The results reaffirm that obese individuals have lower Disgust Sensitivity than lean individuals, as measured by the DPSS-R. Unfortunately, there were no between-group differences in insula grey matter volume. This finding may be explained by the relatively small sample size ($n = 33$), or it is possible that behavioral changes in Disgust Proneness are more strongly associated with functional brain changes and independent of volumetric changes.

Obesity, the related increase in pro-inflammatory cytokines, and development of insulin and leptin resistance, lead to neuroinflammation and parenchymal changes. Diet- and obesity-induced parenchymal density changes have been documented in the rodent mediobasal hypothalamus (MBH) using immunohistochemistry (IHC). Emerging MRI techniques are being developed to quantify these changes in parenchymal density in living humans. Our study sought to explore the viability of using T_1 MRI scans to identify parenchymal density changes in human subjects before and after weight loss and insulin detemir intervention. We extracted signal from the MBH and the insula ROIs reported in the previous disgust papers. Unfortunately, our null results suggest that T_1 -weighted MRI is not a suitable alternative to single or quantitative T_2 mapping. Further study should be done to determine suitable control regions for single echo T_2 if this clinically-applicable method is to be used in future research studies.

The Role of the Insula in the Emotion of Disgust

fMRI

We have identified insula subregions that are both structurally and functionally associated with Disgust Proneness. The insula has been established as a region associated with the disgust response. Insula activation has been associated with individual differences in Disgust Proneness when viewing disgust stimuli, including facial expressions of disgust. (Phillips et al., 1997; Mataix-Cols et al., 2008) Baumann and colleagues showed greater insula activation to disgusting images compared to neutral images in normal weight individuals. (Wicker et al., 2003; Wright et al., 2004; Baumann and Mattingley, 2012) Additionally, there is insula activation when viewing images of disgusting foods, in contrast with images of non-food items or appetizing foods. (Calder et al., 2007)

We created a stimulus presentation task designed to differentiate between insula activation elicited from food-related disgust images and insula activation elicited from contamination disgust images. The stimuli categories included images of food (e.g. crackers, pizza), contaminant (e.g. mold, roaches), and contaminated food (e.g. moldy crackers, pizza with roaches). The study revealed several overlapping insula subregions with a positive association of Disgust Sensitivity scores with BOLD activation while viewing images from each category. The majority of the overlapping insula subregions were located bilaterally in the anterior insula, a region commonly associated with object valence. (Britton et al., 2006; Viinikainen et al., 2010) Therefore, it is possible that the positive association of Disgust Sensitivity scores with BOLD activation within this subregion of the anterior insula is driven by the need to assign a positive or

negative valence to a viewed image. This is especially likely considering the rapid-event-related design of the stimuli presentation. When rapidly viewing and appraising whether one would eat the items presented in various images of food, contaminant, and contaminated foods, one must quickly discern whether a food item is edible or would pose a health risk through contamination. This concept may be translated to animal foraging behavior, where an animal needs to identify fresh and edible items from rotting, contaminated, or poisonous items.

Results from the same analysis reveal some distinct areas within the insula with a positive association of Disgust Sensitivity scores with BOLD activation while viewing images from only the contaminant or contaminated food categories. One area of activation associated with contaminated food images was only slightly posterior to the primary anterior insula subregions discussed above. In fact, this subregion is still considered to be part of the anterior insula. However, an area of activation associated with contaminant images was located in the far posterior insula. This suggests that the insula may be organized along a gradient, where the valence of food related items is processed in the anterior insula and the valence of pure contaminants is processed in the posterior insula. Further studies are necessary to fully understand the anterior-posterior organization of the insula in terms of valence processing.

VBM

Next we examined the association of structural insula GMV with Disgust Proneness. Identifying the association of insula subregion GMV with behavioral Disgust Proneness will further clarify the neural underpinnings of the emotion of disgust. Combined with functional data, we are able to report the structure to function relationship of the emotion of disgust. Our analysis revealed a

positive association of Disgust Sensitivity and Disgust Propensity with GMV of insula subregions. The subregions reported in the left hemisphere are located in the anterior insula for both Disgust Sensitivity and Propensity. However, the subregions reported in the right hemisphere do not overlap for Disgust Sensitivity and Propensity. The right insula subregion associated with Disgust Sensitivity is located in the anterior insula, while the right insula subregion associated with Disgust Propensity is located in the posterior insula. The location of these subregions spatially overlap with published functional data.

The insula subregions representing a positive association of Disgust Sensitivity with GMV spatially overlap with the subregions reported in the fMRI study. (Watkins et al., 2016) Disgust Sensitivity is associated with left insula GMV at xyz coordinates $[-44, 8, -3]$ (38 voxels) and left insula BOLD activation at xyz coordinates $[-44, 16, 0]$ (73 voxels). Disgust Sensitivity is associated with right insula GMV at xyz coordinates $[44, -9, 6]$ (416 voxels) and right insula BOLD activation at xyz coordinates $[38, 6, 14]$ (135 voxels). It is important to note that xyz coordinates represent peak T values, but the spread of the ROI is based on total voxels. Therefore, the right subregions spatially overlap even though there xyz coordinates vary by up to 15 mm.

Our fMRI study did not analyze the association of Disgust Propensity with BOLD activation. However, Schäfer *et al* did analyze the association of Disgust Propensity with BOLD activation while viewing disgusting compared to neutral images. (Schäfer et al., 2009) Our reported insula subregions spatially overlap with the subregions reported by Schäfer *et al* (2009). Disgust Propensity is associated with left insula GMV at xyz coordinates $[-39, 17, 11]$ (41 voxels) and

left insula BOLD activation at *xyz* coordinates [-36, 24, 6] (voxels not reported). Disgust Propensity is associated with right insula GMV at *xyz* coordinates [36, 30, 0] (268 voxels) and right insula BOLD activation at *xyz* coordinates [36, 27, -3] (voxels not reported).

Combined, the results of our fMRI and VBM papers and Schäfer *et al* (2009) strongly support the notion that Disgust Propensity and Disgust Sensitivity are associated with both the structure (GMV) and function (BOLD) of the reported insula subregions. Identifying insula subregions that are both functionally and structurally associated with the disgust response further clarifies the neural underpinnings of the emotion of disgust. Abnormal Disgust Proneness has been identified as one factor contributing to eating disorders such as anorexia nervosa. (Davey et al., 1998; Troop et al., 2002; Aharoni and Hertz, 2012; Houben and Havermans, 2012) Psychiatric interventions, such as Cognitive Behavioral Therapy, reduce behavioral Disgust Proneness and therefore may be useful in treating individuals with anorexia nervosa. Future research endeavors should conduct longitudinal functional and structural imaging studies to examine possible neurological changes within the subregions outlined above.

The Association of Disgust Proneness with Obesity

Increased levels of food-related disgust have been associated with eating disorders such as anorexia nervosa. (Davey et al., 1998; Troop et al., 2002; Aharoni and Hertz, 2012) Conversely, a recent study found significantly lower Disgust Proneness scores in obese compared to lean individuals. (Houben and Havermans, 2012) These findings suggest that increased Disgust Proneness among individuals with anorexia nervosa may partially account for lower BMI

whereas decreased Disgust Proneness among obese individuals may be associated with higher BMI. Indeed, we report that lean and obese individuals did not significantly differ in how easily they are prone to experience disgust (Propensity), but that obese individuals were less likely to appraise the experience of disgust as negative (Sensitivity). The finding that obese individuals have lower Disgust Sensitivity scores supports the possibility that a diminished disgust response may be one factor associated with obesity. In this sense, it is possible that the experience of the disgust response and an associated negative appraisal are factors necessary to inhibit the drive for overconsumption. Whereas individuals with higher levels of disgust (e.g. individuals with anorexia nervosa) over-inhibit their drive to eat, it is possible that individuals with lower levels of disgust (e.g. obese individuals) may have a reduced ability to inhibit their drive to eat. In other words, obese and lean individuals may recognize they are consuming excess calories (Disgust Proneness) but obese individuals experience diminished negative appraisal (Disgust Sensitivity), which may contribute to their failure to adequately limit caloric consumption.

Our results do not exactly match those reported by Houben *et al* (2012). (Houben and Havermans, 2012) Houben *et al* used the Disgust Scale - Revised (DS-R) to show that obese individuals have lower Disgust Proneness. Although our results report lower Disgust Sensitivity in the obese group, we do not report lower Disgust Propensity, a measure more closely related to what the DS-R measures. We believe that methodological differences may have accounted for the divergent findings - specifically the use of different disgust scales and differences in the BMI stratification method used by Houbens *et al* (2012). The DS-R measures only Disgust Proneness and does not differentiate between the two components of Disgust Proneness (Propensity and Sensitivity). In addition, Houben *et al* stratified their sample into low and high BMI (defined as

± 1 standard deviation from the mean BMI). Their low BMI group consisted of individuals with a BMI range of 13.86-19.73 and their high BMI group consisted of individuals with a BMI range of 28.79-38.96. Therefore, their low BMI group was comprised of a mix of underweight (BMI < 18) and healthy weight (BMI = 18-24.9) individuals. It is not possible to distinguish whether the reported between-group differences for Disgust Proneness are influenced by the underweight nature of the low BMI group, or if they reflect a difference between lean and obese individuals.

The Insula, Disgust Proneness, and Obesity: Tying It All Together

Using fMRI

Delineation of brain regions associated with Disgust Proneness may offer important insights into eating behaviors that contribute to obesity. Although imaging studies have identified the insula as a primary region implicated in Disgust Proneness, much remains unknown about the neural correlates of Disgust Proneness in obesity. Thus, we paired disgusting food-related images with the administration of the DPSS-R to examine group differences in the neurobehavioral correlates of Disgust Proneness. Our results indicate that the insula has decreased activity in the obese group as compared to the lean group when viewing contaminated food images. This was the first identification of reduced insula activation to disgust stimuli in obese relative to lean individuals.

The difference in insula activation was restricted to the right hemisphere. The unilateral results may be explained by two competing ideas. The left insula may be less sensitive to contaminated food stimuli, which would reduce the range of activation measured between groups.

Alternatively, if the observed findings are a consequence of obesity rather than a risk factor for

it, the left insula may be resistant to the effects of obesity. Decreased insula activation was only observed when viewing contaminated foods, and not when viewing food or contaminant alone, suggesting that differences in Disgust Proneness (we are not measuring Sensitivity or Propensity at this point) for lean versus obese individuals are specific to the interaction of food and contaminant. These findings raise the possibility that lower Disgust Sensitivity and reduced insula activation may contribute to the tendency to overeat among obese individuals.

We next explored the association of our behavioral measure of disgust (DPSS-R) with neural activation elicited by the presentation of contaminated food stimuli. The interaction analysis revealed one area of activation within the anterior insula where the association of Disgust Sensitivity scores with BOLD activation while viewing contaminated food images differed by group. Within this area of activation, there was a positive correlation of Disgust Sensitivity scores with BOLD activation in the lean group, whereas there was a negative association of Disgust Sensitivity scores with BOLD activation in the obese group. This finding suggests that there is a functional dissociation between self-report of Disgust Sensitivity (scores) and neural activation in obese individuals. In a typical excitatory neurobehavioral system, neural activation increases as a measurable behavior increases. In this context, the system remains intact in the lean group, but is disrupted in the obese group. This observed uncoupling of disgust-related behavior and neural activation in obese individuals may reflect a fundamental dysregulation in a disgust system that may help mediate appropriate feeding behavior.

The behavioral result of dysregulation in the disgust system has been demonstrated in a previous study showing that lower measures of core and contamination disgust predict a greater likelihood

of eating high calorie food. (Houben and Havermans, 2012) Via this mechanism, lower Disgust Sensitivity in obesity may lead to a greater probability of seeking out and ingesting high calorie foods - a behavior associated with obesity. Reduced Disgust Sensitivity may slightly extend the extent to which obese individuals will consume food. As Houben *et al* discuss, this may present as an increase in the total calories consumed in one meal or as the frequency an individual will consume a high calorie food over a low calorie food. (Houben and Havermans, 2012) This is directly contrasted with individuals with anorexia nervosa - with higher levels of disgust - who are less likely to consume excess calories over an extended timeframe. (Hadigan et al., 2000) The effect of Disgust Sensitivity on diet and food choice does not need to be severe in order to lead to and further cement obesity. An excess of approximately 11 calories per day, or one potato chip, can lead to 1 pound of weight gained per year. In this context, reduced Disgust Sensitivity may contribute to long term obesity.

These findings offer a dimensional complement to previous studies showing that individuals with anorexia nervosa have significantly elevated Disgust Proneness, specifically to food related items, as compared to controls. (Troop et al., 2002; Aharoni and Hertz, 2012) That is, whereas individuals with anorexia nervosa have heightened food related Disgust Proneness, obese individuals have diminished food Disgust Proneness (Anorexic > Lean > Obese). Because this diminished Disgust Proneness was observed at both the level of behavioral report and at the level of neural responses, it might be possible that decreased Disgust Proneness could encourage overeating.

In the context of obesity, the Disgust Proneness may be a potential target for intervention. Indeed, a disgust-based implicit priming task is effective at increasing Disgust Proneness in obese individuals and subsequently decreasing preference for high-calorie foods. (Legget et al., 2015) The logical extension of such studies would be to examine the longitudinal effects of a disgust-based implicit priming task on the structure and function of the insula subregions reported throughout the literature. The proposed study would shed light on whether behavioral change precede neural changes or if neural changes precede behavioral changes.

Using VBM

The above research identifies differences in insula BOLD activation between lean and obese individuals. However, there is no data on insula GMV differences between lean and obese. Unfortunately, we found no evidence that insula GMV is different between lean and obese individuals or that insula GMV is associated with BMI. The lack of between-group differences may be due to a relatively small sample size ($n = 33$). Alternatively, it is possible that behavioral changes in Disgust Proneness are only associated with functional brain changes, and not with GMV.

The disconnect between BMI-related reductions in insula structure and insula function may be explained by differences in brain metabolism and glucose uptake between lean and obese individuals. Insulin facilitates systemic and neural glucose uptake. (Jauch-Chara et al., 2012) Thus, impaired insulin action due to insulin resistance, as observed with obesity, leads to a decrease in neural glucose uptake. (Kahn and Flier, 2000; Kaiyala et al., 2000; Schwartz et al., 2000; Carvalheira et al., 2003; De Souza et al., 2005; Schwartz and Porte, 2005) Because neural

glucose uptake is strongly correlated with functional BOLD activation, an impairment in neural glucose uptake, as observed in obese individuals, would lead to a reduction in functional BOLD activation without reducing GMV within the same region. (Rothman et al., 1999; Hyder et al., 2001) This concept should be further studied and characterized by utilizing spectroscopic measures of cerebral adenosine triphosphate (ATP) and phosphocreatine levels during a Disgust Proneness task.

Not All MRI Sequences Are Created Equal

This study sought to explore the viability of using T₁-weighted MRI scans to identify parenchymal density differences between lean and obese diabetics and parenchymal density changes in human subjects before and after weight loss and insulin detemir intervention. The two regions of interest were the MBH and insula subregions identified in the studies discussed above. Both regions were included based on evidence of structural and functional deficits associated with obesity. The MBH, a major feeding center with a network of both orexigenic and anorexigenic cell populations, is associated with an increase in parenchymal density and T₂ signal intensity in obese rodents and humans. (De Souza et al., 2005; Thaler and Schwartz, 2010; Cai and Liu, 2011; Buckman et al., 2013; Cai, 2013) The insula, a major disgust response center, is associated with reduced functional BOLD activation during a Disgust Proneness task, a behavioral deficit reported in obese humans. (Watkins et al., 2016) Interestingly, neither region is associated with decreased grey matter volume in obese humans. (Carnell S, Gibson C, Benson L, Ochner CN, 2012; Yokum et al., 2012; Shefer et al., 2013; Watkins et al., 2016 *in preparation*) It is possible that the underlying cause of the observed functional and behavioral

changes associated with the insula are driven by cellular level changes similar to those observed in the MBH.

The study was designed to expand upon data suggesting the single and quantitative T₂-weighted MRI is sensitive to quantify signal intensity changes that are positively correlated with BMI in humans. (Thaler et al., 2012; Schur et al., 2015) If T₁-weighted MRI is equally as sensitive to parenchymal density, then researchers could analyze massive MRI repositories to further our understanding of parenchymal changes in obesity and diabetes. Unfortunately, our results did not yield any evidence that T₁-weighted MRI is as sensitive to parenchymal density as single or quantitative T₂-weighed MRI.

There was one statistically significant result in our between-group baseline analysis. The obese group had significantly higher signal intensity values in the right insula when compared to the controls. Unfortunately, this effect likely reflects a lower mean signal in the lean group, rather than a higher signal in the obese group. The mean signals extracted from the bilateral insula ROIs of the lean group are significantly different ($p = 0.01$), whereas the mean signals extracted from the bilateral insula ROIs of the obese group are statistically similar ($p = 0.07$). Thus, we examined both lean and obese group mean signals in the subsequent regression analysis.

The regression analysis did not reveal any significant findings to support the hypotheses that T₁-weighted MRI is sensitive enough to measure differences in baseline signal intensity extracted from lean and obese groups and that this signal intensity would be associated with measures of body composition and insulin sensitivity. This may be due to MRI limitations specifically

related to using T_1 - instead of T_2 -weighted images and relying on single echo over quantitative acquisition parameter.

T_1 - and T_2 -weighed MRI both rely on measuring the changes in interactions between water molecules and tissue macromolecules to assess the spatial distribution between grey and white matter. (Crooks et al., 1987; Tofts and du Boulay, 1990; Miot-Noirault et al., 1997) Small changes in the interactions of molecules can be measured to detect lesions within the gray and white matter of the brain. However, T_1 - and T_2 -weighted images are differentially sensitive to changes in molecule interaction by tissue type. In particular, T_1 is better at detecting the spatial distribution of myelin-bound cholesterol within brain white matter. (Koenig, 1991) Thus, T_1 is most commonly used in clinical populations with white matter lesions or atrophy, such as Multiple Sclerosis. (Neema et al., 2007; Polman et al., 2011) Conversely, T_2 relates to proton transfers, molecular exchange, and diffusion of water, making it most suitable for imaging changes in cellular density changes in grey matter, which is low in cholesterol and fat. (Miot-Noirault et al., 1997; Barkovich, 2000) Our null results, in combination with the positive results reported by Thaler *et al* (2012), support the notion that T_2 -weighted imaging is the preferred imaging sequence for the quantification of small changes in grey matter cellular density.

Not only is T_1 -weighted imaging relatively insensitive to grey matter signal intensity, the use of single echo imaging presents an issue of signal normalization. Single echo scans are susceptible to multiple types of variability that influence the baseline values of the acquired signal intensity. Therefore, each scan must be internally normalized in order to compare the data between groups or within subjects. For this study, we followed the normalization protocol described by Thaler *et*

al (2012). Signal intensity from the ipsilateral amygdala was used to normalize the signal extracted from the MBH and insula ROIs. Although this normalization approach has a mathematical rationale, there is no evidence to support its biologic rationale. Almost all of the studies of diet- and obesity-induced parenchymal changes look at the MBH. There are no IHC studies that examine the amygdala, and therefore no evidence to suggest that the amygdala is unchanged by diet and obesity. The issue extends to the majority of brain regions, with the exception of the hippocampus, which is associated with changes in neural density. (Namavar et al., 2012) Quantitative imaging addresses this issue.

The advantage of quantitative T_2 , compared to single echo imaging, is the unbiased quantitative nature of the values obtained. Quantitative T_2 can reliably detect subtle differences in T_2 signal intensities (relaxation times), which allow researchers to identify subregion-level changes in parenchyma. (Bernasconi et al., 2000; Wendel et al., 2001; Briellmann et al., 2004; Lee et al., 2013; Berkseth et al., 2014; Schur et al., 2015) Multiple echoes allow the researcher to extrapolate a single T_2 signal intensity value that does not need to be normalized to a control region. A T_2 map can be created by calculating T_2 values using an exponential fit function, signal intensity at echo time t , $S(t) = S_0 \times e^{-t/T_2}$. Thus, the T_2 signal intensity value is normalized within the values of the multiple echoes acquired. (Briellmann et al., 2004; Lee et al., 2013; Berkseth et al., 2014; Schur et al., 2015) The fundamental drawback to using quantitative T_2 is the increased time required to acquire each scan, limiting its use in the clinical setting. In research however, quantitative T_2 has been used to successfully identify increases in T_2 signal intensity within the hypothalamus of obese rodents and humans. (Lee et al., 2013; Berkseth et al., 2014; Schur et al., 2015)

Using quantitative T_2 and IHC, an initial study by Lee et al (2013) reports that longer T_2 relaxation times are a marker of increased parenchymal density (gliosis). (Lee et al., 2013) Longer T_2 relaxation times within the MBH are reported in mice fed a 60% high fat diet for 21 weeks, when compared to a control group fed a 12% fat chow diet. IHC shows that the same mice on high fat diet have increased astrocyte and microglia density, compared to controls. For the mice fed high fat diet, astrocyte density is positively correlated with T_2 relaxation times within the MBH. (Lee et al., 2013)

A follow up study in mice also detected diet- and obesity-induced increases in parenchymal density that were measured with quantitative T_2 and confirmed with IHC. (Berkseth et al., 2014) Combined quantitative T_2 MRI and IHC analysis on post-mortem human brains yielded similar results. (Schur et al., 2015) IHC measures of parenchymal density correlated with T_2 relaxation times, suggesting that quantitative T_2 is the best current method available for detecting parenchymal density in human brains.

Although the evidence strongly suggests that T_1 -weighted MRI is not sensitive enough to measure parenchymal density changes in humans, the dataset used in our study lacks appropriate control measures of reduced neuroinflammation and parenchymal density. For clear moral and ethical reasons, we could not remove our human subjects' brains and directly quantify parenchymal density with IHC. This "study weakness" alone prevents us from ensuring that controls at baseline had lower levels of parenchymal density or that weight loss and insulin detemir intervention had any significant effect on parenchymal density. Additionally, markers of systemic and neuroinflammation were not immediately available to compare group level

differences or longitudinal changes in inflammation. However, the combined effects of weight loss (via caloric restriction) and insulin detemir intervention significantly reduced measures of body composition (BMI, weight, adipose tissue, visceral adipose tissue, subcutaneous adipose tissue, and lean tissue). Although the HOMA-IR score was not significantly reduced ($p = 0.11$) by the intervention, there was a biologically relevant reduction in HOMA-IR from week 6 - week 26 (16.93 - 12.00). These are not direct measures of neuroinflammation but both body composition and HOMA-IR have consistently been associated with neuroinflammation and parenchymal density. (De Souza et al., 2005; Thaler et al., 2012; Buckman et al., 2013; Cai, 2013; Berkseth et al., 2014; Schur et al., 2015)

Overall, T_1 -weighted MRI is not recommended to detect changes in parenchymal density. Single echo T_2 -weighted MRI may be sensitive enough for parenchymal density studies, but more research must be done to determine an appropriate control region for signal normalization. Single echo T_2 is an attractive method because it is commonly used in the clinical environment and is a common image type found in research MRI repositories. Quantitative T_2 mapping is the leading method for quantifying changes in parenchymal density, even with the major drawback of increased acquisition times.

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