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EFFECTS OF OBESITY AND WEIGHT LOSS FOLLOWING BARIATRIC SURGERY ON BRAIN FUNCTION, STRUCTURAL INTEGRITY AND METABOLISM

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To my family and friends

“voikaa paksusti”

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ABSTRACT

Obesity is one of the key challenges to health care system worldwide and its prevalence is estimated to rise to pandemic proportions. Numerous adverse health effects follow with increasing body weight, including increased risk of hypertension, diabetes, hypercholesterolemia, musculoskeletal pain and cancer. Current evidence suggests that obesity is associated with altered cerebral reward circuit functioning and decreased inhibitory control over appetitive food cues. Furthermore, obesity causes adverse shifts in metabolism and loss of structural integrity within the brain. Prior cross-sectional studies do not allow delineating which of these cerebral changes are recoverable after weight loss. We compared morbidly obese subjects with healthy controls to unravel brain changes associated with obesity. Bariatric surgery was used as an intervention to study which cerebral changes are recoverable after weight loss.

In Study I we employed functional magnetic resonance imaging (fMRI) to detect the brain basis of volitional appetite control and its alterations in obesity. In Studies II-III we used diffusion tensor imaging (DTI) and voxel-based morphometry (VBM) to quantify the effects of obesity and the effects of weight loss on structural integrity of the brain. In study IV we used positron emission tomography (PET) with [^{18}F]-FDG in fasting state and during euglycemic hyperinsulinemia to quantify effects of obesity and weight loss on brain glucose uptake.

The fMRI experiment revealed that a fronto-parietal network is involved in volitional appetite control. Obese subjects had lower medial frontal and dorsal striatal brain activity during cognitive appetite control and increased functional connectivity within the appetite control circuit. Obese subjects had initially lower grey matter and white matter densities than healthy controls in VBM analysis and loss of integrity in white matter tracts as measured by DTI. They also had initially elevated glucose metabolism under insulin stimulation but not in fasting state. After the weight loss following bariatric surgery, obese individuals' brain volumes recovered and the insulin-induced increase in glucose metabolism was attenuated.

In conclusion, obesity is associated with altered brain function, coupled with loss of structural integrity and elevated glucose metabolism, which are likely signs of adverse health effects to the brain. These changes are reversed by weight loss after bariatric surgery, implicating that weight loss has a causal role on these adverse cerebral changes. Altogether these findings suggest that weight loss also promotes brain health.

Key words: brain, obesity, bariatric surgery, appetite control, structural magnetic resonance imaging (sMRI) functional magnetic resonance imaging (fMRI), voxel-based morphometry (VBM), diffusion tensor imaging (DTI), positron emission tomography (PET).

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TIIVISTELMÄ

Lihavuus yleistyy nopeasti koko maailmassa ja se on yksi suurimmista terveydenhuollon tulevaisuuden haasteista. Lihavuus lisää riskiä sairastua useisiin sairauksiin mm. verenpainetautiin, diabetekseen, tuki- ja liikuntaelinten sairauksiin ja useisiin syöpiin. Lihavilla on havaittu aivojen palkkiojärjestelmän reagoivan yliaktiivisesti ruokaan liittyviin ärsykkeisiin ja toisaalta ruokahalua hillitsevien alueiden toiminnan on havaittu olevan heikompaa kuin normaalipainoisilla. Lihavuus aiheuttaa myös haitallisia muutoksia aivosolujen rakenteessa ja aineenvaihdunnassa. Näiden muutosten palautumispotentiaalia ei voida arvioida poikkileikkaustutkimuksissa. Tässä työssä tutkimme sairaalloisen lihavia lihavuusleikkaukseen valittuja potilaita, joiden aivokuvantamistuloksia verrattiin normaalipainoisten vastaaviin kuvauksiin. Potilaat tutkittiin myös kuusi kuukautta lihavuusleikkauksen jälkeen painonlaskun aiheuttamien aivomuutosten tutkimiseksi.

Ensimmäisessä tutkimuksessa ruokahalun säätelyjärjestelmän toiminnan ja lihavuuden aiheuttamien muutosten selvittämiseksi käytettiin toiminnallista magneettikuvantamista (fMRI). Rakenteellisia muutoksia arvioitiin II ja III osatyössä diffuusiotensorikuvantamisella (DTI) sekä vokseliperustaisen morfometrian avulla (VBM). Neljännessä osatyössä aivojen sokeriaineenvaihduntaa tutkittiin positroniemissiotomografian (PET) avulla käyttämällä [^{18}F]-FDG-merkkiainetta paastotilassa ja insuliini-stimulaation aikana. VBM -ja PET-menetelmiä käytettiin myös lihavuusleikkauksen vaikutusten arviointiin.

fMRI-kokeessa havaittiin, että ruokahalun säätelyyn osallistuu laaja hermoverkko jonka keskeiset osat sijoittuvat otsa- ja päälaenlohkoihin. Lihavilla tutkittavilla havaittiin alentuneet vasteet häntätumakkeessa ja etupihtipoimun alueella. Lisäksi heillä havaittiin voimakkaammat toiminnalliset yhteydet ruokahalun säätelyverkostossa. Lihavuus aiheuttaa aivoissa laajaa aivokudoksen harventumaa ja hermoratojen eheyden alentumaa. PET-tutkimuksessa havaittiin lihavuuden lisäävän aivojen insuliiniherkkyyttä. Puoli vuotta lihavuusleikkauksen jälkeen rakenteelliset ja aineenvaihdunnan muutokset palautuivat osittain.

Lihavilla on muutoksia aivojen rakenteessa ja aineenvaihdunnassa normaalipainoisiin verrattaessa, mitä voidaan pitää aivojen terveyden kannalta haitallisina ilmiöinä. Painon lasku lihavuusleikkauksen jälkeen kumoaa osittain nämä muutokset, joten painon pudottaminen voidaan olettaa olevan myös aivojen terveyden kannalta hyödyllistä.

Avainsanat: aivot, lihavuus, lihavuusleikkaus, ruokahalun säätely, toiminnallinen magneettikuvantaminen (fMRI), rakenteellinen magneettikuvantaminen (sMRI), vokselipohjainen morfometria (VBM), diffuusiotensorikuvantaminen (DTI), positroniemissiotomografia (PET).

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ABBREVIATIONS

BMI = body mass index

MRI = magnetic resonance imaging

fMRI = functional MRI

PPI = psychophysiological interactions (task-related functional connectivity)

VBM = voxel-based morphometry

GM = grey matter (cortical neuronal layer and subcortical nuclei)

WM = white matter (axons that connect neurons between grey matter regions)

DTI = diffusion tensor imaging

FA = fractional anisotropy

MD = mean diffusivity

PET = positron emission tomography

[^{18}F]-FDG = 18-fluorodeoxyglucose (PET tracer, glucose analogue)

Ki = net influx rate (of PET tracer into the tissue)

MNI = Montreal Neurological Institute

GLM = general linear model

FDR = false discovery rate (correction method for multiple comparisons)

FWE = family wise error (correction method for multiple comparisons)

LIST OF ORIGINAL PUBLICATIONS

I. Tuulari, J.J. et al., 2015. Neural circuits for cognitive appetite control in healthy and obese individuals: an FMRI study. PloS one, 10(2), p.e 0116640.

II. Karlsson, H.K., Tuulari JJ et al., 2013. Obesity is associated with white matter atrophy: A combined diffusion tensor imaging and voxel-based morphometric study. Obesity (Silver Spring, Md.), 21(12), pp.2530–2537.

III. Tuulari JJ et al. Weight loss after bariatric surgery induces white and grey matter density recovery in the morbidly obese: a voxel-based morphometric study. (manuscript)

IV. Tuulari, J.J. et al., 2013. Weight loss after bariatric surgery reverses insulin-induced increases in brain glucose metabolism of the morbidly obese. Diabetes, 62(8), pp.2747–2751.

1. INTRODUCTION

Eating is a prerequisite for maintaining health and normal function for all living things. It provides energy for all our actions – from cellular function to locomotion – as well as countless minerals and vitamins to act as buffers and cofactors to our enzymes. The brain governs eating and the maintenance of body weight and adiposity or the amount of stored energy. Humans generally match their energy requirements almost perfectly on day-to-day basis, but this system also allows weight gain (Jéquier 2002). This phenomenon is based on highly conserved genes that have persisted in most species throughout generations. Historically, individuals, who had the capability to store energy, had increased chances of survival through times when food resources were scarce (Sellayah et al. 2014). However, this type of scarcity of resources is seldom encountered in the modern society and thus such genes are a liability in the modern societies where palatable foods are cheap and easily obtainable. This has lead to significant increase in the prevalence of obesity and associated adverse health outcomes such as hypertension and diabetes among others (Mokdad et al. 2003).

Obesity is not easy to treat as the excess food intake has usually lasted for years and each individual exhibits highly conserved eating behavior - both in food choice and amount (Jéquier 2002). Oftentimes, eating offers comfort and pleasure and can be compulsive for some individuals (E. Smith et al. 2011). It is thus important to study the behavioral patterns and brain mechanisms predisposing individuals to aberrant eating behavior in order to better understand the developmental trajectories of obesity and to help to plan future treatments (Davis et al. 2007; Berthoud & Morrison 2007; S. Z. Yanovski & J. A. Yanovski 2014). Among other diseases, obesity is also a contributing factor for age-related brain degeneration and may thus have adverse effects on brain health and ultimately on cognitive performance (E. Smith et al. 2011). The mechanisms of these changes remain poorly understood although the implications from epidemiological studies are well acknowledged and cross sectional studies imply loss of structural integrity within the brain (Mueller et al. 2014; Brooks et al. 2013). Critically, it remains unresolved whether these changes are recoverable after weight loss. Thus, it is important to characterize the obesity-related changes in brain structure and function in further detail and assess their recovery potential after weight loss.

Bariatric surgery is an effective treatment for morbid obesity. It leads to rapid weight loss, and despite some patients regaining weight at the group level the total long-term weight loss is 10-25% (Yu et al. 2015). In this series of studies, we compared healthy individuals to morbidly obese subjects to unravel brain changes of obesity and used bariatric surgery as an intervention to study which structural and metabolic brain effects of obesity are recoverable after weight loss.

2. REVIEW OF LITERATURE

2.1 The prevalence and adverse health effects of obesity

In the United States, the age-adjusted prevalence of obesity [Body mass index (BMI) over 30] is approximately 35% among adults; moreover around 70% of the adult population are overweight (BMI > 25) (Flegal et al. 2012). In Europe there is a large variance in the prevalence of obesity across countries; ranging between 4.0 - 28.3% in men and 6.2% - 36.5% in women (Berghöfer et al. 2008). Currently, in Finland 66% of men and 46% of women are overweight (BMI > 25) and the prevalence of obesity is ca. 20% for both sexes (Männistö et al. 2012). Obesity results in increased risk for premature death and a multitude of diseases: diabetes, high blood pressure and cholesterol levels, asthma, arthritis and musculoskeletal pain (Mokdad et al. 2003) as well as many types of cancers (Calle & Kaaks 2004). Diabetes and elevated blood pressure, glucose and lipids are hazardous to vasculature and thus lead to increased risk for diseases such as coronary heart disease and ischemic stroke (Manson & Bassuk 2015). Consequently, obesity is also a risk factor for cognitive decline (Kivipelto et al. 2005; E. Smith et al. 2011). This poses massive costs and challenges to health care system and the costs are likely to increase in the future (Sellayah et al. 2014). Furthermore, it has become more and more apparent that obesity is associated with altered brain responses and understanding the mechanics behind these changes might help us to design better treatments for overweight and obesity (S. Z. Yanovski & J. A. Yanovski 2014). On the other hand, as the amount of obese individuals is bound to increase substantially, it is also imperative to explore the breadth of metabolic and structural brain changes that accompany obese phenotype.

2.2 The brains reward circuit, eating and obesity

Complex homeostatic mechanisms govern eating and appetite in the brain. The primary satiety signals affect the enteric nervous system and exert their effects to hypothalamus either via the vagus nerve or directly when carried in plasma (Wynne et al. 2005). The hypothalamus is a key brain region in detecting internal satiety signals and integrating this information for processing in higher-order brain areas (Wynne et al. 2005). Importantly, the homeostatic system of energy balance seems to be hardwired to encourage eating (Wynne et al. 2005; Goldstone et al. 2014). The hormonal signaling of satiety is only one of the factors influencing eating choices, as they are ultimately subject to conscious decisions as well as saliency assessment of the brains reward encoding system. Namely, palatable food cues are able to trigger a strong urge to eat independent from current feelings of hunger. Accordingly, a strong activation in the reward system may override

the homeostatic control and even previous conscious decisions regarding eating behavior. In the modern societies, where food cues are abundant, this likely contributes to the increasing prevalence of obesity in concordance with environmental factors (Swinburn et al. 2011).

The reward circuit provides our brain with constant updates on the hedonic properties of encountered cues and pleasantness of sensory stimuli, such as food pictures (Nummenmaa et al. 2012). At a larger scale it forms the basis of motivated behavior and gives us feedback of actions that are of benefit to us from evolutionary perspective, such as exercise, having sex and eating (Berridge & Kringelbach 2013). In general two separate processes drive any motivated behavior, namely, liking that depicts the hedonic aspect of experiences and wanting that depicts the intensity of motivation to obtain the reward (Berridge & Kringelbach 2013). However, the same system also encodes the unnatural pleasures that arise from activities such as substance abuse and gambling (Yau & Potenza 2015; Koob & Volkow 2010). Furthermore the naturalistic pleasure responses themselves may be "overused" as is the case in sex addiction and binge eating (Yau & Potenza 2015; G. K. W. Frank 2015).

The mesolimbic dopamine tracts are important in all reward responses (Wise 2002; Koob & Volkow 2010). The key brain areas of the pleasure circuit are connected to the nucleus accumbens and ventral tegmental area, which form the core components of the mesolimbic dopamine system (Wise 2002; Berridge & Kringelbach 2013). Higher brain areas of the reward circuit include the ventral striatum, hippocampus, amygdala, the orbitofrontal cortex, medial and lateral frontal cortices (Wise 2002; Koob & Volkow 2010). Repetitive stimulation of the reward system may cause permanent changes in its functioning and subsequently drive a variety of compulsive, addiction-like behaviors (Koob & Volkow 2010), which might apply for eating palatable foods as well. This view is supported by studies reporting lowered dopamine receptor levels in obese individuals (Volkow et al. 2008; Wang et al. 2011; Volkow et al. 2013; Volkow et al. 2005). Lowered dopamine receptor levels are a hallmark of many addictive disorders and in regard to obesity may lead to compulsive overeating and unhealthy eating habits together with development and maintenance of obesity (Volkow et al. 2013). Yet, it must be noted that the extant scientific literature does not uniformly support the assumptions of brain level similarities between behavioral addictions and obesity (Ziauddeen et al. 2012; Avena et al. 2012; Karlsson et al. 2015).

Taken together, the reward system is, among other functions, a key determinant of eating choices as it engages in assessing external food related cues and their hedonic value. The key components of reward circuitry (nucleus accumbens, ventral striatum, dorsolateral prefrontal and orbitofrontal cortices) and regions involved in homeostatic encoding (insula) show elevated responses to food pictures as contrasted to non-food pictures (D.

G. Smith & Robbins 2013; Pursey et al. 2014). Furthermore, the responses are higher for palatable foods and obese individuals have higher responses as compared to lean individuals (Nummenmaa et al. 2012; Pursey et al. 2014). External stimuli such as sights, smells and tastes that are received in primary sensory cortices can activate the reward network and the actions taken are determined through integration of homeostatic signals and inhibition efficiency of the frontal lobes (Figure 1). The information is integrated and processed in the whole system, meaning that the flow of information between the sub components is not likely to be linear nor sequential but reciprocal and constant in nature.

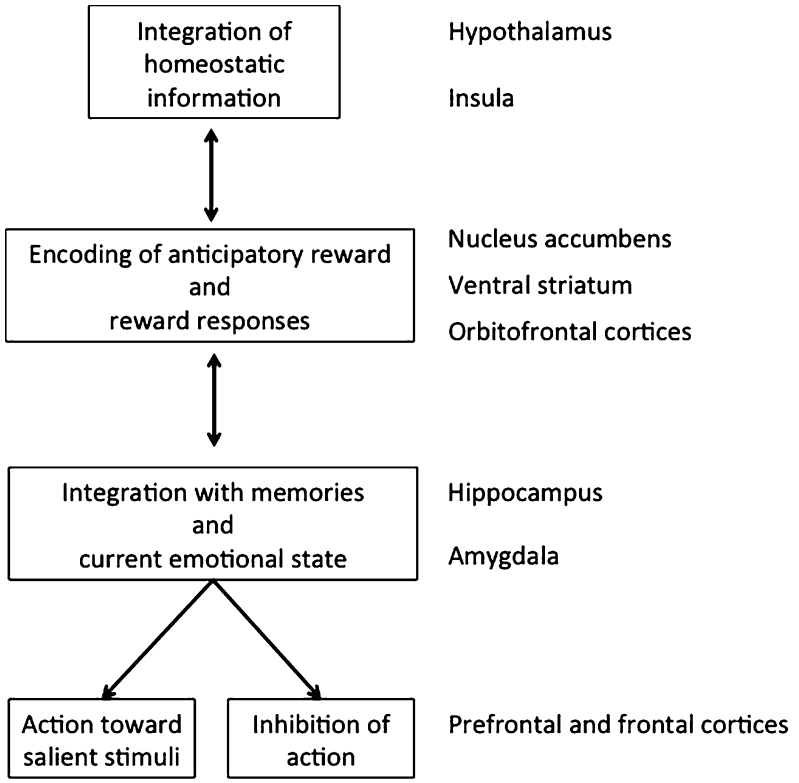

Figure 1. Key regions of reward encoding and action selection in the brain.

2.3 Brain circuits of volitional appetite control

The hypersensitivity of the reward system to visual food cues in obesity is rather well established (Pursey et al. 2014), but the brain mechanisms supporting volitional appetite control over food cues and their potential dysfunctions in obesity have not been extensively studied. Functional imaging studies addressing general cognitive control in the brain have shown that the pre-supplementary motor area (preSMA), dorsal lateral

and inferior frontal as well as parietal areas form the brain's cognitive inhibition network (Simmonds et al. 2008). Increasing the intensity of the cognitive load may recruit even wider frontal circuits (Criaud & Boulinguez 2013). Although these control circuits have been identified in relatively simple paradigms such as go/no-go task, it could be expected that the same regions participate in appetite control as well. This is supported by a recent study, where adolescent subjects viewed visual food cues and thought of the long-term costs or benefits of not eating the food and suppressing cravings for the food. These tasks increased activation in prefrontal and superior frontal regions (Yokum & Stice 2013). However, in that study participants' BMI did not modulate the intensity of brain activation. In another study participants had to either increase or decrease appetite while viewing food pictures (Scharmüller et al. 2012). In the appetite decrease condition the subjects were asked to reappraise the presented stimuli by giving the food pictures new meanings, such as considering them as non-food items. Both appetite decrease and increase tasks activated the striatum, insula and dorsolateral frontal regions as compared to watching across all subjects and more so in the obese subjects (Scharmüller et al. 2012).

In summary, obesity-related effects to frontal brain activations during cognitive appetite modulation have led to conflicting results. Previous reports entail both no modulatory effects in respect to BMI (Yokum & Stice 2013) and increased activations in obesity (Scharmüller et al. 2012). Previous studies used reappraisal tasks that are relatively complex. The brain responses more of faster appetite modulation are unknown. Furthermore, although prior studies have revealed that obesity alters functional connectivity of the reward circuit while subjects viewed food pictures (Nummenmaa et al. 2012), our understanding of how obesity could influence functional connectivity of the cognitive appetite control networks has remained elusive.

2.4 Obesity and structural changes in the brain tissue

Epidemiological studies show that obesity is a risk factor for cognitive decline not only through promoting vascular dementia but also by adding to the decline in other types of dementia such as Alzheimer's disease (Kivipelto et al. 2005). It has been proposed that obesity-related brain volume reductions could be a biomarker for future cognitive decline even prior to any manifest disease (Taki et al. 2008). Indeed, brain density reductions have been systematically found in obese as compared to normal weight individuals in voxel-based morphometry (VBM) studies (Pannacciulli et al. 2006; Pannacciulli et al. 2007; Taki et al. 2008; Hua et al. 2010; Cazettes et al. 2011; Cohen et al. 2011). Corroborating evidence comes from studies showing that obesity is associated with loss of structural integrity of the white matter as measured by diffusion tensor imaging (DTI) (Xu et al. 2013; Mueller et al. 2014; Marqués-Iturria et al. 2015).

Two competing hypotheses for the obesity-related neural degeneration have been put forward. First, the focal density reductions could predispose individuals to overeating, and would thus constitute a biomarker for increased risk of future weight gain (Pannacciulli et al. 2007; Cazettes et al. 2011; Marqués-Iturria et al. 2015). In support of this view, many regions where the obesity-related density reductions are observed also participate in reward function monitoring, maintenance of homeostasis and inhibitory control that as discussed above. Thus, atrophy in these regions might reflect altered reward function that would ultimately lead to overeating and obesity. Second, the loss of tissue integrity in the brain may be due to the obese phenotype. In this view the volumetric atrophy is seen as a diffuse sign of cellular damage caused by metabolic burden that adds to that observed with increasing age (Brooks et al. 2013). It is also possible that these hypotheses might both be correct, meaning that both cause and effect type of changes are seen in the limited scope of cross sectional studies reported to date. Several factors known to cause neuronal and glial damage have been proposed to account for the lowered brain tissue densities in obesity. First, adverse cellular effects may follow vascular damage caused by hypertension and elevated plasma lipids associated with obesity (Messier 2005). Second, increase in adipose tissue mass forms a low-grade inflammatory state through increased production of cytokines, which in turn are known to increase cellular oxidative stress and may impair cellular repair mechanisms (Hotamisligil 2006). Third, brain volume alterations among obese may also result from chronic hyperglycemia and glucose neurotoxicity much in a way that is hypothesized to occur in peripheral diabetic neuropathy (Tomlinson et al. 2008). Thus, multiple factors may mediate the neuronal and glial damage in obesity, but no consensus on their individual contributions exists. Indeed, it may prove challenging to separate them, as all of them manifest simultaneously with weight gain.

The effects of weight gain and weight loss on brain structure remain underspecified. Studying participants who lose weight reveals which of the obesity-related changes are recoverable and thus likely highlights at least some of the changes that are caused by metabolic burden of obese phenotype. To our knowledge, no studies to date have addressed the effects of weight loss after bariatric surgery to brain tissue integrity.

2.5 Brain glucose metabolism in healthy and obese

Brain has a constant, high demand of energy. Adult human brain consumes the most energy as compared to other organs in the body, which is around 20% of our total body energy consumption. A marked regional difference exists, because glial cells in the white matter (WM) consume only about 0,5% of the energy that neurons in the grey matter do (Harris & Attwell 2012). Energy production and metabolism in the brain is glucose driven and the enzymes responsible for energy production are the same for

most cells in the body-including neurons (Sutherland et al. 2012) (Figure 2). The rate of energy production varies according to momentary demands from cell function and external regulating signals. In the case of neurons the momentary demands are high and fluctuating, but the effects of external modulation factors such as insulin can be assessed in resting state, where participants do not perform a task prior or during the scan (Hirvonen et al. 2011).

Insulin is a pancreatic peptide hormone that is one of the main hormones that govern the rate of glucose metabolism in the whole body. Insulin secretion from the pancreas is increased when plasma glucose levels rise. Thus, after meals, insulin increases glucose uptake in most tissues including skeletal muscles, liver and adipose tissue (Henriksen 2002; Kahn & Flier 2000). The increased intake of glucose, in turn, rapidly increases the usage of glucose for energy production and also storage as glycogen and triglyceride molecules. These effects are mediated mainly via stimulation of glucose transporter receptor (GLUT) that has five identified subtypes GLUT 1-5 (Büsing et al. 2013). The predominant receptor type in peripheral tissues such as liver, adipose tissue and skeletal muscle is the GLUT-4 (Schulingkamp et al. 2000). Weight gain causes fat accumulation (Resnick & Howard 2002), and insulin resistance via down regulation or hypo function of GLUT-4 in peripheral tissues, and this is considered one of the main causative factors for obesity-related type 2 diabetes (Altaf et al. 2014). The changes that accompany peripheral insulin resistance within the central nervous system after weight gain are likely more complex. Although, most studies state that brain glucose metabolism is independent of insulin action (Hasselbalch et al. 1999; Tomlinson et al. 2008), insulin seems to have the potential to regulate brain tissue metabolism as well. First, animal studies have shown that insulin enters brain tissue via receptor-mediated transport that has a specific saturation property at relatively low levels of circulating insulin (King & Johnson 1985; Jaspan et al. 1997). Second, insulin receptors are widely expressed in the central nervous system (CNS) (Schulingkamp et al. 2000). Importantly the distribution of insulin receptor subtypes in the brain is not uniform, as insulin independent GLUT 1 and partly insulin dependent GLUT 3 are abundant in the whole brain. Contrasting this, insulin sensitive GLUT 4 receptors are located mainly in pituitary, hippocampus and hypothalamus (Schulingkamp et al. 2000). This fact is often not clearly addressed in the extant literature. For example, brain insulin resistance has been proposed to develop in a similar manner as peripheral insulin does (Tschritter et al. 2006; Sutherland et al. 2012), but the mechanism remains highly speculative and the studies seldom have standard insulin stimulation or methods for assessing brain metabolism directly. For the purposes of the current thesis the direct measurements with PET are the most relevant ones.

The glucose analogue [18F]-FDG is a commonly used PET tracer that can be used to accurate and direct measurement the glucose uptake of cells (Hamacher et al. 1986; Hirvonen et al. 2011) (Figure 2). As discussed above insulin seems to have no effects

on brain glucose uptake in healthy individuals (Bingham et al. 2002; Hirvonen et al. 2011). However, contrary to healthy individuals, obese type 2 diabetics have increased metabolism in response to resting state euglycemic hyperinsulinemia as compared to fasting condition (Hirvonen et al. 2011). Interestingly, this might signal increased glucose neurotoxicity that is one of the proposed factors underlying cellular insult in the brain (Tomlinson et al. 2008). Taken together, the effects of insulin on brain tissue glucose metabolism in obesity are unclear as we do not know, whether the increased insulin responses are a feature of type 2 diabetes or does it occur in all obese. More importantly, it is not known how weight loss affects the glucose uptake within the brain tissue under insulin stimulation.

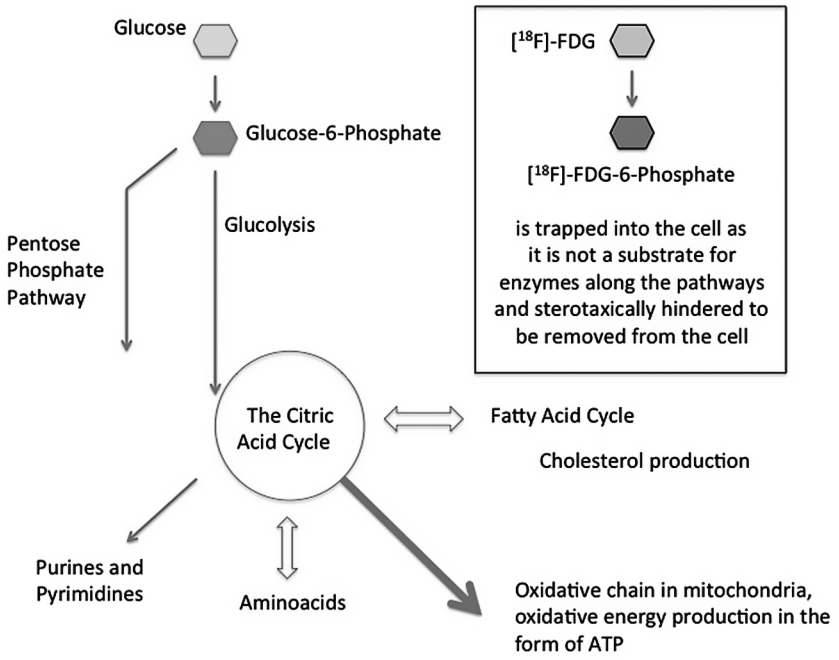

Figure 2. The main pathways of cellular energy nutrient metabolism in the brain and the faith of [18F]-FDG in cellular metabolism.

2.6 Bariatric surgery as a treatment for morbid obesity

Bariatric surgery is an effective means for weight loss in morbid obesity. It induces rapid weight loss and subsequent improvement of metabolic health (Chang et al. 2014). In Finland around 1000 bariatric surgeries are performed every year for morbidly obese patients with a BMI over 40 or over 35 when the patient has obesity-related comorbidities such as type 2 diabetes, hypertension or hypercholesterolemia that are difficult to treat (Koivukangas 2015).

When patients are remitted to the Turku University Hospital for bariatric surgery, they undergo tight screening in endocrinology and surgery policlinics. Patients with eating disorders and abuse of alcohol are excluded. They receive nutritional counseling and before the actual surgery they must lose weight and succeed in following a very low energy diet. Weight loss prior to surgery makes the procedure easier, but it is also an additional safety measure, as uncontrolled eating after surgery might result in death should the sutures break. The surgical procedures used in Finland are called Roux-en-y-gastric bypass (Figure 3A) and Sleeve gastrectomy (Figure 3B). The general fatality rate in these surgeries is around 0.5 %. The details of the procedures are described in detail elsewhere (Helmiö et al. 2012). Nationally, the vast majority of patients undergoing the surgery are female (ca. 85%) and ca. 70% of the source population used in the current studies were female (Helmiö et al. 2012). Despite the efficacy of bariatric surgery it also leads to permanent life changes. Due to the modification of the patient's gastrointestinal tract they eat only small amounts of food and have to consume calcium and vitamin supplements for the rest of their lives. Rapid weight loss results in flaccid areas of skin that sometimes need to be excised by plastic surgeons (in so called post bariatric surgery).

After the surgery some patients regain weight, but at the group level total long-term weight loss is 10-25% (Yu et al. 2015). This effect far surpasses other treatment modalities such as nutritional counseling and sport interventions (Peirson et al. 2014). Surgery also quickly and effectively treats the comorbid conditions especially type 2 diabetes (Ardestani et al. 2015). Importantly for the aims of the studies enclosed in this thesis, bariatric surgery provides an excellent interventional approach for studies on which pathological processes are reversed by weight loss.

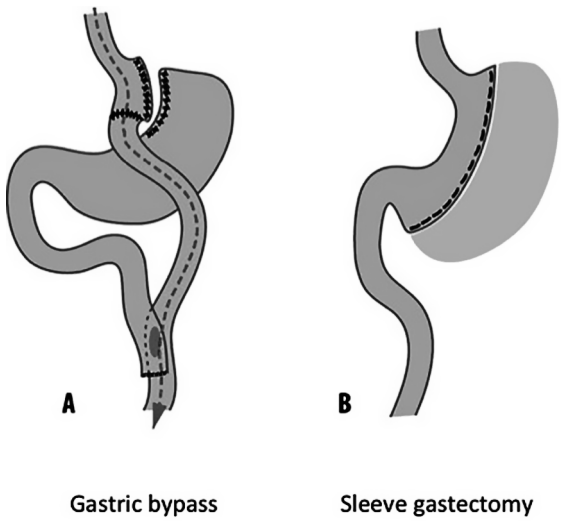

Figure 3. The different methods for bariatric surgery that were employed in the current studies. Image is modified from an open online health information web page: http://www.terveyskirjasto.fi.

2.7 Summary of the literature review

The amount of obese individuals is increasing throughout the world. Due to the vast array of concomitant comorbidities it is one of the major health challenges of the 21st century. In Finland more than 50% of adult population is overweight (BMI > 25) and 20% are obese (BMI > 30).

Evidence from functional brain imaging studies suggests that obese individuals have hyperactive reward circuitry in response to food cues. This may be coupled with decreased ability to control food-related urges due to absolute or relative hypo function of inhibitory frontal areas.

Additionally, obesity is related to adverse changes in brain tissue integrity. Brain atrophy in the reward and control areas may further promote dysfunctions in reward and cognitive control circuits. Another way to explain the obesity-related brain atrophy is to see it as an accumulation of metabolic burden and cellular damage.

Although insulin effects to brain tissues glucose uptake are still unclear, the effects appear minimal for normal weight individuals. However, obese type 2 diabetics have increased insulin responses in the brain. This might cause glucose neurotoxicity, which may be one of the causal contributors to brain atrophy.

The brain effects of weight loss may be addressed in patients undergoing bariatric surgery, which results in rapid weight loss and, in most cases, amelioration of type 2 diabetes. This thesis focuses on differences between non-obese and morbidly obese individuals in brain structure, function and metabolism. This cross sectional design is employed in all sub studies. Second, the effects of bariatric surgery on brain tissues structural integrity and glucose metabolism of brain tissue of the morbidly obese participants is addressed.

3. OBJECTIVES OF THE STUDIES

The specific aims of the studies were:

I. To identify brain circuits responsible for volitional appetite control and determine how obesity modulates the circuit's responses and functional connectivity ***(Study I).***

II. To determine how morbid obesity affects brain tissue's structural integrity as measured by voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) ***(Study II).***

III. To determine how brain structure changes after bariatric surgery as measured by VBM ***(Study III).***

IV. To determine brain glucose uptake by using [18F]-FDG-PET in fasting condition and during euglycemic hyperinsulinemia and how bariatric surgery modulates the metabolic response ***(Study IV).***

4. MATERIALS AND METHODS

All studies were conducted in accordance with the Declaration of Helsinki. They were approved in the joint Ethical Committee of University of Turku and Turku University Hospital. Every participant signed an informed consent form prior to laboratory measurements and scanning. The current data stems from two consecutive studies addressing the effects of bariatric surgery on the brain among measurements made to other organ systems. The studies had identical setting in respect to the timing of the measurements (Figure 4). The subjects that completed brain scans and their amounts in each sub study are summarized in Table 1.

4.1 Participants, inclusion criteria and measurements

The subjects groups were morbidly obese subjects about to undergo bariatric surgery and normal weight controls. They were matched for age, height, and sex. Exclusion criteria for all subjects included binge-eating disorders, neurological and mental disorders. Subjects were also excluded for substance abuse, including excessive alcohol consumption. Laboratory measurements were done during screening visits prior to scanning. PET imaging sessions also required blood sample acquisition during the scans (Study IV). A summary of the measurements is given in Table 2 of the Results-section. Detailed inclusion and exclusion criteria of the studies are presented in the Appendix chapter.

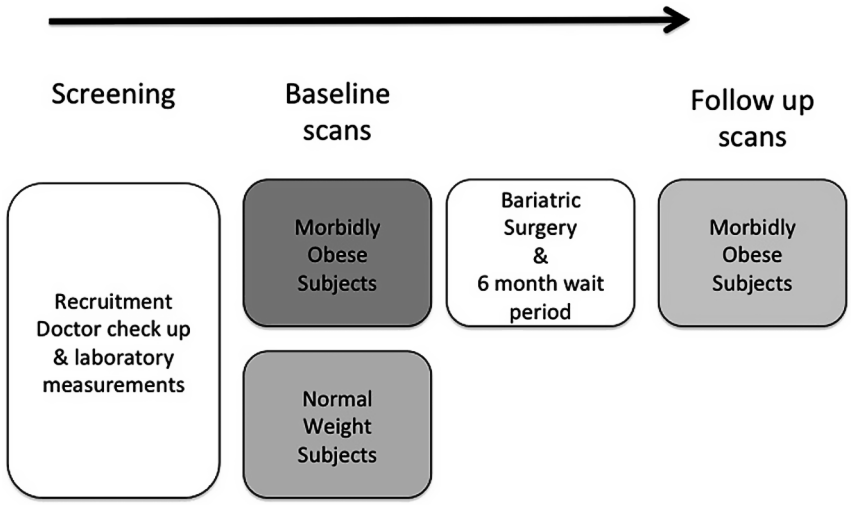

Figure 4. The general design of the Studies enclosed in this thesis.

Table 1. The source population for this thesis was drawn from two studies. NCT trial names appear below the study names on the left (http://www.clinicaltrials.gov).

	Amount of participants	Study I	Study II	Study III	Study IV
Sleevepass/ NCT00793143	25 morbidly obese		23 (**)	23 Postop: 18 (#)	22 Postop: 17 (#)
	10 healthy controls (PET & MRI)		10	10	7 (†)
	12 healthy controls (MRI)		12	4 (***)	
SleevePET2/ NCT01373892	28 morbidly obese	27 (*)		27 Postop: 22 (##)	
	15 healthy controls	14 (*)		14	

Abbreviations: (*) Exclusions were made based on micro vascular findings in brain MRI scans (as assessed by a radiologist). (**) Two scans were lost due to unsuccessful data storage. (***) Only subjects that had completed the oral glucose tolerance test and other metabolic measures were included. (†) We lost two control scans as a result of unsuccessful data storage, and one control subject was excluded because of an elevated BMI (over 25). (#) Two subjects did not undergo bariatric surgery. Three participants opted to drop out from the postoperative studies. (##) One subject did not undergo bariatric surgery. Three participants opted to drop out from the postoperative studies. 1 scan was lost due to overt motion.

4.2 Study I: Volitional appetite control as measured by fMRI

In study 1 we used fMRI to reveal brain circuits supporting volitional appetite control in healthy and obese individuals. The scanning involved measurement of blood oxygenation level dependent (BOLD) contrast, in which the MR images are made sensitive to the state of oxygenation of hemoglobin (Kim & Ogawa 2012). The variations of the BOLD signal reflect variations of the functional activity of neural networks in a millimeter precision and with a temporal resolution that typically varies between 1-3 seconds (Hillman 2014; Huettel et al. 2004). Hence, fMRI can be applied to identify neural activity during various cognitive tasks.

Experimental design: Participants refrained from eating and drinking for 3–4 hours prior to scanning. Before the MRI scans, participants also rated their feelings of hunger. The stimuli were 80 digitized full-color images of foods (Nummenmaa et al. 2012).

During fMRI acquisition, food stimuli were presented at the center of the screen in 16 s blocks intermixed with 1.75 s rest periods. The blocks were presented in pseudo-randomized order and fully counterbalanced across participants. Altogether there were twenty blocks of each condition, and total scanning time was approximately 19 minutes. During each block, participants saw four food pictures for 4 seconds. A colored rectangle around the picture denoted the task the participant had to perform throughout that block (Figure 5): during *inhibition* task, they had to inhibit urges to eat the food, during passive *viewing* task they had to view the foods without appetite modulation, and during *imaginary eating* task they had to imagine eating the foods they saw.

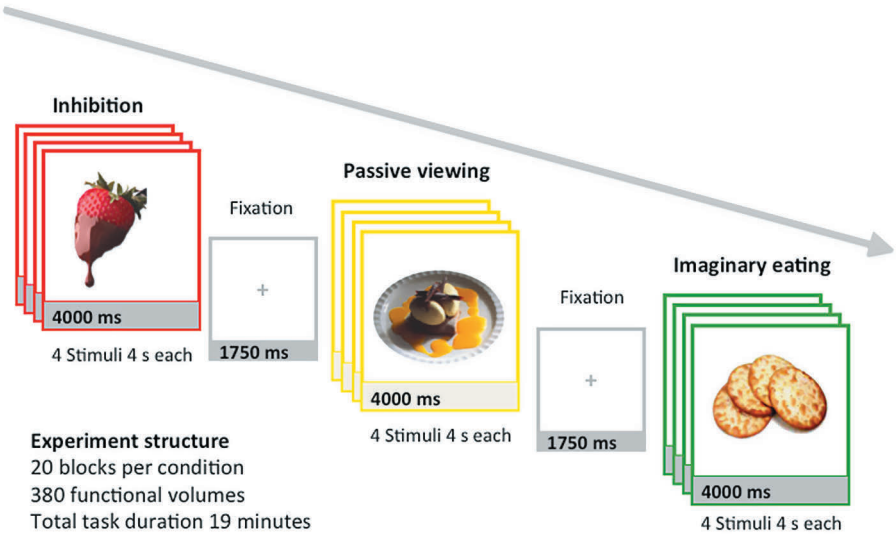

Figure 5. The food control task used in the fMRI experiment (Study I).

MR image acquisition: MR imaging was performed with Philips Gyroscan Intera 1.5 T CV Nova Dual scanner at Turku PET centre. 380 functional volumes covering the whole brain were acquired with T2-weighted echo-planar imaging (EPI) sequence with following parameters: TR = 2987 ms, TE = 50 ms, 90° flip angle, 192 mm FOV, 64 x 64 reconstruction matrix, 62.5 kHz bandwidth, 4.0 mm slice thickness, with no gaps between slices, 30 interleaved slices acquired in ascending order. In addition, High-resolution anatomical images were acquired using a T1-weighted sequence with 1 mm^3 resolution and by following acquisition parameters: TR 25 ms, TE 4.6 ms, flip angle 30°, 280 mm FOV, 256 x 256 reconstruction matrix.

Data preprocessing: Data were pre-processed and analyzed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/) running on Matlab 2011b (Math Works, Natick, MA). The EPI images were first realigned to the first scan image by rigid body transformations. The

data quality was checked from the SPM prints that visualize the amount of movement required for the co-registration and also by viewing a sample of each participants EPI volumes. This step corrects the data for head movements that occur during scanning. Next, EPI images were normalized to the standard MNI space (Montreal Neurological Institute – International Consortium for Brain Mapping) using linear and non-linear transformations obtained from the normalization parameters of T1 images. Finally, the EPI images were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM).

Modeling of task evoked BOLD responses and statistical analysis: A whole-brain general linear model (GLM) was used to assess regional effects of task parameters on brain activation (Friston et al. 1994). Low-frequency signal drift of the BOLD signal was removed using a high-pass filter (cut-off 128 s) and we applied autoregressive AR(1) modeling of temporal autocorrelations. The first level model estimates the individual task related differences in brain activations between the tasks. This model included all three experimental conditions and each realignment parameter from the EPI co-registration step as regressor of no interest. The individual contrast images were generated using the contrasts: inhibition <> viewing, imaginary eating <> viewing, and inhibition <> imaginary eating. We used these contrast images in the second-level model, which is a new GLM that was used to generate statistical t-test images across all subjects and between subject groups. The data were thresholded at $p < 0.05$, false discovery rate (FDR) corrected at the cluster level (Chumbley et al. 2010).

The task related connectivity analysis: The connectivity between brain regions can vary as a function of the psychological context (Friston et al. 1997). This is known as a Psychophysiological Interaction (PPI). PPI analysis reveals which regions have more or less similar activity pattern or "connectivity" with the source region as a function of a specific contrast (Passamonti et al. 2008). Source region selection was hypothesis driven. The key nodes of the brain' s inhibition network were selected from a previous meta-analysis on inhibitory processing in go/no-go tasks (Simmonds et al. 2008; Criaud & Boulinguez 2013) and to the caudate nucleus (Nummenmaa et al. 2012). A spherical 8 mm region of interest (ROI) was drawn at these locations in inhibition minus passive viewing contrast. The time-series for each participant was computed by using the first eigenvariate from all voxel time series in the defined volume around the ROI coordinate and PPI-deconvolution parameter defaults in SPM8 were used (Gitelman et al. 2003). The PPI term was then calculated as the element-by-element product of the ROI in neuronal time-series and a vector coding for the selected contrast (*inhibition > passive viewing*). This product was then re-convolved by the canonical hemodynamic response function (hrf). First-level PPIs were run to generate SPM contrast images similar to the first level GLM model, and these contrast images were analyzed and thresholded in the second-level modeled as described above.

4.3 Study II: Brain changes in obesity as measured by VBM and DTI

In Study II we combined VBM and DTI measurements to unravel brain changes of obesity as compared to healthy controls. Anatomical MR images can be used for a voxel-wise characterization of brain tissue compartments, namely, grey matter (GM) and white matter (WM) via separating these tissues based on intensity differences; for a review see (Ashburner & Friston 2001). VBM was originally developed to detect cortical thinning more reliably than traditional user-defined volumetric analysis via introduction of spatial normalization to a standard space and spatial smoothing. The technique used also corrects for the volumetric changes to yield tissue "density" values. DTI is an MRI technique that measures the diffusion properties of water molecules within body tissues; for a review see (Mori & Zhang 2006). Within the brain diffusion is restricted in the white matter that comprises mainly of axons that connect neurons with one another. Voxel-based analysis of the DTI analysis was used to yield two metrics: the fractional anisotropy (FA) and mean diffusivity (MD) values. Higher MD and lower FA values indicate damage or impaired fiber integrity due to loss of coherence in diffusion directions within white matter tracts (Soares et al. 2013).

MR image acquisition: For VBM, high-resolution anatomical images with 1 mm^3 resolution were acquired using a T1-weighted sequence: TR 25 ms, TE 4.6 ms, flip angle 30°, 280 mm FOV, 256 x 256 reconstruction matrix. For DTI, 32 non-collinear directions of gradients and non-diffusion weighted b0 image were acquired to obtain the whole diffusion tensor, using a single-shot spin-echo echo-planar-imaging sequence (TE 89 ms, TR 5947 ms, 90° flip angle, FOV 240 112 x 112 imaging matrix). The images were reconstructed to 256 x 256 imaging matrix with 3 mm slice thickness and 1 mm gap between slices. The data contained 36 transverse slices.

VBM data preprocessing: Structural images were analyzed with SPM5 (Welcome Department of Cognitive Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm/). The SPM5 software enables automated radio-frequency bias correction, tissue classification and spatial normalization to be combined with the segmentation step (Ashburner & Friston 2005; Good et al. 2001). Prior to segmentation the origo of each T1 volume was set to anterior commissure. This step entailed visual quality control of the data. For the segmentation, we set the cut-off of spatial normalization to 25 mm with medium affine regularization (0.01). Following normalization and segmentation into GM and WM, a modulation step was incorporated to take into account volume changes caused by spatial normalization that causes certain brain regions to shrink or expand. This was done by multiplying the voxel values in the segmented images by the Jacobian determinants that were obtained from the spatial normalization step (Ashburner & Friston 2001). The segmented, normalized, and modulated GM and WM images were smoothed using a Gaussian kernel of 10 mm FWHM, and entered into between-group comparisons (healthy controls <> obese patients) using GLM (Friston et al. 1994). For this model an

absolute threshold mask was set at 0.1 to avoid possible edge effects around the border between GM and WM.

DTI data preprocessing: For DTI (Mori & Zhang 2006), diffusion weighted (DW) images were processed with FDT analysis package in FSL (www.fmrib.ox.ac.uk/fsl) software. First, the DW images were corrected for movements and scanner artifacts termed eddy currents (Jenkinson & S. Smith 2001), and non-brain structures were removed using Brain Extraction Tool (S. M. Smith 2002). Next, the diffusion tensors, FA and MD images were computed using FDT toolbox implemented in FSL (S. M. Smith et al. 2004). FA and MD images were analyzed in SPM5. Images were normalized to the MNI space using linear and nonlinear transformations, smoothed with a Gaussian kernel of 10 mm full-width half-maximum (FWHM), and entered into between-group comparisons (healthy control subjects <> obese patients) using GLM.

Delineation of subcutaneous and visceral fat masses: To obtain complementary metrics to describe the adiposity of the participants, we also determined the volume of abdominal subcutaneous (SAT) and visceral adipose tissue (VAT) from axial T1-weighted images dual fast field echo images covering the abdominal area (TE 2.3 and 4.6 ms, TR 120 ms, slice thickness 10 mm without gaps). Abdominal subcutaneous and visceral fat was counted from top of liver until the head of femoral bone appeared on both sides, and then were analyzed with the SliceOmatic software version 4.3 (http://www.tomovision.com/products/sliceomatic.htm). Sets of images were opened in SliceOmatic and the borders of adipose tissues were determined. SAT and VAT volumes were calculated from these manually drawn slices.

Statistical analysis: A between-subjects model (independent samples t-test) was used in SPM to compare the differences between the groups in all derived brain metrics. Effects of age and gender were modeled as a regressor of no interest for all analyses. Statistical threshold was set at $p < 0.05$; family-wise error (FWE) corrected (Nichols & Hayasaka 2003) at the cluster level (Hayasaka et al. 2004). An uncorrected threshold of $p < 0.001$ was used to highlight smaller differences. To determine which metabolic variables best predict brain abnormities GM and WM as well as both diffusion parameters (FA and MD values), the peak values from between group comparisons were extracted using Marsbar software (http://marsbar.sourceforge.net). Further statistical analyses were done using SPSS 18 Windows (SPSS, Chicago, IL) including definition of linear associations between each brain metric and metabolic variables.

4.4 Study III: Structural brain changes after bariatric surgery (VBM)

In study III, we used VBM for studying the changes in brain tissue densities after bariatric surgery and concomitant weight loss. We included participants from both consecutive studies (Table 1).

Statistical analysis: MR image acquisition and data preprocessing were the same as in Study II. The smoothed GM and WM images were analyzed using GLM in SPM8 with identical settings to Study II. First, a between-subjects model (independent samples t-test) was used to compare GM and WM densities for non obese versus obese patients, as well as for non-diabetic obese versus diabetic obese subjects in the baseline state before surgery. Second, within-subjects model (paired sample t-test) was employed to estimate GM and WM changes following bariatric surgery by comparing patients' postoperative and preoperative scans with each other. Subjects' ages in years were entered into the between groups comparison models as a regressor of no interest to account for age-related changes across subjects. Statistical threshold was set at $p < 0.05$ whole brain FDR corrected.

4.5 Study IV: Obesity-related changes in brain insulin sensitivity ([18F]-FDG-PET)

[18F]-FDG provides us with highly accurate estimate of cellular rate of glucose uptake that in the case of brain tissue approximates the cellular metabolic rate. In resting state brain scans the technique has a temporal resolution of tens of minutes (Wadsak & Mitterhauser 2010).

Experimental design: All measurements were performed after a 12 h fast. PET scans were performed under two conditions: under fasting condition and during euglycemic hyperinsulinemic clamp (DeFronzo et al. 1979; Voipio-Pulkki 1992; Nuutila et al. 1996), on separate days less than 2 weeks apart. In brief, during insulin infusion plasma glucose concentration is held normal (5.0 mM) with glucose infusion. Hyperinsulinemia prevents physiological liver gluconeogenesis and whole body insulin sensitivity can be defined based on glucose infusion demand (DeFronzo et al. 1979). In terms of central nervous system clamp mimics to some extent a prolonged postprandial state (Nummenmaa et al. 2012; Hirvonen et al. 2011). Arterialized blood samples were drawn every 30 min during the scan and analyzed for radioactivity concentration in plasma using an automatic g counter (Hirvonen et al. 2011).

PET image acquisition: The scans were performed on GE Advance PET camera (General Electric Medical Systems, Milwaukee, WI). [18F]-FDG-PET was synthesized with standard computer-controlled manufacturing procedure of the Turku PET Centre (Hamacher et al. 1986). The tracer (187 +/- 9 MBq) was injected intravenously over 15 s, and radioactivity in brain was followed thereafter for 40 min (4 x 30, 3 x 60, and 7 x 300 s frames). All data were corrected for dead time decay, and measured photon attenuation and reconstructed using a Hann filter with a cutoff frequency of 0.5 (Alenius & Ruotsalainen 1997).

Data preprocessing: The influx constant (Ki) was calculated for each voxel separately using the linear Gjedde-Patlak plot with arterial plasma input function, with a linear phase start time of 20 min. Glucose uptake estimate of the cerebral metabolic rate (CMR) (CMRglu [μmol x 100 g – min-^{1}]) was then calculated at voxel level as follows: CMRglu = Ki x C_p / LC; where C_p is the plasma glucose concentration and LC is the lumped constant (which was set at 0.80). Summed PET images were normalized spatially to a ligand-specific into and in house template in MNI space (MNI International Consortium for Brain Mapping) using SPM5 (www.fil.ion.ucl.ac.uk/spm/) running on Matlab (Math Works, Natick, MA). Normalization parameters were subsequently applied to corresponding parametric glucose uptake images (Ashburner & Friston 1997). Finally, images were smoothed at 10 mm FWHM.

Statistical modeling: Preoperative data were analyzed with a 2 (fast, clamp) x 2 (non-obese, obese) ANOVA and the follow-up data for morbidly obese patients with a 2 (fast, clamp) x 2 (preoperative, postoperative) ANOVA in SPM5. The statistical threshold in SPM analysis was set at $p < 0.05$, FDR corrected (Chumbley et al. 2010). Regional CMRGlu values were extracted using Marsbar (http://marsbar.sourceforge.net). Further statistical analyses were done using SPSS, version 18.0, for Windows (SPSS, Chicago, IL).

5. RESULTS

Characteristics of the groups that participated in both studies as well as changes after the surgery are presented in Table 2. Bariatric surgery resulted in expected weight loss and subsequent improvement in some metabolic variables such as fasting glucose and blood pressure, but the improvement was not statistically significant for all the measured variables (Table 2).

Table 2. Characteristics of the participants as in Study III. Data are presented from all the participants in Sleevepass & Sleevepet 2 studies. *Note: * p < 0.05; *** p < 0.001*

	Non-obese N=28, 2 males		Morbidly Obese Preoperative N=50, 6 males			Morbidly Obese Postoperative N=40 all female		
	Mean	SD	Mean	SD		Mean	SD	
Age (years)	45.7	10.4	44.2	9.7	NS			
Weight (kg)	67.2	10.2	117.8	14.3	***	90.3	14.3	***
BMI (Body mass index)	23.3	2.5	42.3	3.9	***	32.6	5.0	***
Waist circumference (cm)	76.7	9.2	119.9	10.4	***	100.4	13.5	***
Fat percent	30.5	6.4	49.1	5.6	***	42.7	6.3	***
Systolic blood pressure (mmHg)	126.1	13.2	132.7	17.6	NS	125.7	11.7	*
Diastolic blood pressure (mmHg)	79.2	8.6	85.6	9.3	NS	79.7	9.5	**
HbA1c(%)	5.5	0.03	5.9	0.06	NS	5.6	0.06	NS
Fasting glucose (mmol/l)	5.3	0.05	6.2	1.2	***	5.3	0.6	NS
Triglycerides (mmol/l)	0.07	0.03	1.2	0.04	***	4.2	1.0	NS
HDL (mmol/l)	1.8	0.4	1.2	0.2	***	1.3	0.3	NS
LDL (mmol/l)	2.5	0.8	2.4	0.7	NS	1.1	0.4	NS
HDL / Kol ratio (%)	41.0	8.6	30.1	7.2	***	32.8	9.6	NS
sensitive CRP (mg/l)	0.9	0.9	4.4	3.7	***	2.4	0.9	NS
Thyroid stimulating hormone (mU/l)	1.12	0.4	1.7	1.4	*	1.3	1.4	NS
Beck depression inventory II	3.9	3.8	5.4	4.9	NS	3.7	3.4	NS

5.1 Study I: Brain networks of appetite control in healthy and obese

In Study I, fMRI was used to delineate the brain circuits of volitional appetite control and measuring whether functioning of this network would be altered in morbid obesity. Participants viewed pictures of foods for 19 minutes, while their task was to volitionally *inhibit* their urges to eat, *imagine eating* the foods or *view* the foods passively. We hypothesized that volitional modulation of appetite would engage the frontal cortical circuits. Obese individuals were expected to have lower frontal activations during appetite control (*inhibit* > *imagine eating*) reflecting failure to volitionally inhibit their

urges to eat. We further predicted that functional connectivity of the volitional appetite control and reward circuit would be lowered in obese individuals.

Volitional appetite modulation across all participants: Comparison between the *inhibition* and passive *viewing* revealed widespread activation in frontal cortical regions, including bilateral superior, superior and medial frontal gyri, cingulate cortex and precentral gyrus bilaterally (Figure 6A). Increased activation was also observed in the left inferior frontal gyrus and temporal pole. Supplemental motor area (SMA), thalamus and cerebellum were activated bilaterally. Additional activations were observed in visual areas in the bilateral occipital cortices. When *imaginary eating* was contrasted with passive *viewing*, similar brain areas were activated (Figure 6B). Finally, to reveal the brain circuit of *appetite control*, we contrasted the active tasks (*inhibition* and *imaginary eating*) with each other. The areas showing increased activation during inhibition versus imaginary eating included right inferior frontal gyrus, bilateral medial frontal cortices, posterior cingulate cortex, precuneus, cuneus, right hippocampus and superior parietal areas (Figure 6C).

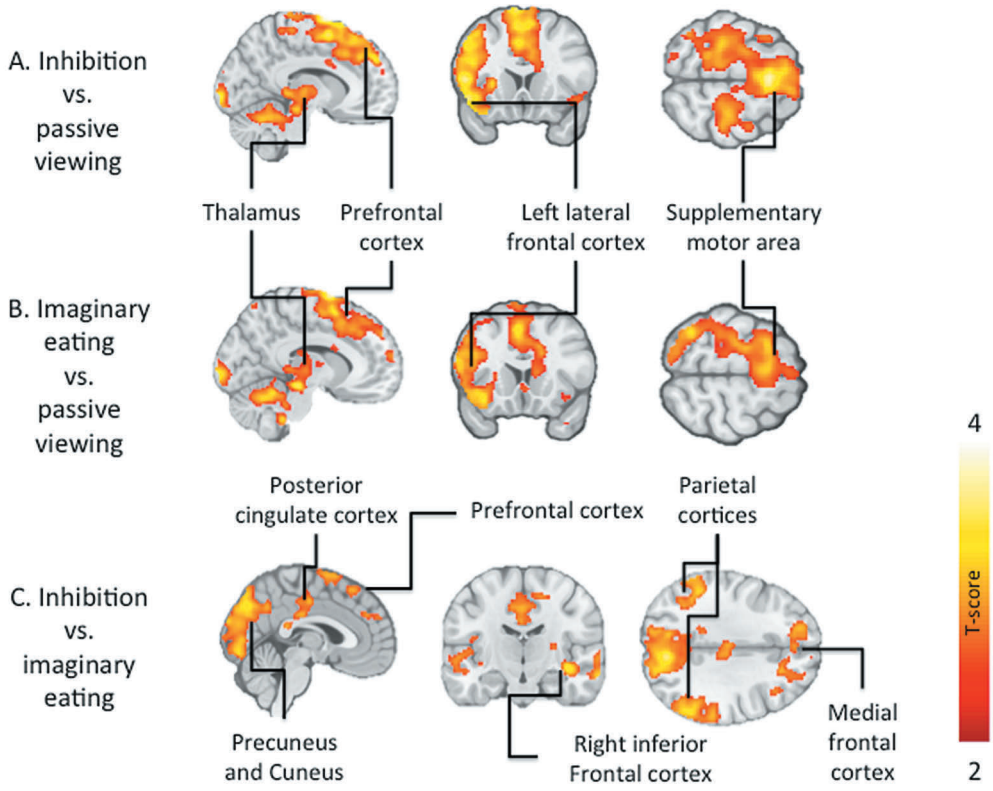

Figure 6. Regional brain responses across all subjects. Brain regions showing stronger responses during inhibition minus passive viewing condition (A), imaginary eating minus passive viewing condition (B) and inhibition condition minus imaginary eating (C). The data are thresholded at $p < 0.05$, FDR corrected at cluster level. The figure also appears in the original publication of study I.

Differences between obese and healthy participants: In the inhibition minus imaginary eating comparison, normal-weight subjects showed stronger activations in bilateral dorsal caudate nuclei and anterior medial frontal areas (Figure 7C). Other contrasts of interest revealed no significant activations at our a priori statistical threshold ($p < 0.05$, FDR corrected). Using a more lenient statistical threshold ($p < 0.005$, uncorrected at the cluster level), we found that in inhibition vs. passive viewing comparison, normal-weight subjects had stronger responses in the left middle and inferior frontal gyri, and right orbitofrontal cortex (OFC) (Figure 7A). When contrasting imaginary eating with passive viewing, healthy subjects showed stronger activation of right insula (Figure 7B).

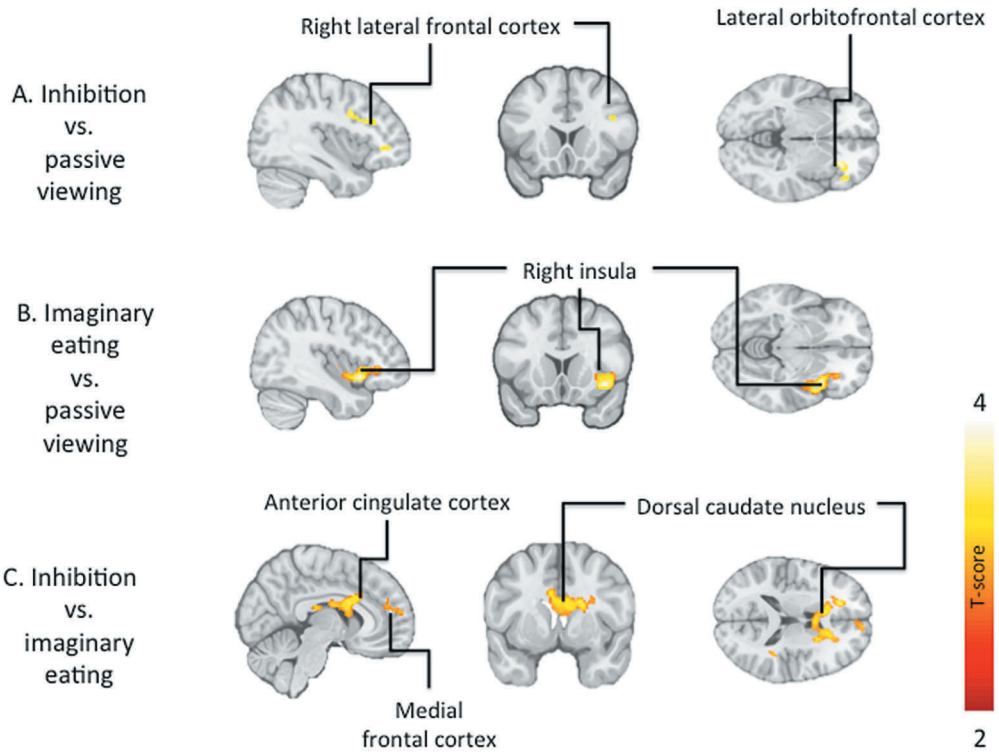

Figure 7. Regional differences in brain activations between normal-weight and obese subjects. Brain regions showing stronger activation in normal-weight versus morbidly obese subjects in inhibition minus passive viewing (A), imaginary eating minus passive viewing (B) and inhibition minus versus imaginary eating (C) contrasts. The data in C are thresholded at $p < 0.05$, FDR corrected at cluster level ($p < 0.005$, uncorrected at A and B). The figure also appears in the original publication of study I.

Functional connectivity analysis: We used PPI to study task-driven (*inhibition* versus *passive viewing*) functional connectivity of the control circuit. Across all subjects the right caudate nucleus showed increased connectivity with bilateral precunei/cunei

and parietal cortices (Figure 8). The left preSMA showed decreased connectivity with bilateral cerebellum and left superior parietal gyrus. The cluster extended to left insula, thalamus and caudate (Figure 8). The right insula showed decreased connectivity with the left precentral gyrus and paracentral lobule, with an extending cluster to preSMA (Figure 8).

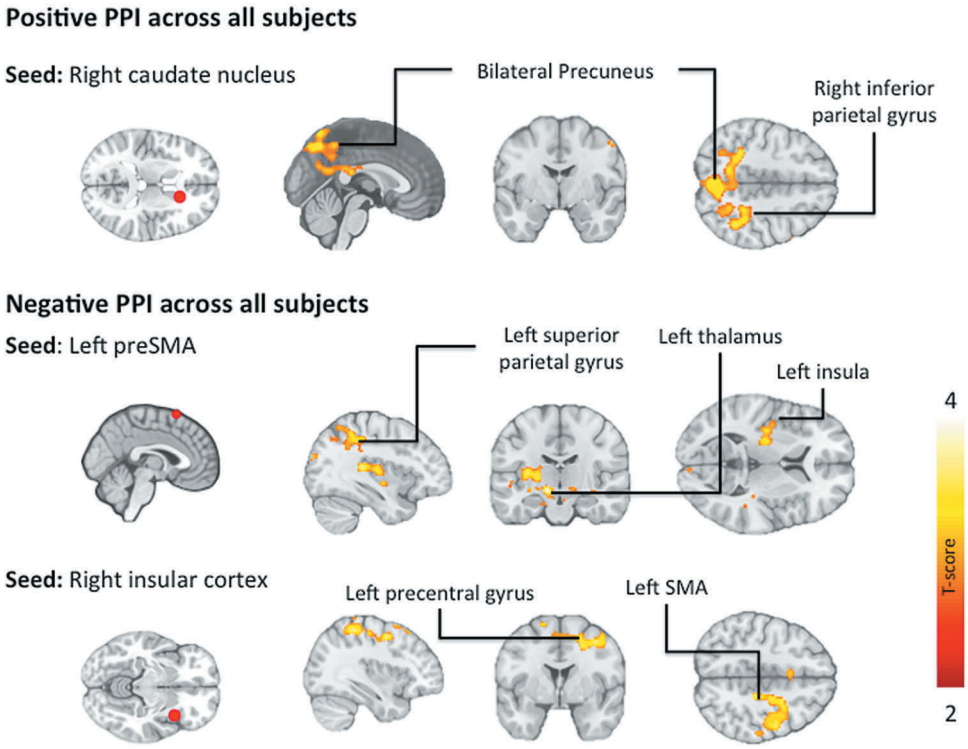

Figure 8. Functional connectivity (PPI) across all subjects. The data are thresholded at $p<0.05$, FDR corrected at cluster level. The figure also appears in the original publication of study I.

Between-group comparisons revealed that obese subjects had stronger functional connectivity between middle frontal cortex and bilateral SMA, right putamen and right middle temporal gyrus (Figure 9). Obese participants also showed stronger connectivity between the right precuneus and bilateral supplemental motor area, bilateral thalamus, bilateral pre-central gyri and right inferior frontal gyrus extending to right insula (Figure 9). In addition, obese participants showed stronger connectivity between preSMA and bilateral middle cingulate cortex, bilateral medial and lateral superior frontal cortices, thalamus, right inferior parietal lobule and right angular gyrus (Figure 9).

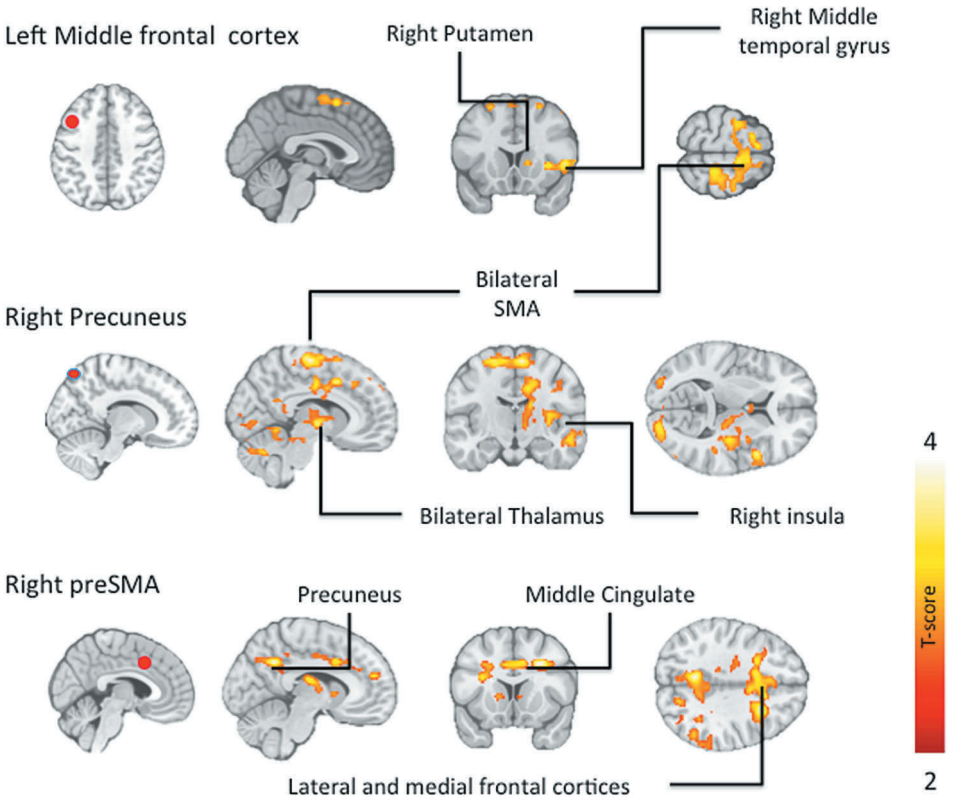

Figure 9. Differences in functional connectivity (PPI) in normal-weight versus obese individuals. The data are thresholded at $p < 0.05$, FDR corrected at cluster level. The figure also appears in the original publication of study I.

Summary: Altogether, the results suggest that premotor areas, superior frontal cortices and the precuneus support cognitive modulation of appetite upon encountering visual food cues. Although most of these areas appear to function similarly in obese and normal-weight individuals, dorsal striatal and medial frontal responses during volitional *appetite control are* reduced in obese individuals. Yet contradicting our hypothesis, functional connectivity of the control circuit was increased, rather than decreased, in morbidly obese versus control subjects. These results offer some support to the hypothesis that obesity is linked with impaired cognitive inhibition capability in regard to food cues.

5.2 Study II: Adverse structural changes accompany obesity

A combined VBM and DTI analysis was used to delineate the obesity-related changes in brain tissue's structural integrity. Full-volume analysis was used to compare grey (GM) and white matter (WM) density between the groups as well as fractional anisotropy (FA) and mean diffusivity (MD) values. Standard linear regression models were used to

determine metabolic factors that associate with the structural metrics that were extracted from preprocessed images. We hypothesized that GM/WM densities and white matter integrity would be lowered in the morbidly obese subjects in comparison with healthy controls and the metabolic factors would explain the degree of these adverse changes.

Differences between obese and healthy participants: Obese subjects had lower focal and global GM and WM densities than non-obese control subjects did (Figure 10), as well

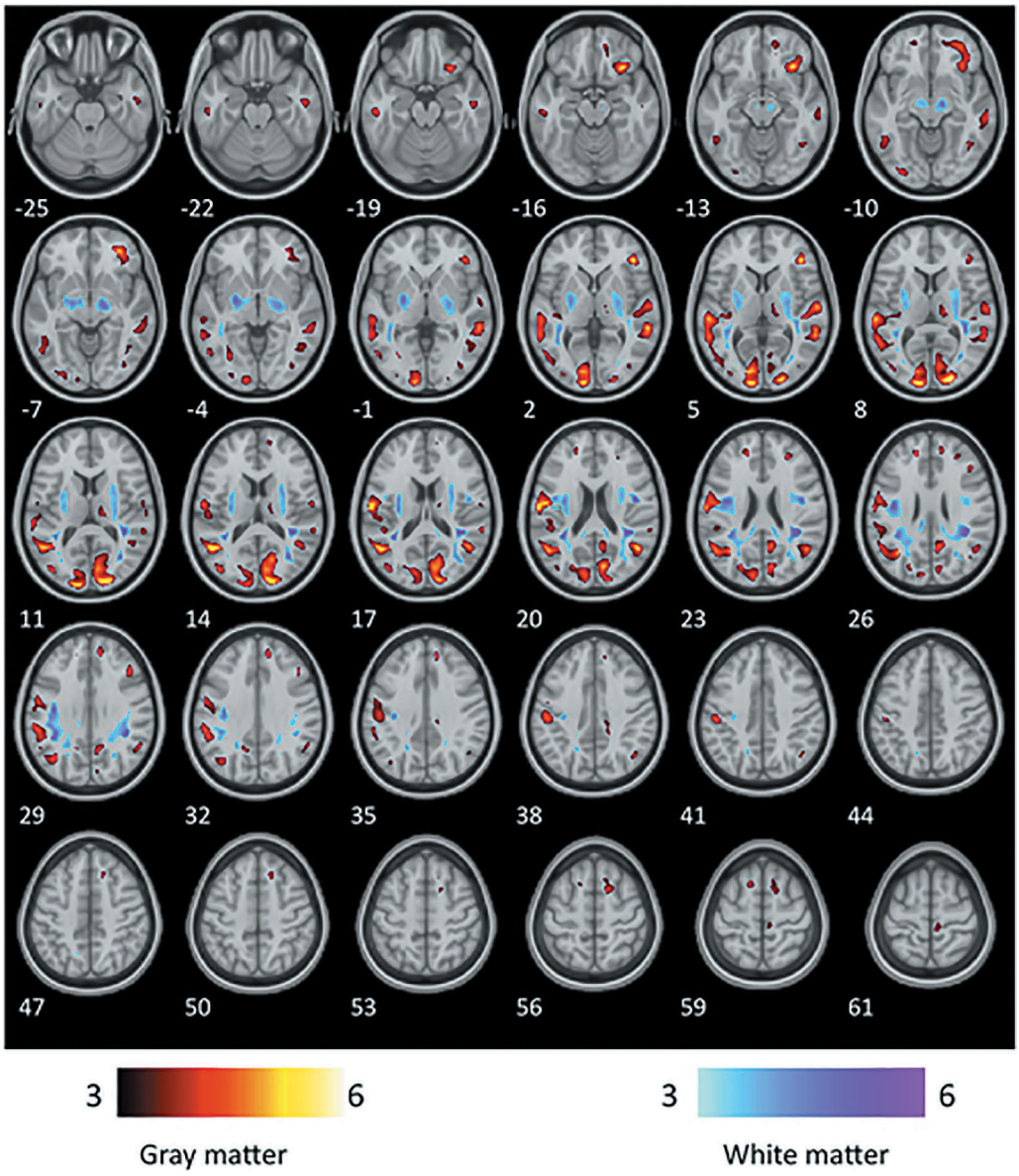

Figure 10. Regions with decreased grey (red to yellow) and white (turquoise to pink) matter volumes in non-obese > obese subjects. The data are thresholded at $P < 0.001$, uncorrected, for visual inspection. The figure also appears in the original publication of study II.

as lower FA and MD values (Figure 11). Grey matter density was significantly lower in right inferior frontal gyrus, temporal lobes (inferior temporal gyri, right middle temporal gyri), somatosensory areas (left postcentral gyrus) and visual cortices (occipital gyri). WM density was lower near the limbic regions that convey emotional and homeostatic signaling (insular region, near amygdalae) and below superior and middle temporal cortices. Obese subjects had lower FA values in corticospinal tracts, mammillary bodies, optic radiations, corpus callosum, and right inferior occipitofrontal fascicle, and lower MD values in both uncinate fascicles and inferior occipito-frontal fascicles (Figure 11).

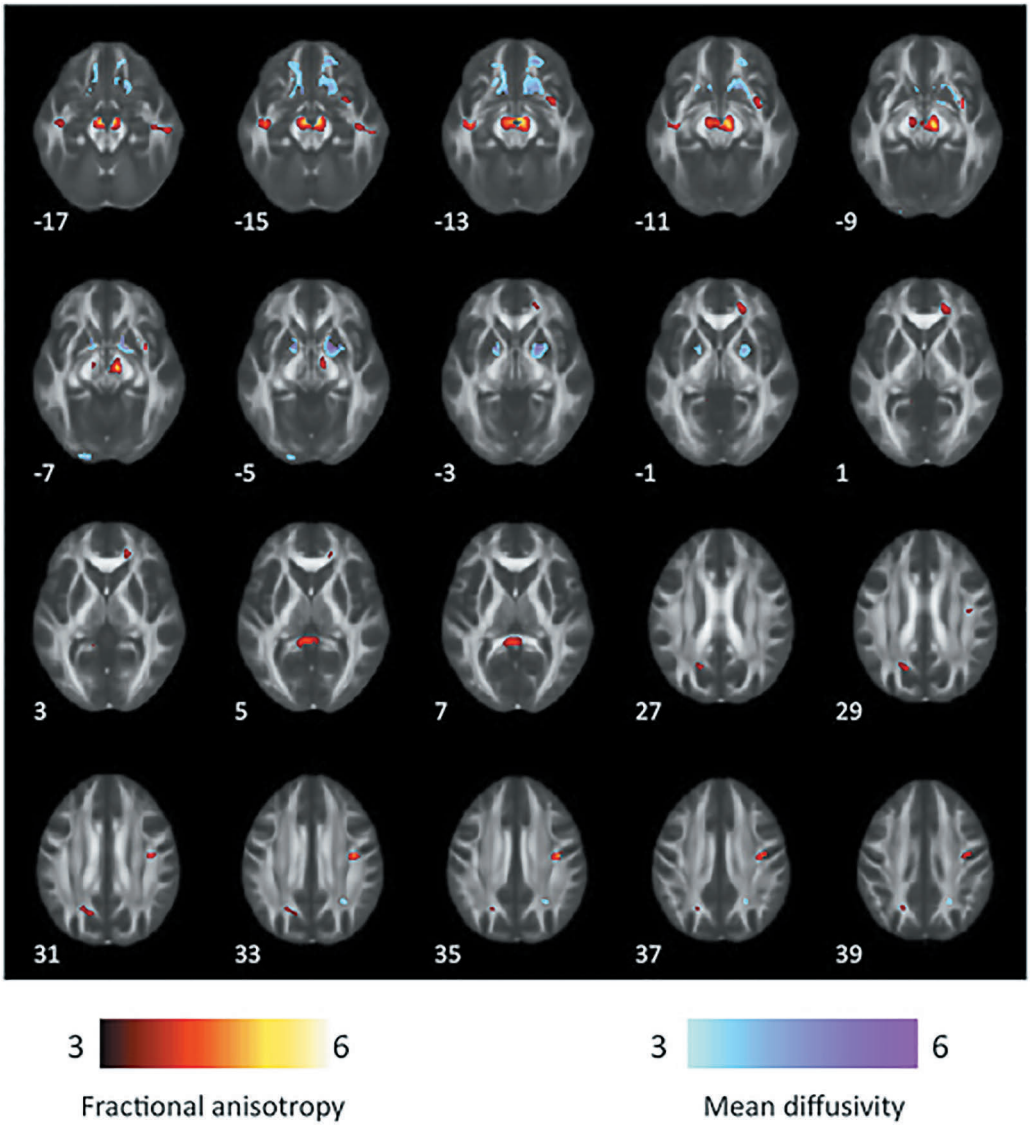

Figure 11. Regions with decreased FA (red to yellow) and MD (turquoise to pink) values in non-obese > obese subjects. The data are thresholded at $P < 0.001$, uncorrected, for visual inspection. The figure also appears in the original publication of study II.

Linear associations between brain metrics and metabolic variables: Regression analysis showed that focal brain metrics were negatively associated with BMI: GM density in right thalamus and right middle frontal gyrus, WM density below the left insula and in locus coeruleus, FA values in right callosal and maxillary bodies as well as MD values in left putamen and right inferior orbitofrontal fascicles. Second, we found that the changes in brain structure were systematically associated with fat percentage, and that triglyceride levels had a minor association in some regions. Also, subcutaneous adipose tissue volumes correlated negatively with most regional GM volumes.

Summary: The focal obesity-related structural changes were observed in brain regions governing reward seeking, inhibitory control of appetite as well as connecting white matter tracts. Furthermore, these changes are negatively associated with BMI. It is possible that these abnormalities reflect vulnerable phenotype that increases the risk of developing obesity. Alternatively, it is possible that some structural changes reflect neural degeneration caused by obesity.

5.3 Study III: Bariatric surgery recovers brain atrophy

Building up on the findings in Study II, we quantified the effects of weight loss after bariatric surgery to brain densities by using VBM. Baseline scans included non-obese and morbidly obese participants. Obese participants were scanned again six months after the surgery. Local GM and WM densities were quantified using voxel-based morphometry (VBM). Full-volume analyses were used for comparing baseline between-group differences as well as the effect surgery-induced weight loss in the morbidly obese. Metabolic variables were used in linear models to predict WM and GM densities in the clusters identified from the preoperative full-volume comparisons. First, we predicted that GM and WM densities would be initially lowered in the morbidly obese subjects in comparison with healthy controls. Second, we hypothesized that the rapid weight loss after bariatric surgery would recover at least some of the obesity-related volume reductions.

Differences between healthy and obese participants: Preoperatively the morbidly obese subjects had decreased grey matter density in bilateral inferior orbitofrontal regions, right middle frontal gyrus, left dorsolateral prefrontal cortex and right middle cingulate gyrus (Figure 12). We also found reductions in right insula, right inferior temporal gyrus, bilateral precunei and occipital regions. White matter reductions were observed beneath bilateral orbitofrontal gyri, posterior in the left middle occipital gyrus, right cuneus, cerebellar regions and the midbrain/medulla (Figure 12). The diabetics did not differ from the non-diabetic obese participants.

Linear associations between brain metrics and metabolic variables: In general, most metabolic variables linked with obesity (BMI, waist and abdominal circumference, fat percent, abdominal and visceral fat volumes, systolic blood pressure, fasting glucose, cholesterol levels and triglycerides) were negatively associated grey and white matter densities. Plasma HDL cholesterol levels were positively associated with both GM and WM in most brain regions

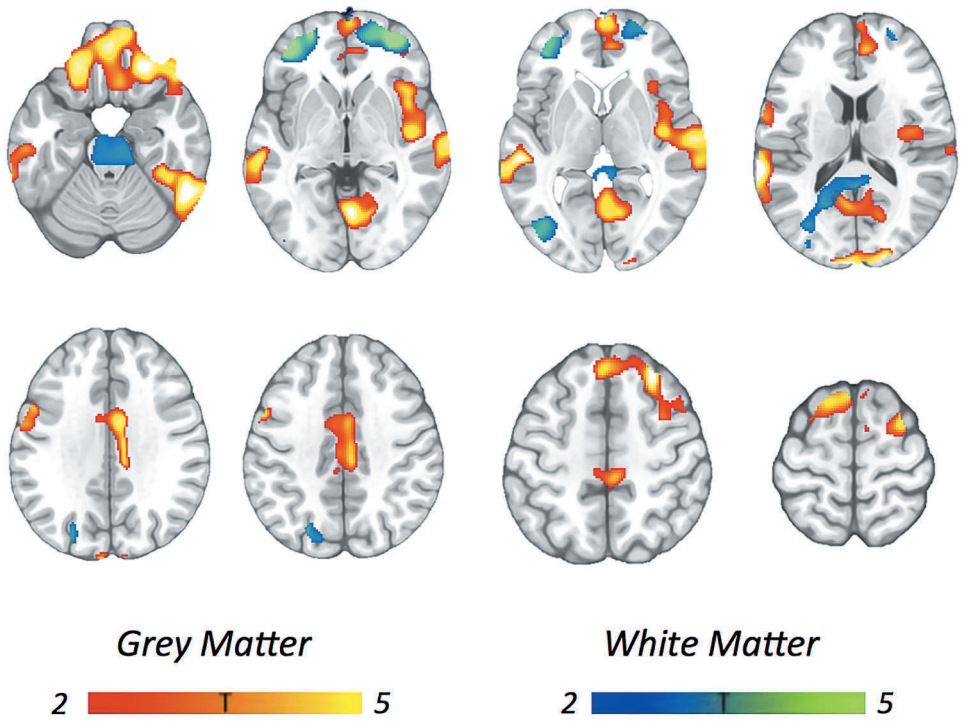

Figure 12. Brain density changes of morbid obesity. The brain areas that show lower densities in morbidly obese subjects in preoperative state as compared to non-obese subjects. Results are thresholded at $p < 0.001$, FDR corrected at the cluster level. The z-coordinates in MNI space from left to right are: -22, -2, 2, 15 for the top panel and 32, 40, 52, 65 for the lower panel.

Changes after bariatric surgery: We found extensive volume recovery, spanning throughout the white matter (Figure 13). Grey matter recovery was observed in frontal and posterior brain regions near the sulcal folds (Figure 13). These effects only partially overlap with initial volume reductions observed in the non-obese versus obese comparison in the preoperative state (Figures 12 and 13). Preoperative diabetic state did not predict the magnitude of GM/WM density changes. Correlation analysis between the postoperative increases in brain densities and decreases in metabolic variables revealed a positive association between brain density increases and decreases in fat percent and waist circumference. Yet, surprisingly, there were no systematic associations between weight change or change in BMI.

Summary: Obesity was associated with decreased brain densities, which replicated findings from Study II. Central adiposity and accompanying cardiovascular risk factors (age, systolic blood pressure, cholesterol) were predictors for the initial regional atrophy as well as its recovery after weight loss (systolic blood pressure, HDL cholesterol). Weight loss resulted in global recovery in white matter volume as well as more local recovery of grey matter. These changes likely represent improved brain integrity, which suggests that weight loss has a positive causal influence on brain atrophy.

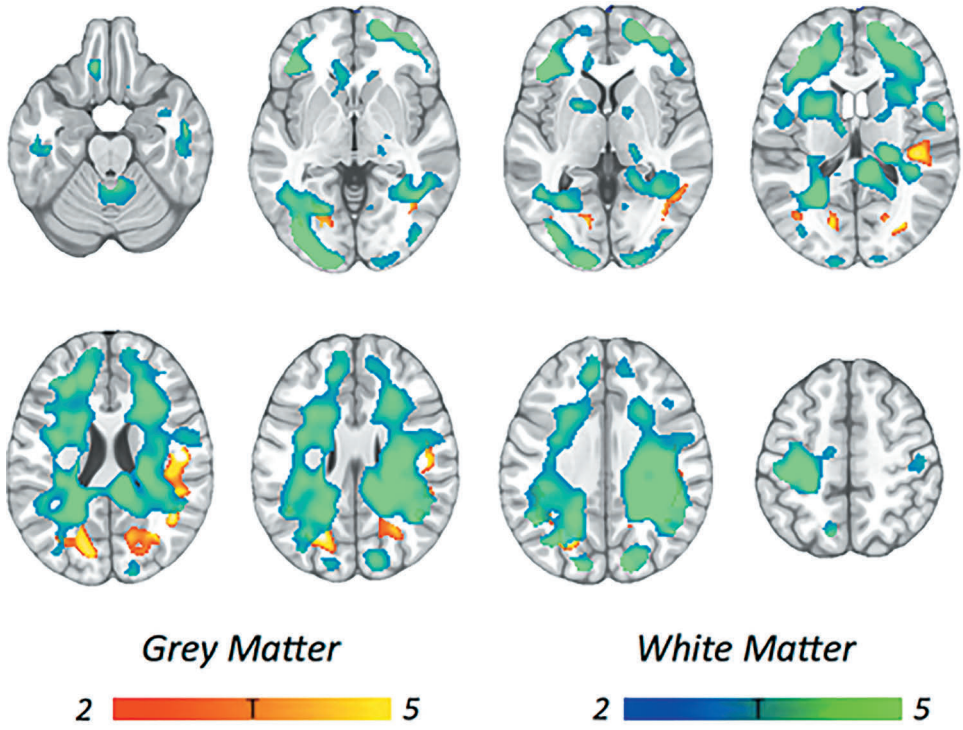

Figure 13. Brain density recovery after bariatric surgery. The brain areas that show brain density recovery in morbidly obese after bariatric surgery as compared to the preoperative state. Results are thresholded at $p < 0.001$, FDR corrected at the cluster level. The z-coordinates in MNI space from left to right are: -22, -2, 2, 15 for the top panel and 22, 28, 32, 52 for the lower panel.

5.4 Study IV: Insulin increases brain glucose uptake in obese before surgery

To characterize obesity-related changes in brain insulin responses, we measured brain glucose uptake with and without insulin stimulation (hyperinsulinemic-euglycemic clamp) using [18F]-fluorodeoxyglucose PET scanning. We expected that obese individuals would have higher glucose metabolism during insulin stimulation and that rapid weight loss after bariatric surgery would result in decreased brain glucose metabolism during insulin stimulation.

Differences between healthy and obese participants: We found that during fasting condition, there was no between groups difference in brain glucose uptake. However, the hyperinsulinemic clamp increased brain glucose uptake in the obese in a widespread manner as compared to healthy subjects (Figure 14A). Thus, the hyperinsulinemia increased brain glucose metabolism only in the morbidly obese subjects.

Changes after bariatric surgery: After the surgery and weight loss, the increase in glucose metabolism under insulin stimulation was no longer observed (Figure 14B and 14C), and this attenuation was coupled with improved peripheral insulin sensitivity.

Summary: Obesity is associated with increased insulin-stimulated glucose metabolism in the brain and that this abnormality can be reversed by bariatric surgery. The results of our study support the evidence showing that insulin stimulates brain glucose metabolism of the morbidly obese. Altogether, the results imply that bariatric surgery not only results in weight loss but also improves overall metabolic health likely including the brain.

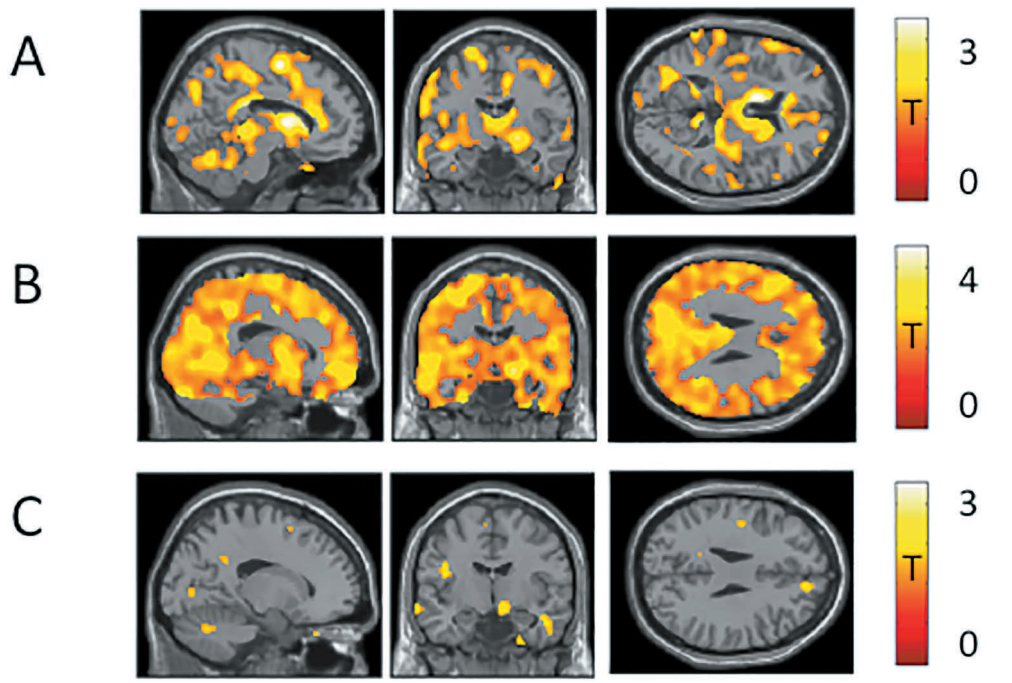

Figure 14. Insulin-induced changes of brain glucose metabolism before and after bariatric surgery. A: Preoperative ANOVA (obese > non-obese x clamp > fast). MNI coordinates 29, 2, and 10 are chosen so that regional maximum in right caudate nucleus can be seen. The data were thresholded at $P < 0.05$, FDR corrected. B: Preoperative comparison of morbidly obese patients for clamp vs. fast. MNI coordinates: -16, 8, and 28. The data were thresholded at $P < 0.05$, FDR corrected. C: Postoperative comparison of morbidly obese patients for clamp vs. fast. MNI coordinates: -16, 8, and 28. The data were thresholded at $P < 0.05$, uncorrected. The figures also appear in the original publication of study VI.

6. DISCUSSION

6.1 Functional brain changes of obesity (Study I)

By using fMRI, we were able to characterize a brain circuit of volitional appetite control that is activated while viewing visual food cues. Both inhibition and imaginary eating tasks engaged superior frontal and prefrontal, parietal and inferior cerebellar cortical regions. These findings are in line with published imaging studies (Scharmüller et al. 2012; Yokum & Stice 2013). As both volitional *inhibition of appetite* and *imaginary eating* tasks require working memory in retrieving the task and verbal instructions, activation of left-sided lateral inferior areas (Broca's area) and superior frontal cortices can be expected in both tasks (Simmonds et al. 2008; Criaud & Boulinguez 2013). The cerebellum was also activated and likely participates to control functions and maintenance of task engagement (Koziol et al. 2012). When we compared inhibition to imaginary eating, we observed activation of a more limited set of regions, particularly in superior medial frontal areas and the precuneus. This implies that medial frontal areas are specifically involved in inhibition of appetite. They are likely assisted by the precuneus, which participates to a vast array of cognitive functions and is also activated during self-referential tasks such as appetite control (Utevsky et al. 2014). It has also been proposed that precuneus might be a "hub" region for the assessment of the intensity of expectant reward encoded in the reward areas (Cavanna & Trimble 2006; Rushworth et al. 2004). Supporting this view, PPI across all participants revealed increased functional connectivity between the caudate nucleus and parietal cortices encompassing the precuneus. The caudate nucleus is known to participate both to encoding of the hedonic value of the visual stimuli and in action planning (Apicella et al. 1991; Staudinger et al. 2011). Decreased functional connectivity was observed between bilateral preSMA and left posterior insula, which might mean that the influence of the interoceptive areas is actually reduced during the cognitive appetite inhibition (de Araujo et al. 2012; de Araujo & Simon 2009; S. Frank et al. 2013; Diekhof et al. 2008).

Obesity influenced brain responses during appetite control. Normal weight subjects had stronger brain responses during appetite control (*inhibition* minus *imaginary eating* contrast) than obese individuals in medial frontal and orbitofrontal cortices, as well as in dorsal caudate nucleus. Furthermore, with more lenient thresholding, we found that normal weight individuals had stronger responses in the left dorsolateral prefrontal cortex and right orbitofrontal cortex during inhibition versus passive viewing comparison. They also had stronger responses in right insula in imaginary eating minus passive viewing

comparison. Thus, our results offer some support to the hypothesis that dysfunctions in the frontal circuits underlie pathological eating in obesity (Nummenmaa et al. 2012; Koob & Volkow 2010; Volkow et al. 2005). Decreased engagement of striatum in the obese during appetite control and lower responses in the right insula during imaginary eating may reflect diminished incentive value and impaired homeostatic encoding over visual food cues (Volkow et al. 2013; Pursey et al. 2014; de Araujo et al. 2012). Contradicting our predictions, functional connectivity analysis implicated enhanced instead of decreased functional connectivity between most regions of the cognitive control network in the obese subjects. Obese individuals had stronger functional connectivity between pre-SMA, anterior cingulate and thalamus. Second, the obese individuals showed increased connectivity between the precuneus, SMA, left postcentral -and precentral gyri. Functional connectivity analysis (PPI) does not allow us to determine causal relationships between the activated brain region activations (O'Reilly et al. 2012), but it is possible that the increased connectivity reflects increased neuronal demands and/or compensatory activation that is required to maintain volitional appetite control that is trait-specific for obese individuals.

Taken together a wide neural circuit is activated in cognitive appetite modulation. Supporting the inhibitory brain circuit function may prove effective to reduce eating and aid weight loss. Indeed, most currently available anti-obesity pharmaceuticals modulate widespread neurotransmitter systems (Adan 2013). For example, naltrexone-buproprion therapy has been shown to enhance the activity of brain areas implicated in Study I and other appetite control studies (Scharmüller et al. 2012; Yokum & Stice 2013), namely, anterior cingulate, superior frontal areas, insula, superior parietal areas (Wang et al. 2014). It may be that pharmacological therapy with bupropion and naltrexone might convey its effects partly through enhanced inhibitory brain function upon encountering food cues. The effects of pharmacological interventions to brain responses to food stimuli and appetite control appear as a viable setting for future studies.

6.2 Structural brain changes of obesity (Study II)

VBM analysis revealed that brain densities were lower in obese individuals. The GM regions included right orbitofrontal cortex, amygdala and insula that are considered key regions of the reward circuitry (Koob & Volkow 2010). Obese subjects also had lowered WM densities in regions underlying insula and amygdala. Thus, there may be an obesity-related impairment of homeostatic processing in insula and impaired hedonic assessment in the orbitofrontal cortices (Pannacciulli et al. 2007). On the other hand in Study II, the brain atrophy was mainly located in parietal and occipital areas. These areas are not considered hedonic hotspots of the brain (Berridge & Kringelbach 2013) and the atrophy may thus be a sign of more diffuse cellular damage. Indeed, most previous

studies report predominantly frontal locations for obesity-related brain atrophy (Taki et al. 2008; Pannacciulli et al. 2006).

Voxel-based analysis of the DTI data was carried out to study effects of obesity on white matters structural integrity in more detail. Damaged or impaired fiber integrity leads to increased diffusion and is indicated by higher MD and lower FA values (Soares et al. 2013). FA reductions in obese subjects were found in corticospinal tracts, mamillary bodies, optic radiations and corpus callosum. These white matter areas encompass major white matter tracts and atrophy within them might cause impeded integration of information and cause impairment of cognitive processes (Stanek et al. 2011). Lowered FA values in the obese were mostly located in posterior brain regions just as the volumetric atrophy detected in the VBM analysis. MD reductions in the obese were observed in both uncinate fascicles and inferior occipitofrontal fascicles. Unlike FA values, which are higher in coherent WM, MD values are higher where water diffusion is less restricted by fibers and structure (Soares et al. 2013). Thus, this finding does not imply obesity-related loss of integrity. They may be related to the hypothesized oversensitive reward circuit detected in the morbidly obese in functional studies and accord well with a recent study that reported increased tract integrity between reward circuit components in healthy obese participants (Gupta et al. 2015). However, opposite findings have also been reported in young adult population (Marqués-Iturria et al. 2015). Thus the effects of obesity on white matter integrity remain controversial.

In summary, the identified density reductions in obese individuals are in parallel with the current body of literature. However, the DTI findings imply mixed effects of WM integrity. Finally, the GM/WM densities and DTI metrics were all negatively associated with BMI and fat percentage, so it appears that the degree of obesity is also systematically and negatively related to all measured brain tissues structural metrics. Future studies addressing the effects of weight change and recovery potential of brain tissue are of importance in unraveling the obesity-related structural brain changes in more detail.

6.3 Structural brain changes after weight loss (Study III)

VBM was further used to measure the structural brain changes that occur after bariatric surgery and weight loss. Morbidly obese subjects had initially decreased grey and white matter volumes. However, the distribution of the changes was now more frontally placed than in Study II and thus more in line with previous findings (Taki et al. 2008). Alterations were observed in regions involved in homeostatic integration (insula) (S. Frank et al. 2013), hedonic encoding (orbitofrontal cortex) (Pursey et al. 2014; Nummenmaa et al. 2012) and the brain areas implicated in cognitive control in Study I (preSMA, SMA,

anterior cingulate and the precuneus) and previous studies (Scharmüller et al. 2012; Yokum & Stice 2013; Simmonds et al. 2008).

In line with Study II, GM and WM brain densities were negatively associated with metabolic factors. However, the associations were partly different in GM and WM areas. As expected, age was negatively associated with GM densities (Good et al. 2001). Body adiposity, blood pressure; hyperglycemia and hyperlipidemia were most associated with GM and WM densities. Additionally, plasma HDL cholesterol levels, which are considered a protecting factor against vascular damage, were positively associated with both GM and WM brain densities. These findings accord with the proposal that adverse cellular effects in brain could follow worse systemic metabolic healthy. Possible mediating factors include vascular damage caused by hypertension, hyperlipidemia and diabetes (Breteler et al. 1994; Korf et al. 2007; Cohen et al. 2011). Low-grade systemic inflammation, is a well known features of obesity and may ultimately lead to increased metabolic stress and impaired cellular repair (Hotamisligil 2006; Lumeng & Saltiel 2011; Pannacciulli et al. 2007). Direct cellular insult may also result from hyperglycemia and glucose neurotoxicity (Tomlinson et al. 2008; Messier 2005; Biessels et al. 2008). However, in a complementary analysis, no differences in brain densities were found between diabetic and non-diabetic obese participants. Taken together, in Study III, the associations between metabolic variables and brain tissue integrity accord with well-known risk/protecting factors for atherosclerosis, vascular diseases (Friedman et al. 2014) and cognitive decline with increasing age (Jagust et al. 2005; Pannacciulli et al. 2006; Walther et al. 2010). Although, their specific and individual contributions to cerebral atrophy cannot be resolved the results underline the multifactorial relationship between brain tissues integrity and systemic metabolic health.

Given that obesity is associated with brain tissue volume reductions, the subsequent hypothesis was that weigh loss after bariatric surgery would reverse some of these changes. Indeed, widespread recovery of WM and spatially more limited recovery of GM densities in frontal and posterior brain areas were detected after the surgery. WM density recovery extended considerably beyond the frontal and midbrain areas, where atrophy was observed preoperatively. Correlation analysis between the postoperative increases in brain densities and changes in metabolic variables showed only limited correlations with each other and the changes did not appear directly proportional to BMI change.

The focal density reductions could indeed predispose an individual to overeating (Pannacciulli et al. 2007; Cazettes et al. 2011), and appear as a biomarker for increased risk for obese phenotype that is detected early on – even before weight gain. This is implied in prospective studies, in which GM density reductions located in areas involved in reward functions, monitoring, maintenance of homeostasis and inhibitory control.

(Yokum et al. 2012; Smucny et al. 2012; Kurth et al. 2013). These areas were implicated in our results as well. Importantly, in Study III, GM densities in these regions did not recover after bariatric surgery and weight loss. It may be that neurons within the cortical grey matter are not able to recover after cellular insult. It is also possible that recovery within the grey matter needs more time than six months. Animal studies on stroke models imply peaking of the recovery within 4 months (Pearse et al. 2007), but the time or the mechanisms through, which neurons recover after subtotal damage as the one caused by metabolic stress are not known. WM and glial cells within it may have greater capacity for regeneration than neurons do, namely, through recovery in myelination (Bhatt et al. 2014) or restoration of other types of glia (Allen & Ben A Barres 2009; Tomassoni et al. 2013).

Study III was first to report obesity-related cerebral atrophy is recoverable by weight loss after bariatric surgery. This suggests that a causal relationship exists between body adiposity and brain tissue integrity. However, from the current results it is not possible to infer, which one of the two main hypothesis (cause or consequence) for obesity-related atrophy is more prominent for a given brain region. This could be better assessed in true longitudinal studies with more than two scanning measurements. For example in observational studies, in which the relationship between weight change is compared to changes in brain structural metrics.

6.4 Brain glucose metabolism in obesity and after weight loss (Study IV)

In study IV, participants were scanned twice; once after a 12 h fast and once during euglycemic hyperinsulinemia. These two scans were compared with each other to yield insulin responses in [18F]-FDG uptake that is a proxy for glucose metabolism within the brain tissue. Preoperative comparison included comparison of insulin responses between morbidly obese and normal weight participants. Obese subjects had higher insulin responses during insulin stimulation in striatum and cerebellum, but no differences between the groups were detected in the fasting condition. Thus the basal glucose metabolism in the brain appears unchanged in obesity. Clinically, patients with chronic type 2 diabetes often need insulin treatment. Insulin improves the overall glycemic control and reduces glucose neurotoxicity in peripheral organs. The opposite might be true for the brain tissue through increased oxygen radical production (Tomlinson et al. 2008). Thus, in Study IV we identified one mechanism that might drive brain atrophy detected in Studies II and III. Although this may not result in brain improvement of brain tissue integrity following surgery as the insulin effect were mainly cortical (located in GM). Furthermore, the same obese participants have increases in functional connectivity between the caudate nuclei, insula and amygdala with fMRI responses to food pictures

(Nummenmaa et al. 2012), which implies that regional insulin responses in the striatum are also connected to reward encoding.

In a previous study, insulin-induced elevations have been detected in obese type 2 diabetics but not in healthy participants (Hirvonen et al. 2011). Thus, the increased insulin sensitivity in the brain appears to be a feature of obesity even in the absence of clinically elevated glucose levels that would lead to a diagnosis of diabetes or pre-diabetes. Nevertheless, it must be noted that insulin resistance is likely present to some extent in most obese individuals. Diabetes is diagnosed from plasma glucose levels that do not accurately depict insulin secretion (Sutherland et al. 2012). Intuitively, the mere increased amount of insulin in the plasma during hyperinsulinemia might explain the increases in brain glucose uptake (Hasselbalch et al. 1999; Bingham et al. 2002). However, in Study IV the difference in insulin levels was not statistically significant between the groups and because the transport of insulin to brain tissue is saturated at relatively low physiological levels of circulating insulin (Hasselbalch et al. 1999), we do not consider this to be the main explaining factor behind the detected changes (Hirvonen et al. 2011). The fact that obesity seems to be associated with increased sensitivity to insulin in brain tissue is new and challenges the traditional views of brain being totally independent of insulin actions.

Prior studies have shown that the ratio of plasma-to-cerebrospinal fluid decreases in obesity (Kern et al. 2006), which suggests that the insulin transport to CNS is impeded. This might lead to sensitization of insulin receptors in the brain, which is supported by the fact that obese individuals have diminished metabolic responses following intranasal insulin administration that bypasses the blood brain barrier (BBB) (Hallschmid et al. 2008). These findings could be a sign of "brain insulin resistance" (Pagotto 2009), which would then actually locate on the BBB rather than in the brain tissue as the term points out (Hallschmid & Schultes 2009). Regional brain insulin resistance, however, is most likely to be present in hypothalamus, hippocampus and other brain regions that express GLUT-4 (Sutherland et al. 2012). Hypothalamus is of special interest, because the cells express insulin sensitive GLUT-4 receptors and they may convey satiety signals (Wynne et al. 2005) and consequent central modulatory signals to pancreas, liver and adipose tissue (Kreier et al. 2013). Thus insulin resistance in hypothalamus might impede these functions and participate in the development of systemic insulin resistance (Kreier et al. 2013). Unfortunately, due to the limitations in resolution in PET imaging measuring the specific signal from small structures such as the hypothalamus is not reliable and this matter needs to be clarified further in animal studies.

Our findings challenge the view that brain insulin resistance appears as a global phenomenon (Hallschmid et al. 2008). First, in the fasting state global brain glucose utilization is not decreased in obese subjects, which implies that between meals

the brain basal insulin sensitivity is similar in normal weight and obese individuals. Correspondingly, under insulin stimulation, obese individuals show globally elevated glucose metabolism within the brain. An alternate model of insulin stimulation might also be proposed, where elevated plasma levels of insulin would increase the delivery of insulin across the BBB. If the cells in the brain then adapt to this change by increasing the rate of insulin clearance, then decreased cerebrospinal fluid–to–plasma insulin ratio of the obese individuals (Kern et al. 2006) would actually reflect chronically elevated insulin usage rate. This would also explain the diminished catabolic responses to intranasal insulin (Hallschmid & Schultes 2009), as the given amounts of insulin would presumably be too small for cells accustomed to increased insulin levels. We do not currently have a PET tracer that would bind to insulin receptors. Having such a tracer would help us clarify the molecular mechanics of insulin responses in further detail. With currently available methods, the brain insulin responses might be studied while performing an oral or intravenous glucose tolerance test. This would yield information how brain glucose uptake is modified by simultaneous rise in blood glucose and insulin levels, which approximates the true postprandial state more accurately.

Study IV was the first to report pure obesity-related increased insulin sensitivity in the brain. Importantly, obesity decreases in glucose utilization in most tissues (Altaf et al. 2014); thus, these changes are opposite to those described in peripheral organs. These results also challenge the standard textbook notion that still describes brain glucose metabolism to be insulin independent and theories on brain insulin resistance that is similar to other organs (Sutherland et al. 2012).

6.5 Methodological consideration

The strength of the current studies includes the use of morbidly obese participants that reflect the extreme pathology of obesity. This design makes the studies sensitive in finding features that accompany obesity even in small sample sizes. The powerful weight loss intervention - bariatric surgery - is one of the main strengths of the study. It offers means to systematic successful weight loss over relatively short period of time. Importantly, weight loss after the surgery does not necessitate special dieting, medication or exercise regimes that are challenging to standardize across all participants. There are also caveats to this, as it is uncertain, whether our findings can be generalized to less obese individuals or whether the positive effects also occur when the weight loss is non-surgical. The bariatric surgery permanently limits the amount the patients are able to eat and leads to improvement in glycemic status even before the weight loss ensues and the mechanics behind the alterations of whole body metabolism are yet to be delineated in detail (Ardestani et al. 2015).

The sample sizes have to be considered in respect to the ones generally used in the field and are thus modality-specific. For the PET study (***Study 4***) and for the fMRI normal (***Study 1***) the sample size can be considered to lie within typical range (***Study 4***). In critical view sample sizes would ideally be larger and the groups balanced in size (Button et al. 2013; David et al. 2013). Postoperative comparison necessitated participation in several scans, and because of failures in even single data acquisition and persons withdrawing from the study due to personal reasons we had a relatively large dropout rate (10/47 obese participants in Study III and 5/22 obese participants in Study IV) as reported in Table 1.

The studies involved mostly females due to the low availability of morbidly obese men opting and qualifying for surgery (ca. 30% of all patients participating to the studies), it is possible that the results may not be generalizable to males. The age range of our obese subjects was fairly broad (Mean ca. 45 years; range 29-60). However, some changes (functional, structural and metabolic) may unfold only after decades of obesity, hyperglycemia, hyperlipidemia and micro inflammation - or may manifest only with more extreme degrees or prolonged length of these states. The obese subjects diagnosed for type 2 diabetes, hypertension and hypercholesterolemia were using oral medication, which may have resulted in flattened associations to brain densities and DTI metrics as the medication improves the metabolic values toward healthy levels.

6.6 The Clinical significance of the findings

The findings suggest a significant obesity-related cascade of adverse brain changes in structure and metabolism. The results from Study I show that obese individuals not only have altered brain responses during appetite inhibition but also increase in functional connectivity between a large set of brain regions. These findings further encourage us to find pharmacological and behavioral treatments that affect wide neuronal networks via multiple neurotransmitter systems (Adan 2013; Wang et al. 2014). Furthermore, decreased brain densities (as observed in VBM studies) are associated with many vascular risk factors. This encourages a thorough individual risk assessment and the use of glucose, blood pressure and cholesterol lowering medication (Greenlund et al. 2004). Of special note is the increased glucose metabolism in the brain after insulin stimulation that underlines the importance of glycemic control in diabetics with minimal insulin doses. This may be better achieved by adhering to nutritional and sports guidelines (Colcombe et al. 2006; Balady et al. 2007). In everyday patient education, telling about adverse brain changes may help in motivating life style changes and in maintaining compliance. The recovery of the brain changes after weight loss might be especially motivating to point out.

7. FUTURE DIRECTIONS

The results suggest a causal link between body adiposity brain metabolism and structural integrity, but only in the context of surgical weight loss. The structural and metabolic abnormalities are likely caused by obese phenotype, as they appear recoverable after the weight loss. Longitudinal studies with scanning and other measurements in more than two time points would be ideal for investigating either brain function and tissue integrity alterations in future weight gain/loss. These types of close follow up studies are ultimately needed to assess any causal relationships between structural brain abnormalities and obesity. Namely, repeated scans (performed eg. yearly) would help us to differentiate the brain changes that precede obese phenotype from the ones that occur after prolonged obesity. The combination of structural, functional and molecular brain imaging modalities would help us characterize brain changes more thoroughly. In this regard combinations of fMRI and PET (Dougherty et al. 2008), fMRI and DTI (Wagner et al. 2015) and even fMRI and VBM/DTI appear the most viable ones (Oertel-Knöchel et al. 2014). As an adjunct to this setting recording of personality traits, the level of food addiction together with sleep, eating and exercise habits would be more optimal for revealing the modifying factors of weight changes in further detail. This type of prospective study with repeated scanning could be implemented within a cohort study, where a plethora of additional metabolic and behavioral variables would be available from a longer period of time. In such a setting, even single scan of subjects, would enable us to better estimate the relationship between the cumulative exposure and observed changes. As an example, life time glycemic index can be used to assess the degree of hyperglycemia throughout years (Mäkimattila et al. 2002). Similar metrics could be defined to other parameters of metabolic health such as hypertension and cholesterol levels as well. Finally, selection of morbidly obese subjects with pathological eating behavior (binge eating, impulsive personality or recent fast weight gain at a young age) could add to the results obtained in the current studies in helping to characterize brain features of different traits of aberrant eating behavior.

To unravel the obesity-related functional brain changes we also need robust physiological studies that address the homeostatic appetite regulation. For example, hormonal measurement and/or administration during brain scanning could provide us with new homeostatic factors that we should control for when running the functional experiments (Goldstone et al. 2014). These studies might then be appended to include pharmacological manipulation with the candidate drugs such as phentermine and topiramate-ER against obesity (S. Z. Yanovski & J. A. Yanovski 2014). Standard visual stimuli sets food pictures are not used and thus the fMRI studies reported to date have a large variety within the stimuli sets (Pursey et al. 2014). Standard stimuli sets would help to assure

the comparability between studies. For intervention studies such as those employing hormone or nutrient administration or pharmacological manipulation standardization of the experimental designs would be especially important. In addition to standard food pictures, we might benefit from standard reappraisal strategies for appetite control studies that are controlled with training (Giuliani et al. 2013).

8. CONCLUSIONS

The major findings of the studies are as follows:

I. Obesity results in hypoactive functioning of caudate nucleus and anterior cingulate during volitional appetite control and increases functional connectivity within the food control circuit. These changes may ultimately drive excessive eating and contribute to development and maintenance of obesity.

II. Morbid obesity is associated with widespread adverse effects brain tissue's structural integrity as measured by voxel-based morphometry and diffusion tensor imaging. This may reflect vulnerability to developing obesity or may reflect neural degeneration caused by obesity.

III. Bariatric surgery recovers white matter atrophy globally, whereas grey matter recovery is more limited. This suggests that weight loss has a positive causal influence on brain structure.

IV. Obese individuals have increased glucose metabolism under insulin stimulation and this abnormal response is ameliorated by bariatric surgery. This further implies that improvement in overall metabolic health might include the brain.

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Also, vielen herzlichen Dank für alle!
Achtung!

10. APPENDIX: INCLUSION AND EXCLUSION CRITERIA OF THE STUDIES

Inclusion criteria for the patient population

1) BMI > 40 kg/m2 or > 35 if there is an additional risk factor
2) Age: 18-60 years
3) Previous, carefully planned, conservative treatments for obesity have failed

Exclusion criteria for the patient population

1) BMI over 60 kg/m2
2) Weight more than 170 kg
3) Waist circumference > 150 cm
4) Mental disorder or poor compliance
5) Eating disorder or excessive use of alcohol
6) Active ulcus-disease
7) Diabetes requiring insulin treatment or fasting glucose more than 7 mmol/l

Inclusion criteria for the control group

1) BMI 18-27 kg/m2
2) Age 18-60 years
3) Fasting plasma glucose less than 6.1 mmol/l
4) Normal glucose tolerance test (OGTT)

Exclusion criteria for healthy participants

1) Blood pressure > 140/90 mmHg
2) Any chronic disease
3) Mental disorder or poor compliance
4) Any chronic medical defect or injury which hinder/interfere everyday life
5) Eating disorder or excessive use of alcohol

Exclusion criteria for both groups

1) Pregnancy
2) Past participation in nuclear medicine imaging studies
3) Any other condition that in the opinion of the investigator could create a hazard to the subject safety, endanger the study procedures or interfere with the interpretation of study results
4) Presence of any ferromagnetic objects that would make MR imaging contraindicated

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