Turku PET Centre
Turku University Central Hospital

CLINICAL STUDY PROTOCOL

(The intervention studied is not a drug substance)

Date: 28.09.2010
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Study No: SLEEVEPET 2

Study title: Prospective, randomised study: Metabolic and functional effects of bariatric surgery accessed using PET and MRI

Phase: (when applicable)

PET ligands: [^{18}F]FTHA
[^{11}C]raclopride
[^{11}C]carfentanil

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1. OVERVIEW

Diet and nutrition are important factors in the maintenance of good health throughout the entire life course. Their role as determinants of chronic diseases such as obesity, diabetes and cardiovascular diseases is well established. The annual increase in the prevalence and the severity of obesity in both adults and children is currently substantial (1), and in Finland 40% of adults are overweight or obese. Identifying the mechanisms that make some individuals vulnerable to overeating, as well as pinpointing how obesity changes the functioning of the human mental and bodily functions would be critical for understanding the current high prevalence of obesity.

Animal studies suggest that the brain extensively coordinates the adaptive mechanisms and the alterations of energy intake and expenditure. Accumulating evidence suggest that obesity is associated with changes in brain morphology and function, thus the source for obesity may lie in the brain. In the present project we test this hypothesis by implementing a multimodal neuroimaging approach with positron emission tomography (PET), functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI) and voxel-based morphometry (VBM). By studying brain anatomy and function in lean individuals as well as obese patients undergoing weight loss surgery, we can evaluate whether the observed differences between lean and obese individuals are causes or consequences of obesity. In PET imaging of the brain, we will focus on two specific neurotransmitter systems, dopamine and opioid, which are largely been unexplored in obesity research.

Obesity is related to elevated plasma glucose levels and endothelial dysfunction. Elevated plasma glucose and endothelial dysfunction are known risk factors for diabetes and coronary heart disease. Weight reduction modifies risk factors such as blood pressure and lipid profile for chronic diseases. On this study we are able to further investigate changes in fatty acid metabolism and hormones affecting feeding and energy balance. In addition, changes in brain activation in response to food stimuli will be assessed. As far as we know there are no previous positron emission tomography (PET) studies that investigate these risk factor variables from one study population pre-operatively and after bariatric surgery.

The objectives of this study are to measure effect of obesity on brain structure and molecular pathways, food-stimuli mediated brain activation response, on hormones affecting both feeding and energy balance as well as on bone metabolism and bone marrow fat. The study consists of two phases. In the first phase the studies are performed at baseline before bariatric surgery and in the second phase post-operatively after 6 months. Regional free fatty acid uptake in myocardium, skeletal muscle, subcutaneous fat, visceral fat, pancreas, liver, brain, intestine and the bone are studied with PET and 14(R, S)-[^{18}F]-fluoro-6-thia-heptadecanoid acid ([^{18}F]FTHA). Changes in body fat distribution, in ectopic fat and fat content of key organs are investigated with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS).). Cardiac functioning is studied with echocardiography and functional MRI (fMRI) and ectopic fat in and around the heart using MRI and MRS. Brain reward system response to food stimuli is assessed using functional MRI (fMRI) and white and grey matter volumes using diffusion tensor imaging (DTI). Brain neurotransmitter system will be measured with [^{11}C]raclopride and [^{11}C]carfentanil and PET. In the second part of the study the same variables are studied after bariatric surgery: either laparoscopic sleeve gastrectomy or Roux-en-Y gastric bypass. The objective is also to study whether less invasive sleeve gastrectomy is as beneficial in terms of weight loss and co-morbid diseases as more invasive Roux-en-Y gastric bypass and to compare the changes in tissue metabolism between these two surgical procedures.

The study consists totally of 60 study subjects. Of these 40 are morbidly obese adults, BMI ≥ 40 kg/m^2 or if there is additional risk factor BMI ≥ 35 kg/m^2. The morbidly obese patients are randomized either for laparoscopic sleeve gastrectomy or Roux-en-Y gastric bypass. For a control group 20 lean and healthy subjects will be recruited.

2. BACKGROUND

2.1. Obesity

Obesity has reached epidemic proportions worldwide. In the last three decades there has been a dramatic increase in prevalence of obesity. In the United States obesity prevalence among adults (defined as BMI ≥ 30 kg/m^2) has doubled and at the same time among adolescents age 12-19 the prevalence has tripled (2). In the USA, obesity is the second most common preventable factor causing premature deaths right after smoking: it is estimated that in the USA obesity and its complications cause 400 000 deaths annually (3). Body mass index (BMI: weight divided by height in meters squared, kg/m^2) can be used to estimate the degree of obesity. Normal weight is defined as BMI < 25 kg/m^2, because BMI higher than this is associated in increased risk of having many diseases (4). Overweight, also defined as mild obesity, is defined as BMI 25.0-29.9 kg/m^2. Corresponding values for remarkable - and difficult obesity are 30.0-34.9 kg/m^2 and 35.0-39.9 kg/m^2. Morbid obesity is defined as BMI 40 kg/m^2 or more.

Both the BMI and the prevalence of obesity have increased in Finland during the last two decades. In the year 2002 the average BMI among working men was 27 kg/m^2 and among working women 25.9 kg/m^2. At least 66 % of men and 49 % of women were slightly overweight (BMI ≥ 25 kg/m^2) and one fifth were remarkable obese (BMI ≥ 25 kg/m^2) (5). In the future overweight and obesity rates will increase substantially (6) contributing to the increase in number of people with diabetes by more than 2.5-fold, from 84 million in 1995 to 228 million in 2025 in developing world (7). Behavioural and dietary interventions have not resulted in significant improvement among morbidly obese patients.

Obesity is associated with increased morbidity and mortality. Obesity results in increased risk of having other diseases such as type 2 diabetes, high blood pressure, metabolic syndrome, coronary heart disease, stroke, obstructive sleep apnoea, gout, fatty liver, gallstones, knee arthrosis, asthma and some forms of cancer (postmenopausal breast cancer, cervical cancer, colon cancer and kidney cancer) (8,8). The increase in mortality is mainly due to these co-morbid diseases such as insulin resistance, type 2 diabetes, high blood pressure and changes in lipid profile (3). Mortality due to obesity and diabetes is showing a worrying trend, not only because both obesity and diabetes are already affecting a large proportion of the population, but also because they have started to appear earlier in life. Alarmingly, not only the prevalence but the severity of obesity has increased in both adults and children (2).

2.2. Bariatric surgeries

Over the past decade, bariatric surgery has gained increasing acceptance as a treatment modality for morbid obesity and significant co-morbid diseases among patients who have failed conventional dietary or behavioural interventions. Bariatric surgery is considered to be more effective among morbidly obese patients (BMI ≥ 40 kg/m^2) than conservative treatment options. Surgical treatment results in more pronounced weight loss and have also beneficial effects on co-morbid diseases (9). Two comprehensive, recently published, long-term follow-up studies indicate that surgical treatment is more effective to decrease mortality among morbidly obese patients compared to

conventional treatment modalities (10)(11). The most used surgical technique in the world is gastric bypass, comprising over 65 % of all bariatric procedures over half of them done by using laparoscopy (12). In this procedure a pouch of ~30 ml is formed from the upper part of the stomach, in which small intestine is connected. 150 cm away from this connection of the small intestine (so-called efferent intestinal limb) and gastric pouch, a part of the small intestine (so-called afferent intestinal limb) is connected. This afferent limb of intestine conducts bile and pancreatic juices.

Gastric bypass restricts food intake and leads to a mild malabsorptive state due to partial (150 cm) duodenal bypass. In addition, there are changes in endocrine functions due to changes in secretion of hormones (13). The mean percent of excessive weight loss in 10 year-follow-up after surgery was approximately 25 % while conservative treatment led to 1.6 % increase in weight. In the same study, surgery was also considered to have more pronounced beneficial effect on co-morbid diseases (type 2 diabetes, hypertriglyceridaemia, low HDL, blood pressure, hyperuricaemia) after both 2- and 10 year follow-up compared to conservative treatment (11). In gastric bypass procedure the risk of mortality is 0.5-2.5 % and morbidity 10-20 % (14)(9).

Gastric sleeve procedure (SG) is quite new technique and was originally developed as the first part of a “two-stage” gastric bypass procedure among so-called super-obese (BMI $\geq$ 60) patients (15). As a restrictive operation it has the advantage of not influencing in absorption (16). SG also results in restriction of food intake but also changes in secretion of appetite-stimulating hormone called ghrelin due to resection of the upper part of stomach (fundus) from where ghrelin is normally secreted. Lately the SG has been used actively as such for the surgical treatment of obesity among patients with BMI between 35-60. The short-term results are promising and are equivalent in terms of weight loss and co-morbid diseases when compared to gastric bypass. Data published to date show an average excess weight loss of 33-83 % after 1 year follow-up. In spite of the wide range of data, results suggest that SG might be as effective as traditional weight loss surgeries that commonly have an average excess weight loss of 60-85 % after 1 year of surgery(17) . SG as a single operation for morbid obesity has gained attention, for it is “less complex” than some other alternative techniques like Roux-en-Y gastric bypass, having no gastrointestinal anastomosis (18). In addition no mesenteric defects are created eliminating the risk of internal hernia, no foreign material is used as in the case of gastric banding, the whole digestive tract remains accessible to endoscopy, it is not associated with dumping syndrome, the risk of peptic ulcer is low and the absorption of nutrients, vitamins, minerals and drugs is not altered (19). As a result, studies published on SG to date show fewer postoperative morbidities when compared to RYGB or BPD-DS (biliopancreatic diversion duodenal switch) (20). This makes SG an attractive choice for the treatment of high BMI patients in whom complications are more prevalent (21). In addition of its technical simplicity and lower risk ratios, the conversion of SG to RYGB or BPD-DS is relatively straight-forward. Thus after 12-18 months from SG, if the post-operative BMI still classifies patient as morbidly obese (BMI $\geq$ 40 kg/m^2) SG can be converted into RYGB or BPD-DS (20). However long-term benefits are still unknown (22,23) and thus many questions remain about the current state and long-term outcome of SG.

Recent studies suggest that both gastric sleeve and bypass surgery affect endocrine responses via changes in secretion of hormones. These hormones are secreted in periphery but have also central effects in regulation of feeding and energy balance. Numerous studies have proven the beneficial effects of bariatric surgery on the circulating levels of ghrelin and glucagon-like-peptide 1 (GLP-1). (24,25) However it is likely that also other, yet unidentified factors play a role in the weight loss after SG. One aim of this study is to compare possible changes in hormone secretion after surgery and if the change in hormone secretion is different between the two surgical techniques.

Physiological states associated with energy balance are primary determinants of eating behaviour. Comparative studies have identified an interconnected network comprised by amygdala, ventral striatal and midbrain regions in various aspects of food reward processing (26-28). Excessive sensitivity to foods in obese individuals has been found to be mediated by hyper-activation of the reward system (29), and individual differences in reward drive are known to predict how this system responds to perceiving of foods (30). Exaggerated sensitivity to high-calorie food-cues may be a critical factor explaining obesity (31).The reward system responds more vigorously to high-calorie (appetizing) vs. low-calorie (bland) foods (32), especially in obese vs. normal weight individuals (33). Measuring brain responses with functional MRI (fMRI) while viewing food images can thus be used to study how the surgical treatment of obesity modifies the responsiveness of the reward system, which in turn will provide the foundations for a neural model of energy balance control in the brain. Obesity is found to affect brain white and grey matter volumes compared to lean individuals (34,35). Weight loss seems to normalise some of these changes (36). One aim is to study if weight loss induces changes in brain grey and white matter volumes.

Bariatric surgery has complex effects on bone tissue. Several reports have shown that malabsorption surgery such as RYGB leads to increased rate of bone turnover when assessed by serum markers of bone turnover (37,38). However, the data on bone density or on actual fracture risk are either somewhat inconsistent or nonexistent, respectively (37). There is very limited published data on the effects of gastric sleeve procedure on bone metabolism. Bariatric surgery could affect bone by multiple mechanisms. First, weight loss reduces the mechanical stimulation on the skeleton and thus may lead to bone loss. Especially RYGB has been suggested to impair calcium and vitamin D absorption while both RYGB and SG affect the secretion of many incretins such as ghrelin, GLP-1 and -2 that have been proposed to modify bone cell activities (39). In addition, the reduction in fat mass leads to decreased circulating levels of adipokines adiponectin and leptin that are also involved in regulating the bone homeostasis (37). In addition to these effects on bone tissue, bariatric surgery and subsequent weight loss likely induces changes in bone marrow fat. Obesity results in increased levels of bone marrow fat but so does caloric restriction such as that in patients with anorexia nervosa (40). These findings highlight how the functional role of bone marrow fat remains elusive and suggest that bone marrow fat is under very complex regulatory control. Studies presented in this protocol would greatly elucidate the effects of bariatric surgery and specifically the possibly differing effects of RYGB and SG surgeries to 1) the overall bone metabolism and bone mineral density measured by serum markers of bone metabolism and quantitative computed tomography with PET-CT and 2) the metabolic functions and responses to weight loss of the bone marrow fat as measured by PET imaging, MRI and MRS.

2.3. Free fatty acid metabolism and obesity

2.3.1. Free fatty acid metabolism in adipose tissue

Fatty acid acids (FFA) are stored in the form of triacylglycerol, i.e. triglycerides mainly in adipocytes, but also other organs including skeletal muscle, heart and liver. During fasting state, insulin level falls and permit the release of FFA and glycerol (41,42). Arterial plasma FFA concentration closely correlates with abdominal subcutaneous FFA release (41). In the plasma, FFA are transported in a nonesterified form attached to albumin, or bound covalently to triglycerides, which are transported in chylomicrons and very-low-density lipoproteins FFA are hydrolyzed from circulating chylomicrons or very-low-density lipoproteins by lipoprotein lipase, which locates in the capillary endothilium. FFA is taken up by the tissue passively and by facilitated transport (43) and lipoprotein lipase expression is the rate limiting step in this uptake process (44). Insulin stimulates lipoprotein lipase in adipose tissue (45). FFA is re-esterified to triglycerides in adipocytes if

glycerol 3-phosphate is abundant. Glucose level inside the cell can affect FFA release via glycerol 3-phosphate supply. Insulin promotes energy storaging by stimulating triglyceride synthesis, such as lipogenesis, and inhibiting lipolysis in adipocytes (46).

2.3.2. Free fatty acid metabolism in skeletal muscle

The majority of FFA present in human body is derived from dietary sources. At resting condition, FFA availability usually exceeds the whole body resting FFA oxidation (47) and the excess FFA are stored primarily in adipose tissue. In the fasting state, circulating FFA are derived from adipose tissue via lipolysis (48). The major regulator of the adipose tissue lipolysis rate is the hormone sentitive lipase (49). Further, the activity of this hormone is stimulated by the sympathetic nervous system, catecholamines and human growth hormone and inhibited by insulin. In addition to the rate of lipolysis and plasma FFA concentration, the rate of plasma FFA oxidation is affected by the nutritional state and preceding dietary intake, exercise mode, intensity, and duration and the state of training (50). After an overnight fast under resting conditions, leg muscle plasma FFA uptake has been suggested to be 4-5 µmol/kg/min and around 20% of the whole body's systemic FFA uptake (51).

2.3.3. Free fatty acid metabolism in myocardium

The myocardium is an aerobic organ and it has an extremely high metabolic rate. Its main energy source is oxidative phosphorylation and substrate usage depends on the arterial substrate availability, the state of body is in (fasted or fed), and the energy demand (52). In the fasting state when the blood FFA concentration is high, the normal healthy heart mainly utilises FFA (60-70%), whereas only 30% of energy is derived from glucose and 10% from lactate. In the fasted state FFA is released by adipose tissue lipolysis. The oxidation of FFA inhibits glucose oxidation and glucose is stored as glycogen; the glucose-sparing effect of FFA oxidation (53). Inside the myocardial cell, FFA is activated to acyl CoA by an acyl-CoA synthetase. Acyl-CoA enters the mitochondrial membrane with the carnitine system which forms acyl carnitine. Within the mytochrondia acyl carnitine enters B-oxdation to form acetyl CoA, which further enters the TCA cycle (52).

2.3.4. Free fatty acid metabolism in the liver

Blood enters the liver via the hepatic artery and the portal vein. A large amount of blood (75-85%) and the nutrients perfuse through the portal vein while the hepatic artery carries oxygen-rich blood (48). A major part of initial degradation of FFA occurs in the liver as it converts carbohydrates into FFA, consumes, stores and releases lipids as part of lipoprotein and thus, control blood lipid levels. However, from the total amount of FFA oxidation occurring in the liver, the liver uses only a small portion for its own intrinsic metabolic processes. In the fasting state, the liver mainly utilises FFA and amino acids as fuels (54).

2.3.5. Free fatty acid metabolism in the intestine

It has been shown in some studies that besides other tissues, intestinal mucosa also utilises free fatty acid and this implies a unique duality of fatty acid sources for the intestine (lumen and plasma) (55). To our knowledge, there has not been any study investigating the free fatty acid uptake in morbidly obese and also the changes in free fatty acid uptake after bariatric surgery. This study is designed to investigate the free fatty acid uptake by the intestine among morbidly obese before and after bariatric surgery.

2.3.6. Free fatty acid metabolism in the bone and bone marrow fat

Bone marrow is a mixed tissue comprised of bona fide bone cells (osteoblasts and osteoclasts) as well as bone marrow adipocytes and cells of the hematopoietic lineage. There is no prior information on the FFA metabolism in the bone or bone marrow fat. However, based on the findings in adipose tissue in other organs, majority of the FFAs accumulating in bone are likely taken up by the bone marrow adipocytes. It has been postulated that bone marrow adipocytes could provide energy to osteoblasts and/or osteoclast by locally releasing the stored FFAs while some reports suggest that FFAs could have direct effects on bone cells (40,56).

2.4. Reward system, obesity and food addiction.

Overeating likely depends on the balance between the reward circuit that involves the ventral striatum and amygdala and several other interconnected brain regions, and networks that inhibit reward-seeking that involve regions such as the dorsolateral prefrontal and orbitofrontal cortices, insula and the dorsal striatum (57). In other words, altered brain structure and functioning might be a key factor that would explain obesity. Comparative studies have identified an interconnected network comprising of amygdala, striatal and midbrain regions in various aspects of reward processing (26). This reward system plays a key role in guiding appetitive as well as addictive behaviours. Addictions result from adaptations in specific neurons caused by repeated exposure to a drug of abuse (58). There are phenomenological similarities between obesity and addictive behaviors that have led researchers to suggest that addictions may provide a framework for understanding some of the phenotypic characteristics of overeating and obesity (59). Feeding and drug use involve learned preferences and habits that have been established by powerful, repeated reinforcing rewards, and the neural circuitry involved in drug addictions and obesity is strikingly similar (60). The most clearly established commonality of the mechanisms of drug and food intake is their ability to activate the dopamine containing link in the brain's reward systems(61). Functional neuroimaging studies in humans have revealed that drug-related sensory cues may trigger drug seeking behaviour by eliciting hyperactivity in the brain's reward circuit and similarly, food-related cues may trigger food-seeking behaviour via the same system (62,63). Reward circuit's exaggerated sensitivity to high-calorie food cues may be a critical factor explaining obesity (64,65) and accordingly, excessive sensitivity to foods in obese individuals has been found to be mediated by hyperactivation of the reward system (29).

2.4.1. Altered neurochemistry of the reward circuit in obesity

The findings discussed above may be related to altered neurochemistry and structure of the reward circuit in obesity and addictive disorders. Patients with addictive disorders show lower baseline D_2 receptor (D_2R) density in the striatum, and blunted dopamine release following the administration of the drug of abuse. These abnormalities may reflect vulnerability endophenotypes that increase the risk of developing addictive disorders (57). Similar to drugs of abuse, food consumption is associated with dopamine release in the dorsal striatum in healthy subjects, and the amount of dopamine released is correlated positively with ratings of food pleasantness (66). Similar to patients with addictive disorders, obese subjects have lower baseline striatal D_2R density, which is directionally proportional to BMI (67). Accordingly, the low D_2R density may make individuals prone to overeating by rendering the brain reward system unselectively responsive to food-induced reward. Another key neurotransmitter system intimately involved in reward functions is the endogenous opioid system, particularly the μ-opioid receptor (MOR) which mediates the effects of endogenous β-endorphins and various exogenous opioid agonists and antagonists (68). Animal

studies have established that opioid agonists increase and opioid antagonist decrease food intake and hedonic liking of palatable foods, respectively (69). Imaging studies in humans have also provided evidence for altered MOR signaling in addictive disorders. MOR density was found to be increased in the ventral striatum in patients with alcohol dependence (70), and increased MOR density was associated with the personality trait of reward dependence in healthy subjects (71). Increased MOR density has also been associated with high cocaine craving (72). Accordingly, changes in MOR density could contribute to the elevated craving of foods in obesity, yet this remains to be established.

2.4.2. Obesity surgery as a novel way for studying the causes and consequences of obesity

Over the past decade, bariatric weight loss surgery has gained increasing acceptance as a treatment modality for morbid obesity. Bariatric surgery is a very effective means for weight loss among morbidly obese patients. Surgical treatment results in pronounced weight loss in short time and has also beneficial effects on co-morbid diseases (9). Obesity surgery also provides a novel methodological approach for studying whether specific brain-level differences between obese and lean individuals are causes or consequences of obesity. A longitudinal study on individuals undergoing obesity surgery allows us to assess whether the observed differences between obese and lean individuals are associated with the obese phenotype and are thus recoverable by the surgery, or whether they represent stable individual differences that constitute as risk factors which explain why some people become obese or addicted. Such dissociation has a major impact on the appropriate psychological and pharmacological treatment of obesity.

2.5. Positron Emission Tomography (PET) and tracers

Positron emission tomography (PET) combined with 14(R, S)-[^{18}F]-fluoro-6-thia-heptadecanoid acid ([^{18}F]FTHA) with PET is used to measure free fatty acid uptake and oxidation in myocardium and skeletal muscle (73). [^{18}F]FTHA is metabolically trapped tracer. 89% [^{18}F]FTHA taken up by the heart enters mitochondria and the rate of radioactivity accumulation of [^{18}F]FTHA reflects the β-oxidation in the heart (74). 36% of the [^{18}F]FTHA accumulated in skeletal muscle, enters mitochondria suggesting that in skeletal muscle [^{18}F]FTHA traces FFA uptake but not specifically FFA beta-oxidation(74). By using simple graphical analysis (75) the free fatty acid uptake rates in the tissue can be calculated.

In addition to its role in studying tissue metabolism and blood flow, PET can also be used to study specific neurotransmitters in the brain. In this study, we will focus on two such neurotransmitter systems, dopamine and opioid, given their prominent role in reward functions in humans and previous preclinical evidence suggesting that they are implicated in obesity. Striatal and thalamic dopamine D_2 receptors are measured with the antagonist [^{11}C]raclopride, which is probably the best validated PET neuroreceptor radioligand (76). On the other hand, μ-opioid receptors can be measured with the high-affinity agonist radioligand [^{11}C]carfentanil, which is also well validated for human studies (77). These receptors are found throughout the brain, with the exception of the occipital cortex. Both radioligands can be quantified without arterial blood samples, using a simple reference tissue method (cerebellum for [^{11}C]raclopride and occipital cortex for [^{11}C]carfentanil.

3. OBJECTIVES AND HYPOTHESES

3.1. Objectives:

The objectives of this study are to measure effect of weight loss and the possible differences between sleeve gastrectomy and gastric bypass:

1. On whole-body and fasting free fatty acid metabolism in myocardium,skeletal muscle, liver, pancreas, intestine, bone, brain and subcutaneous and visceral fat.
2. On total body fat and abdominal fat distribution & abdominal fat volumes
3. On liver fat content
4. On glycemic control and on biochemical markers (leptin, ghrelin, adiponectin, HDL, LDL, triglycerides, cholesterol, calcium, B12-vitamin, cytokines, bile acids and high sensitivity C-reactive protein).
5. On brain white and gray matter volumes and distribution
6. On brain activation response to food stimuli before and after surgery
7. On the two specific neurotransmitter systems, dopamine and opioid
8. On correlation between brain activation to food stimuli and hormonal changes especially of the gastrointestinal peptides (e.g. ghrelin)
9. On correlation between the subjective feelings of hunger and brain activation
10. On markers of bone metabolism (Vitamin D, PTH, S-Ctx, bsAP, PINP and osteocalcin) and bone mineral density
11. On quantitative and metabolic changes in the bone marrow fat

We also aim:

12. To take DNA samples for further investigation on genetic markers related to cardiovascular diseases, type 2 diabetes and obesity risk factors as a part of CMGene-study
13. To investigate the effect of morbid obesity on the histology and thus take biopsies
    a. During endoscopy from gastric mucus, duodenum, antrum and corpus
    b. During surgery from liver, abdominal subcutaneous and visceral fat
11. To investigate the effect of morbid obesity and the surgery-induced weight loss on the histology of abdominal subcutaneous adipose tissue by taking a biopsy from abdominal adipose tissue
12. To measure the effects of bariatric surgery on gene and protein expression in these tissue biopsies
13. To compare the general risk factors between sleeve gastrectomy (SG) and gastric bypass
14. To obtain information about the hormonal benefits and long-term results of SG
15. To investigate the effects of bariatric surgery on intestinal perfusion and metabolism.
16. To investigate the effects of bariatric surgery on the rate of bone turnover and bone quality using paired bone biopsies
17. The rate of bone turnover (bone biopsy),

3.2. Hypotheses:

3.2.1. The main hypotheses of the PET study with [18F]FTHA

is that the free fatty acid uptake among obese in the brain, heart, pancreas, liver, intestine, skeletal muscles, abdominal fat tissue and in the bone is increased and then decreased after weight loss.

3.2.2. The main hypotheses of MRI/MRS

We predict that the liver and mediastinal fat content is effectively decreased due to surgery.

Brain white matter volumes among obese are expected to be larger and to decrease during surgery-induced weight loss. The bone marrow fat content should decrease upon surgery induced weight loss.

3.2.3. The main hypotheses of the brain activation studies (fMRI) and molecular imaging studies (neurotransmitter PET):

We predict that the neural responses of the reward circuit (amygdale, ventral striatal and midbrain regions) will be decreased post-operatively for the appetizing vs. bland foods in surgical groups. The differential activity is expected to be more profound in the patient group before surgery vs. healthy controls [54]. This between-groups difference in the activity of the reward system is expected to diminish in the post-operative scan. Furthermore, it is expected that this change in responsiveness is due to decreased responses to appetizing food only. In addition the changes of brain activation due to surgery-induced weight loss are expected to correlate with changes in gastrointestinal hormone secretion and with subjective feelings of hunger. Obesity is predicted to be associated with lowered grey and white matter volume (as measured by voxel-based morphometry), as well as altered connectivity between the subcomponents of the reward circuit (as measured by diffusion tensor imaging).

We predict that striatal D_2 receptors, as measured with [^{11}C]raclopride, are decreased in obese individuals in comparison with lean control subject, and that weight loss following surgery results in increase in the number of these receptors. This would be consistent with lowered sensitivity to actual food reward in obesity. These receptors are also expected to predict functional responses in fMRI, especially receptors in the ventral striatum. Given the role of μ-opioid receptors in reward functions, we predict that these receptors are increased in the brain reward network in obesity, consistent with hypersensitivity to anticipatory reward in obesity. Again, these receptors are hypothesized to correlate with fMRI responses – and with dopamine D_2 receptors. Finally, we predict that weight loss will normalize the brain opioid dysfunction in obesity.

3.2.4. The main hypothesis of the bone biopsy and bone mineral density studies

We hypothesize that surgery-induced weight loss leads to increased rate of bone turnover where the balance is tilted towards increased bone resorption. This could result in decreased bone mass.

4. STUDY DESIGN

4.1. Study type

The study is a prospective, randomised study.

4.2. Study design

Patient Group (will be studied pre and 6 months after the surgery):

Visits before Surgery	Examinations (table 1 and table 2 in the laboratory test)
Preliminary visit	Doctor's checkup Oral glucose tolerance test Visit to the PET and MRI scan
1. PET-visit	Calorimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride ja 11C-carfentanil tracers
MRI-visit	MRI-scan

Visits after Surgery	Examinations
Preliminary visit	Oral glucose tolerance test
1. PET-visit	Calorimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride and 11C-carfentanil tracers
MRI-visit	MRI-scan

Control group (will be studied only once expect brain fMRI):

Visit times	Examinations
Preliminary visit	Doctor's checkup Oral glucose tolerance test Blood samples
1. PET-scan	Calorimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride and 11C-carfentanil tracers
1. MRI-visit	MRI-scan
2. MRI-visit (after 6 months)	MRI-scan

5. PATIENT/SUBJECT SELECTION

5.1 Source population

The study consists totally of 60 study subjects. Of these 40 are morbidly obese adults (male of female, BMI $\geq$ 40 kg/m^2 or if there is additional risk factor BMI $\geq$ 35 kg/m^2) and they will be recruited from patients undergoing the surgical procedure as a part of normal treatment mode. The morbidly obese patients are randomized either for laparoscopic sleeve gastrectomy (n=20) or Roux-en-Y gastric bypass (n=20). For a control group 20 lean, healthy, age and sex matched subjects will

be recruited. The control subjects will be recruited according to personal contacts, electrical and traditional bulletin boards, and newspaper advertisements.

5.2 Inclusion and exclusion criteria

Inclusion criteria for the patient population

1) BMI > 40 kg/m^2 or ≥ 35 if there is an additional risk factor (lean control group BMI 18-27 kg/m^2)
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/m^2
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
8) Pregnancy
9) Past dose of radiation
10) Presence of any ferromagnetic objects that would make MR imaging contraindicated
11) 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

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 the control group

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
6) Pregnancy
7) Past dose of radiation
8) 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
9) Presence of any ferromagnetic objects that would make MR imaging contraindicated

6. ASSESSMENTS

6.1 General study outline

Patient Group: *(will be studied pre and 6 months after the surgery):*

Visits before Surgery	**Examinations**
Preliminary visit	Doctor's checkup Oral glucose tolerance test Visit to the PET and MRI scan
1. PET-visit	Calarimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride and 11C-carfentanil tracer
MRI-visit	MRI-scan

Visits after Surgery	**Examinations**
Preliminary visit	Oral glucose tolerance test
1. PET-visit	Calorimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride and 11C-carfentanil tracer
MRI-visit	MRI-scan

Control group: *(will be studied only once):*

Visit times	**Examinations**
Preliminary visit	Doctor's checkup Oral glucose tolerance test Blood samples
1. PET-scan	Calorimetry (fasting) Perfusion-PET PET-scan with 18F-FTHA -tracer
2. PET-visit	PET-scan with 11C-raclopride ja 11C-carfentanil tracer
1. MRI-visit	MRI-scan
2. MRI-visit (after 6 months)	MRI-scan

Pre-operative studies in the surgery policlinic

The history of carefully planned, conservative treatments for obesity is assessed when patients seeking surgical treatment come in policlinic. A possible endocrinolocigal disease is excluded by the endocrinologist. Eating disorder is excluded by dietician using eating questionnaires BES and BITE. In addition to ECG the following laboratory values are measured pre-operatively: CBC (complete blood count), TSH, urate, fasting plasma glucose, GHbA1c, INR (blood coagulation), albumin, ALAT, AFOS, GT, total cholesterol, HDL, LDL, TG, creatine, Na, K, Ca, Pi, B12, D25,

folate, hs-CRP, plasma cytokines, IL1β, adiponectin, leptin, bile acids, gastrointestinal peptides (CCK, bombesin, gastrin-releasing peptide, GIP, GLP1, peripheral PYY, pancreatic polypeptide, oxyntomodulin, ghrelin, obestatin), lipoprotein fractions, serum fatty acid composition, non-cholesterol sterols (squalene, cholestenol, desmosterol, lathosterol, campesterol, sitosterol, cholestanol, avenasterol) and the sizes of LDL - & HDL-particles. In addition oral glucose tolerance test (OGTT) of 75 g with insulin, fatty acid and c-peptide measurements (time points 0 and 120 minutes) and dexamethasone test of 1 mg, are done.

Pre-operative gastroscopy is performed to all patients in the policlinic of surgery. During gastrointestinal endoscopy routine biopsies are taken from duodenum, antrum and corpus. This visit includes the decision if the patient is eligible for surgery. If the patient is accepted, the surgical risk factors and the study information are carefully explained. Randomisation either in the gastric sleeve or gastric bypass group is done using closed envelopes. Pre-operative quality of life is assessed using BAROS-survey.

After this, selected patients will visit the dietician. The dietician gives first instructions about the pre-operative very-low-calorie-diet (VLCD) phase and second nutritional advice about the post-operative phase. During the VLCD-phase patients usually lose weight approximately about 1.5-2.5 kg in a week. Before the surgery an abdominal ultrasound is performed to exclude gallstones. If gallstones are found, they are removed during the bariatric surgery.

6.2 PET studies

Pre operative PET studies will be performed before the preoperative VLCD phase (see chapter 6.1). PET studies are done in two separate PET visits both after a 10-12 hour overnight fast. In the first PET visit calorimetry measurements (see chapter 6.5), perfusion of intestine using $^{15}[O]H_2O$ and tissue (brain, heart, liver, pancreas, intestine, abdominal subcutaneous and visceral fat, bone and skeletal muscle) FFA uptake with [^{18}F]FTHA PET will be studied. In the other PET visit brain striatal D_2R density will be measured with radioligand [^{11}C]raclopride (78) and brain MOR density using [^{11}C]carfentanil (79). In both PET visits two catheters are inserted, one in an antecubital vein for injection of tracers and another in the opposite antecubital arteliazed vein for blood sampling. The subjects will be lying in a supine position throughout the studies.

The PET visit 1 PET studies start with myocardial perfusion measurements at rest. For the measurement of myocardial blood flow, 900 MBq of $[^{15}O]$-H_2O will be injected intravenously and a dynamic scan will be performed for 6 min. Then injection of [^{18}F]FTHA (185 MBq) is given and dynamic PET scanning starts with brain (40 min) and follows with heart/bone (20 min), liver/pancreas (20 min), abdominal area/intestine (20 min), and finally skeletal muscle/bone (10min, static) scan is performed. Radiotracers $[^{15}O]H_2O$ (80) and [^{18}F]-FTHA(81) (73) are produced and myocardial perfusion (82)(83) and FFA uptake (75) in different tissues analysed as previously described. Plasma radioactivity is measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland). Plasma glucose and free fatty acids are frequently measured during the PET study.

The PET visit 2 studies start with the injection of [^{11}C]raclopride (300 MBq) and dynamic brain scanning will be performed for 51min Receptor binding will be assessed as the binding potential (BP_{ND}), which is the ratio at equilibrium of specific binding to non-displaceable binding in the brain, using cerebellum as the reference region. 100 min after [^{11}C]raclopride injection the injection of [^{11}C]carfentanil (300 MBq) will be given and dynamic brain scanning performed for 60min. Receptor binding will be assessed as the binding potential (BP_{ND}), using the occipital cortex as the

reference region. Both ROI-based statistics and statistical parametric mapping (SPM) (84) will be used in the statistical analyses.

An integrated PET/CT, GE Discovery^{TM} ST System (General Electric Medical Systems, Milwaukee, WI, USA) with resolution of 3.75 is used for PET studies. All data will be corrected for dead-time, decay and measured photon attenuation. Dynamic PET-scans will be reconstructed with MRP reconstruction method (85). Brain scan is performed with a covering the whole brain in 3D mode. CT scans will be conducted to correct for photon attenuation and to evaluate fat masses and tissue density(86) . Plasma radioactivity is measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland. Plasma glucose and free fatty acids are frequently measured during the PET study.

According to the study protocol the patients will get two FTHA, two raclopride and two carfentanil injections. These will result in 14.8 mSv of radiation dose. Moreover, the measurement from CT will result in 6.7 mSv of effective dose. Altogether the radiation dose to study subjects will be 22,5 mSv which equals to exposure to natural background radiation in Finland for 4 years and 4 months. Dosimetry calculations done by the hospital physicist can be found from the appendix. So in general, the total dose accounting all PET-studies for patients is less than 22.5 mSv and for control group less than 11.25 mSv.

6.3 MRI/MRS/fMRI studies

Subjects are instructed to fast for 7-8 hours prior to the 1H MRS examination. Data from MRI/MRS/fMRI studies is obtained using either 1.5 Tesla – or 3.0 Tesla system (Intera, Philips Medical Systems, Best, the, Netherlands).

6.3.1 Liver, adipose tissue, and myocardium

Liver studies
Axial T1-weighted dual fast field echo images (TE 2.3 and 4.6 ms, TR 120 ms, slice thickness 10 mm without gap), covering the area of the liver are acquired during standardized breath-hold instructions. A MR imager (Gyroscan Intera CV Nova Dual, Philips Medical Systems, the Netherlands or Magnetom Verio, Siemens Medical Systems, Germany) with a flexible surface coil and body coil is used for liver volume and tiglyceride content measurement. A single voxel with a volume of 27 cm^3 is positioned in the liver outside the area of the great vessels. To ensure similar voxel placement before and after the intervention, the voxel location is recorded in each patient. A PRESS 1H MRS sequence is used with the following parameters: TR = 3000 ms, TE = 25 ms with data acquired during breath-hold intervals. 1H MRS findings of the liver have been validated in both animal and human studies (87,88)(89). Using a local workstation, liver margins are outlined manually on each individual image. Total liver volume is calculated by multiplying the measured surface areas of each slice by the slice thickness, as previously described (89).

Adipose tissues
Additionally T1W FFE images are obtained at the level of the intervertebral disc L2-L3 for analysis of abdominal adipose tissue masses as previously described (90) and to obtain anatomical reference for PET. Thigh area is scanned for the anatomical reference for PET.

Myocardium
Mediastinal fat is analysed from thoracal image. Heart function is imagined as in routine clinical heart-studies: Left ventricular function and dimensions are measured from continuous short axis

slices by using the balanced turbo field echo (bTFE) sequence on 1.5 T or 3.0 T MRI-system (91). Ten to fourteen slices are acquired during serial breath holds and vectorcardiographic (VCG) or electrocardiographic (ECG) gating to cover the left ventricle completely from apex to atrium. Slice thickness is 6-8 mm without gap between slices. Imaging parameters are set depending of used MRI-unit, for 1.5 T MRI these are e.g. repetition time (TR) of 3.4 ms, echo time (TE) of 1.7 ms, flip angel of 60º and matrix of 256 x 256. Images are stored digitally in DICOM-format. Afterwards, when all subjects have undergone MRI-scan, image analysis is performed using validated post-processing software (e.g. ViewForum R5.1; Philips Medical Systems) and validated analysis method (92) . Cine loops of every slice are reviewed to identify end-diastolic and end-systolic frames. Epicardial and endocardial contours are outlined manually. Papillary muscles are separately outlined and included in myocardium. End-diastolic volume (EDV) and end-systolic volume (ESV) are calculated from which cardiac output (CO), stroke volume (SV) and ejection fraction (EF) are computed. Myocardial mass is calculated from diastolic images. In addition triglyceride content in myocardium will be studied as previously reported(93)

Echocardiography
A Vivid 7 (GE Vingmed Ultrasound AS, Horten, Norway) ultrasound equipment will be used for cardiac measurements. The subjects will be lying in left recumbent position throughout the study.

The standard tomographic views of the left ventricle (parasternal long- and short-axis and apical 4-chamber, 2-chamber, and long-axis views) are obtained. LV and RV diameters, LV wall thickness and left atrial diameter are measured from M-mode tracings in the parasternal long axis as done routinely.

Using pulsed-wave Doppler, mitral inflow velocities (peak early diastolic velocity (E), peak late diastolic velocity (A), E/A ratio, and isovolumetric relaxation time (IVRT)) and pulmonary vein flow velocities are measured. With tissue Doppler imaging, longitudinal myocardial velocity and deformation are measured by use of strain rate and strain calculations. Apical long-axis and parasternal short-axis imageloops are acquired with frame rate over 60 Hz to be able to do speckle tracking with off-line analysis software. With speckle tracking radial and longitudinal myocardial velocities and deformations are calculated. All calculations and the most of the measurements are done afterwards with standard analysis software (EchoPac PC, GE Vingmed Ultrasound AS, Horten, Norway). Each representative value is obtained from the average of three measurements.

6.3.2 Brain

The following conventional image sequences are acquired: (1) sagittal T1-weighted sequence (TR/TE 650/15 [repetition time/echo time msec]), (2) coronal fluid attenuated inversion recovery (FLAIR) sequence (TR/TE/TI 11000/140/2800 [repetition time/echo time/inversion time msec]), (3) transverse T2-weighted spin-echo sequence (TR/TE 4500/100 [repetition time/echo time msec]). All these sequences are acquired with slice thickness of 5 mm and 1 mm interslice gap. Moreover a transverse T1-weighted turbo gradient-echo sequence (TR/TE 25/4.6 msec, flip angle of 30º, contiguous sections and section thickness of 1mm) is obtained. Cerebral white matter signal intensity changes are rated on FLAIR images by using the scale proposed by Fazekas et al (92), which has a maximum score of 6 and has shown a “very good” inter-observer reproducibility in a prior study (94).

6.3.2.1 Brain diffusion tensor imaging (DTI)

For DTI, 32 non-colinear directions of gradients are acquired to obtain the whole diffusion tensor with an echo-planar imaging (EPI) single-shot sequence. Diffusion imaging acquisition parameters

are as follows: TE/80 ms, 30 ms, 45 ms, 32 transverse slices, slice thickness 5 mm with a gap of 0.5 mm. ROIs are placed individually for each patient by one experienced neuroradiologist blinded to the diagnosis of morbid obesity. Contamination of adjacent structures and T2-visible focal lesions are avoided. ROI:s are placed in the following areas bilaterally: frontal, parietal, occipital white matter as well as middle thalamus, pulvinar and putamen. ROIs are also placed in the genu and splenium of corpus callosum. The FA and MD values of each ROI are measured by using Pride software (Philips Medical Systems, Best, The Netherlands). Brain volume index (BVI) is calculated from MD maps by dividing the brain volume by the sum of brain volume and cerebrospinal fluid (CSF) volume. Brain tissue and CSF are separated by using a threshold value of 2.4 x 10-3 mm2/s i.e. pixels with MD value of 2.4 x 10-3 mm2/s or more were considered as CSF.

6.3.2.2 Functional MRI (fMRI) studies

The aim of the fMRI is to assess how bariatric surgery (sleeve/bypass) influences the brain reward system response to food stimuli. The main interest is whether bariatric surgery can specifically reduce the responses to appetizing, high-calorie foods and is there a difference in responses between the two surgical procedures. Reward drive will be measured using BIS/BAS-questionnaire. Self-reported hunger level will be measured with a visual analogue scale, and food craving with the Food Craving State / Trait (FCS-FCST) questionnaire (31). Before a control subject can participate in the fMRI study block, the possible factors affecting to the study outcome (i.e. eating disorder) are excluded using the same question forms used by the dietician for the obese patients.
Echo-planar imaging (EPI) protocol with Philips Gyroscan Intera 1.5T scanner will be used. Functional and anatomical volumes are collected with Philips Gyroscan Intera 1.5T CV Nova Dual scanner. High-resolution, anatomical images (1 mm^3 resolution) will be acquired using a T1-weighted sequence (TR=25 ms, TE=4.6 ms, flip angle 30º, scan time 376 s). Whole-brain functional volumes are acquired using blood oxygenation level dependent (BOLD) -weighted EPI sequence (TR=2998 ms, TE=50 ms, flip angle 90º, FOV=192 mm, matrix=64x64, bandwidth=62.5 kHz, slice thickness=4.0 mm, gap between slices=0.5 mm, 30 interleaved slices acquires in ascending order) sensitive to BOLD contrast. Manual responses will be acquired with a MRI compatible button box.

The stimuli consists of digitalized full-colour photographs depicting

I. Appetizing, high-calorie foods (e.g. chocolate cake, sweets)
II. Bland, low-calorie foods (e.g. cabbage, lentils)
III. Non-food objects (e.g. cars, tools)

Fifty images from each category will be used. Stimulus presentation will be controlled with Presentation computer program (Neurobehavioral Systems Inc.) Stimuli are projected from an LCD projector onto a non-magnetic screen mounted at the foot of the scanner tube, and an angled mirror reflects the images on the screen to the participants' field of vision.
The experiment will run with a classic blocked (box-car) design. Appetizing foods, bland foods and non-food objects will be presented in separate 15 second -blocks that consist of presentation of five pictures from the respective category. A 15 s rest period (fixation) will be intermixed between the stimulation blocks to reduce the activation of the reward systems back to baseline level and to enhance the power of the design. Each stimulus will be displaced slightly to the left or to the right from the centre of the screen and the participant has to detect the direction of the displacement and respond with a button press. This ensures that the participant has to pay attention to the stimuli. A total of 10 blocks per condition will be run, and the order of the blocks will be counterbalanced across the participants. The experiment lasts about 15 minutes. After the experiment, the participants will rate how appetizing the food stimuli were, using a scale ranging from 1 (not appetizing at all) to 10 (extremely appetizing).

SPM5 software (www.fil.ion.ucl.ac.uk/spm/software/spm5) is used for the data analysis (84). First, functional images are sinc (sine cardinal) interpolated in time to correct for slice time differences and realigned to the first scan by rigid body transformations to correct head movements. Next the images are unwarped and a mean functional image is generated. The mean functional images are inspected for excessive signal dropout. EPI and structural images are co-registered and normalized to the T1 standard template in MNI space (95) using linear and non-linear transformations, and smoothed with a Gaussian kernel with 8 mm FWHM. A random effects model is implemented using a two-stage process of within (first level) and between (second level) –subjects modelling. This random-effects analysis assesses effects on the basis of inter-subject variance and thus allows making inferences from the population from where the participants were selected. For each participant a General Linear Model (GLM) is used to assess regional effects of task parameters on BOLD indices of activation. The model includes four experimental conditions (appetizing foods, bland foods, objects, rest) and effects of no interest (realignment parameters) to account for motion-related variance. Low-frequency signal drift is removed using a high-pass filter (cut-off 128 seconds) and AR(1) modelling of temporal autocorrelations is applied. The individual voxel-wise t-contrast images are generated using the following contrasts:

(1) Appetizing vs. bland foods
(2) Bland vs. appetizing foods
(3) Appetizing and bland foods vs. objects

These images are subsequently entered into a second-level model, subjected to a voxel-wise contrast and t-test using Gaussian Random Field Theory to assess whether or not the reward systems respond to high and low calorie foods in the experimental and the control group. A mixed ANOVA will be used to assess whether the responses of the reward system are different in the experimental vs. in the control group. The appetizing vs. bland foods contrast is used to asses the activation of the reward system in the experimental groups. The appetizing food vs. objects and bland foods vs. objects contrasts will be used to assess whether the gastric sleeve or gastric bypass surgery reduces the responses to appetizing foods only.

6.4. Analysis of brain studies

6.4.1. Acquisition and analysis of functional MRI data

Stimuli for fMRI experiments will be photographs of appetizing (high-calorie) and bland (low-calorie) foods, attractive and unattractive male and female faces, and nude male and female bodies. A number of fMRI studies indicate that the contrasting the viewing of such stimuli is a reliable way of activating the reward circuit (96)(97,98)(63). fMRI data will be acquired during a fasting state to maximize the hedonic value of the food stimuli. We will implement blood oxygenation level dependent (BOLD) contrast echo-planar imaging, in which the MR images are made sensitive to the state of oxygenation of haemoglobin (99). The variations of the BOLD signal reflect variations of the functional activity of neural networks (100). Hence, fMRI can be applied to localize ongoing neural activity during various cognitive and affective tasks. SPM5 software (www.fil.ion.ucl.ac.uk/spm/software/spm5) is used for the data analysis (84). First, functional images are sinc interpolated in time to correct for slice time differences and realigned to the first scan by rigid body transformations to correct head movements. Next the images are unwarped and a mean functional image is generated. EPI and structural images are co-registered and normalized to the MNI space using linear and non-linear transformations, and smoothed with a Gaussian kernel with 8 mm FWHM and analyzed with the general linear model (GLM).

6.4.2. Acquisition and analysis of structural MRI Data for VBM

High-resolution, anatomical images (1 mm^3 resolution) will be acquired using a T1-weighted sequence. Spatial normalization, tissue classification and radio-frequency bias correction are combined into a single step with segmentation to grey and white matter and cerebro-spinal fluid (101). Smoothed (10mm FWHM) and modulated grey and white matter images will be analyzed with GLM in SPM5 software.

6.4.3. Acquisition and analysis of DTI data

For DTI, 32 non-colinear directions of gradients are acquired to obtain the whole diffusion tensor with an echo-planar imaging (EPI) single-shot sequence. Data analysis will be carried out with FSL software (http://www.fmrib.ox.ac.uk/fsl/). Following brain extraction, the diffusion weighted images will be corrected for eddy currents and head motion. Subsequently, FA maps will be computed. Nonlinear registration will be employed to align the FA data across all subjects. FA maps will be analyzed using statistical parametric mapping (84). Probabilistic fibre tracking will be conducted using individually defined ROIs as source regions. Tract-based spatial statistics will be employed to compare the regions of the fibre tract skeleton where obese and lean individuals show different FA values.

6.4.4. Self-report measures

Reward drive will be measured with the BIS/BAS questionnaire (102). Self-reported hunger level will be measured with a visual analog scale, and food craving with the Food craving State / Trait (FCS-FCST) questionnaires (31). Depression will be measured with BDI-II (43), anxiety with the STAI form B (103), and external food sensitivity with the Dutch Eating Behavior Questionnaire (DEBQ) (104) . Self-reports of hunger and mood will be obtained with the visual analogue scale. Questionnaires will be completed pre- and postoperatively and in all experiments we will analyze the effects of the obesity surgery on the self-report measures, and correlate the self-reports with functional and structural brain data.

6.4.5 Visual stimulation for fMRI

During the primary fMRI experiment, anticipatory food-related reward activity will be induced by showing the participants alternating 16s blocks of appetizing, high-calorie and bland, low-calorie food pictures. Periodically the participants are asked to evaluate their i) hunger level and ii) heart rate. In two control experiments, rewarding and nonrewarding control stimuli (bodies, faces and objects) will be presented using a similar design. All experiments will last approximately 12 minutes. Additional resting state block of 10 minutes will also be included. fMRI will be performed pre- and postoperatively. In the preoperative scan, testing for an interaction between the food type (appetizing vs. bland) and group (patients vs. controls) will reveal the components of the reward circuit that show enhanced responses to appetizing foods in obesity. BOLD signal changes due to viewing appetizing and bland foods will be correlated with striatal D_2R density.

6.4.6. Predictions: D2R and MOR

For D2R, testing for the main effect of group (obese vs. lean) as well as the interaction between group (obese vs. lean) and measurement (pre- vs. postoperative) will reveal whether lower D_2R availability – particularly in the striatum - in obesity will be reversible upon successful weight loss following surgery. For MOR, testing for the main effect of group (obese vs. lean) and an interaction

between group (obese vs. lean) and measurement (pre- vs. postoperative) will reveal whether obesity is associated with higher MOR availability – particularly in the striatum - , and whether this will be reversible upon successful weight loss following surgery. Additionally, self-reported trait craving for foods will be correlated with striatal MOR density. In line with the animal work (69,105), we predict that food craving will be positively associated with MOR availability in the striatum.

6.4.7. Predictions: fMRI

We predict that in the preoperative scan, the patients will show elevated responses to high vs. low-calorie foods in the reward circuit (ventral striatum, amygdala, medial prefrontal cortex). However, this hyperactivation predicted to be abolished after weight loss surgery. Regarding MOR and D_2R, we hypothesize that increased anticipatory reward activity (as indexed by fMRI) due to foods in the ventral striatum is associated with decreased striatal MOR and D_2R density.

6.4.8. Predictions: DTI and VBM

Prior to surgery, patients are expected to have lower GM and WM density in the regions related to reward processing including medial prefrontal cortex and striatum. If these differences in tissue composition are due to adiposity, they are expected to be abolished by the weight loss following the surgery. However, if they are markers of increased propensity for gaining weight, weight loss surgery will not have an effect on these regional differences. On the basis of prior PET studies(106) we predict that connectivity of the striatum and frontal cortical regions will be decreased in obese vs. lean individuals. Again, comparison of the postoperatively acquired tract skeletons reveals whether these changes are reversible.

6.5 Indirect calorimetry

Calorimetry is the measurement of energy expenditure (EE). Direct calorimetry measures total heat loss from the body; indirect calorimetry measures total energy produced by the body. In this study, the metabolic rate measurements (VO_2, VCO_2 and EE) will be made using an open-system indirect calorimeter Deltatrac®(107) . For the measurement, the subject's head is placed under a plastic transparent hood (canopy) connected to the analyzer. The collection of expiratory gases is performed in the resting, fasting state and during the OGTT (supine position). Energy expenditure, glucose, lipid and protein oxidation will be calculated as described (108) .

6.6. Bioimpedance

Bioimpedance is done for the measurement of body fat content using electrical scale (Omron BF400). Bioimpedance is based on measuring electrical signals passing through the fat, lean mass and water in the body. The actual impedance or conductivity of various tissues in the body is known so by measuring current between two electrodes and applying this information to complex proven scientific formulas, body composition (i.e. body fat content) can be determined.

6.7. Timing of laboratory measurements

Screening laboratory tests include CBC (complete blood count: platelet count, red blood cell count, white blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration), urate and thyrotropin (TSH). In

addition a dexamethasone of 1 mg test is done. Laboratory tests in this study include basic tests and additional tests. Tests are done according to Table 1 and Table 2 below. Basic and additional laboratory test –packages are taken pre-operatively before VLCD and 6 months post-operatively

Table 1: Laboratory tests and other measurements

SCREENING TESTS	**PREOPERATIVE TESTS***	**POSTOPERATIVE TESTS**
Exclusion tests	Preoperative tests*	Post operative tests: 6 months
Basic tests	Basic tests	Basic tests
Additional tests	Additional tests	Additional tests
D-25, B12, fE-folate	Calorimetry	Calorimetry
Calorimetry	Bioimpedance	Bioimpedance
Bioimpedance	PET samples	PET samples
**Subject has been accepted for the surgical treatment of obesity*		
In addition, weight, BMI and waist circumference are measured in every time point. Bioimpedance is measured using electrical scale (Omron BF 400).		

Table 2: Laboratory test- kits

EXLUSION TESTS	**BASIC TESTS**	**ADDITIONAL TESTS**	**PET SAMPLES**
PVK+T	INR(coagulation)	Oral glucose tolerance test with insulin, fatty acid and c-peptide measurements (OGTT, 75g glucose load, time points 0 and 120 minutes)	Blood radioactivity
Thyrotropin (TSH)	Albumin	High sensitivity C-reactive protein (hs-CRP)	With ^{18}F-THFA: blood free fatty acids
Dexamethasone test of 1mg (DXM)	Fasting plasma glucose (fd-gluk)	Non-cholesterol sterols (squalene, cholesterol, desmosterol, lathosterol, campesterol, sitosterol, cholesterol, avenastarol)	
Urate	Glucosylated haemoglobin-A-fraction c (GHbA1c)	Bile acids	
	Alanin aminotransferase (ALAT)	P-cytokines (IL-1β, IL-1βab..)	
	Alkaline phosphatise (AFOS)	S-adiponectin	
	Glutamyl transferase (GT)	S-leptin	
	Total cholesterol	Serum fatty acid composition	
	HDL-cholesterol	Lipoprotein fractions	
	LDL-cholesterol	Size of LDL and HDL particles	
	Triglycerides	Gastro-intestinal peptides (CCK, bombesin, gastrin-releasing peptide, GIP, GLP1, peripheral PYY, pancreatic polypeptide, oxyntomodulin, ghrelin, obestatin)	
	Creatine	Lipodomics (not in 24 months)	
	Electrolytes(Na, K, Ca)	Proteomics (not in 24 months)	
	Phosphate (Pi)		
The samples of all study groups are collected and stored in a similar way. The order of analysis is random. Spare serum samples will be collected for future analysis.			

6.8. Biopsies, proteomics and DNA samples

Human tissue biopsies
During endoscopy routine biopsies are taken from duodenum, antrum and corpus. Biopsies from liver, subcutaneous and visceral fat will be taken during bariatric surgery and subcutaneous fat biopsies 6 months postoperatively under local anaesthesia using 1 % lidocaine without adrenaline. Tissue samples will be cut into samller pieces and immediately stored in formalin or frozen in liquid nitrogen and stored in -70ºC.

Bone biopsies will give very important insight into the bone metabolism in morbid obesity as well as after bariatric surgery. Dynamic and static histomorphometric analysis is the golden standard to analyse changes in bone turnover. Transiliacal bone biopsies are taken from the anterior iliac crest in conjunction of the surgery as well as 6 months after the surgery (from the opposite side). The biopsies will be harvested under local anaesthesia in the operation theatre as described in Tamminen et al. 2007. Samples will be fixed in ethanol and analyzed in the bone biopsy unit of the Kuopio University Hospital. Separate written informed consent is done for bone biopsy sampling.

RNA isolation and expression analysis
Total RNA will be isolated from human tissues with Trizol (Invitrogen, Carlsbad, CA), from HepG2 and C2C12 using RNeasy with DNase 1 treatment (Qiagen, Valencia, CA). cDNA will be synthesized using random hexamers (Advantage, Clontech, Mountain View, CA), for subsequent real-time qPCR (ABI Prism 7000 or 7700 Sequence Detection System, Applied Biosystems, Inc., Foster City, CA). PCR products will be detected using SYBR Green or Taqman pre-designed Assays-on-Demand.

Protein isolation and expression analysis
Frozen tissue samples will be lysed in buffer containing protease inhibitor cocktail (Sigma), sodium fluoride (100 mM), sodium orthovanadate (2mM) and 1 % Triton X-100. Protein concentrations will be determined using a BCA kit (Pierce Biotechnology, Rockford, IL) and proteins will be separated by SDS-PAGE for subsequent Western blotting.

DNA samples
DNA samples are collected and used according to another study protocol: CMGene. Separate written informed consent is done for DNA sampling. Results from the DNA studies are not given to the subjects.

7. SAFETY

During PET studies: Heart rate and blood pressure will be monitored during the studies. Chemical purity of [^{11}C]raclopride, [^{11}C]carfentanil and [^{18}F]FTHA are measured before administration. Stable isotopes are commercial sterile products.

8. ADVERSE EVENTS

8.1. Reporting of Adverse events (AE's)

All adverse events will be registered, and when necessary, the ethical committee and the National Agency for Medicine will be notified. All adverse events will be fully documented and followed in order to determine the final outcome (until the event has subsided or the condition as stabilised).

8.2. Expedited Reporting

According to Finnish national regulation, serious adverse event* will be reported to suitable authorities within 3 days whereas unexpected or unusually severe adverse experience** will be reported within ten days.

*A serious adverse event is any event that is fatal, life-threatening, disabling, incapacitating, results in hospitalisation, or prolongs hospitalisation.
**A severe adverse experience is any event that prevents normal everyday activity.

8.3. Emergencies

Emergencies may occur during the experiments. A medical doctor will be present during all the measurements in the study. Vital functions will be monitored, and materials and drugs needed for first aid/resuscitation are readily available.

9. ETHICS

9.1. Ethical considerations

The studies will be performed using standard procedures that have been in clinical or research routine use for years in the Turku PET Centre. This study will be conducted according to Good Clinical Practice and the Declaration of Helsinki. Written informed consent for the study will be obtained from all subjects before the beginning of the study. Subjects will be informed of their right to withdraw from the study at any time. At least one medical doctor will be available at all times during the study. All necessary investigator contact information will be provided along with a description of the study. The radiation dose fof patients will be around 22.5 mSv which is approximately 4.4 times the natural background radiation in Finland for one years. Although there is no exact knowledge about the risk for developing cancer after this dose of radiation, the risk of fatal cancer is estimated to correspond to the risk for dying when driving 22500 kilometres in a car or smoking 225 cigarettes (109). As the control group is studied only once the radiotion dose will be around 11.25 mSv.

9.2. Ethical Review

Study protocol, patient information, and informed consent form will be submitted to the Ethical Committee of the Hospital District of the South-Western Finland and studies will not start before getting ethical permission. The ERC/IRB will be informed by the Investigator of all subsequent protocol amendments and of serious or unexpected adverse experiences occurring during the study, which are likely to affect the safety of the subjects or the conduct of the study.

9.3. Subject information and informed consent

Information about the study procedures and design will be given in both oral and written form to the study subjects. All necessary investigator contact information will be provided along with a description of the study. Written informed consent for the study will be obtained from all subjects before the beginning of the study. The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented. Subjects will be informed of their right to withdraw from the study at any time.

10. DATA COLLECTION AND MANAGEMENT

10.1. Case Report Forms

One set of case report forms will be filled during the initial clinical examination in order to collect subjects' personal information and their medical status and history relevant to the aims and exclusion criteria of this study.

10.2. Electronic data collection

Data from the case report forms, clinical observations (such as adverse effects or changes in medical status) as well as results from the study (biological and PET results) will be gathered in electrical form.

10.3. Data management

All data collected is strictly confidential. It will be archived on paper or electronically at least for fifteen years

11. DATA ANALYSIS

11.1. Analysis of PET-data

For calculation of skeletal muscle, myocardial, liver, pancreas, and adipose tissue FFA uptake the three compartment model (110) and graphical analysis (75) are employed. Plasma and tissue time-activity curves are analysed graphically to quantify the fractional rate of the tracer transport, KI (73).

11.2. Analysis of MRI data

The software SliceOmatic is used to measure the adipose tissue mass before and after the surgery. Then data will be extracted to SPSS for further analysis.

12. STATISTICS

12.1. Sample size

The number of subjects to be recruited is based on power analysis. Joint analysis based on all the variables of interest suggest that sample size of 20 plus 20 patients and 20 controls is sufficient for detecting statistically significant differences at alpha level of 0.05.

12.2. Statistical plan

Appropriate statistical analyses will be performed corresponding to current standards.

12.3. Possible interim analyses and stopping rules

No interim analysis is intended.

13. QUALITY ASSURANCE

13.1. Information of study personnel and training

All the involved personnel at the participating centres will be informed about the aims and the practical issues of the study. The measurements are routinely performed at the Turku PET Centre and have been applied for years.

13.2. Protocol amendments

According to Finnish regulations, protocol amendments can be made if all investigators agree. They are presented in a written form and dated. They include the original chapter of the study protocol and the amended chapter with and explanation of this change. All important protocol amendments will be submitted for review to the ethical committee and the National Agency for Medicine.

14. STUDY SCHEDULE

The clinical part of the project will be spread for three years (01.01.2011 to 31.12.2013). Analysis and reporting of the data will be started as soon as possible.

15. CRITERIA FOR PREMATURE STUDY TERMINATION

No interim analysis is intended.

16. FINANCING

The study will be financed with project grants of Pirjo Nuutila (EFSD 300 000€), Pirjo Nuutila and Jussi Hirvonen (Sigrid Juselius 140 000€). Other research grants will be applied for study financing from the CoE funding from Academy of Finland (the study and the researchers are part of "Finnish Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic Research", Ministry of Education), and from several foundations.

The study has neither a straight nor indirect financing from the industry. The financing of the study is governed through the TYKS and University of Turku.

Study budget	**Unit**	**Year 2011**		**Year 2012**		**Year 2013**		**Year 2014**		**Total n of studies**	**TOTAL (€)**
PET (18F)FTHA (2x40+20)	328	50	16400	50	16400		0		0	100	32 800
PET (11C)RAK (2x40+20)	302	50	15 100	50	15 100		0		0	100	30 200
PET (11C)CAR (2x40+20)	348	50	17400	50	17400		0		0	100	34 800
MRI+FMRI+MRS	400	50	20000	50	20000		0		0	100	40 000
FMRI controls 6 mo	246	10	2460	10	2460		0		0	20	4 920
Echocardiography(all subjects pre and post)	200	60	12000	60	12000					120	24 000

OGTT	60	50	3000	50	3000		0		0	100	6 000
Travelling cost of study subjects	30	320	9600	320	9600		0		0	640	19 200
Lipidiomics	300		0		0	100	30000		0	100	30 000
Analysis of tissue biopsies	150		0		0	80	12000		0	80	12 000
Travelling cost of researchers	2000		0		0	7	14000	8	16000	15	30 000
Publication costs	700		0		0	5	3500	5	3500	10	7 000
Salary of one research nurse	2465	12	29580	12	29580		0		0	24	59 160
Salary of 3 PhD student	2600	36	93600	36	93600	36	93600	36	93600	144	374 400
Salary of one senior researcher	6200	12	74400	12	74400	12	74400	12	74400	48	297 600
			293540		**293540**		**227500**		**187500**		**1 002 080**

The study subject fee will be paid only for the control subjects. The study subject's fee will be totally 300€. If the patient consents to the bone biopsies 100€ will be paid. Travelling expenses will be paid according to prices for public transportation

17. INSURANCE

The subjects are covered by the patient insurance.

18. STUDY REPORT AND PUBLICATION(S)

The results will be reported in international peer-review journal.

19. ARCHIVING

All data collected will be strictly confidential. It will be archived on paper and electronically for at least fifteen years.

20. REFERENCES

21. APPENDICES

Appendix 1 World Medical Association Declaration of Helsinki
Appendix 2 Written information to study subjects
Appendix 3 Informed consent form
Appendix 4 Radiation dose calculations

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21. APPENDICES

Appendix 1 World Medical Association Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002
Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles,

be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally

authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists. **See footnote**.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study. **See footnote**.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Note: **Note of clarification on paragraph 29 of the WMA Declaration of Helsinki**

The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or

- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious

or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

Note: **Note of clarification on paragraph 30 of the WMA Declaration of Helsinki**

The WMA hereby reaffirms its position that it is necessary during the study planning process to identify post-trial access by study participants to prophylactic, diagnostic and therapeutic procedures identified as beneficial in the study or access to other appropriate care. Post-trial access arrangements or other care must be described in the study protocol so the ethical review committee may consider such arrangements during its review.

The Declaration of Helsinki (Document 17.C) is an official policy document of the World Medical Association, the global representative body for physicians. It was first adopted in 1964 (Helsinki, Finland) and revised in 1975 (Tokyo, Japan), 1983 (Venice, Italy), 1989 (Hong Kong), 1996 (Somerset-West, South Africa) and 2000 (Edinburgh, Scotland). Note of clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002.

9.10.2004

APPENDIX 2

SLEEVEPASS: versio 1.4
11.1.2010

SUOSTUMUS: SLEEVEPET 2

Laihdutusleikkauksen metaboliset ja funktionaaliset vaikutukset PET- ja MRI-menetelmillä tutkittuna.

PET-keskuksen tutkimuslääkäri on pyytänyt minua osallistumaan tutkimukseen, johon kuuluu PET- ja magneettikuvauksia, sokerirasitustesti ja sydämen ultraäänitutkimus. Olen saanut tutkimusta ja sen yhteydessä suoritettavaa tietojen keräämistä ja käsittelyä kuvaavan tutkimushenkilötiedotteen. En ole lääketieteellisessä tutkimuksessa annetun lain 488/1999 7-10 §:n tarkoittama henkilö (alaikäinen, raskaana/imettävä nainen, vanki tai kehitysvammainen). Suostun vapaaehtoisesti osallistumaan yllä mainittuun tutkimukseen ja annan suostumukseni tutkimuksen yhteydessä tapahtuvaan tietojen keräämiseen ja niiden käsittelyyn. Minua informoidaan siitä, mistä tietojani hankitaan. Annan suostumukseni niiden tietojen keräämiseen, jotka ovat tarpeellisia tätä tutkimusta varten. Minulle on selvitetty, että minusta kerättäviä tutkimustietoja tullaan käsittelemään luottamuksellisina siten, että niistä ei voida tunnistaa henkilöllisyyttäni.

Tästä tutkimuksesta saatavat tiedot saattavat olla hyödyllisiä sairaalloisen lihavuuden kirurgisten hoitotulosten tulkinnassa ja lihavuuteen liittyvien säätelytekijöiden ymmärtämisessä. Tutkimuksesta saatavat tiedot saattavat myös edistää lihavuuden hoitomahdollisuuksia. Suostun, että tietojani voidaan viranomaisten luvalla käyttää tätä tarkoitusta varten. Minulle on selvitetty, että tutkimuksessa kerättyjä tietoja säilytetään mahdollisten viranomaistarkastuksen vuoksi, kunnes voimassa oleva laki sallii niiden hävittämisen.

Ymmärrän, että osallistumiseni tutkimukseen on täysin vapaaehtoista. Voin keskeyttää osallistumiseni missä tutkimuksen vaiheessa tahansa syytä ilmoittamatta

Allekirjoituksellani vahvistan osallistumiseni tutkimukseen sekä annan suostumukseni vapaaehtoisena tutkimushenkilönä olemiseen.

_____ _____
Potilaan allekirjoitus Päiväys

_____
Nimen selvennys

_____ _____
Lääkärin allekirjoitus Päiväys

_____
Nimen selvennys

Alkuperäinen allekirjoitettu suostumuslomake säilytetään tutkimuksesta vastaavan lääkärin tiedostoissa.

APPENDIX 3

SUOSTUMUS: SLEEVEPET 2 - LUUBIOPSIA

Laihdutusleikkauksen metaboliset ja funktionaaliset vaikutukset PET- ja MRI-menetelmillä tutkittuna.

PET-keskuksen tutkimuslääkäri on pyytänyt minua osallistumaan tutkimukseen, johon kuuluu luukoepalan otto. Olen saanut tutkimusta ja sen yhteydessä suoritettavaa tietojen keräämistä ja käsittelyä kuvaavan tutkimushenkilötiedotteen. En ole lääketieteellisessä tutkimuksessa annetun lain 488/1999 7-10 §:n tarkoittama henkilö (alaikäinen, raskaana/imettävä nainen, vanki tai kehitysvammainen). Suostun vapaaehtoisesti osallistumaan yllä mainittuun tutkimukseen ja annan suostumukseni tutkimuksen yhteydessä tapahtuvaan tietojen keräämiseen ja niiden käsittelyyn. Minua informoidaan siitä, mistä tietojani hankitaan. Annan suostumukseni niiden tietojen keräämiseen, jotka ovat tarpeellisia tätä tutkimusta varten. Minulle on selvitetty, että minusta kerättäviä tutkimustietoja tullaan käsittelemään luottamuksellisina siten, että niistä ei voida tunnistaa henkilöllisyyttäni.

Tästä tutkimuksesta saatavat tiedot saattavat olla hyödyllisiä sairaalloisen lihavuuden kirurgisten hoitotulosten tulkinnassa ja lihavuuteen liittyvien luuston aineenvaihdunnan muutosten ymmärtämisessä. Tutkimuksesta saatavat tiedot saattavat myös edistää lihavuuden sekö luustosairauksien hoitomahdollisuuksia. Suostun, että tietojani voidaan viranomaisten luvalla käyttää tätä tarkoitusta varten. Minulle on selvitetty, että tutkimuksessa kerättyjä tietoja säilytetään mahdollisten viranomaistarkastuksen vuoksi, kunnes voimassa oleva laki sallii niiden hävittämisen.

Ymmärrän, että osallistumiseni tutkimukseen on täysin vapaaehtoista. Voin keskeyttää osallistumiseni missä tutkimuksen vaiheessa tahansa syytä ilmoittamatta

Allekirjoituksellani vahvistan osallistumiseni tutkimukseen sekä annan suostumukseni vapaaehtoisena tutkimushenkilönä olemiseen.

_____ _____

Potilaan allekirjoitus Päiväys

_____

Nimen selvennys

_____ _____

Lääkärin allekirjoitus Päiväys

_____

Nimen selvennys

Alkuperäinen allekirjoitettu suostumuslomake säilytetään tutkimuksesta vastaavan lääkärin tiedostoissa.

APPENDIX 4

VALTAKUNNALLINEN PET-KESKUS SOP7505/LIITE 3 1
(1) versio 04

APPENDIX **SÄTEILYANNOSLASKU**
Tuula Tolvanen 28.9.2010
Sleeve 2

TUTKIMUSKOODI:
GE D690 PET/TT, 3D-moodi

Tutkimusprojektin aikana tutkimushenkilölle aiheutuu säteilyaltistusta seuraavista PET-merkkiaineista.

Merkkiaine	Injisoitava tai inhaloitava aktiivisuus (MBq)	Efektiivinen annos (mSv/MBq)	Keskimääräinen efektiivinen annos injektiota kohti (mSv)
^{15} O-H_2O	900 MBq	0,0011 mSv/MBq	1,0 mSv
^{18} F-FTHA	185 MBq	0,019 mSv/MBq	3,5 mSv
^{11} C-raklopriidi	300 MBq	0,0067 mSv/MBq	2,0 mSv
^{11} C-karfentaniili	300 MBq	0,0046 mSv/MBq	1,4 mSv

Tutkimussuunnitelman mukaisesti tutkittavalle annetaan yksi (2) ^{15} O-H_2O, kaksi (2) ^{18} F-FTHA, kaksi (2) ^{11} C-raklopriidi ja kaksi (2) ^{11} C-karfentaniili injektiota. Tästä aiheutuva säderasitus on 14,8 mSv. Lisäksi tutkittavasta mitataan vaimennuskerroin tietokonetomografia mittauksella iteratiivisella rekonstruktiomenetelmällä aivoista kuusi kertaa ja neljästä eri kehon alueesta (rintakehä, vatsa, alavatsa, reidet) kaksi kertaa ja niistä aiheutuu arviolta 6,7 mSv efektiivinen annos. Yhteensä tutkimuksesta aiheutuu 22,5 mSv säderasitus, joka vastaa noin neljän vuoden neljän kuukauden aikana saatua taustasäteilyn määrää Suomessa.

Tämä arvio sisältää ^{15} O-H_2O, ^{18} F-FTHA, ^{11} C-raklopriidi ja ^{11} C-karfentaniili-injektioista sekä aivojen, rintakehän, vatsan, alavatsan ja luurankolihaksen alueen vaimennus-korjausmittausten aiheuttaman säderasituksen.

Vertailuarvoja tutkimuksesta aiheutuvalle säteilyannokselle:
Vuotuinen taustasäteily Suomessa 4 – 5 mSv.
Keskimääräinen efektiivinen annos tutkimusta kohti
Vatsan TT-tutkimuksesta 12 mSv.
Pään TT-tutkimuksesta 1.3 mSv.
Luuston gammakuvauksesta (^{99m}Tc) 3.7 mSv.
Aivojen SPECT (^{99m}Tc) 6.9 mSv.
Sydänlihasperfuusion SPECT (^{99m}Tc) 9.1 mSv.
FDG-PET tutkimus 5 mSv.

Kirjallisuusviitteet
Vertailuarvot muista lääketieteellisistä säteilylle altistumisista: Lääketieteellinen aikakauskirja Duodecim 2000;116:664-669.

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