An obese brain and an inflamed body: Central and peripheral consequences of obesity

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Citation for published version (APA):
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General Introduction
CHAPTER 1

Introduction

According to the World Health Organization the prevalence of obesity has nearly doubled since 1980. Approximately 1.9 billion adults were overweight (BMI ≥ 25 kg/m² and < 30 kg/m²) in 2014 while at least 600 million were obese (1). In the Netherlands, 31.5% of the population is overweight and 10.1% is severely obese (statline.cbs.nl). The potential medical consequences of obesity include development of the metabolic syndrome (2) with complications such as cardiovascular disease, diabetes mellitus type 2 (T2DM), non-alcoholic fatty liver disease (NAFLD), respiratory complications (obstructive sleep apnoea), osteoarthritis of large and small joints and some types of cancer (3; 4; 5). Obesity itself has recently been recognized as a disease by the Obesity Society Council (6).

Not all obese people develop disease since about 20-30% of the obese population remains relatively healthy, and is characterized by preserved insulin sensitivity, absence of hypertension and a favourable lipid, inflammation, hormonal, liver enzyme and immune profile (7; 8). This group is often referred to as metabolically healthy obese (MHO), in contrast with obese people that develop complications referred to as the metabolically abnormal obese (MAO). The MHO group shows a lower lean body mass (LBM), lower visceral adipose tissue content, less fat accumulation in the liver and lower muscle fat infiltration compared to the MAO group, whereas no difference in amount of subcutaneous adipose tissue has been reported (7; 9). Moreover, high birth weight and childhood/adolescence-onset obesity are associated with a MHO profile (10; 11). Mechanisms explaining the differences between the two metabolic phenotypes are largely unknown. Dynamic aspects of adipose tissue (AT) may explain part of the differences because many metabolic abnormalities in obesity-induced insulin resistance correlate with adipocyte characteristics, including adipocyte-size, degree of inflammation within adipose tissue and the secretion of adipokines involved in insulin sensitivity, energy expenditure and fuel handling (12). A causal relationship between dysfunctional AT in the setting of obesity and the metabolic phenotype therefore seems plausible.

To study whether metabolic function and phenotype of adipose tissue contributes to the metabolic derangements in obesity, we performed several studies in morbidly obese subjects undergoing bariatric surgery. We performed in depth metabolic phenotyping combined with tissue analyses and studied independent variables associated with metabolic health.

Obesity has a multifactorial pathogenesis and studies in twins, analyses of familial aggregation, adoption studies and animal models of obesity all indicate that both genetic and environmental factors are determinants of obesity (13; 14; 15; 16). Obesity results from an energy imbalance between calories consumed, i.e. food intake and calories burned, i.e. energy expenditure. The two main reasons why numbers of obesity grow globally are the increased availability of foods high in sugars and fat, and a decrease in physical activity due to the ever more sedentary nature of many jobs, changing modes of transportation, and upcoming urbanization. It has become clear that the decrease in energy expenditure is not
accounted for in energy intake in large percentages of the population leading to increasing body weight. Why food intake is not adjusted in accordance with caloric need is subject of extended basic and clinical research. Studying the underlying mechanisms obviously involves the brain because the brain is the master regulator of energy intake and metabolism. Extended brain areas and circuits control food intake by integrating metabolic and hormonal signals of hunger and satiety and dysregulation of this feedback system contributes to obesity (17).

To study whether there are differences in brain regions involved in food intake between lean and obese subjects and whether the assumed differences are reversible after weight loss, we performed brain imaging and analysed feeding behaviour questionnaires in lean and obese subjects after weight loss.

PART I Obesity and the brain

Food intake is essential for survival and it is not surprising that there are multiple pathways, which are coordinated by the brain, that regulate energy intake (figure 1). Food intake is initiated by both external (smell and sight of food) and internal (metabolic and hormonal signals) factors. The homeostatic control of food intake is driven by hunger and satiety signals which inform the brain on energy status resulting in an appropriate feeding response and adjustment in energy expenditure (17; 18). The central homeostatic control of energy metabolism is orchestrated by the hypothalamus. The major neurons involved in homeostatic control are the orexigenic neuropeptide Y (NPY) and agouti related protein (Agrp) neurons and the anorexigenic proopiomelanocortin (POMC) and cocaine amphetamine related transcript (CART) neurons in the arcuate nucleus (ARC). Additionally, input from the nucleus of the solitary tract (NTS) in the brainstem and the afferent vagal nerves from the gut provide information on energy status (19). The control of food intake through homeostatic mechanisms is disturbed in obesity. For example, in rodent models of obesity the anorexigenic effects of leptin are reduced showing a state of leptin resistance. Indeed, despite the fact that most adults are aware of the consequences of eating too much food, it has proven to be difficult to maintain a healthy energy balance illustrated by the obesity epidemic. This might be due to the fact that food is a powerful positive reinforcer caused by the rewarding or hedonic properties of (palatable) food. Individual differences in the reinforcing value of food may provide a mechanism to explain the excess intake and positive energy balance in some, but not all persons (20, 21). Using functional magnetic resonance imaging (fMRI) it has been shown in obese individuals that images of high caloric food trigger an enhanced response in brain areas involved in reward (22) independent of hunger and satiation compared to lean controls (23).

The augmenting value of food is related to activity of the dopaminergic system and it has been shown that dopamine (DA) is the primary neurotransmitter involved in food reinforcement (24; 25). DA is synthesized from phenylalanine in the midbrain ventral tegmental area (VTA) and the substantia nigra. There are 5 dopamine receptor subtypes (D1R-
D5R) besides dopamine transporters responsible for DA re-uptake. The main DA projections run from the VTA towards the nucleus accumbens (NAc) and other regions including dorsal striatum (caudate and putamen), cortical (orbitofrontal cortex (OFC), the cingulate gyrus (ACC), the limbic regions (hippocampus and amygdala) and the lateral hypothalamus (for review 26). Drugs that block dopamine D2 receptors (D2R) increase appetite and result in significant weight gain in rats and humans (27; 28), on the other hand, drugs that increase brain dopamine concentration act anorexigenic (29). Also in humans, using single photon emission and positron emission computed tomography (SPECT/PET) imaging, a decrease in striatal D2/3R was observed in obese individuals compared to lean controls (30) and lower striatal D2/3R availability has been linked to decreased activity in the OFC and ACC in obese humans (31). Moreover, in obese individuals D2/3 receptors negatively correlate with BMI and some but not all human brain imaging studies showed an increase in D2/3R availability after weight loss but the results are inconsistent and the sample sizes low (30; 32; 33). The relationship between feeding behaviour and reward has been studied extensively in rodents and to a smaller extent in humans (for review 34) and those studies show that macronutrient composition, eating pattern and exposure to high palatable food all can modulate the brain circuitry involved in food related reward resulting in overeating. A second neurotransmitter involved in the regulation of food intake is serotonin (5-hydroxytryptamine, 5-HT) (35). An inverse relationship between brain serotonin and food intake has been described (36). We earlier showed that serotonin transporter (SERT) binding within the diencephalon in lean men decreases after a short term hypercaloric high fat high sugar snacking diet while an increase in meal size did not (37; 38). The recently approved 5-HT2c receptor agonists further show the involvement of brain serotonin signalling in regulation of food intake and body weight. Discussion of how serotonin modulates food intake, body weight and energy metabolism is outside the focus of this theses (34).

In summary, obesity is the result of a disbalance between energy intake and expenditure. Overeating results from disrupted hedonic and homeostatic brain circuits and although many underlying mechanisms have been studies in rodent models, translational studies are relatively scarce due to the relative inaccessibility of the human brain. Imaging studies showed that dopaminergic and serotonergic systems might be altered in human obesity but the pathogenesis of these observations remains unclear. Moreover whether the differences between lean and obese subjects are caused by obesity or induce obesity remains matter of debate.

In this thesis, we describe studies on striatal dopamine receptor availability (D2/3R) in lean and obese subjects before and after weight loss to investigate whether lower receptor availability is reversible and whether that relates to healthier eating behaviour (assessed with questionnaires).
PART II Obesity, metabolism and inflammation

Glucose metabolism

Glucose is the major fuel for many organs including the brain and therefore blood glucose concentrations are tightly regulated through peripheral hormones and the autonomic nervous system. After blood glucose levels rise in response to exogenous nutrient supply from the gastrointestinal tract, insulin is secreted from the pancreatic islets and flows via the pancreatic vein through the portal vein exposing the liver to a high concentration of insulin, while a lower concentration of insulin is presented to peripheral tissues. In insulin sensitive tissues like adipose tissue and skeletal muscle insulin binds to its receptor and activates an insulin signalling cascade resulting in recruitment of the glucose transporter 4 (GLUT
4) on the cell membrane resulting in cellular glucose uptake. Glucose is then either stored as glycogen or oxidized. In the liver, insulin suppresses glucose production and activates glycogen synthesis. Lipolysis, the process of degradation of triglycerides within adipocytes resulting in FFA and glycerol efflux is also regulated by insulin. Insulin suppresses lipolysis through inhibition of hormone sensitive lipase. Insulin release is augmented by incretins, gut hormones released after food ingestion. About 50% of the total amount of insulin released in response to ingestion of glucose is attributed to mainly glucagon-like peptide (GLP-1) (39). During fasting, hypoglycemia is prevented by an increase in glucose production, a reduction in peripheral glucose uptake and an increase in lipolysis induced by increased glucagon, cortisol and growth hormone levels as well as activation of the autonomic nervous system. Obesity increases the risk of insulin resistance (IR), which is defined as an impairment of insulin’s capacity to increase peripheral glucose uptake into insulin responsive tissues such as adipose tissue, and muscle, a reduction in suppression of liver endogenous glucose production (EGP) and a reduction in insulin-mediated suppression of lipolysis. IR might also be present in the brain although conflicting data exist (40; 41). IR increases the risk for impaired fasting glucose, glucose intolerance and finally diabetes mellitus type 2. Insulin resistance in obesity is caused by several factors, including increased release of FFA and pro-inflammatory cytokines from adipose tissue, genetic factors (42), lipotoxicity, ectopic lipid accumulation, mitochondrial dysfunction, ER stress (43) as well as eating pattern and macronutrient composition (38; 44). Although many of these pathways have been shown in rodent models of obesity, in humans it is less clear whether and to what extent these pathways contribute to whole body insulin resistance. Finally, as described above brain circuitries involved in glucose metabolism might also be affected by obesity and hyperphagia. In conclusion, obesity-induced insulin resistance has a multifactorial etiology and the exact contribution of each component still needs to be elucidated.

Bariatric surgery

Caloric restriction and weight loss are well known to improve insulin sensitivity (45). Treatment of obesity by a calorie restricted diet, physical exercise, a combination of both, or medication results in a partial reversal of the obesity-induced metabolic alterations when subjects adhere to the intervention. The sequential order of restoration of healthy metabolism is unknown since most studies are performed in a cross-sectional way. Longitudinal studies would be of help, but are very difficult to perform because repeated measurements within a limited time frame are time-consuming, expensive and might overburden the study subjects. Moreover, weight reduction with above mentioned interventions is often modest and difficult to maintain. A more effective method to rapidly lose weight and maintain significant weight loss is bariatric surgery. At present, bariatric surgery is the most effective treatment modality to induce sustained weight loss and reversal of the obesity-induced changes in lipid and glucose metabolism. It reduces cardiovascular risk factors and decreases mortality rates (46; 47; 48). Bariatric procedures result in either reduced food intake (restrictive surgery) and/
or reduced food uptake (malabsorptive surgery). Roux-en-Y gastric bypass surgery (RYGB) and biliopancreatic diversion are the most effective methods in terms of sustained control of weight loss and glucose homeostasis (46). RYGB is the most commonly performed bariatric procedure, and is considered the ‘gold standard’ treatment for morbid obesity (BMI > 40 kg/m² or > 35 kg/m² with obesity-related complications) (49; 50). Current techniques involve the use of a surgical stapler to create a small and vertically oriented gastric pouch, the volume of which is usually less than 30 cm³. The pouch is completely divided by the gastric remnant and is anastomosed to the jejunum (between 30 and 75 cm from the ligament of Treitz), through a narrow gastrojejunal anastomosis in a Roux en-Y fashion (Fig. 2) Bowel continuity is restored by an entero-entero anastomosis between the excluded biliary limb and the alimentary limb. This anastomosis is usually created at 75–100 cm distal to the gastrojejunostomy, although it has also been performed at 100–250 cm in patients with BMI above 50 kg/m². RYGB usually results in 60–70% excess weight loss and most of this effect is maintained (51; 52; 53; 54). Therefore, it can be concluded that bariatric surgery for morbid obesity is associated with long-term weight loss and decreased all-cause mortality and a reduction in morbidity such as cardiovascular disease, hypertension and diabetes.

Figure 2. Schematic representation of RYGB.
The included figure is the property of Johnson and Johnson and Ethicon Endo-Surgery (Europe). Reprint with permission from: KJ Neff, T Olbers and CW le Roux. Bariatric surgery: the challenges with candidate selection, individualizing treatment and clinical outcomes. Copyright © 2013 Neff et al; licensee BioMed
Because of its major effect on weight loss and metabolism, bariatric surgery can be used as a model to study alterations that contribute to improvement in insulin sensitivity. Recently, reports have been published on amelioration of insulin sensitivity within 10 days after malabsorptive bariatric surgery. This phenomena occurred in the absence of significant weight loss (55; 56). Alterations in more traditional glucoregulatory factors, like free fatty acids (FFA) and adiponectin could not explain this early improvement. Compared to similar weight loss during a very low caloric diet (VLCD), RYGB has a greater effect on glucose metabolism which indicates that other mechanisms besides weight loss must be involved (57). One concept is that by bypassing the proximal gastrointestinal tract from the nutrient flow an increase in release of gut hormones such as GLP-1 and peptide YY (PYY) is induced (58; 59). GLP-1 has insulinotropic effects by stimulating proliferation of β-cells of the pancreas and additionally GLP-1 inhibits glucagon secretion, suppresses endogenous glucose production (EGP), slows gastric emptying and promotes satiety (60; 61). Although the effect on weight loss is not dependent on GLP-1 since GLP-1 knock out animals still show weight loss after bariatric surgery (59; 62). PYY has an anorectic action on the central nervous system (CNS) via the vagal nerve and reduces gastric emptying and inhibits pancreatic exocrine function (63). Another result of bypassing a part of the gastrointestinal (GI) tract is adaptation and restructuring of cells of the intestine (for review 64). Duodenal-jejunal bypass, leaving the stomach intact and bypassing the upper gut, in Zucker rats leads to atrophy of the bypassed gut and hyperplasia in the jejunum (65). In normal physiology the duodenum and jejunum resorb most macronutrients, whereas the ileum absorbs micronutrients. As such the proximal intestine takes up most of the dietary glucose by the sodium-D-glucose co-transporter 1 (SGLT1). SGLT1 overexpression is associated with obesity in murine models (66), leading to increased glucose transport. Furthermore, SGLT1 is also overexpressed three- to four fold in human and animal models of DM2 (67; 68). In a rodent RYGB model it was shown that intestinal glucose uptake is reduced (69), providing a possible mechanism for some of the antidiabetic effects of RYGB.

Another mechanism of bariatric surgery-induced amelioration of glucose metabolism is through changes in bile acids (BA). Recently bile acids (BA) have been recognized as hormones affecting whole body metabolism (70). Bile acids are required for the uptake of lipids from the intestine, they are produced in the liver and released in the duodenum in response to fat ingestion. Most bile acids are reabsorbed in the ileum by apical sodium-dependent bile acid transporters (ASBT). Serum BA levels are increased after RYGB in human and animal models (71; 72; 73). Two BA receptors have been identified, i.e. the farnesoid X receptor (FXR) (74) and the Takeda G-protein-coupled receptor-5 (TGR5) (75). TGR5 is considered to increase GLP-1 release thereby improving insulin secretion and insulin sensitivity (76). FXR is the main regulator of BA metabolism and is involved in lipid metabolism, energy homeostasis and insulin sensitivity (for review 77). FXR exerts its effects on BA secretion through fibroblast growth factor 19 (FGF19 in humans and FGF15 in mice) and FGF21 (78; 79). In mice FGF19 increases metabolic rate and energy expenditure in response to a high fat diet (80). Whether
BA and FGF 19 or FGF21 mediate the quick improvements in energy metabolism observed after bariatric surgery is unclear since in humans no changes in FGF19 or BA were observed in the first week after RYGB (81). Finally, malabsorptive bariatric surgery inevitable leads to an alteration in communication between the brain and the gut, thereby possibly affecting regulation of body weight and aspects of glucose metabolism (82; 83).

In short, bariatric surgery has a sustained effect on body weight loss, obesity-related morbidity and insulin sensitivity. The short-term and long-term beneficial effects of RYGB on body weight and glucose metabolism are partly elucidated and include malabsorption, reduced food intake, gastrointestinal enteroplasticity, change in gut hormone secretion, bile acids and altered communication between the gut and the brain. Moreover it is not clear whether the improvement in glucose metabolism is due to an increase in \( \beta \)-cell function, an increase in hepatic or peripheral insulin sensitivity, or a combination of these factors. In the present thesis, we aimed to investigate the short-term effect of bariatric surgery on basal glucose metabolism, insulin sensitivity, lipolysis and striatal D2/3R availability.

**Adipose tissue as an endocrine organ**

Adipose tissue is composed of pre-adipocytes, mature lipid filled adipocytes and the stromal vascular fraction, containing adipose tissue macrophages (ATM), lymphocytes and vascular endothelial cells (84; 85). The primary function of adipose tissue is to store excessive calories as triglycerides (TG) and to release free fatty acids during fasting. In the 1990’s adipose tissue was also recognized as an endocrine organ (86) because of its ability to secrete molecules acting in distant tissues. The cytokines and hormones secreted by adipose tissue are referred to as adipokines. They exert their biological functions both in a local and systemic manner, influencing many biological processes including glucose metabolism (87). The first adipose hormone to be discovered was leptin (86). Leptin is mainly produced in adipocytes, and correlates with body fat content (88). It plays an important role in regulating and controlling food intake and it is referred to as ‘the satiety hormone’. Since then, many new adipokines have been identified (89) among which the adipokine adiponectin, which is solely produced by adipose tissue, has a role in improving insulin sensitivity. In the contrary to leptin, adiponectin levels are inversely correlated with adipose tissue mass (90).

More recently retinol-binding protein 4 (RBP\(_4\)) has been added to the list of adipokines. RBP\(_4\) is a transport protein for vitamin A and its main function is delivering retinol to tissues. It is synthesized mainly by hepatocytes and adipose tissue (91). Recent studies revealed that RBP\(_4\) is increased in obesity and that overexpression of RBP\(_4\) induces insulin resistance (Graham 2006 NEJM). The precise role of RBP\(_4\) in regulating peripheral and hepatic insulin sensitivity in obese human subjects remains to be elucidated (92).

Besides adipokines, cytokines originating from adipose tissue have been shown to be associated with insulin sensitivity. A link between adipose tissue inflammation, insulin resistance and obesity was made a decade ago (84; 93) while tumor necrosis factor alpha (TNF-\(\alpha\)) was recognized as one of the first pro inflammatory cytokines secreted by
adipose tissue (94). Hereafter other cytokines were identified including interleukin-6 (IL-6), monochemoattractant protein-1 (MCP-1), IL10, serum amyloid A (SAA), interleukin-1β (IL-1β) and others (86; 95; 96; 97; 98; 99).

Pro-inflammatory profiles of adipose tissue, high serum leptin and RBP4 and lower adiponectin all have been linked to insulin resistance and most studies show that adipose tissue function is important in maintaining glucose homeostasis. Disrupted secretion or function of adipocytes hampers whole body insulin sensitivity. Whether tissue specific inflammatory phenotypes independently contribute to insulin resistance in obesity in humans is still not known.

This thesis describes studies on mRNA expression levels of inflammatory proteins and RBP4 in insulin sensitive tissue in relation to basal glucose metabolism, insulin sensitivity and lipolysis in morbidly obese women undergoing bariatric surgery.

**Inflammation in obesity**

Obesity is associated with a state of chronic low grade inflammation with higher circulating C-reactive protein (CRP) (100). In the early nineties Hotamisligil et al reported TNF-α to be locally produced in adipose tissue in states of obesity and interfere with insulin sensitivity (94). Since then it has been shown in numerous studies in animals and humans that adipose tissue in obesity is characterized by an influx of bone marrow-derived immune cells including macrophages resulting in a shift in balance between anti-inflammatory and pro-inflammatory cytokines favoring the pro-inflammatory state (84; 101; 102). In the lean condition, adipose tissue resident macrophages (ATM) are alternatively activated, referred to as an M2 state, with predominant production of anti-inflammatory proteins aimed for tissue repair, extracellular matrix modeling and adipogenesis (103).

When obesity develops, adipose tissue expands due to hypertrophy of adipocytes. When the adipocyte reaches a critical cell size it starts to secrete growth factors to induce pre-adipocyte proliferation (104), leading to hyperplasia. The efficiency and capacity of adipocytes to store lipids within the lipid droplets are controls by lipid droplet proteins like CIDEA (105) perilipin and adipophilin (also known as adipocyte differentiation relate protein, ADRP) (106). It has been shown that macrophage content of adipose tissue is positively related to adipocyte size with macrophages organized in crown like structures surrounding adipocytes (84). Whether cell size is the triggering factor in the induction of inflammation is still matter of debate but blocking hormone sensitive lipase in adipose tissue resulting in large lipid-loaded adipocytes also triggers an inflammatory state (107) The overall hypothesis is that when adipose tissue becomes dysfunctional in terms of balancing lipid storage and lipid breakdown due to the inflammatory state, fatty acids are released from adipose tissue and stored in non-adipose tissue like muscle and liver. This so called ectopic lipid accumulation contributes to whole body insulin resistance.

There are several pathways that contribute to the inflammatory state of adipose tissue in obesity. One pathway involves tissue hypoxia because of inadequate absence of increased
vascularization and vascular endothelial growth factor (VEGF) (108) despite an increase in adipose mass. This leads to a cascade of events including induction of hypoxia induce factor-1 (HIF-1) (109) recruitment of numerous immune cells and production of pro-inflammatory proteins (110). The pro-inflammatory cytokines negatively impact the insulin signaling pathway either directly or indirectly by stimulating inflammatory pathways (111) and reduce insulin-mediated suppression of lipolysis (112) (figure 3). However, despite tissue hypoxia, adipocyte cell death is increased some (107) but not all studies in obese humans (113). Besides, ATM can cause tissue damage and inhibit cell proliferation (114). Other pathways involved in triggering an inflammatory state in adipose tissue in obesity include among others ER-stress, adipocyte necrosis, altered adipokine secretion, upregulation of MCP-1 and abnormal extracellular matrix remodeling leading to fibrosis of adipose tissue (for review: 115).

Besides recruited and resident macrophages, other immune cells are invading adipose tissue in obesity. Mast cells and natural killer T cells (NKT) within adipose tissue contribute to IR in diet-induced obesity (116; 117) and recent data showed an increase in recruited B and T lymphocytes into adipose tissue in obesity (figure 3) (118). Regulatory T cells (Tregs), especially the CD4+ population, serve to suppress the immune response of other inflammatory cells in order to protect the human body from excessive innate immune reactions. Therefore it is not surprising that Tregs are increased in adipose tissue in obese conditions (118). Tregs positive for CD4, FOXP3 and CD25 cells represent activated T cells and secrete the anti-inflammatory cytokine IL10. IL10 inhibits TNF-α production by macrophages and surprisingly it has been shown that in obese visceral adipose tissue the amount of Tregs is diminished (118; 119; 120) therefore contributing to an ongoing pro-inflammatory state. Additionally, high levels of insulin inhibit IL10 production by Tregs (121), suggesting that the hyperinsulinemic state occurring in obesity might further contribute to the development of the state of low-grade inflammation.

Finally it has been shown that modulation of inflammation in rodents and weight loss in humans reduces adipose tissue inflammation and increases insulin sensitivity (122). Inflammatory changes within adipose tissue occur both in visceral and subcutaneous compartments but most mechanistic studies in rodents focus on visceral fat. It has been shown that increased visceral fat increases the risk for insulin resistance and comparing subjects matched for BMI, those with higher percentage of visceral adipose tissue (VAT) are more insulin resistant compared to subjects with a higher percentage of subcutaneous adipose tissue (SAT) (123). VAT shows a higher expression of MCP-1, which is produced by macrophages and endothelial cells, and recruits monocytes, leukocytes and other inflammatory cells (for review 124). In addition, the macrophage markers CD68 and CD14 show greater expression in VAT compared to SAT (125). It is of interest studying whether inflammatory changes within VAT are more pronounced compared to subcutaneous adipose tissue and whether that predicts IR in humans. On the other hand one could hypothesize that as long as subcutaneous adipose tissue is able to sequester the caloric surplus adequately,
insulin sensitivity is preserved and the occurrence of inflammation in adipose tissue offsets IR. In this thesis we therefore study inflammatory changes within both adipose tissue compartments and relate these to insulin sensitive metabolic fluxes. In summary, caloric excess is stored as triglycerides within adipocytes that become enlarged. Long term obesity is associated with influx of immune cells into adipose tissue, creating a pro-inflammatory environment and a low grade inflammatory state. Inflamed adipose tissue is associated with adipose tissue dysfunction and insulin resistance. Ongoing lipolysis leads to ectopic lipid accumulation resulting in further reduction of insulin sensitivity. Most mechanistic studies on this topic are performed in rodent models of obesity and it remains to be clarified if and to what extent inflammation in different adipose tissue compartments contribute to insulin sensitivity in obese humans. Therefore in this thesis we aimed to study inflammatory expression profiles in omental and subcutaneous adipose tissue compartments and to correlate these findings with basal glucose metabolism as well as whole body, liver and adipose tissue insulin sensitivity.

Liver steatosis, hepatic insulin sensitivity and inflammation in obesity

The liver plays an essential role in glucose and lipid metabolism and disrupted liver function in the setting of obesity leads to hyperglycemia and dyslipidemia. Obesity is associated with increased storage of liver fat, i.e. liver steatosis defined as a triglyceride storage of > 5.7% (126). Liver steatosis increases the risk for nonalcoholic fatty liver disease (NAFLD),
nonalcoholic steatohepatitis (NASH) and cirrhosis, which can lead to liver failure and hepatocellular carcinoma (127). An non-invasive method to quantify the lipid content of the liver is magnetic resonance spectroscopy (1H-MRS) (126). Lipid accumulation in the liver can be higher due to an increased uptake of FA from dietary fat, increased FA released from adipose tissue and from de novo lipogenesis or reduced FA oxidation (128; 129). In addition, adiponectin, an insulin sensitizing adipokine, is lower in subjects with NAFLD compared to BMI matched controls (130; 131) and replenishment of adiponectin in obese mice reverses insulin resistance and alleviates NAFLD (132). In animal models short term high fat feeding leads to NAFLD and hepatic insulin resistance without peripheral insulin resistance (133). Excessive TG storage in the liver is associated with insulin resistance in some but not all studies (134). A major role for diacylglycerol with subsequent activation of protein kinase C (PKC) has been described underlying the association between liver steatosis and insulin resistance. (135; 136). And recently heme oxygenase-1 has been proposed as a pro-inflammatory signal linking inflammation to insulin resistance in mice on a high fat diet (137). Moreover lipid-induced endoplasmic reticulum (ER) stress has been associated with insulin resistance in mice (138). In parallel with inflammatory changes occurring in adipose tissue, obesity is associated with inflammation in liver in rodents. In vivo models in mice showed that chronic systemic inflammation leads to increased lipid accumulation in liver (139; 140). Whether liver steatosis per se contributes to liver inflammation or vice versa remains a matter of debate. So far it has been shown that inflammatory changes in NAFLD have been attributed to a reduction in peroxisome proliferator-activated receptor α (PPARα) expression, lower circulating adiponectin, exposure to cytokines derived from adipose tissue as well as lipotoxicity induced by fatty acid overload (141; 142). Finally, Kupffer cells (the resident macrophages of the liver) decrease in rodents on a high fat diet while recruited myeloid cells invade the liver through a mechanism involving the C-C chemokine receptor type 2 (CCR2). Subsequently the invaded immune cells trigger hepatic fat accumulation which is dependent on chemokine (C-C motif) ligand 2 (CCL2)/CCR2 (15). In support, short term infusion of oleate increases hepatic recruitment of myeloid cells, suggesting that increased portal delivery of FFA both induces inflammation and triglyceride (TG) accumulation through induction of chemotaxis (143). Finally, increased levels of TNFα induce upregulation of the nuclear transcription factor SREBP-1 and the enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) and increase de novo lipogenesis (139; 141).

To study the relation between liver inflammation, hepatic fat content and hepatic insulin sensitivity in humans, we assessed glucose metabolism, liver fat and expression profiles of inflammatory markers in liver biopsies from morbidly obese women undergoing bariatric surgery and describe the outcome of these studies in this thesis.
Aims and outline of this thesis

The main aims of this thesis were to study:

1. Striatal dopamine receptor availability in obese women and controls as well as the reversibility of lower striatal dopamine receptor availability after short and long term weight loss.

2. The role of inflammation in insulin resistance.

Part 1 of the thesis describes three studies on striatal dopamine D₂/D₃ receptor availability. In chapter two we compared striatal dopamine D₂/D₃ receptor availability between obese and lean women. In chapter three we studied changes in striatal dopamine D₂/D₃ receptor availability in obese women before and shortly after Roux-en-Y gastric bypass surgery, and in chapter four we studied long-term changes in striatal dopamine D₂/D₃ receptor availability, i.e. more than three years after bariatric surgery in the same cohort.

Part 2 of this thesis describes four studies on inflammation in adipose tissue and liver of obese women and its relation to insulin sensitivity. In chapter five we focused on short-term effects of RYGB surgery on hepatic, peripheral and adipose tissue insulin sensitivity. In chapter six we studied the expression of pro- and anti-inflammatory markers in adipose tissue in relation to metabolic fluxes. In chapter seven we describe the results of the relation
between the adipokine retinol binding protein 4 (RBP4) in adipose tissue and liver and insulin sensitivity in obese women. Finally, in chapter eight we studied the relationship between inflammatory changes in liver and hepatic insulin sensitivity as well as liver fat content.

References


Chapter 1
GENERAL INTRODUCTION


