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

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Summary and General Discussion
CHAPTER 9

Summary
This thesis addresses three topics in obesity research: a. alterations in the obese brain and b. insulin resistance and inflammation. The studies described in this thesis are performed in humans with the major aim to translate findings from rodents and explore new pathways that might be targeted for future medical treatment.

PART I Obesity and dopaminergic signaling in the brain
The brain has gained interest in human obesity and metabolism research because of its role as master regulator of energy metabolism. Previous studies have shown lower striatal dopamine receptor availability (D<sub>2/3</sub>R) in obese subjects compared to lean controls. It is unknown whether this is a cause or consequence of obesity. We first reproduced these findings (chapter 2) and next we studied whether striatal dopamine receptor availability (D<sub>2/3</sub>R) changes after bariatric surgery induced weight loss. To this end, we determined striatal D<sub>2/3</sub>R availability in the brain using ([123 I]IBZM SPECT two weeks before and six weeks after Roux-en-Y gastric bypass (RYGB) surgery in morbidly obese women. We found no increase in striatal D<sub>2/3</sub>R availability despite clinically significant weight loss, suggesting that a short-term hypocaloric period, i.e. a negative energy balance per se, does not induce major alterations in striatal dopaminergic neurotransmission. Moreover, striatal D<sub>2/3</sub>R availability did not correlate with measures of insulin sensitivity (chapter 3). To determine the effects of long-term weight loss on striatal D<sub>2/3</sub>R binding we repeated ([123 I]IBZM SPECT to assess D<sub>2/3</sub>R availability at least 2 years after RYGB and the first ([123 I]IBZM SPECT. We also assessed metabolic parameters and eating behavior. We found an increase, but not normalization, in D<sub>2/3</sub>R availability compared with the first assessment, indicating that reduced striatal D<sub>2/3</sub>R availability in obesity is reversible to some extent, suggesting that it is merely a cause of obesity. To our knowledge we are the first to show intra-individual changes in striatal D<sub>2/3</sub>R availability after long-term surgery induced weight loss. Interestingly, the improvements in insulin sensitivity and food craving after RYGB did not correlate with changes in striatal D<sub>2/3</sub>R availability, challenging an important role for dopamine receptor availability (D<sub>2/3</sub>R) in these RYGB induced effects (chapter 4).

PART II Obesity, metabolism and inflammation
To further unravel the association between obesity and glucose metabolism, we studied metabolic parameters in morbidly obese women before and two weeks after bariatric surgery (chapter 5). We assessed insulin sensitivity during a two-step hyperinsulinemic euglycemic clamp with a stable glucose isotope tracer. We found a decrease in endogenous glucose production (EGP), lower plasma insulin and glucose levels and increased free fatty acids (FFA) two weeks after bariatric surgery. There was no effect on peripheral or hepatic insulin sensitivity. Thus, the early beneficial metabolic effects reported in morbidly obese adults after RYGB surgery do not include increased insulin sensitivity. Lower EGP shortly after
RYGB probably is a physiological adaptation to the semi-starvation postoperative period and does not reflect improved insulin action. Most studies use HOMA-IR as a surrogate index of insulin sensitivity and the reduced postoperative insulin and glucose levels inevitably led to a lower HOMA-IR. However, using a hyperinsulinemic euglycemic clamp, which is considered the gold standard for determining insulin sensitivity, we did not detect any improvement in insulin sensitivity, suggesting that HOMA-IR does not reflect true insulin sensitivity in this setting. The lack of effect on insulin sensitivity may be explained by higher plasma levels of FFA, which are known to impair insulin signaling. Since tapering down of insulin treatment shortly after RYGB has been reported repeatedly, other factor besides insulin sensitivity must account for that effect. Gut related factors, like gut peptides and the gut-brain axis are likely candidates. Indeed it has been shown that meal-induced elevations in GLP-1 are increased shortly after RYGB.

Next we focused on the role of inflammation in adipose tissue on insulin action in skeletal muscle and adipose tissue, measured as insulin-mediated glucose uptake and suppression of lipolysis respectively. In chapter 6 we show extensive inflammatory changes in adipose tissue in insulin resistant obese women compared to healthy lean controls. Insulin resistance was confirmed by low peripheral insulin sensitivity ($R_d$), high basal insulin levels, low QUICKI and high HOMA-IR, and additionally by low GLUT 4 mRNA expression in adipose tissue. The latter correlated positively with $R_d$. A state of low grade systemic inflammation was shown by an increase of C-reactive protein (CRP) and adipose tissue was characterized by an increased influx of macrophages and T cells. Subcutaneous adipose tissue (SAT) displayed a predominant pro-inflammatory phenotype, whereas visceral adipose tissue (VAT) showed higher expression levels of anti-inflammatory markers. Finally, we show that the influx of activated T cells was higher in visceral fat, which might account for the higher expression of anti-inflammatory markers since T cells dampen local inflammation. We did not find any correlations between adipose tissue inflammatory markers and insulin sensitivity of adipose tissue or muscle. In conclusion, inflammation in adipose tissue of insulin resistant, morbidly obese women is characterized by increased influx of macrophages and activated T cells, but this is not associated with insulin action. Further long-term follow up studies are needed to elucidate the role of inflammation in insulin resistance in humans.

We then focused on the recently identified adipokine retinol-binding protein 4 (RBP4). RBP4 is a transport protein, mainly synthesized by hepatocytes and adipose tissue, and its main function is delivering retinol to tissues. Recent studies revealed that RBP4 is increased in obesity and that overexpression of RBP4 induces insulin resistance. In chapter 7 we show that in morbidly obese women, besides increased expression of RBP4 in adipose tissue, liver tissue is characterized by increased RBP4. While tissue levels did not correlate with insulin sensitivity, serum RBP4 inversely correlated with hepatic, adipose tissue and skeletal muscle insulin sensitivity. These results suggest that RBP4 acts as a circulating hormone on insulin sensitive metabolic pathways. Lowering serum RBP4 might be an attractive treatment strategy in reducing insulin resistance.
Finally, in chapter 8 we studied whether hepatic inflammation and accumulation of hepatic fat contribute to hepatic insulin resistance in morbidly obese subjects. Intrahepatic triglycerides (IHTG) were assessed using $^1$H-MR Spectroscopy ($^1$H-MRS) and liver biopsies were scored for steatosis and criteria for non-alcoholic fatty liver disease (NAFLD). Gene expression of pro- and anti-inflammatory markers and CD68 (macrophage marker) in liver tissue were markedly increased in obese subjects compared to lean controls. In the obese group with liver steatosis IL10, an anti-inflammatory cytokine, showed an inverse correlation with IHTG. Thus, IL10 may play a role in the interaction between liver steatosis and inflammation. This is in line with studies showing that influx of immune cells in mice on a high fat diet worsen hepatic steatosis. Interestingly, we found no correlation between IHTG, inflammation and hepatic or peripheral insulin resistance. In conclusion, morbid obesity is associated with hepatic inflammation independently of liver fat content and insulin sensitivity. Further studies are needed to determine the pathogenesis of hepatic inflammation in obesity and to explore the role of specific inflammatory pathways in hepatic glucose and lipid metabolism.

**General Discussion**

The obesity epidemic poses a threat for individuals and society because of its devastating consequences for our health in general. Treatment of obesity is difficult and many diet intervention studies showed regain of the lost weight after longer term follow up. Maintenance of health despite the presence of obesity might be a more realistic goal and therefore it is important to study determinants of metabolic health by dissecting the multiple pathways that are involved in development of the metabolic syndrome. Despite the overall modest effects of hypocaloric diets on long-term weight loss, the ultimate goal in the treatment and prevention of obesity would be to achieve a state of chronically reduced food intake. This could be accomplished in the future by targeting brain areas involved in body weight regulation since accumulating evidence in rodents and humans shows that many brain circuits involved in food intake and energy expenditure are altered in the obese state. Since many studies on causes and consequences of obesity are performed in rodents, we aimed to perform translational studies and focused on central (striatal dopaminergic system) and peripheral (inflammation, insulin sensitivity) changes in the obese state.

**PART I Obesity and the brain**

Previous studies showed that striatal dopamine receptor 2 and 3 ($D_{2/3}R$) availability is lower in obese humans (1, 2) and although we reproduced these findings (chapter 2) and showed that striatal $D_{2/3}R$ availability is lower in obese women compared to lean controls, $D_{2/3}R$ availability did not correlate with BMI per se. This means that excessive adipose tissue itself is not directly related to lower striatal $D_{2/3}R$. Other direct factors that might be involved are (excessive use of) nutrients and eating habits besides genetically determined expression or functionality of striatal dopamine receptors. In other words it is currently unknown whether obesity induces lowering of striatal $D_{2/3}R$ or whether lower striatal dopamine receptors...
predispose to obesity in an obesogenic environment. As discussed in chapter 3, it has been hypothesized that overeating in subjects susceptible to obesity constitutes a compensatory response to make up for decreased dopaminergic signaling in the reward circuitry caused by reduced expression of dopamine receptors due to genetic factors (3, 4). In contrast, downregulation of striatal D2/3 R occurring after the onset of obesity in animal studies suggests changes in the striatal dopaminergic system to be a consequence of a persistent increase in palatable food consumption, positive energy balance and/or fat mass (5, 6). To study whether lower striatal D2/3 R availability in obesity is reversible and thus a consequence of obesity, we studied striatal D2/3 R availability before, 6 weeks and >2 years after surgery-induced weight loss. While D2/3 R availability does not change 6 weeks after RYGB surgery despite clinically significant weight loss (chapter 3), we demonstrated that striatal D2/3 R availability increases >2 years after RYGB surgery (chapter 4). Surprisingly, the change in striatal D2/3 R availability did not correlate with the change in body weight, body fat mass or BMI, again pointing to a body weight independent relation. It remains to be established if peripheral factors (and if so, which ones) modulate striatal D2/3 R availability. Furthermore, striatal D2/3 R availability did not normalize, i.e. increase to the levels of lean controls, which might be explained by the fact, that despite massive weight loss, BMI did not return to normal, i.e. < 25 kg/m2. Also at present it is unknown whether an increase in striatal D2/3 R availability represents an increase in dopamine receptors or a decrease in synaptic striatal dopamine in humans. Studying food cues or amphetamine induced dopamine release before and after weight loss could shed light on these questions. Moreover, it would be informative to repeat the SPECT study once the participants reach a BMI < 25 kg/m2. In addition, it would be of interest to study whether diet-induced weight loss or different types of diets as well as changes in eating patterns would differentially affect striatal D2/3 R availability. Finally, studying the changes in striatal D2/3 R availability upon nutrient excess and weight gain would clarify the change over time and its relation to either specific nutrients or a critical increase in body weight. Further studies in rodents and humans are needed to elucidate whether and how striatal dopamine and striatal D2/3 R receptors contribute to the obese state.

Body weight is determined by energy intake and energy expenditure. Energy, or food, intake is a result of a complex interplay between hedonic and homeostatic brain circuits. When obese subjects consume food or imagine consuming food, they show less striatal activation compared to lean individuals (4, 7). This might be explained by hypo-responsiveness of the reward (hedonic) circuitry. This is in line with the lower striatal D2/3 R availability observed in obese versus lean individuals and rats (chapter 2, 1, 6, 8) and lower basal dopamine levels in obesity-prone rats (9). Upon surgery-induced weight loss, our study participants showed less craving for food and disinhibition but these changes were not related to changes in D2/3 R availability, nor to the amount of body weight loss (chapter 4). Because we did not study food cue induced striatal dopamine release, we are unable to address the question whether reduced craving and disinhibition after weight loss are related to dynamic changes in striatal dopamine release. Correlating specific aspects of eating behavior, eating patterns
and food preferences in relation to the striatal dopaminergic system in addition to studying the changes occurring during weight gain and weight loss might unravel the role of striatal dopamine/receptors in food intake and thus body weight regulation. Although lower striatal D_{2/3}R availability is a consistent finding in obesity, it is still unknown whether this represents a hypodopaminergic or hyperdopaminergic state. While children at high risk for obesity show increased responsiveness to consumption of palatable food (10) obese (adult) individuals show low responsivity (4). The propagated reward deficiency might therefore be a consequence of overconsumption/obesity itself rather than a cause of obesity. This is in line with the contradictory results on the relation between a dopamine receptor polymorphism leading to lower receptor expression and BMI (11) and with rodent studies showing that eating high fat diets or diets with an increased fat/carbohydrate ratio decreases striatal D_{2/3}R availability not explained by obesity (6, 12, 13). However it was recently shown that lentivirus-mediated knockdown of striatal D_{2/3}R rapidly accelerated the development of addiction-like reward deficits and the onset of compulsive-like food seeking in rats (14). Therefore more studies in rodents and humans showing that striatal dopaminergic signaling or receptors contribute significantly to body weight regulation are needed, before designing novel drugs, or other therapies, targeting the striatal dopaminergic system.

In conclusion, the striatal dopaminergic system is altered in obese rodents and humans with lower receptor availability and less striatal activity. Although these changes are partially reversible after weight loss, it remains to be determined whether these alterations are a cause or a consequence of obesity and whether they represent a hyper- or hypodopaminergic state. Further insight is needed before development of treatments targeted at striatal dopaminergic signaling in obese humans.

**PART II Obesity, metabolism and inflammation**

a. Bariatric surgery and insulin resistance

A better understanding of the pathogenesis of obesity-induced insulin resistance is a vital step in developing new paradigms for a) maintenance of metabolic health, specifically insulin sensitivity and b) treatment of insulin resistance and diabetes mellitus type 2 (T2DM). Bariatric surgery, in particular Roux-en-Y- gastric bypass (RYGB) surgery has proven to be the most effective treatment for obesity, leading to sustained weight loss and improved glucose metabolism. RYGB has rapid clinical effects but whether these are related to the bariatric intervention itself, or to the semi-starvation period after surgery remains matter of debate. An improvement in insulin sensitivity would suggest that creating a small gastric pouch, bypassing the first part of the proximal gut has body weight loss-independent effects on glucose metabolism while a reduction in basal glucose metabolism only would imply an effect of food restriction because it has been shown that starvation reduces glucose output (15). Dissecting these effects is of importance because if the former is true, more research should be directed towards gut factors or the gut-brain axis in modulation of glucose metabolism. This could lead to new treatments in the battle against obesity-induced insulin
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resistance and T2DM. We found that in the first two weeks following RYGB, basal endogenous glucose production, as well as fasting insulin and glucose levels decrease, without a significant effect on peripheral or hepatic insulin sensitivity (Chapter 5). This suggests that the beneficial rapid metabolic effect of RYGB is merely an adaptation to fasting. The observed higher FFA levels in our subjects support this since fasting strongly induces lipolysis and our results are in line with an earlier report (16). Increased FFA might explain the lack of improvement in insulin sensitivity in our study since FFA are known to impair insulin signaling (17). Although we show, using golden standard techniques, that there is no improvement in insulin sensitivity, clinical studies reported on rapid tapering down of insulin requirements and lower postprandial blood glucose. Therefore other mechanisms, besides pure effects on insulin sensitivity, must play a role. RYGB comprises both a restriction and a malabsorption component. The restrictive component follows from the creation of a small gastric pouch, resulting in reduction of caloric intake while malabsorption is caused by bypassing the duodenum and the proximal jejunum. Although this has been the classical view for years, it has been shown recently that malabsorption could not account for the effects on weight loss and other factors should be considered to be responsible for the decline in body weight (for review 18). Therefore the question arises whether restriction, i.e. reduced food intake, alone could explain the major beneficial effects of RYGB. Several studies in patients with T2DM and/or obesity reported a rapid decrease in insulin and glucose levels after a calorie restricted diet (19, 20). However, Foo et al showed that with comparable weight loss RYGB has a more pronounced effect on glucose metabolism than a very low calorie diet alone (21). Alternatively, reduced postoperative levels of the stomach derived hunger hormone ghrelin might contribute to the reduction in food intake and therefore account for the effects on glucose metabolism as well, but ghrelin levels in the longer term tend to increase despite sustained weight loss (22). This indicates that the additional effect of RYGB surgery is related to the second aspect of the procedure, which is rerouting of food directly from the small gastric pouch into the jejunum bypassing the duodenum and the first part of the jejunum (150 cm). Several mechanisms have been proposed to contribute independently to the metabolic improvements after bariatric surgery, including a) an increase in gastrointestinal hormones like GLP-1 leading to an enhanced β-cell responsivity and insulin release, b) remodeling of the cell structure of the intestine, c) changes in bile acid metabolism accompanied by a change in the gut microbiome, d) altered communication between the gut and the brain and finally in the longer term e) the decrease in fat mass leading to a reduction in low grade inflammation. The latter requires clinically significant weight loss and is unlikely to be a specific effect of the bariatric intervention itself as it can also be achieved by low calorie diets. Our data as well as reports in the literature indicate that the relation between improved insulin sensitivity and RYGB is less straightforward than previously suggested. In conclusion, we primarily aimed to explore whether the reported early improvements in glucose metabolism are explained by an increase in insulin action and conclude that in the short term, i.e. 2 weeks after RYGB, insulin-mediated suppression of endogenous glucose
production, representing hepatic insulin sensitivity and insulin-mediated glucose uptake, indicative of skeletal muscle insulin sensitivity, are not increased while basal endogenous glucose production, glucose and insulin are reduced. The latter probably represents the metabolic adaptation to the semi-starvation postoperative period. Lower postprandial glucose and insulin requirements in T2D patients in the early postoperative phase can be explained by higher insulin secretion either induced by higher nutrient-induced GLP-1 secretion or other gut or gut-brain axis related factors. This requires further studies in humans using study designs including a very low calorie diet control group.

b. Obesity and inflammation

Obesity is associated with systemic low grade inflammation and invasion of various immune cells into adipose tissue has been described (23, 24, 25). Many studies in rodents show that inflammation in adipose tissue affects adipose function and insulin sensitivity resulting in insulin resistance/glucose intolerance. During the development of obesity the number of macrophages in adipose tissue can increase up to four fold (26, 27). Chemotaxis induced by MCP-1 secreted from enlarged adipocytes attracts circulating monocytes that differentiate into macrophages within adipose tissue (28, 29). Depending on the local environment and only partly understood, adipose tissue macrophages (ATM) can be classically (M1) or alternatively (M2) activated and produce either predominantly pro- or anti-inflammatory cytokines, respectively. Rodent and human studies found that in the lean state, most macrophages show a M2 phenotype but when adipose tissue expands they switch towards a pro-inflammatory profile (30, 31, 32). Rodent studies show that increased lipolysis induced by the inflammatory state as well as release of cytokines into the systemic circulation both can hamper insulin action resulting in insulin resistance or T2DM. Conversely, reducing or preventing inflammation restores insulin sensitivity in different rodent models (33, 34). Although human studies confirm the presence of inflammation in adipose tissue in obesity, less is known on the effects of these inflammatory changes on insulin sensitivity in humans. We show extensive pro- and anti-inflammatory changes in adipose tissue of morbidly obese subjects compared to lean controls (chapter 6) but surprisingly no direct correlation was demonstrated between any of these markers and insulin action in adipose tissue, liver and skeletal muscle. Notably, different inflammatory profiles were present between subcutaneous (SAT) and visceral adipose tissue (VAT) compartments with subcutaneous adipose tissue showing a more pro-inflammatory state. This is in line with an earlier published paper (35). Since VAT is associated with insulin resistance (for review 36) we hypothesized that pro-inflammatory changes in that compartment might contribute to insulin resistance independently of the inflammatory state in SAT and as long as the overall VAT phenotype is shifted towards a more anti-inflammatory pattern, no clinically significant effect on insulin sensitivity might be expected. Our study showed no correlation between inflammation in either SAT and VAT and insulin action in adipose tissue but since this was measured on the whole body level we cannot exclude that inflammation in VAT affected local
lipolysis specifically in that compartment. Study designs including the use of micro dialysis, metabolic tracers, AV differences and portal blood analyses could shed more light on these questions.

Finally, activation of macrophages in adipose tissue attracts circulating T cells to dampen the inflammatory response (37). Regulatory T cells (Tregs), which are of an immunosuppressive nature are reduced in adiposity (37, 38). Tregs are CD4+ cells that express CD25+ and FOXP3, a forkhead transcription factor required for their specific development and function (CD4+CD25+FOXp3 regulatory T cells) (39). Tregs secrete the anti-inflammatory cytokine IL10, which inhibits TNF-α production by macrophages, preventing local tissue damage and counteracting inflammation. We show that T-cell markers are increased in both adipose tissue compartments in the obese subjects compared to the lean controls. Higher expression of CD4 suggests increased influx of T cells in general and higher expression of CD25 shows increased levels of activated T-cells. FOXP3, a marker of regulatory T cells, was not significantly different between lean and obese or within compartments but it has been shown in rodents and humans that they play an important role in counteracting the pro-inflammatory macrophages (40). Furthermore, the higher expression levels of activated T cells in VAT might be an explanation for the predominantly anti-inflammatory environment in that compartment. Studies using FACS analysis are needed to further characterize the subpopulations of T-cells and future rodent studies should be aimed at studying their role in obesity-induced inflammation and insulin sensitivity by modulating expression of different T-cell populations. In humans, additional longer term follow up studies are needed to get insight in T-cell dynamics during the development of obesity and insulin resistance. Also, it remains to be studied whether weight loss reduces T-cell influx and activation. If regulatory T cells play an important role in maintaining adipose tissue homeostasis by counterbalancing the pro-inflammatory response induced by obesity, treatment strategies aimed at increasing Tregs might be a promising option in the treatment of obesity-associated metabolic disturbances.

Besides low grade inflammation and reduced insulin action in adipose tissue and skeletal muscle, obesity is also associated with non-alcoholic fatty liver disease (NAFLD) and hepatic insulin resistance. Hepatic steatosis occurs in states of increased de novo lipogenesis, reduced fatty acid oxidation or a combination of both, but its contribution to obesity-associated hepatic insulin resistance is uncertain. In our cohort, no correlation between IHTG and hepatic and peripheral insulin sensitivity was present (chapter 8) and we recently confirmed this lack of a correlation between IHTG and insulin resistance in a large obese cohort (Ter Horst et al, unpublished data) which is in line with others (41). In addition, we showed earlier that severe hepatic steatosis in subjects with a genetic lipid disorder was not associated with peripheral or hepatic insulin resistance (42). In contrast, a number of other studies in humans and rodents have reported that increased IHTG is associated with hepatic and adipose tissue insulin resistance (43, 44, 45) but whether these subjects had uncomplicated NAFLD
or progressed to non-alcoholic steatohepatitis is unknown. In addition, several studies in humans and animal models of NAFLD have revealed that increases in hepatic diacylglycerol (DAG) leads to activation of protein kinase C (PKC), which in turn attenuates insulin signalling (46), and this mechanism is thought to be a major determinant of hepatic insulin resistance. We did not measure PKC and DAG in our tissue samples and therefore we cannot rule out an effect of steatosis on these parameters but since the obese subjects did not show a correlation between IHTG and insulin resistance, this is less likely. Conflicting results on correlations between IHTG and insulin sensitivity may further be due to the various methods used to measure insulin sensitivity. We used the two-step euglycemic hyperinsulinemic clamp and a stable glucose isotope tracer, which is the gold standard for measuring insulin sensitivity, but not all investigators use this method.

The role of hepatic inflammation in hepatic insulin sensitivity in humans remains unclear. Inflammatory changes associated with hepatic steatosis have been described (47) but it is unclear what the mutual underlying mechanism is. Human data are limited and we here show that gene expression of pro- and anti-inflammatory markers in liver tissue of morbidly obese women are markedly increased compared to matched lean controls. However, inflammation of liver tissue did not correlate with intrahepatic triglyceride (IHTG) accumulation or insulin sensitivity (chapter 8). Only one person in our cohort showed signs of NASH, which might have affected our results since it has been shown that steatosis and inflammation are related in the presence of NASH (48). However only a smaller percentage of subjects with NAFLD progress to NASH meaning that other mechanisms besides liver fat must contribute to hepatic inflammation in the setting of obesity. Excessive (dietary) fatty acids trigger an inflammatory response leading to lipotoxicity, ER stress and reactive oxygen species (ROS) formation (49), and fructose and dietary derived lipids might provoke a hepatic stress response by activating the NFkB pathway (50), in turn leading to the production of pro-inflammatory cytokines by activated Kupffer cells (51). Obstfeld et al (47) showed that systemic immune cells invade the liver in mice on a high fat diet and that they promote hepatic steatosis in a chemokine receptor 2 dependent manner. Finally, we did find that hepatic expression of IL-10, an anti-inflammatory cytokine, inversely correlates with hepatic steatosis and recently it has been shown that IL10 secreted by M2 activated Kupffer cells selectively promotes apoptosis of pro-inflammatory M1 polarized Kupffer cells (52). This shows a complex interplay between diet, inflammation and liver fat. More detailed studies on the role of specific cytokines and immune cells involved in hepatic steatosis (or vice versa) and hepatic insulin resistance in rodents and humans are needed to develop treatment strategies aimed at reducing steatosis, inflammation or both.

Furthermore, studies in rodents consistently show that hepatic steatosis and hepatic insulin resistance can be induced before the onset of obesity in response to a high fat diet (HFD) (53). We did not have information on the dietary fat or carbohydrate/fructose content in our subjects neither on meal frequency, which are known contributors to hepatic steatosis in a hypercaloric setting. We recently showed that hypercaloric diets (high-fat-high-sugar; HFHS)
with increased meal frequency increases both IHTG and abdominal fat in lean men, whereas similar diets with increased meal size do not (54), suggesting that snacking independently contributes to IHTG accumulation. In addition fructose intake in excess of caloric need has been shown to trigger hepatic steatosis through an increase in de novo lipogenesis (for review 55). Taken together, both meal composition and meal frequency can contribute independently to the development of hepatic steatosis and inflammation. The range in IHTG was broad and did not correlate with BMI in the obese subjects. This might be related to genetic factors, as several genes have been described in rodent models and in humans that predispose for the development of NAFLD (56, 57, 58, 59).

In conclusion, our results show that morbidly obese women are characterized by an inflamed liver independent of liver fat and hepatic insulin resistance. This suggests that hepatic inflammation per se does not trigger hepatic insulin resistance in obesity. Studies on changes in inflammatory phenotype during the course of obesity with emphasis on different immune cells are needed to explore whether, how and when inflammation affects hepatic glucose metabolism. In addition, no correlation between liver fat and either inflammatory markers or hepatic insulin sensitivity was found. More detailed studies in rodents and humans are needed to clarify the interaction between disturbed hepatic lipid and glucose metabolism in obesity and inflammation.

**Clinical implications and further studies**

Current obesity management strategies include lifestyle changes to induce weight loss. Unfortunately this strategy shows a high failure rate with weight regain in the long term. Bariatric surgery is associated with significant long-term weight loss and improved metabolic health, but this treatment is not available for all obese subjects. A third option is medical pharmacotherapy in addition to increased physical activity and dietary changes. Lower striatal D2/3 R availability in obesity (chapter 2) and the observed increase >2 years after RYGB surgery (chapter 4) suggests that the dopaminergic system might be a potential target in the treatment of obesity with the aim to increase dopamine release, receptor expression and/or signaling. Several anti-obesity drugs that act directly in the central nervous system are already available. Drugs like lorcaserin, a serotonin 2C receptor agonist, and phentermine/topiramate, a sympathomimetic amine with anorectic effect, affect body weight by reducing appetite (60). However, these drugs are only approved for short term use and as an adjunct to a reduced-calorie diet and increased physical activity. A number of other anti-obesity drugs have been removed from the market due to serious side effects. Therefore pharmacological targeting of the dopaminergic system may be a promising alternative strategy. Alternatively, the dopaminergic system can be influenced by deep brain stimulation (DBS). This method has already been successfully used to treat severe resistant depression, obsessive compulsive disorder (OCD) and addiction (61, 62, 63). In rats it has been shown that DBS of the nucleus accumbens (Nac) modulates feeding behavior (64, 65) as well as glucose metabolism in an intensity dependent matter (66). The nucleus accumbens (NAc) is involved in food related
reward (67). Thus, DBS of the NAc could be a potential target for the treatment of refractory morbid obesity (68).

Obesity is characterized by low grade systemic and tissue inflammation. In our studies this did not correlate with insulin sensitivity but since these studies were cross-sectional we cannot rule out that modulation of inflammation will affect insulin sensitivity. In chapters 7 and 8 we show that morbid obesity is associated with invasion of macrophages and T cells in adipose tissue leading to a mixed inflammatory phenotype in both adipose tissue compartments. Moreover we show an inflamed liver in these obese women. Since rodent studies show protective effects of reducing inflammation on the development of insulin resistance and diabetes, it might be of interest to develop compounds that interact with local immunity to prevent metabolic derangements in obesity. Also, the potential protective role of T cells in adipose tissue needs to be further elucidated. Of interest, therapies are now being developed to specifically target M1-activated macrophages using hybrid nanoparticles (69), without affecting anti-inflammatory macrophages (M2). These techniques are not yet applicable in the clinical setting and more research is needed to further develop this approach.

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