CHAPTER 8

Summary and General Discussion of the Thesis

This final chapter summarises and discusses the findings of the work presented in this thesis. The main aim of this thesis was to investigate the role of glucagon-like peptide-1 (GLP-1) in the neuronal control of feeding in obesity and type 2 diabetes mellitus (T2DM), and the mechanisms by which GLP-1 receptor agonists cause weight loss.

Food ingestion activates the secretion of the hormone GLP-1 by enteroendocrine L-cells, located in the distal part of the gut (1). GLP-1 stimulates insulin secretion and inhibits glucagon release, thereby lowering blood glucose (2). In addition, several observations suggest that GLP-1 has a role in the regulation of feeding (2-4). In rodents, GLP-1 receptors have been demonstrated in brain areas controlling feeding behaviour and energy balance (4-7). Recent additional data in rodents showed that central GLP-1 receptors are involved in the anorectic effects of GLP-1 (8). GLP-1 receptor agonists are currently used for the treatment of T2DM and improve glycaemic control, but also stimulate satiety leading to reductions in food intake and body weight (9-11). We hypothesised that the effects of GLP-1 receptor agonists on food intake are, at least partly, mediated via brain areas regulating appetite and reward.

SUMMARY OF THE MAIN FINDINGS

First, in **Chapter 2**, we gave an overview of the knowledge regarding the physiological role of GLP-1 in the central regulation of feeding behaviour and the proposed routes of action. We also provided an overview of the effects of GLP-1 receptor agonists on body weight, showing consistent and dose-dependent weight loss in both obese T2DM patients and obese non-diabetic individuals. We concluded that treatment-related weight loss can be attributed to a decrease in energy intake rather than an increase in energy expenditure. In addition, we concluded that the effects of GLP-1 on the central nervous system (CNS) may be due to both direct (mediated via central GLP-1 receptors) and indirect routes of action (via GLP-1 receptors on vagal afferents originating in the intestine and portal circulation).

In **Chapter 3**, we demonstrated using in situ hybridization in post-mortem brain material, that the GLP-1 receptor is ubiquitously expressed in the human hypothalamus, a key brain area in the regulation of feeding behaviour and peripheral glucose metabolism. In addition, we demonstrated that T2DM patients compared with control subjects have reduced GLP-1 receptor expression in the paraventricular nucleus (PVN) and infundibular nucleus (IFN), important areas in the regulation of feeding behaviour and peripheral glucose metabolism. We hypothesised that this decreased expression of the GLP-1 receptor may contribute to dysregulation of feeding behaviour and glucose homeostasis in T2DM patients.

In the following chapters we explored using functional MRI (fMRI) whether the effects of GLP-1 receptor agonists on food intake may be mediated via brain areas regulating appetite and reward. In **Chapter 4**, we determined the acute effects of GLP-1 receptor activation on CNS activations in response to visual food cues. We therefore studied obese T2DM patients, obese normoglycaemic individuals and lean individuals (n=48) in a randomised, placebo-controlled cross-over study. Each subject underwent three fMRI sessions at separate visits with intravenous infusion of A) the GLP-1 receptor agonist exenatide, B) exenatide with prior GLP-1 receptor blockade by exendin 9-39 or C) placebo. We used exendin 9-39 to determine whether the effects of exenatide are GLP-1 receptor mediated. To study the effects of GLP-1 receptor activation *per se*, i.e. independent of hormonal or metabolic changes induced by GLP-1 receptor activation, all measurements were performed during a somatostatin pancreatic-pituitary clamp. Somatostatin was infused to suppress endogenous insulin, glucagon, growth hormone and GLP-1 production. Glucagon, growth hormone and insulin were infused at constant rates to achieve stable levels. Glucose was infused at a variable rate to clamp blood glucose at 5.0 mmol/l. We found that obese T2DM patients and normoglycaemic obese versus lean individuals have increased CNS responses to watching food pictures in appetite- and reward-related brain regions (insula and amygdala). Intravenous infusion of the GLP-1 receptor agonist exenatide versus placebo decreased these food-related CNS responses in T2DM patients and obese subjects in the insula, amygdala, putamen and orbitofrontal cortex (OFC), which correlated with reductions in subsequent food intake during a choice buffet. We found that the effects on food intake and food-related CNS responses are GLP-1 receptor mediated, as these were largely blocked by prior GLP-1 receptor blockade with exendin 9-39.

In **Chapter 5**, we described the acute effects of GLP-1 receptor activation on brain reward-system activation in response to anticipation and receipt of chocolate milk in our cohort of obese T2DM patients, normoglycaemic obese subjects and lean controls. It has previously been suggested that obese individuals have increased brain reward system activation while anticipating food intake, which may lead to cravings for food, and decreased reward system activation during actual food consumption, which may induce overeating. In line with previous studies, we found that body mass index (BMI) is negatively correlated with CNS responses to chocolate milk receipt (consummatory food reward) in putamen. In addition, we found a positive correlation between BMI and CNS responses to anticipation of receipt of chocolate milk (anticipatory food reward) in putamen, caudate nucleus and insula. Infusion of exenatide versus placebo increased CNS responses to receipt of chocolate milk and decreased anticipation of receipt of chocolate milk in brain areas regulating reward, appetite and motivation (amygdala, putamen, caudate nucleus, insula and OFC). These effects were largely prevented by pretreatment with the GLP-1 receptor antagonist exendin 9-39. We concluded that GLP-1 receptor activation decreases anticipatory

food reward, which may reduce cravings for food, and increases consummatory food reward, which may prevent overeating. In **Chapter 6** we investigated the neural correlates of emotional eating, a tendency to eat in response to negative emotions, which is an important aspect of overeating. Using the Dutch Eating Behaviour Questionnaire (DEBQ) we determined whether emotional eating is associated with altered CNS responses to visual food-cues and with altered sensitivity to the effects of GLP-1 receptor activation on these CNS responses. We observed in our cohort that emotional eating scores were positively correlated with CNS responses to visual food-cues in food-related brain areas, independent of BMI. Interestingly, emotional eating scores were negatively correlated with exenatide-induced reductions in CNS responses to food-cues in normoglycaemic obese individuals and in obese T2DM patients. We concluded that emotional eaters, independent of their BMI, have increased CNS responses to food-cues in appetite- and reward-related brain areas and are less sensitive to the central effects of GLP-1 receptor activation.

Finally, in **Chapter 7** we determined structural changes in white matter integrity using diffusion tensor imaging (DTI) and white matter volume using voxel based morphometry (VBM) in T2DM patients, normoglycaemic obese and lean individuals. We found that both white matter integrity, as measured by axial diffusivity, and white matter volume are decreased in obese T2DM patients compared with lean normoglycaemic subjects. In normoglycaemic obese compared with lean subjects, axial diffusivity as well as white matter volume tended to be reduced, whereas there were no differences between normoglycaemic obese and T2DM subjects. Decreased white matter integrity and volume were related to higher age, being male, higher BMI, HbA1C and fasting glucose and insulin levels. However, multivariate analyses demonstrated that only BMI was independently related to white matter integrity, and age, gender and BMI to white matter volume loss. We concluded that obese T2DM patients have reduced white matter integrity and volume, but that this is largely explained by BMI, rather than T2DM *per se*.

DISCUSSION AND FUTURE PERSPECTIVES

Expression of the GLP-1 receptor in the human hypothalamus

In **Chapter 3** we determined GLP-1 receptor expression in the human hypothalamus using in situ hybridization in post-mortem brain material. We observed profound expression of the GLP-1 receptor throughout the hypothalamus. These findings in the human hypothalamus are in line with findings in rodents and non-human primates (5;12). Because of the established role of the paraventricular nucleus (PVN) and arcuate nucleus (ARC) (or infundibular nucleus (IFN) as it is called in humans) in the regulation of glucose metabolism and feeding behaviour in rodents, we quantified GLP-1 receptor mRNA expression in the PVN and IFN and compared this between patients with T2DM and control subjects. We found reduced expression of the GLP-1 receptor in T2DM patients in both PVN and IFN. From rodent studies is it clear that GLP-1 affects energy metabolism by acting on the ARC (IFN) and the PVN. Chemical ablation of the ARC in rats prevents the inhibitory action of GLP-1 on feeding (13); GLP-1 infusion into the ARC lowers hepatic glucose production, thereby reducing blood glucose (14); and direct injection of GLP-1 in the PVN of rats leads to decreased food intake (15). The observed decrease in GLP-1 receptor expression in our study could contribute to dysregulation of glucose homeostasis and feeding behaviour in T2DM patients. However, it is unclear if the observed reduction in GLP-1 receptor expression is either a cause or a consequence of the presence of obesity and/or T2DM. Reduced GLP-1 receptor expression may be caused by alterations in GLP-1 levels in T2DM. However, a meta-analysis of studies on post-prandial GLP-1 secretion did not show a clear alteration in meal-related GLP-1 levels in T2DM patients (16). In individuals without T2DM, fasting GLP-1 levels were shown to be positively associated with body fat mass (17). It could therefore be speculated that higher fasting GLP-1 levels in individuals with a higher body fat mass (as often observed in T2DM) could lead to down regulation of the GLP-1 receptor. On the other hand, it has been shown in rats that a hyperglycaemic state, as is present in T2DM, decreases GLP-1 receptor expression in pancreatic islets (18), which could indicate that decreased GLP-1 receptor expression is rather a consequence of a hyperglycaemic state.

Future studies are needed to confirm decreased GLP-1 receptor expression in the hypothalamus of T2DM patients in a larger sample. Furthermore, new imaging techniques using radio labelled GLP-1 and positron emission tomography (PET) could be used to determine GLP-1 receptor binding throughout the human brain in vivo.

Alterations in food-related CNS responses in obesity and T2DM

It has been hypothesised that changes in CNS responses to food in satiety- and reward-related areas are crucial in the development of obesity, comparable to the role for changes in CNS responses in drug addiction (19;20). Several studies in obese individuals demonstrated alterations

in CNS activations in response to food-related stimuli in appetite- and reward-related areas (21-24). In our study we have used two fMRI paradigms to determine food-related CNS responses, i.e. watching food versus non-food pictures and anticipation/receipt of chocolate milk versus a tasteless solution. In **Chapter 4** we demonstrated that obese T2DM patients and normoglycaemic obese versus lean subjects have increased CNS responses to watching food pictures in appetite- and reward-related brain regions (insula and amygdala). This is consistent with previous studies demonstrating increased CNS responses to visual food cues in obese compared with lean individuals (21;23;25). We expanded these observations by showing that obese T2DM patients also have increased CNS responses to visual food cues in the insula. Although the viewing of food pictures is often used as a relatively simple and interesting food stimulus in fMRI studies investigating the regulation of food intake and the development of obesity, the central responses to actual food consumption may be even more important from a pathophysiological perspective (24). During our experimental protocol, we therefore also measured CNS responses during actual food consumption and during anticipation of actual food consumption**.** In **Chapter 5** we demonstrated that BMI is positively correlated with CNS responses to anticipation of receipt of chocolate milk (anticipatory food reward) in the caudate nucleus, putamen and insula. This is in line with our findings of increased CNS responses to watching food pictures in appetite- and reward-related brain regions in obese compared with lean subjects in **Chapter 4**.

In addition to increased CNS responses to watching food pictures and anticipation of chocolate milk receipt, we demonstrated in **Chapter 5** that BMI is negatively correlated with CNS responses to receipt of chocolate milk (consummatory food reward) in right putamen. This finding is consistent with data from previous studies that showed blunted striatal (caudate nucleus and putamen) responses to chocolate milkshake receipt as a function of BMI (24;26).

Taken together, it seems that obesity is associated with decreased CNS responses to palatable food receipt and increased CNS responses when viewing visual food cues or when anticipating food intake. It has been hypothesised that decreased reward-system activation in response to palatable food receipt in obese subjects may induce compensatory overeating to compensate for this reward deficit (24). Increased anticipatory food reward when viewing food pictures or when anticipating food intake, may reflect that obese individuals experience increased anticipation of the palatability of a food reward compared with lean individuals (20), which may lead to cravings for food. Another theory posits that overeating leads to hyperactivation of brain reward areas in response to anticipation of palatable food via conditioning (27). Whether altered food-related CNS responses are a cause or a consequence of overeating, is still unclear (26). Two prospective studies showed that altered CNS responses to food cues can predict future weight change, suggesting that changes in food-related brain responses may be causal in the development of obesity (28;29), but future studies are needed to further elucidate this issue.

Effects of GLP-1 receptor activation on food-related CNS responses

Using acute intravenous administration of the GLP-1 receptor agonist exenatide, with or without prior GLP-1 receptor blockade using exendin 9-39, we determined the effects of GLP-1 receptor activation on food-related CNS responses. In **Chapter 4** we described that exenatide versus placebo decreases CNS responses to watching food pictures in obese T2DM patients and normoglycaemic obese subjects (in insula, amygdala, putamen and OFC). This is consistent with our observation that exenatide reduces CNS responses to anticipation of receipt of chocolate milk (anticipatory food reward) in putamen, insula, amygdala and OFC (**Chapter 5**). The effects of GLP-1 receptor activation on CNS responses to visual food cues and anticipatory food reward may reduce cravings for food, which could lead to reductions in food intake. Interestingly, we also found that exenatide increases CNS responses to receipt of chocolate milk (consummatory food reward) in putamen, insula, amygdala, OFC and caudate nucleus. These effects of GLP-1 receptor activation on consummatory food reward may counteract overeating.

The effects of GLP-1 receptor activation on food-related CNS responses were present in brain areas that are implicated in the regulation of reward and appetite. The amygdala has a key role in emotional learning and in encoding reward value predicted by conditioned stimuli (30;31). The putamen, caudate nucleus and OFC are likewise implicated in reward processing (24;32), whereas the OFC is also involved in decision making (33). The insula was shown to be involved in gustatory perception (34), which is represented in the processing of visual food cues (35), tasted or smelled food stimuli (36) and also in food craving (37). The observed effects of GLP-1 receptor activation on these brain areas are of interest since they are expanding findings from previous fMRI studies showing effects of leptin and the gut-derived hormones peptide YY and ghrelin on brain responses to food-cues in similar brain areas (38-41). Future studies could determine whether combined administration of GLP-1 and other anorexigenic hormones may have additive effects on food-related CNS responses and food intake. Proof of concept studies have already demonstrated that glucagon and GLP-1, as well as peptide YY and GLP-1, act synergistic in reducing food intake (39;42).

We found in **Chapter 4 and Chapter 5** that the exenatide-induced effects on food-related CNS responses were largely inhibited by exendin 9-39, suggesting that the effects of exenatide are GLP-1 receptor mediated. Whether the CNS effects of exenatide are mediated via central or peripheral GLP-1 receptors cannot be determined from our study. In rodents, it was shown that exendin-4 (a peptide closely resembling exenatide structurally) is able to cross the blood-brain barrier (43) and to reduce the rewarding value of food mediated via mesolimbic GLP-1 receptors (44) pointing towards direct effects on the CNS. However, other studies in rodents have shown that the effects of GLP-1 on the CNS may be partly mediated via indirect routes of action i.e. via GLP-1 receptors on vagal fibres signalling to the CNS (45). Vagotomy attenuated the effects of

peripheral administered GLP-1 on food intake and neuronal activation in central food intake regulating areas in rodents (46). Furthermore, peripheral administration of the GLP-1 albumin fusion protein (albugon), which is unable to cross the blood-brain barrier, reduced food intake and induced neuronal activation, but these effects were less robust in comparison to exendin-4 (47). Since in our study exendin 9-39 largely blocked the CNS effects of exenatide, and in rodents the uptake of exendin 9-39 in the brain is low (48), this may imply that the observed CNS effects are mostly mediated via peripheral GLP-1 receptors. Future studies using administration of GLP-1 fused to albumin (making it unable to cross the blood brain barrier, such as albiglutide or dulaglutide) in humans could further elucidate the contribution of peripheral and central GLP-1 receptors in the effects of GLP-1 (receptor agonists) on the CNS.

Interestingly, GLP-1 and its receptors have also been demonstrated on mouse taste buds, suggesting an involvement of GLP-1 in gustatory perception (49). GLP-1 signalling modulates taste sensitivity in mice and increases the responsiveness to sucrose (50). In dietary obese rats, exenatide altered sweet taste preference and sweet taste information processing in the brain (51). Whether these effects of GLP-1 are mediated via direct actions on taste buds or via actions on the brain is still unclear. In addition, it is unknown whether possible direct effects on gustatory perception play a role in the observed effects of GLP-1 receptor activation on the CNS in our study.

In line with our findings on anticipatory and consummatory food reward, GLP-1 receptor activation was shown to modulate the rewarding properties of amphetamine, cocaine, alcohol and nicotine in rodents (52;53). Exenatide reduced motivated behaviour for a drug reward and reduced dopamine release in the nucleus accumbens (54). Dopamine is a key neurotransmitter involved in (drug and food) reward signalling in the brain (20;55). The mechanisms of GLP-1-mediated alterations in food and drug reward are largely unknown and this field of study is in its infancy (52;56). New insights into these mechanisms could provide therapeutic targets for both obesity and substance abuse disorders. An ongoing study (NCT02302976) in cocaine users is currently determining the acute and sub-chronic (5-day) effects of treatment with exenatide on subjective (euphoric) and behavioural (self-administration) effects of cocaine. Additional studies in alcohol or nicotine dependent subjects are of interest.

Effects of GLP-1 receptor activation on appetite and food intake

In contrast to previous studies (9), we found no statistically significant effects of GLP-1 receptor activation on scores of hunger, fullness, appetite, prospective food consumption and desire to eat (**Chapter 4**). In individuals eating their normal diets in their normal environment, appetite scores have been shown to correlate with, but not reliably predict, energy intake to the extent that they could be used as a proxy of energy intake (57). Under experimental conditions, such

as those in our complex experimental fMRI study, appetite scores may be less sensitive (57). In addition, since we also wanted to measure hunger scores while subjects were lying in the MRI scanner (and subjects had two intravenous catheters making it difficult to write), subjects were asked to verbally express their feelings on a 10-point scale while in other studies subjects filled out the questionnaire by themselves. This may have reduced the sensitivity for detecting subjective effects of GLP-1 receptor activation.

Although we did not observe effects of GLP-1 receptor activation on subjective feelings of hunger, we did observe a significant difference in caloric intake between test days (**Chapter 4**). Exenatide versus placebo infusion reduced caloric intake with 23% ± 8%, 24% ± 10% and 14% ± 5% in the lean, obese and T2DM group respectively. These effects were GLP-1 receptor mediated, as these were largely blocked by prior infusion of the GLP-1 receptor antagonist exendin 9-39. Furthermore, we demonstrated that the exenatide-induced reductions in food intake are associated with exenatide-induced changes in CNS responses to watching food pictures.

Effects of GLP-1 receptor activation on gastric emptying

In previous studies GLP-1 has been shown to decrease gastric emptying (58) and it can induce nausea, which may contribute to its satiety-inducing effects. However, it is not likely that the observed effects of GLP-1 receptor activation on the CNS in our study were related to differences in gastric emptying. All measurements were performed in fasting subjects and the total amount of chocolate milk consumed during our fMRI task was only 8 ml. Unfortunately, after the lunch when all infusions were terminated some subjects experienced nausea and vomiting, which may have been caused by exenatide-induced effects on gastric emptying. However, there were no significant effects of exenatide on nausea scores during the fMRI measurements and before and directly after the lunch. There are several other reasons to assume that delayed gastric emptying and nausea are not the sole cause of reductions in food intake and body weight during GLP-1 receptor agonist treatment. The weight reduction seen during trials with GLP-1 receptor agonist treatment is also observed in the absence of nausea (59;60) and the effects on gastric emptying are subject to tachyphylaxis (rapid desensitization) (61), while the effects on body weight persist over periods up to 3 year (59;62).

Effects of GLP-1 receptor activation in emotional eaters

Emotional eating, a tendency to eat in response to negative emotions, may have an important role in overeating. Emotional eating was shown to be associated with obesity (63;64) and with less weight loss during a weight loss program as well as following bariatric surgery (65). In addition, emotional eating is associated with weight regain after weight loss accomplished by treatment interventions (66). The neural correlates and pathophysiology of emotional eating

are however not well delineated. In **Chapter 6** we demonstrated that higher emotional eating scores are associated with increased CNS responses to watching food pictures in appetite- and reward-related brain areas (insula, amygdala and OFC), independent of BMI. Our findings expand findings from a previous study that showed that emotional eaters have increased activity of parieto-occipital regions in response to watching food pictures, measured with event related potentials (ERP) (67). In addition, a previous study that used fMRI demonstrated that emotional eaters have greater activation in reward-related areas (parahippocampal gyrus and anterior cingulate gyrus) during anticipation of palatable food receipt during a negative mood (68).

We also determined in **Chapter 6** whether emotional eating scores are associated with the effects of exenatide on food-related CNS responses and food intake. We found that emotional eating scores are negatively correlated with exenatide-induced reductions in CNS responses to watching food pictures in normoglycaemic obese subjects in the amygdala and in T2DM patients in the insula. It could be speculated that emotional eaters are less sensitive to physiological signals that regulate satiety, such as GLP-1. However, although we observed that emotional eating is associated with exenatide-induced changes in food-related CNS responses, we found no correlation between emotional eating and exenatide-induced changes in food intake. This may be due to the fact that in emotional eaters, an *ad libitum* lunch buffet in an experimental setting is not a good proxy for caloric intake in daily life. However, our data suggest that it is important to investigate the relation between emotional eating and body weight loss in clinical trials with GLP-1 receptor agonists. Our findings may provide a mechanistic explanation for the fact that treatment with GLP-1 receptor agonists is only associated with significant body weight loss in a subset of patients (11;69). Insights in the relation between emotional eating and treatment-related weight loss could be clinically relevant for deciding which patients would benefit most from treatment with GLP-1 receptor agonists with respect to body weight loss.

The use of somatostatin pancreatic clamps

We demonstrated the acute effects of GLP-1 receptor activation on CNS responses to food-cues and food intake during a somatostatin pancreatic-pituitary clamp (**Chapter 4, 5 and 6)**. To determine the effects of GLP-1 receptor activation independent of glucometabolic changes, we infused somatostatin to suppress endogenous insulin, glucagon, growth hormone and GLP-1 production. Glucagon, growth hormone and insulin were infused at constant rates to achieve stable levels. Glucose was infused at a variable rate to clamp blood glucose at 5.0 mmol/l. The use of pancreatic clamps provides the unique opportunity to determine the effects of GLP-1 receptor activation *per se* and to tease out the effects of GLP-1-induced increases in insulin levels and decreases in glucagon and glucose levels. Changes in glucose and insulin levels may confound the effects of GLP-1 receptor activation on the CNS, since

insulin and glucose have been shown to modulate brain activity in areas controlling feeding behaviour (10). However, as a consequence of the pancreatic clamps, our measurements were performed under non-physiological circumstances. Therefore it may be difficult to directly translate our results to e.g. clinical use of GLP-1 receptor agonists. However, the observed food intake suppressive effect of exenatide in our study is in line with studies using intravenous GLP-1 in a more physiological setting (10) and with observed weight loss during chronic treatment with GLP-1 receptor agonists (11). Long-term studies using fMRI measurements of food-related CNS responses during chronic treatment with GLP-1 receptor agonists are ongoing (NCT01363609).

The use of functional MRI

We used fMRI to measure food-related CNS responses and the effects of GLP-1 receptor activation on these responses (**Chapter 4, 5 and 6**). fMRI is based on the blood oxygen level dependent (BOLD) effect, which depends on changes in local deoxyhaemoglobin concentrations in the brain as a result of neuronal activity, leading to alterations in MRI signal intensity (70). FMRI analysis involves thousands of simultaneous tests on discrete voxels in collected brain volumes. Therefore the use of multiple comparisons correction is important in order to prevent Type 1 errors (71). An illustrative example comes from an fMRI study in which a dead salmon was scanned while showing it pictures of humans in social situations (72). Without using multiple comparisons corrections a significant cluster was found within the brain of the dead salmon. Random noise in the fMRI time series may yield spurious results if multiple comparisons are not controlled for. In order to prevent Type 1 errors in our study, we used family-wise error (FWE) corrections and we determined a priori regions of interest based on previous studies (insula, putamen, caudate nucleus, amygdala and OFC) (21-23). We only reported brain activations that survived FWE correction for multiple comparisons at the voxel level within the regions of interest using a small volume correction or across the entire brain for regions not a priori of interest.

A limitation of standard fMRI is the limited spatial resolution, making it difficult to measure food-related CNS responses in small brain areas such as the hypothalamus. Since we observed decreased hypothalamic GLP-1 receptor expression in T2DM patients in **Chapter 3**, it would be of interest to determine the effects of exenatide on food-related CNS responses in the hypothalamus. Unfortunately, the spatial resolution of most fMRI sequences, with whole-brain coverage, is regarded too low to measure reliable hypothalamic activation (73). Furthermore, due to its location adjacent to air-filled sinuses which can cause signal drop-out, the hypothalamus is often subject to artefacts (73). Studies using specialised imaging protocols with the hypothalamus as region of interest could determine effects of GLP-1 receptor activation on hypothalamic activity.

The use of structural MRI

In **Chapter 7**, we used structural MRI to determine alterations in white matter integrity and volume in obese T2DM patients and obese normoglycaemic versus lean subjects. White matter tract integrity can be measured with use of the MRI technique Diffusion Tensor Imaging (DTI), which quantifies microstructural alterations in white matter. With use of voxel-based morphometry (VBM), structural changes in white matter volume can be differentiated and localised. Using these techniques we found that both white matter integrity, as measured by axial diffusivity, and white matter volume are decreased in obese T2DM patients compared with lean subjects. In normoglycaemic obese compared with lean subjects axial diffusivity as well as white matter volume only tended to be reduced. Our findings of lower axial diffusivity in T2DM are in line with previous studies showing lower axial diffusivity in T2DM patients (74) and in type 1 diabetes patients (75). Studies in animal models and humans have suggested that axial diffusivity might represent axonal integrity, although careful interpretation is required (76;77). In a mouse model of multiple sclerosis it was demonstrated that greater decreases in axial diffusivity were associated with greater amounts of axonal damage and with more neurological disability (78). Further studies are needed to determine the clinical relevance of loss of axial diffusivity in T2DM patients.

Our findings of reduced white matter volume in T2DM patients are in line with a previous study in a large cohort of T2DM patients (79). Alterations in brain white matter integrity and volume may play a key role in T2DM-related cognitive impairment (79;80).

Higher BMI independently predicted decreased white matter integrity and white matter volume. Interestingly, higher HbA1C, fasting plasma glucose and insulin levels were no independent predictors of decreased white matter integrity and volume. Our findings indicate that obese T2DM patients have reduced white matter integrity and volume, but that this is largely explained by BMI, rather than the presence of T2DM *per se*.

To conclude, in this thesis we demonstrated the effects of GLP-1 on the neuronal control of feeding in obesity and T2DM. Using in situ hybridization, we observed reduced hypothalamic GLP-1 receptor expression in post-mortem brain material of T2DM patients. Using intravenous exenatide and fMRI, we showed that GLP-1 receptor activation alters food-related CNS responses, paralleled by reductions in subsequent food intake. Our findings provide novel insights into the mechanisms by which GLP-1 regulates food intake and how GLP-1 receptor agonists cause weight loss. Further insights into the central regulation of feeding may help to develop new treatment strategies for obesity and T2DM.

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