CHAPTER 9

Summary, general discussion & future perspectives

The aim of this thesis was to investigate the role for glucagon-like peptide-1 (GLP-1) in the central nervous system (CNS) regulation of food intake in humans. We tested the hypothesis that endogenous glucagon-like peptide-1 (GLP-1) has a physiological role in the CNS regulation of food intake in humans, and we assessed if this effect may be altered in in obese patients with type 2 diabetes (T2DM). We also investigated if the central GLP-1 receptor expression is altered in these patients. In addition, we investigated if Roux-en-Y gastric bypass (RYGB) increases the effects of endogenous GLP-1 in areas in the CNS involved in the regulation of food intake, which may contribute to the large intake reduction and sustained weight loss after RYGB. Finally, we tested the hypothesis that treatment with a GLP-1RA affects the regulation of food intake via effects in the CNS and that this may be a mechanism which contributes to the observed weight loss during this treatment. Our premise was that in humans GLP-1 is an important physiological player within the gut-brain axis and in the regulation of food intake, and that treatment effects of GLP-1RA on food intake and body weight reduction are mediated via this same mechanism within the CNS.

SUMMARY OF THE MAIN FINDINGS

In **chapter 2**, we summarised in a review the hitherto available literature on the CNS regulation of food intake and the effects of GLP-1 on energy balance. We focused on the available literature indicating that effects of GLP-1 and GLP-1RA on food intake are mediated via effects on the CNS. Clinical studies demonstrate that treatment with GLP-1RA results in significant weight loss which is mainly due to reductions in food intake, and not due to increased energy expenditure. Studies in rodents demonstrated that GLP-1 affects food intake via effects in the CNS (1-7). Results from these studies indicate that different routes of action are involved, as not only direct effects of GLP-1 in the CNS, but also indirect effects, via vagal afferents to the CNS, affect the regulation of food intake. Nonetheless, evidence for (physiological) effects of GLP-1 in the CNS in humans remained sparse.

In **chapter 3**, we investigated the GLP-1 receptor expression in the post-mortem human hypothalamus, using in situ hybridisation. We found GLP-1 receptor expression throughout the hypothalamus, including the hypothalamic paraventricular nucleus (PVN) and infundibular nucleus (IFN), both involved in the regulation of energy metabolism. Our main finding was that the GLP-1 receptor expression density in both the PVN and IFN was significantly decreased in T2DM patients compared with control subjects, which may be related to the dysregulation of feeding behaviour and glucose homeostasis in T2DM patients.

The physiological effects of GLP-1 in the CNS in both healthy lean individuals and obese patients with T2DM are described in **chapter 4**. We used functional magnetic resonance imaging (fMRI) to measure CNS activation and used an fMRI paradigm with visual food stimuli, consisting of the presentation of food pictures. In line with the available literature, we found that obese T2DM patients have increased activation in CNS areas involved in the regulation of food intake (i.e. the insula, amygdala and orbitofrontal cortex (OFC)) in response to food pictures compared to healthy lean individuals. We also confirmed that intake of a standardised meal leads to reduced activation in the insula in response to food pictures in both healthy lean individuals and obese T2DM patients. Finally we showed that blockade of endogenous GLP-1 effects, using infusion with the GLP-1 receptor antagonist exendin 9-39, blunted the effects of the meal intake, i.e. a smaller meal related reduction in insula activation in response to food pictures during GLP-1 antagonist infusion

compared with placebo infusion. This effect was however only statistically significant in obese T2DM patients. Our finding indicates that endogenous GLP-1 has a physiological role in mediating satiety effects in the human CNS, thereby contributing to the central control of food intake.

In **chapter 5** we addressed the question if treatment with GLP-1RA affects CNS activation in response to visual food stimuli in obese T2DM patients. We again measured CNS activation with fMRI and compared treatment with the GLP-1RA liraglutide with insulin treatment after short-term treatment (10 days) and longer-term treatment (12 weeks). We found, as expected, that liraglutide, compared with insulin, resulted in a significant weight loss after 12 weeks and that both treatments reduced glucose levels. Liraglutide, compared with insulin, numerically reduced the caloric intake during *ad libitum* lunch buffet, but this was not statistically significant. CNS activation in response to food pictures was reduced with liraglutide treatment, compared with insulin treatment, in both fasted and postprandial condition after short-term treatment. However, these effects were not observed after longer-term treatment. This suggests that the effects of GLP-1RA treatment on the CNS may contribute to the induction of weight loss, but not necessarily to its maintenance.

Not only viewing food items, but also tasting palatable food items can induce strong rewarding effects and activation in the CNS. We therefore extended our investigation and in **chapter 6** we describe the effects of endogenous GLP-1 in healthy lean individuals and obese T2DM patients on CNS activation in response to the taste of palatable food. We also investigated in obese T2DM patients the effects of GLP-1RA (liraglutide) on CNS activation in response to the taste of palatable food. FMRI was used to measure CNS activation and we employed a gustatory food stimuli paradigm, which consisted of the consumption of chocolate milk during scanning. In line with observations by others (8;9), we found that obese T2DM patients have decreased CNS activation in response to chocolate milk consumption compared with healthy lean individuals. This indicates that obese T2DM patients have a reduced responsiveness to the consumption of palatable food, which may induce larger palatable food consumption in order to compensate for this deficit. We also found that blockade of endogenous GLP-1 effects with exendin 9-39 infusion blunted the activation in the insula to the receipt of chocolate milk in healthy lean individuals, but this was not observed in obese T2DM patients. This suggests that endogenous GLP-1 is a physiological signal contributing to central rewarding effects of the consumption of palatable food in humans. Finally, we found that short-term treatment with liraglutide, compared with insulin, increased the activation in the insula and putamen to chocolate milk receipt in obese T2DM patients, which indicates that short-term treatment with GLP-1RA may improve the observed deficit in responsiveness to palatable food consumption in obese T2DM. However, comparable to the effects in response to visual food stimuli, the effect of GLP-1RA treatment ceased to be significant after longer-term treatment.

In **chapter 7**, we assessed the effects of RYGB on CNS activation in response to food stimuli, both visual and gustatory, and evaluated the role of enhanced GLP-1 secretion after RYGB in these effects. We performed fMRI scans before and 4 weeks after RYGB. We found that RYGB reduced the CNS activation in response to food pictures and high-calorie food pictures specifically in the fasted condition and in response to chocolate milk receipt in the postprandial condition. As expected, we found elevated GLP-1 levels after RYGB. Our main finding was that the effect of blockade of endogenous GLP-1 was larger after RYGB compared to before on CNS activation in responses to visual food stimuli in the fasted condition and in response to chocolate milk consumption in

the postprandial condition. This suggests that enhanced endogenous GLP-1 effects after RYGB play a role in the reduced CNS activation to food stimuli observed after RYGB. This mechanism may contribute to the decrease in food intake and sustained weight loss observed after this procedure.
In **chapter 8** we evaluated the potential beneficial effects of GLP-1RA treatment on cerebral perfusion. We first described the difference in cerebral perfusion, measured with arterial spin labelling (ASL), between healthy lean individuals and obese T2DM patients. We found a generalised decrease in cerebral blood flow (CBF) in obese T2DM patients, as this decreased CBF was observed not only in feeding regulating areas (i.e. in insula, putamen and nucleus caudatus), but also in the hippocampus, in grey matter and in whole brain. This decreased CBF in obese T2DM patients was statistically independent of differences in BMI, systolic blood pressure and cholesterol levels. However, increased glucose levels (i.e. fasting plasma glucose levels and HbA1c) were clearly associated with the decrease in CBF, which may indicate that the decreased cerebral perfusion in T2DM patients is mainly driven by increased glucose levels. The decreased cerebral perfusion in T2DM may contribute to the increased risk for cognitive impairment associated with diabetes. Others have shown that GLP-1 and GLP-1RA improve peripheral blood flow (10-12) and we therefore investigated if treatment with GLP-1RA in T2DM patients may improve CBF. However, we did not observe effects of treatment with GLP-1RA on whole brain perfusion nor in the predefined regions of interest.

GENERAL DISCUSSION

The central regulation of food intake and obesity

It has been shown that obese individuals (without diabetes) have altered CNS activation in response to food stimuli, both visual and gustatory (8;9;13;14). In this thesis, we found that obese patients with T2DM also have increased activation in the insula, orbitofrontal cortex (OFC) and amygdala in response to viewing food pictures and decreased activation in the insula and OFC in response to the receipt of chocolate milk, compared with healthy lean individuals. It has been suggested that overeating in obese individuals shares similarities with the loss of control and compulsive drug taking behaviour observed in drug-addicted subjects. Therefore, theorists have proposed that addictive processes may be involved in the aetiology of obesity (15;16). The model of 'food addiction' is consistent with the fact that both drugs and food have powerful reinforcing effects that, under certain circumstances or in vulnerable individuals, could overwhelm the CNS homeostatic control mechanisms. Such parallels have generated significant interest in understanding the shared vulnerabilities and trajectories between drug-addiction and obesity (17). If we compare the findings in this thesis to findings in patients with drug-addiction, the increased responsiveness to viewing pictures of food in areas involved in reward circuits, resembles cue exposure (craving-inducing) effects seen in drug-addiction. The reduced responsiveness to the actual receipt of palatable food in the caudate nucleus and putamen, may represent hyposensitivity which may reflect tolerance of the substance, similar to that seen in drug addiction. This deficit in responsiveness of the reward circuitry to palatable food intake may lead to an increase in food intake, as a means to compensate for the deficit in the central reward system, in an effort to achieve a sufficient degree of satisfaction (18;19). However, we could not determine if these abnormalities are premorbid and possibly causal for overeating, or are the consequence of overeating. Findings by others suggest that there may be

a causal role for dysregulation in CNS responses to food stimuli in the susceptibility for overeating and the development of obesity (20;21). On the other hand, others showed that the reduced CNS responsiveness to the actual palatable food consumption can also be a result of overeating (22). Further research is needed to determine the causality of dysregulated CNS activation to food stimuli in the development of obesity, as this may represent a target for the prevention of obesity (see also 'Future perspectives')

The physiological role of GLP-1 in the central regulation of food intake

Hitherto, the physiological role of endogenous GLP-1 in the regulation of feeding was not fully established, especially not in humans. In rodents, although not found consistently (23), peripheral treatment with the GLP-1 receptor antagonist exendin 9-39, used to block the effects of endogenous GLP-1, resulted in significant increases in food intake (24). Moreover, central secreted endogenous GLP-1 was shown to affect food intake and body weight in rodents (25;26). In humans, endogenous GLP-1 was shown to affect prospective food consumption (27). In addition, an association in humans between the postprandial increase in endogenous GLP-1 levels and cerebral blood flow in areas involved in feeding behaviour has been observed (28). However, our studies are the first to investigate the effects of endogenous GLP-1 in an interventional setting in humans. In this thesis, we showed that endogenous GLP-1 in humans contributes to the regulation of CNS responsiveness to food stimuli, thereby probably contributing to the control of food intake.

In **chapter 4**, we describe our finding that endogenous GLP-1 contributes to the meal induced effects in the CNS, as blockade of endogenous GLP-1 blunted the reduction in responsiveness to viewing of food pictures in the insula after meal intake. This finding was paralleled by effects of endogenous GLP-1 on hunger scores, with a blunted postprandial reduction in hunger scores during GLP-1 receptor antagonist infusion. However, both these findings were only statistically significant in obese T2DM patients. In **chapter 6** we showed that endogenous GLP-1 also affects the responsiveness of the insula to the consumption of chocolate milk, as blockade of endogenous GLP-1 effects reduced the responsiveness in healthy lean individuals to chocolate milk consumption. We thus found effects of endogenous GLP-1 in both healthy lean individuals and T2DM patients, but these effects were depending on the type of fMRI food stimuli task. We therefore propose that the difference between the groups in the CNS activation during the different food stimuli tasks can be explained as follows. We did not found a significant effect of endogenous GLP-1 in the healthy lean group during the visual food stimuli task, which may be due to the lower CNS activation during this task in healthy lean individuals compared to obese T2DM patients, therefore reducing the power to detect a further reduction in CNS activation due to endogenous GLP-1. The same holds true for the absence of a significant effect of endogenous GLP-1 during the gustatory food stimuli task in obese T2DM patients, as these patients had lower CNS activation during the receipt of chocolate milk compared to healthy lean individuals. GLP-1 levels were similar between healthy lean individuals and T2DM patients, therefore differences in GLP-1 levels between the groups cannot explain our findings. Furthermore, blockade of endogenous GLP-1 resulted in altered glucometabolic effects in both groups, indicating that endogenous GLP-1 also has peripheral effects in both groups.

Given the results of our fMRI studies, it could be concluded that endogenous GLP-1 signalling and effects are not altered in obese T2DM patients, thus not contributing to the development or

maintenance of obesity and T2DM. This finding may seem to be in contrast to **chapter 3**, where we describe our finding that T2DM patients have decreased GLP-1 receptor expression in two hypothalamic nuclei (i.e. the PVN and the IFN) pivotal in the regulation of energy metabolism. It could be suggested this may lead to a reduced (central) sensitivity to GLP-1 in T2DM patients compared with control subjects. However, it should be noted that in the fMRI studies (**chapter 4** and **6**) we investigated effects of endogenous GLP-1 in the reward areas of the CNS (amygdala, insula, OFC, putamen, caudate nucleus), but not in the hypothalamus, which is involved in the central homeostatic control of food intake. Unfortunately, this areas is often subject to artefact using fMRI (which will be discussed in detail further on).

It could be suggested that decreased GLP-1 receptor expression in the PVN and IFN in T2DM patients could causally contribute to dysregulation of glucose homeostasis and feeding behaviour. On the other hand, it has been shown in rats that a hyperglycaemic state decreases GLP1 receptor expression in pancreatic islets (29), indicating that the decreased GLP-1 receptor expression is rather a consequence of hyperglycaemia.

We observed, as described in **chapter 7**, that elevated endogenous GLP-1 levels, after RYGB surgery, increased the effect of endogenous GLP-1 on CNS activation in response to both visual and gustatory food stimuli, which was associated with weight reduction. Possibly, enhancement of endogenous GLP-1 secretion and signalling may lead to improved CNS responses to food stimuli and by this mechanism may reduce food intake and body weight.

It could be argued that the effects of endogenous GLP-1 in our fMRI studies are mediated via concomitant GLP-1 induced glucometabolic or hormonal changes. Glucose and glucagon levels were indeed increased due to GLP-1 receptor blockade. Both glucose and glucagon have satiating effects which may be mediated by the CNS (30;31). However, despite the higher glucose and glucagon levels, we observed *higher* activation in the patients with diabetes compared to healthy lean individuals and *higher* CNS activation following exendin 9-39 administration during the presentation of food pictures. Hence, differences in glucose and glucagon levels cannot explain our findings. The insulin levels did not differ between groups nor between infusion, therefore this can also not explain our findings. It could also be suggested that the difference in GLP-1 levels between infusion may affect our findings. However, despite *higher* GLP-1 levels during exendin 9-39 administration, we observed that exendin 9-39 *blocked* GLP-1 effects.

Pharmacotherapy with GLP-1 receptor agonists and the effects on the CNS, food intake and body weight

Large clinical studies demonstrated that treatment with GLP-1 receptor agonists (GLP-1RA) results in body weight and food intake reduction (32;33), as described in **chapter 2**. Different mechanisms have been suggested to contribute to these effects of GLP-1RA treatment, among others the effects in the CNS (1;3;4;6;7;34). In **chapter 5** and **6**, we compared treatment with the GLP-1RA liraglutide to treatment with insulin glargine, in order to achieve an isoglycaemic state. We investigated the effect of treatment on CNS activation in response to food stimuli. We found that treatment with liraglutide resulted in reduced activation in the insula and putamen in response to viewing food pictures and increased CNS activation in the caudate nucleus and the insula in response to chocolate milk receipt, which both may lead to reduced food intake. We found that liraglutide

reduced food intake, although not statistically significant, and resulted in significant weight loss. However, the effects of liraglutide in the CNS were only observed after short-term treatment and ceased to be significant after longer-term treatment. This may indicate that the effects of GLP-1RA in the CNS may contribute to the induction of weight loss during this treatment, especially since the effects in the CNS after short-term treatment with liraglutide were associated with the weight loss after longer-term treatment, as described in **chapter 6**. However, given the absence of effects of liraglutide after longer-term treatment in the CNS, it is unclear if effects of liraglutide in the CNS also contribute to the maintenance of weight loss. It could be suggested that the long-term effect of liraglutide on the CNS may be more subtle compared to the short-term effects and therefore may be more difficult to detect. Regardless, the difference in effect size between short- and long-term treatment could very well explain why weight loss is induced after short-term treatment, but is in general only maintained after 12 week treatment with liraglutide 1.8mg (35).

In the current study we investigated the effects of liragluitde 1.8 mg, which is the therapeutic dosage for the treatment of diabetes. Recent studies investigating effects of liraglutide 3.0mg showed larger effects on body weight compared to liraglutide 1.8mg (36). In addition, weight loss also progresses during a longer period with the treatment dosage of 3.0mg (36) It could be suggested that due to the increased dosage of liraglutide the effect on weight is paralleled by larger and longer term effects on the CNS, but this awaits empirical confirmation.

Weight loss, as observed after longer-term treatment, may also affect the CNS responses to food stimuli as measured in our studies. It could be argued that this may explain the longer-term findings of treatment with liraglutide in our studies described in **chapter 5** and **6**. However, fMRI studies investigating the effect of weight loss on CNS responses to food pictures show that, if anything, weight reduction may be associated with *decreased* CNS activation in areas involved in food motivation and reward in response to viewing food pictures (37;38), therefore rather increasing the effects after longer-term treatment with liraglutide. It remains however unknown why the short-term effects in the CNS of treatment with liraglutide do not persist. Further research is needed to investigate which mechanisms are responsible for this finding and if this mechanism can be counteracted. This may help to develop treatment strategies resulting in effects during a longer treatment period, therefore possibly increasing the amount of body weight reduction during treatment (see also 'Future perspectives').

It is known that GLP-1 and especially treatment with GLP-1RA delays gastric emptying and is associated with transient nausea (39;40). The role of gastric motility in appetite and satiation regulation and the effects of GLP-1RA on gastric and gut motility has been extensively demonstrated (41;42). It could therefore be argued that this may explain our observed findings in the CNS during treatment with GLP-1RA, described in **chapter 5** and **6**. In our study, seven patients reported mild nausea during beginning of treatment with liraglutide or following dose escalation and one patient reported moderate nausea. However, after exclusion of these patients from the analyses, the effect of liraglutide on CNS activation was similar. Moreover, nausea scores did not differ between treatments. In addition, although it has been shown that liraglutide exerts an acute reduction in gastric emptying, this effect is markedly diminished after short-term repeated dosing of liraglutide, whereas the body weight loss continues (43), indicating that the inhibitory effect of GLP-1 on gastric emptying is subject to rapid tachyphylaxis, contrary to the effects on weight. Finally, weight loss

during GLP-1RA treatment is also observed in absence of nausea (44-49). Taken together, we believe that our results in the CNS of GLP-1RA treatment cannot be explained by effects on gastric emptying.

Effects of Roux-en-Y gastric bypass and the role of GLP-1 in the central control of food intake

Others have previously shown that RYGB leads to reduced CNS activation in response to food pictures (50-52), which is paralleled by changes in food preferences, taste perception and body weight reduction (53-56). In **chapter 7** we confirmed these previous findings, but also describe the novel results showing alterations in CNS activation in response to palatable food consumption after RYGB in humans. We found that RYGB decreased the activation in the insula in response to chocolate milk consumption. This effect may be associated with the change in food preference, taste perception and to higher susceptibility for sweet taste perception observed after RYGB (57-60). Together, this may explain the finding that patients after RYGB have decreased interest in sweet food and find it less enjoyable or even unpleasant (60-62). However, the finding of *reduced* CNS activation in response to chocolate milk receipt after RYGB may considered to be at odds with findings in our other studies, showing *increased* responsiveness in the CNS to chocolate milk in healthy lean individuals compared with obese T2DM patients and enhancement of this responsiveness due to GLP-1. This seeming discrepancy in results may be explained by the change in taste perception and 'dislike' of sweet palatable food in patients after RYGB. Instead of an increase in rewarding effect of palatable food consumption, this may result in reduced rewarding effects, therefore reduced responsiveness in the CNS in response to chocolate milk receipt (Figure 1). This is in contrast to the other studies, i.e. the healthy lean individuals and obese T2DM patients, who are assumed to all experience 'liking', synonymous with pleasantness, thus a rewarding effect from palatable food consumption, but who differ in the CNS activation induced by this palatable food consumption, which may affect their feeding behaviour.

In **chapter 7** we not only describe the effect of RYGB in general on CNS activation in responses to food stimuli, but also describe the role of endogenous GLP-1 specifically in the altered CNS responses to food stimuli observed after RYGB. We showed that the higher levels of GLP-1 after RYGB lead to larger effects in the CNS and that this mechanism may contribute to the improvement in satiety and to the reduction in food intake and body weight after RYGB. Although it is clear that the reduction in food intake and body weight after RYGB are in part attributable to the restrictive and/or absorption-limiting results of this procedure, our finding support the hypothesis that neuroendocrine changes after RYGB are also involved. Further support for this hypothesis comes from studies comparing RYGB, associated with changes in levels of gut-hormones, with gastric banding procedures, anatomically restricting the ingestive capacity without changes in gut hormones (63;64). It has been shown that RYGB is more effective in weight loss and results in larger reductions in CNS responsivity to visual food cues compared with gastric banding (65). In addition, a favourable response in terms of appetite and weight reduction after RYGB is associated with higher levels of GLP-1 (66;67). Taken together, we conclude that neuroendocrine changes after RYGB contribute to weight and food reducing effects of this procedure and that GLP-1 may be largely involved in this mechanism.

We found a larger effect of endogenous GLP-1 after RYGB on CNS activation in response to palatable food consumption in the postprandial condition and to the viewing food pictures in the fasted condition, but not in the postprandial condition. The fMRI task with the chocolate milk consumption

was, for logistical reasons, only performed in postprandial condition and we are therefore unable to determine the effect of endogenous GLP-1 after RYGB in fasted condition on palatable food consumption. The fact that we did not observe an enlarged effect of endogenous GLP-1 after RYGB in response to viewing food pictures in postprandial condition could be explained by the fact that we only blocked effects of endogenous GLP-1 with administration of the selective GLP-1 receptor antagonist exendin 9-39, but other gut-hormones which are also largely elevated after RYGB, such as PYY, are still able to exert their (enhanced) effects. Especially in postprandial condition, effects of other gut hormone may overrule the effect of blockade of endogenous GLP-1 and largely affect the CNS activation to food pictures, as has been described by others (68). However, we did observe a larger effect of endogenous GLP-1 in response to palatable food consumption in postprandial condition. This may suggests a larger role for GLP-1 in the central rewarding evaluation of taste perception compared to the evaluation of visual food cues. This theory is supported by the presence of GLP-1 receptors in mammalian taste buds and by a reduced sweet taste sensitivity in GLP-1 receptor knock-out mice (69).

Although the levels of GLP-1 were not significantly increased during the fasted condition after RYGB compared to before surgery, we did observe larger effect of endogenous GLP-1 in the CNS in fasted condition in response to viewing food pictures after RYGB. This may be explained by an increased sensitivity for GLP-1 after RYGB, as has been shown in rats (70). In accordance, BMI is correlated with an impaired incretin effect of GLP-1 in humans (71), suggesting that reductions in BMI may enhance the sensitivity for GLP-1. In addition, the activity of the enzyme dipeptidyl peptidase 4 (DPP-4), which degrades native GLP-1 to its inactive form, was shown to be decreased after RYGB, which may lead to increased active GLP-1 levels independent of differences in overall GLP-1 and therefore in a measurable increased effect in the CNS after RYGB. In our current study, we only measured total GLP-1 and are therefore unable to determine the difference in active GLP-1 due to RYGB.

We only performed measurements approximately one month after RYGB and are therefore unable to determine if the enlarged effect of GLP-1 on CNS responses to food stimuli is sustained on long term. Of note, however, one month after surgery complaints of the intestinal anastomoses may still be present and may lead to problems with a number of food products, which patients may be able to tolerate more than a year after surgery. Nonetheless, others did observe reduced CNS responses several years after RYGB (52;72). Levels of endogenous GLP-1 rapidly increase after RYGB, starting 1-3 days after surgery (66;73), with progressive increase during the first year (73-75) and persisting on long term (76). Because the increase in GLP-1 levels is sustained, it is tempting to speculate that the effects in the CNS also persist in long-term, but further research is needed to determine the role for GLP-1 in these longer-term CNS changes.

Signalling routes of GLP-1 to and in the CNS

Endogenous GLP-1 is secreted into the circulation by enteroendocrine L-cells located in the distal jejunum and ileum. However, also GLP-1 producing neurons are supposed to secrete GLP-1 centrally. Different routes of action could be proposed for the action of endogenous GLP-1 in the central regulation of food intake and the activation of GLP-1 receptor in the CNS, as described in **chapter 2**. Endogenous gut-derived GLP-1 may enter the brain through the area postrema or median eminence, at the level of which the blood-brain barrier is permeable. However, due to its short circulating half-life (77;78), it is likely that only a small amount of gut-derived GLP-1 reaches

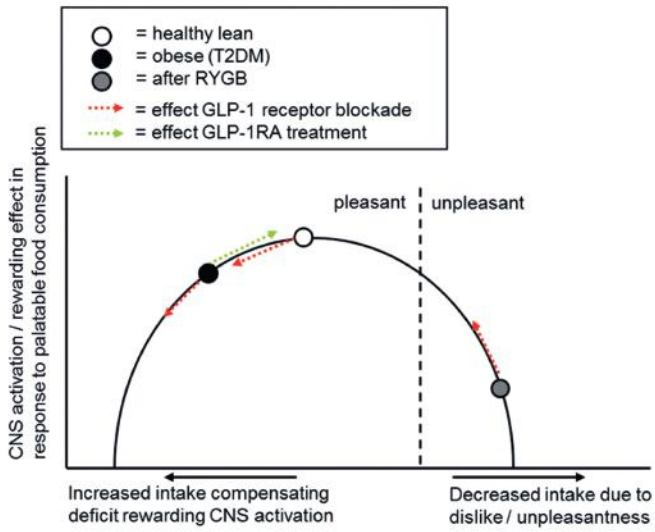

Figure 1: CNS activation in response to palatable food consumption, GLP-1 and related intake effects. Based on our findings in chapter 6 and 7, we postulate that the CNS activation or rewarding effect of palatable food consumption can be described with an inverted u-shape. Individuals located on the left side of the curve, i.e. both healthy lean individuals and obese T2DM patients, experience liking, synonymous with pleasantness, from palatable food consumption. However, if CNS activation in response to palatable food consumption is lower than the top of the curve, food intake may increase to compensate for the deficit in responsivity in order to achieve enough satisfaction. Blocking GLP-1 reduces the CNS responses in these individuals, thereby reducing the rewarding effect but may increase compensating feeding behaviour. Treatment with GLP-1RA increased the CNS responses, thereby preventing compensating feeding behaviour for deficit in CNS rewarding responses. However when on the right side of the curve, when palatable food consumption is experienced as unpleasant, therefore inducing reduced CNS responses, blockade of GLP-1 may decrease the dislike, therefore increase the CNS responses but also food intake. This finding is described in chapter 7. It remains however undetermined which mechanisms may cause the unpleasant sensation of palatable food consumption, as has been described in patients after RYGB.

the brain. Therefore, it is unclear whether peripherally released endogenous GLP-1 needs to enter the brain to affect food intake, or whether other routes of action are involved in its effects on feeding behaviour, such as the activation of vagal afferents (79-81). Activation of vagal afferents may on its turn activate GLP-1 producing neurons or may induce secretion of and/or signalling by other neurotransmitters, such as dopamine and serotonin. Liraglutide and other GLP-1RA were shown to cross the blood brain barrier (82-84), therefore able to exert effects in the CNS via direct activation of central GLP-1 receptors and indirectly via vagal afferent activation. Although studies in rodents show that peripheral administration of GLP-1RA gives rise to measurable concentration in the cerebrospinal fluid and the brain (82;84;85), a small pilot study in humans indicated that during treatment with liraglutide 1.8mg the transfer from blood to cerebrospinal fluid is only minimal (86). It should however be noted that it is unknown what amount of GLP-1 in cerebrospinal fluid is needed to induce actions in the CNS by direct central GLP-1 receptor activation. We cannot compare this to peripheral concentrations, as peripheral liraglutide is 99% bound to albumin (87).

It is not fully clear via which central pathways GLP-1 induces changes in CNS activation to food stimuli and reduction in food intake and body weight. The GLP-1 receptor expression in the hypothalamus,

a pivotal area in the homeostatic control of feeding behaviour, suggests that this is an important pathway. However, GLP-1 also clearly shows effect in areas in the CNS involved in reward processing. Therefore, GLP-1 could also affect transmission of signals induced by other neuropeptides involved in this pathway, e.g. serotonin and/or dopamine, both involved in the reward circuits. In our fMRI studies, we demonstrated the effects of GLP-1 in the activation in several parts of the reward circuitry (i.e. caudate nucleus, insula, putamen, OFC). Interestingly, GLP-1 was shown to attenuate the rewarding power and self-administration of addictive substances such as alcohol, cocaine and amphetamine (88-92). This was also related to changes in dopamine release within the central reward system (88;89;92). This may indicate that GLP-1 affects rewarding effects of substances, therefore the central reward system, independent of the homeostatic regulation of feeding. Further research is needed to further explore which central pathways and specifically neurotransmitter signalling are influenced by GLP-1 and if GLP-1RA treatment and may be proposed as treatment option for drug dependency and addictive food intake.

Cerebral perfusion

Cerebral perfusion was shown to be decreased in patients with mild cognitive impairment and Alzheimer disease (93). The presence of diabetes is also associated with increased risk for developing cognitive impairment or of mild cognitive impairment to dementia (94;95). Moreover, diabetes is associated with vascular complications and cerebrovascular disease, thus the presence of diabetes could be suggested to compromise cerebral perfusion by altering cerebrovascular function. In **chapter 8**, we measured cerebral blood flow (CBF) using arterial spin labelling (ASL) and we confirmed previous findings (96-98) of decreased CBF in obese patients with T2DM compared to healthy lean individuals. We also hypothesized that altered CBF in feeding regulating areas in the CNS may be present in T2DM patients, which may contribute to the observed altered CNS responses to food stimuli. We found reduced CBF in reward processing areas (insula, putamen, caudate nucleus), but also in whole brain, grey matter and in the hippocampus, a key area related to memory functioning. We therefore believe the reduced CBF in obese T2DM patients is a generalised phenomenon. We attempted to disentangle the contribution of various risk factors to the reduced CBF in T2DM patients. The difference between healthy lean individuals and T2DM patients was not driven by differences between the groups in BMI, systolic blood pressure or cholesterol levels, but by differential glucose levels. A role for hyperglycaemia in the development of reduced CBF is supported by the finding of reduced CBF in (lean) patients with type 1 diabetes (96). Our finding may emphasise the benefit of well-controlled glucose regulation in patients with diabetes.

Because GLP-1 and GLP-1RA were shown to affect peripheral blood flow (10-12;99;100), we explored the potential effects of GLP-1RA treatment in enhancing CBF in patients with T2DM. We however did not find an effect of treatment with liraglutide on CBF in these patients. The presence of endothelial dysfunction or structural microvascular damage, both associated with diabetes, may hamper the possibility to demonstrate effects of treatment with liraglutide on CBF. Moreover, the cerebrovascular autoregulation of blood pressure and perfusion in the CNS may compensate potential effects of GLP-1RA treatment on perfusion.

Limitations

The study presented in **chapter 3**, which describes the GLP-1 receptor expression in the human hypothalamus and shows the difference in GLP-1 receptor expression between control subjects and T2DM patients, contained only a modest number of observations, as the availability of well-documented material is often limited. We therefore have adopted a conservative approach by performing non-parametric statistical analyses. Despite the small sample size, we did observe significantly reduced GLP-1 receptor expression in patients with diabetes in two key areas of the hypothalamus involved in the regulation of food intake. Due to missing data, we are however unable to relate our findings to BMI or obesity, but only to the presence of T2DM. However, it is questionable if BMI at death is well representative, as this may be influenced by severe illness. In this same study we did not quantify the total number of neuronal cells in each group, but only determined the receptor expression density. It is therefore unclear if T2DM patients show less expression of the GLP-1 receptor on each cell or if there are fewer cells positive for GLP-1 receptors due to less neuronal cells in general in T2DM patients. Nevertheless, the decrease in GLP-1 receptor expression in T2DM patients suggests a reduced capacity of GLP-1 action in the hypothalamus in these patients compared to control subjects. Finally, we only measured mRNA expression of the GLP-1 receptor, using in situ hybridisation. Immunohistochemistry using a monoclonal antibody for the GLP-1, in order to determine the actual protein expression on the neuronal cells, has been performed in nonhuman primate brain (34). Unfortunately, we were unable to obtain consistent, reproducible results using this monoclonal antibody on human brain material, as performed in our pilot study. Although speculative, this may be related to the fixation procedures that we used for our tissues, which may have caused masking of the antigen.

The groups in our fMRI studies consisted of 20 individuals. This sample size was calculated for our main outcome, i.e. the difference in CNS activation due to intervention. The sample size may have been too small for other outcomes, such as differences in caloric intake or correlations of CNS responses with changes in caloric intake and weight over time .

In our studies we did not determine gender differences. Most of the studies investigating CNS activation in obese individuals in response to food stimuli focus on female subjects (8;13;14). Although we cannot exclude a difference between genders in these CNS activations, large studies investigating the effects of GLP-1RA treatment on glucose control and body weight do not indicate that these effects differ between genders (32;46;47;101-103) and we therefore do not expect this has affected our results on effects of GLP-1 in the CNS. We have chosen to include both genders in our studies in order to extrapolate our findings in general, except for the study described in **chapter 7**, showing the effects of RYGB, as the majority of patients undergoing RYGB are female.

In **chapter 5** and **6** we compared treatment with the GLP-1RA with insulin glargine. This active comparator was chosen to achieve an isoglycaemic state between treatments, as glucose may affect CNS activations in response to food stimuli (31) and we thereby attempted to minimize glycaemic effects on our measurements. However, it also has been shown that insulin is a satiating signal to the CNS (104-107). Although it has been suggested that in obese individuals central insulin resistance is present, insulin treatment may have affected CNS responsiveness to food stimuli. However, despite possible satiating effects of insulin treatment on CNS responsivity, we still observed a larger satiating effect of short-term treatment with liraglutide.

The hypothalamus is known to be an important central area in homeostatic control of feeding (108). Therefore, it is of interest to investigate the effects of physiological postprandial signals such as GLP-1 on the activation in this area. Unfortunately, the location of the hypothalamus within the CNS is often subject to artefacts with fMRI measurements, as it is adjacent to air-filled sinuses, which can cause signal dropouts (109-111). Furthermore, given the small size of the hypothalamus, the spatial resolution of most fMRI sequences, with whole-brain coverage, is considered suboptimal. A specialized imaging protocol is required to measure reliable hypothalamic activation (109-112). In our study protocol, we used a whole-brain coverage, as we were also interested in areas involved in feeding regulation other than the hypothalamus. In **chapter 3** we focussed on the GLP-1 receptor expression in the hypothalamus and showed expression throughout the hypothalamus in humans, suggesting GLP-1 is involved in hypothalamic signalling and activation.

A limitation of the studies investigating the role of endogenous GLP-1 on the CNS activation to food stimuli, described in **chapter 4** and **6**, was that we included only a group of healthy normoglycaemic lean individuals and obese patients with T2DM. We are therefore unable to distinguish the effects of obesity from diabetes per se, but we believe that our findings may extend to healthy obese individuals for several reasons. We previously showed that CNS activation in response to viewing food pictures was similarly increased in healthy obese and obese patients with T2DM and that acute GLP-1RA administration reduced CNS activation in response to food pictures, and reduced food intake in both healthy obese individuals and obese patients with diabetes. Furthermore, effects of GLP-1RA treatment on body weight and food intake are similar in healthy obese individuals and obese patients with type 2 diabetes. Finally, we found effects of endogenous GLP-1 in both healthy lean individuals and in obese T2DM patients, suggesting that these effects are not confined to one study group per se and may therefore also extend to healthy obese individuals.

As also mentioned above and described in **chapter 4** and **6**, we did observe an effect of endogenous GLP-1 in both groups (healthy lean individuals and obese T2DM patients), but this effect differed between the groups depending on the implemented fMRI food stimuli paradigm, i.e. an observed effect of endogenous GLP-1 in the group with the higher baseline CNS activation in response to the used food stimuli. This suggests that a certain amount of CNS activation at baseline is needed to detect changes in activation induced by endogenous GLP-1. This may indicate that fMRI may not provide sufficient sensitivity to detect relatively subtle changes induced by changes in hormonal levels when activation at baseline in a subject group is low.

In our studies investigating treatment effects of GLP-1RA, described in **chapter 5** and **6**, we used a crossover design. A limitation of this design is that the results may be confounded by carryover effects. We therefore included a wash-out period of 12 weeks, which was considered long enough to eliminate possible carryover effects. We investigated if order of treatment affected our findings in both the fMRI data and the clinical data, but this was not the case. Another possible limitation is that, due to the crossover design, the participants underwent six test visits including the fMRI food stimuli tasks, which may induce habituation to the food stimuli. However, since order of treatment did not affect the observed effects of treatment, we do not believe that habituation affected our results. In addition, we created a different visual food stimuli version (with different pictures) for each visit and each fMRI scan performed.

In the study described in **chapter 8**, showing the difference in cerebral perfusion between healthy lean individuals and obese T2DM patients, we did not perform assessments of cognitive performance

and we are therefore unable to relate our findings of decreased cerebral perfusion to possible impaired (sub-clinical) cognitive performance. It is difficult to estimate the clinical implications of our finding of generalised reduced cerebral perfusion in obese T2DM patients, although the association between diabetes and the increased risk for development of mild cognitive impairment has been described (95).

FUTURE PERSPECITVES AND CONCLUSIONS

The findings described in this thesis provide insight and evidence for the role of GLP-1 in the central regulation of food intake in humans. However, as could be expected, other questions have evolved or remain to be investigated. It would be of interest to explore if combining GLP-1 based therapy with a therapy consisting of another hormone involved in the regulation of feeding may synergistically increase the effects in the CNS and therefore the effects on food intake and body weight. In addition, if we would assume that weight loss itself induces compensatory mechanisms preventing larger long-term effects of liraglutide treatment on food intake and body weight reduction, combining GLP-1RA treatment with treatment counteracting these compensatory mechanisms may increase the long-term effects on food intake and weight.

An important question which remains is if altered CNS activation in response to food stimuli is causal for or rather a consequence of overeating and obesity. As also described in the general discussion, previous studies suggested a causal role for the CNS (20;21), but this evidence remains sparse. On the other hand changes in eating habits were shown to consequently affect CNS activation to food stimuli (22), but this study specifically evaluated the effect for the intake of chocolate ice cream only. Large prospective studies with assessment of CNS activation to different food stimuli at baseline and evaluating during a long follow-up period in an observational design feeding behaviour, caloric intake, weight changes and physical activity may provide further insights in the causal role of altered CNS responses to food stimuli in feeding behaviour and in the development of obesity. In order to investigate if CNS activation to food stimuli may adapt or change according to specific feeding behaviour and caloric intake, prospective studies evaluating the effect of diet (both high caloric and caloric restriction) on CNS activation in response to food stimuli should be performed, as this may indicate to which extent CNS responsiveness to food stimuli is affected by dietary habits.

The study described in **chapter 3**, showing a decreased GLP-1 receptor expression in the hypothalamus of T2DM patients, was a post-mortem study. It is therefore impossible to determine causality. It remains unknown if hyperglycaemia may lead to the observed reduced central GLP-1 expression or if hyperglycaemia is a consequence of the decreased hypothalamic GLP-1 receptor expression and signalling. It therefore would be of interest to compare individuals with a higher risk to develop T2DM, such as individuals with a family history of T2DM, but before the onset of hyperglycaemia, to assess if reductions in central GLP-1 receptor expression precede the possible development of T2DM. To assess if hyperglycaemia may affect central GLP-1 receptor expression, it would be of interest to investigate if patients with type 1 diabetes, therefore another pathophysiological mechanism for the development of diabetes and without the known association with higher body weight, show decreased central GLP-1 receptor expression, as this may indicate that reduced central GLP-1 receptor expression is rather a consequence of hyperglycaemia.

The signal routes by which GLP-1 and especially treatment with GLP-1RA exert their central effect are still not fully understood. Both the direct route, by crossing the blood-brain barrier, and the indirect

route, i.e. via vagal afferent activation, are considered to be involved. It is however unclear which route is most important. Direct comparison of a GLP-1RA able to cross the blood-brain barrier with a larger molecule GLP-1RA, i.e. unable to cross the blood-brain barrier, may elucidate the contribution of each signal route in the observed CNS effects. Comparison in humans of peripheral administered GLP-1RA with intranasal administered GLP-1RA, therefore with direct CNS effects, may also contribute to further insight. Furthermore, it is unclear whether the effects in the CNS in response to food cues may induce changes in secretion or receptor expression of other neurotransmitters, such as dopamine or serotonin, which are both known to be importantly involved in reward circuits within the CNS and could thus affect CNS responsivity to food cues and rewarding effects. Research in humans exploring the effects of GLP-1RA treatment on the signalling and receptor expression of these other neuropeptides needs to be performed to elucidate the exact central effects of GLP-1.

It is of interest that GLP-1 administration is not only associated with reductions in food intake, but also with reduced addictive behaviour to other addictive substances, such as cocaine, alcohol and amphetamine (88-92). This effect is however only investigated and observed in rodents. Further research in humans is needed to explore this interesting finding, as this may expand the treatment potential of GLP-1RA and may therefore be clinically relevant.

Although on average a reduction in body weight and food intake is observed during GLP-1RA treatment, not all patients show this to the same extent. It is however unknown which characteristics may predict if an individual will respond or not to treatment with GLP-1RA. It has been shown that patient with emotional eating behaviour are less susceptible to the effects of GLP-1RA administration on the CNS activation to food pictures (113). However, it has not been investigated whether or not emotional eating behaviour may predict which patients may respond best to GLP-1RA treatment regarding food intake and body weight.

In our study investigating patients undergoing RYGB, we only performed measurements four weeks after RYGB. However, it should be noted that in this phase after surgery, patients may still have complaints of the intestinal anastomoses. At this time, they may have problems with a number of food products, which they can tolerate better on the longer term after surgery. Although others also have found reduced CNS responses several years after RYGB (52;65), further research is needed to determine the role for GLP-1 in these longer-term CNS changes.

CONCLUSIONS

To conclude, this thesis provides evidence for GLP-1 as an important player in the gut-brain axis and in the regulation of food intake in humans. Our observations indicate that endogenous GLP-1 contributes to the physiological regulation of food intake via effects in CNS. Although we found decreased GLP-1 receptor expression in the hypothalamus of T2DM patients, we did not find altered effects of GLP-1 on activation in reward and satiety areas in response to food stimuli in obese patients with T2DM. In obese individuals, we observed that enhancement of endogenous GLP-1 levels after RYGB increased the effects of GLP-1 in the CNS. Our findings during pharmacotherapy with GLP-1RA indicate that effects of this treatment in the CNS may contribute to the induction of weight loss. But in view of the absence of an effect in the CNS after longer-term treatment, we did not find that the effects of GLP-1RA in the CNS contribute to the maintenance of weight loss.

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