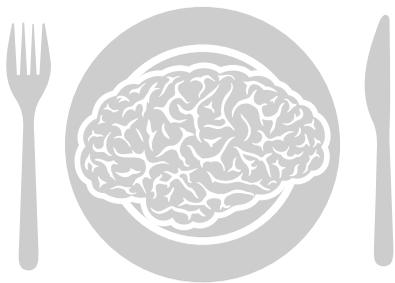


CHAPTER 1

General introduction and outline of the thesis



Obesity is a major public health concern, with a significant burden of morbidity and mortality (1). The worldwide prevalence of obesity has more than doubled between 1980 and 2014 (2). Nowadays 39% of adults are overweight (Body Mass Index > 25 kg/m²) and 13% is obese (Body Mass Index > 30 kg/m²) (2). The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. The increasing prevalence of obesity is related to recent environmental changes, such as an increased access to energy-dense foods (2). In addition, there has been a global decrease in physical activity due to the increasingly sedentary nature of many types of work, changing modes of transportation, and increasing urbanisation.

Obesity is associated with several adverse consequences including an increased risk of cardiovascular diseases, musculoskeletal disorders, cancer and type 2 diabetes (T2DM). Paralleled by the increased obesity prevalence, the global prevalence of T2DM rose to 9% in 2014 (2). T2DM is characterised by progressive pancreatic beta-cell failure against a background of obesity-related insulin resistance (3;4). Achieving and maintaining a healthy body weight is the most important strategy in the prevention and treatment of T2DM (2;5;6).

The neuroendocrine control of feeding

The central nervous system (CNS) has a major role in maintaining body weight and energy balance within a narrow range by regulating energy intake and energy expenditure. Over the past 30 years, it has been established that hormones produced by the gut, pancreas, and adipose tissue are key players in the control of body weight, by acting through a complex neuroendocrine system (7;8). The specific neuronal pathways and mechanisms underlying excessive calorie consumption are not completely understood. A better understanding of neuronal mechanisms that interact with metabolic regulation is needed for further development of preventive and therapeutic strategies for obesity.

The control of food intake can be divided in homeostatic and non-homeostatic feeding (9). Homeostatic feeding controls energy balance by adjusting food intake to energy needs. This balance is regulated in a complex manner by peripheral signals, such as feeding-related hormones (i.e. the anorexigenic hormones insulin, leptin, cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) and the orexigenic hormone ghrelin), which convey information on hunger and satiety to the brain (10;11). The area postrema and nucleus tractus solitarius in the brainstem convey these peripheral signals to the hypothalamus, a key brain area in the homeostatic control of feeding (12). Non-homeostatic or hedonic factors, such as the rewarding properties of food, can override the homeostatic pathway which may result in overeating. Stress, negative moods and emotions can stimulate eating even in absence of energy needs. Corticolimbic circuits (including striatum, amygdala, insula, nucleus accumbens and orbitofrontal cortex (OFC)) are implicated in non-homeostatic eating. The above-described hormonal regulators of homeostatic feeding, may also influence brain reward systems and may increase or decrease the rewarding value of food (13;14).

The neuroendocrine control of feeding as therapeutic target for obesity

In the search of pharmacological treatments for obesity, the above listed anorexigenic and orexigenic signals have been explored as therapeutic targets, however, to no avail. Administration of a ghrelin antagonist (based on the orexigenic effects of ghrelin) resulted in an unexpected increase in food intake and body weight in rats and dogs (15). The adipose-tissue derived anorexigenic hormone leptin has also been explored for therapeutic purposes. However, peripheral leptin administration did not reduce body weight in obese subjects and in T2DM patients (16;17). Also, treatment with a CCK-receptor agonist in obese subjects had no effects on food intake and body weight (18;19). In addition, melanocortin-4 receptor agonists, designed to interact with the anorexigenic pathway in the hypothalamus, resulted in reductions in food intake but were also associated with several side effects such as increased penile erection and blood pressure (20). Currently, novel selective melanocortin-4 receptor agonists are being tested (21). Taken together, these efforts hitherto have been disappointing, only showing the complexity of appetite and energy balance regulation. The GLP-1 system seems to be the first successful therapeutic target for obesity, as GLP-1 receptor agonists showed promising results with regard to weight reduction in humans.

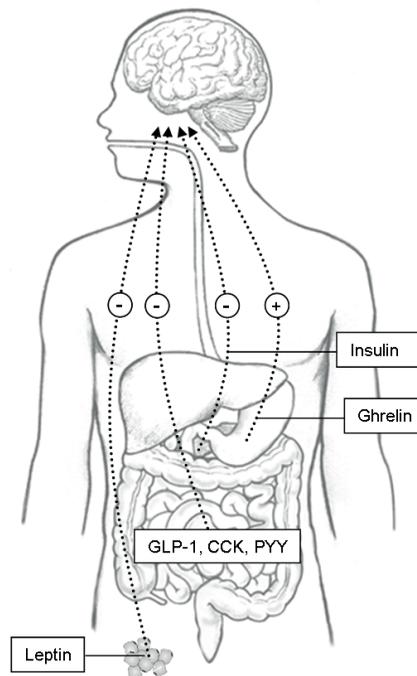


Figure 1 | The CNS integrates input from long-term energy stores (for example, adipose-tissue derived leptin) and short-term meal-related signals (glucose and hormones such as ghrelin, insulin, PYY, CCK and GLP-1) that signal satiety to the CNS in order to regulate food intake. Ghrelin is released by the stomach and stimulates appetite, whereas gut-derived GLP-1, PYY and CCK stimulate satiety. PYY, peptide YY; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin. (Adapted from ref 12. Morton et al.)

Glucagon-like peptide-1, from physiology to pharmacology

In the beginning of the 20th century, it was posited that after ingestion of a meal, a gastrointestinal “messenger” stimulated the enhancement of carbohydrate processing in the pancreas (22). In the early 1980s, the hormone GLP-1 was discovered, which is released from the gut upon meal ingestion (7). GLP-1 is produced by enteroendocrine L-cells, located in the distal part of the gut (23). Oral intake of a meal results in a rapid increase in plasma GLP-1 concentration with a peak after approximately one hour and accounts for 50-70% of total meal-related insulin secretion. In T2DM patients the insulinotropic effect of GLP-1 is lost (24), therefore GLP-1 regained focus of interest for the development of blood-glucose lowering therapy. Indeed, GLP-1 was shown to lower blood glucose via stimulating glucose-dependent insulin secretion, inhibiting glucagon release and delaying gastric emptying (25). Interestingly, GLP-1 was also shown to promote satiety, leading to reductions in food intake and body weight (26;27). In 2002 the first study was published in which native GLP-1 was administered using continuous subcutaneous infusion in T2DM patients (28). After six weeks of treatment, patients showed improved glycaemic control, but also reductions in appetite and body weight. However, native GLP-1 is rapidly degraded by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4) resulting in a circulating half-life of less than 2 minutes, making it unsuitable for therapeutic purposes. Therefore, degradation-resistant GLP-1 receptor agonists have been developed, such as exenatide. Exenatide is a synthetic form of the polypeptide exendin-4, which is secreted by the salivary gland of the Gila monster lizard (*Heloderma suspectum*) (29;30). Eng and Raufman discovered exendin-4 in 1990 in the venom of this lizard and named the peptide exendin since it was isolated from an exocrine gland and was subsequently shown to have endocrine actions. Exenatide was approved by the US Food and Drug Administration (FDA) in 2005 and the European Medicines Agency (EMA) in 2006 and requires to be injected subcutaneously. Several other GLP-1 receptor agonists have been developed for the treatment of T2DM, such as liraglutide, lixisenatide, albiglutide and dulaglutide. In addition to the glucose lowering effects, treatment with GLP-1 receptor agonists is associated with sustained dose-dependent weight loss in T2DM patients (31). Consequently, GLP-1 receptor agonists have been proposed as anti-obesity drug and in 2014 liraglutide was approved by the FDA for the treatment of obesity.

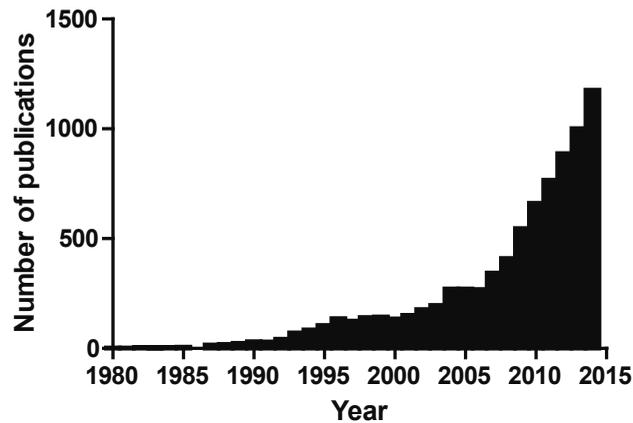


Figure 2 | The number of publications on Pubmed with search terms “GLP-1” shown per year.

Effects of GLP-1 on neuronal control of feeding

The anorectic effects of GLP-1 and GLP-1 receptor agonists are as yet not fully understood, but GLP-1 actions on the brain may partly mediate satiety and weight effects in humans. In rodents, GLP-1 receptors are present in brain areas controlling feeding behaviour and energy balance, such as the hypothalamus, nucleus tractus solitarii, area postrema, dorsal striatum and nucleus accumbens (32). Intracerebroventricular as well as peripheral administration of GLP-1 was shown to reduce food intake in rodents (33). In addition, blocking of the GLP-1 receptor with the GLP-1 receptor antagonist exendin 9-39 increased food intake in satiated rats (34). Recent additional data in rodents showed that central GLP-1 receptors are involved in the anorectic effects of GLP-1 receptor agonists (35;36) and that the rewarding value of food is altered through mesolimbic GLP-1 receptors (37). In humans, using positron-emission tomography (PET), an association was shown between post-prandial endogenous GLP-1 response and changes in neuronal activity of brain areas implicated in satiation and food intake regulation (dorsolateral prefrontal cortex and hypothalamus) (38). However, intervention studies in humans determining the effects of GLP-1 on the neuronal control of feeding and the mechanisms by which GLP-1 receptor agonists cause weight loss are of interest.

Alterations in food-related CNS responses in obesity

It has been hypothesised that excessive eating due to changes in food-related CNS responses in appetite- and reward-related areas underlies the development of obesity, comparable to the role for altered CNS responses in drug addiction (39;40). Functional magnetic resonance imaging (fMRI) can be used to measure regional brain activation in response to specific stimuli in humans (41). This method makes use of the blood-oxygen-level-dependent (BOLD) contrast, in which imaging contrast results from the ratio of oxy- to deoxyhaemoglobin in venous blood (42). When

a specific brain region is activated, the increased consumption of oxygen by neurons during this activation is accompanied by an increase in the supply of fully oxygenated blood, resulting in an increased BOLD signal.

Several studies using fMRI in obese individuals have demonstrated alterations in reward and appetite related circuits in response to food(-related) stimuli. Obese compared with lean women showed increased BOLD responses, while viewing high-calorie food pictures, in regions related to reward anticipation (the dorsal striatum). In addition, these food-related brain responses were positively correlated with Body Mass Index in regions associated with appetite, motivation and emotion processing (insula, OFC, posterior cingulate cortex) (43). Another fMRI study showed increased brain activation in response to food pictures in obese compared with lean subjects in a large number of brain areas hypothesised to mediate motivational effects of food cues (44). Furthermore, obese compared with lean children (age 10-17 years) showed increased food-related brain responses in regions associated with emotion processing and motivation (prefrontal cortex and OFC) (45). In addition, in obese children these food-related brain responses failed to attenuate after a meal in reward-processing areas (nucleus accumbens and prefrontal cortex), whereas the normal weight children showed a significant reduction in these areas after eating. Also alterations in CNS responses to actual food consumption have been studied using fMRI. Stice *et al.* found a negative correlation between Body Mass Index and brain responses in the left caudate nucleus and bilateral putamen to receiving a milkshake versus a tasteless solution, indicating that obesity is associated with decreased food-related reward-system activation (46;47).

Effects of gut-derived hormones on food-related CNS responses

The mechanisms underlying alterations in CNS responses to food-cues are not clear, but multiple metabolic and hormonal factors seem to be involved (8-10). Studies using fMRI have been used to determine the effects of gut-derived hormones on food-related CNS responses. Intravenous administration of the orexigenic hormone ghrelin in healthy subjects increased neural responses to food pictures in the amygdala, OFC, insula and striatum (48), indicating that ghrelin may induce food consumption by enhancing the hedonic and incentive responses to food-cues. In addition, the anorexigenic gut-derived hormone peptide YY was shown to reduce CNS responses to food pictures in an area involved in appetite regulation (insula) (49). Further insights into the neuroendocrine control of feeding may help to develop new treatment strategies for obesity and T2DM.

Aim and outline of the thesis

The aim of this thesis was to investigate the role of GLP-1 in the neuronal control of feeding in obesity and T2DM, and the mechanisms by which GLP-1 receptor agonists cause weight

loss. In **Chapter 2** of this thesis, an overview of the knowledge is presented regarding the physiological role of GLP-1 in the central regulation of feeding behaviour and the proposed routes of action. In addition, an overview is provided of the available data on pharmacological stimulation of GLP-1 pathways leading to reductions in food intake and body weight. In **Chapter 3**, we used in situ hybridization in post-mortem brain material to provide a detailed description of GLP-1 receptor expression in the human hypothalamus. To investigate whether or not GLP-1 receptor expression is altered in T2DM patients compared with control subjects, we used relative quantifications of GLP-1 receptor mRNA in key areas of the hypothalamus involved in glucose and feeding regulation. In **Chapter 4** we tested the hypothesis that GLP-1 receptor agonists reduce food intake by affecting brain areas regulating appetite and reward, and that these effects are GLP-1 receptor mediated and independent of other hormonal and metabolic changes. Therefore, we assessed the acute effects of intravenous exenatide, with or without prior GLP-1 receptor blockade with intravenous exendin 9-39, on food intake and CNS responses to visual food-cues using fMRI in obese T2DM patients, normoglycaemic obese and lean individuals. In order 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. In **Chapter 5** we determined whether GLP-1 receptor activation alters CNS responses to anticipation and consumption of chocolate milk in appetite- and reward-related areas. 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. We used the Dutch Eating Behaviour Questionnaire (DEBQ) to determine 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 responses. **Chapter 7** describes structural changes in white matter integrity and white matter volume in T2DM patients compared with normoglycaemic obese and lean individuals. Finally, **Chapter 8** provides a summary of the results with the general discussion and future directions.

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