CHAPTER 1

General introduction and outline of the thesis

INTRODUCTION

Obesity and the regulation of food intake

The prevalence of obesity has grown to pandemic proportion and nearly 600 million adults globally are affected by obesity (1). Obesity is an established risk factor for diseases such as type 2 diabetes (T2DM), cardiovascular diseases, and several cancers (2;3). Simply stated, obesity is the result of an imbalance between energy consumed and energy expended. An important driver of the increased prevalence of obesity is the current food system (4), with large availability of cheap, palatable, energy-dense food items, improved accessibility of food and persuasive food marketing (5). Studies have shown that increased food supply can indeed explain the weight gain and the rise in obesity in the general population (6-8). However, within a given environment, not all individuals react similarly with regard to their feeding behaviour, which may result in differences in weight between individuals within the same environment. This points to interindividual differences in susceptibility to environmental factors. In the search to prevent and/or treat obesity, it is important to determine which factors contribute to the regulation of energy balance and feeding behaviour in humans and how these factors or mechanisms can be influenced.

The amount of caloric intake varies from day to day and from one meal to another, partly depending on the convenience, time of day, social factors, cost and current mood. However, despite possible short-term mismatches in energy balance, regulating mechanisms are important in matching the long-term energy intake to energy expenditure, promoting stability in the amount of energy stored in the body. The central nervous system (CNS) has been identified as a major player in the regulation of energy balance and feeding behaviour (9;10). Interestingly, it has been suggested that in humans altered CNS activations and responses may play a role in dysregulations of food intake and consequently in the development and/or maintenance of obesity (11;12). Altered activations in the CNS in response to food stimuli have been observed in obesity (13-15), but the mechanisms underlying these altered CNS responses are not fully understood. Gaining further insight in the central signalling involved in the processing and regulation of food intake in humans is therefore important.

The central nervous system in the regulation of food intake

The CNS receives and integrates a multitude of signals related to the perception of food and food intake. Several areas within the CNS are involved in these processes. For example, when we taste food, the primary taste cortex, i.e. the insula, receives neuronal signals, which originate from the tongue and palate and are transmitted along the brainstem and thalamus. The insula encodes different taste modalities and projects to the secondary taste cortex, the orbitofrontal cortex (OFC) with further projections to the striatum, which is part of the corticolimbic system. This corticolimbic system encodes the reward value of food cues (16). In addition, viewing of food also leads to inputs from the visual pathway to the corticolimbic system, which encodes the reward value of the visually perceived food. These interconnected circuits enable appropriate behavioural responses to a given food stimulus (17;18).

The CNS is however not only involved in the processing of tasting or viewing food stimuli, but it also receives signals from the periphery which convey information about the nutritional status affecting feelings of hunger and satiety (9;19). The regulation of feeding behaviour can be divided in

homeostatic and hedonic (or reward-driven) feeding (20). The homeostatic regulation of feeding is important for a stable energy balance and adjusts food intake in order to achieve stability in the amount of body energy stores. It consists of the perception and integration within the CNS of changes in nutrients, hormones and neuropeptides, reflecting alterations in nutrient ingestion or energy stores. Changes in these signals were shown to be involved in feelings of hunger and/or satiety (9;19). The brainstem and hypothalamus are important CNS areas within the regulatory circuit of homeostatic feeding, as they receive and convey signals from the periphery (21;22). Hedonic feeding behaviour is predominantly regulated by the rewarding properties of foods, which involves reward, cognitive and emotional factors and includes corticolimbic circuits in humans (i.e. striatum, amygdala, insula, and orbitofrontal cortex). The central circuits for the homeostatic and hedonic control of food intake include interconnected areas within the CNS. Palatable food can activate reward areas in the CNS, which can be a powerful motivation for food consumption and may overrule signals regulating homeostatic feeding. For instance, in our modern world we no longer eat only when we are (metabolically) hungry, but we often eat in absence of hunger and in spite of large fat reserves. On the other hand, peripheral nutrient and hormonal regulators of homeostatic feeding may also influence the central reward systems and may affect rewarding value of food depending on energy requirements (23;24). It is known that food deprivation or restriction, which induces alterations in food related nutrient and hormonal signalling, increase the reinforcement value of food reward (25;26). Taken together, these two regulating circuits can jointly be considered as a complicated neuroendocrine network of appetitive control (Figure 1) (27).

Circulating peripheral hormones (adipose-tissue and gut-derived hormones) signal information about the nutritional status and energy stores. These are able to directly influence activation in

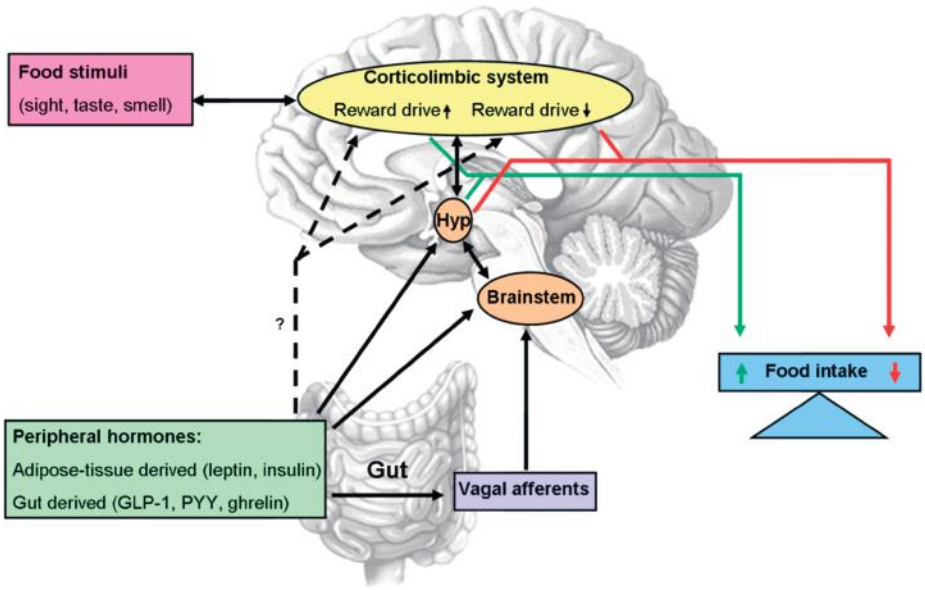

figure 1: Schematic representation of the neuroendocrine network of the control of food intake (*Adapted from Salem & Dhillo* (27))

pivotal CNS areas involved in the homeostatic regulation of energy balance and food intake (i.e. the hypothalamus (Hyp) and the brainstem). Peripheral gut hormones (such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY)) also modulate vagal sensory input into the brainstem, but may also directly access the brainstem and the hypothalamus via areas with a permeable blood-brain barrier (i.e. the area postrema at the level of the brainstem and the median eminence at the level of the hypothalamus). On the other hand, hedonic reward drivers are present within the corticolimbic CNS areas and may overrule the homeostatic system in the regulation of feeding behaviour. These reward drives are stimulated by sensory (food) stimuli and may also be modulated by circulating peripheral hormones.

Glucagon-like peptide-1

Homeostatic signals, such as hormones arising from peripheral organs, convey information about the nutritional status to the CNS. Several peripheral hormones have been identified as regulators in the central control of feeding behaviour, e.g. the adipose-tissue derived hormone leptin, insulin and the gut-hormones ghrelin, cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (19;28-31). The interplay between the gut and the CNS is also known as the gut-brain axis. Gut hormones can directly influence activation in pivotal CNS areas involved in the regulation of feeding by entering the CNS via areas with a permeable blood-brain barrier (i.e. the area postrema at the level of the brainstem and the median eminence at the level of the hypothalamus), but can also indirectly modulate CNS signalling by activation of vagal afferents (Figure 1). In the search for treatments for obesity, these hormones have been studied for their effect on food intake and weight loss, however mostly without success (32-37), except for GLP-1.

GLP-1 is a gut-derived hormone secreted following food ingestion into the circulation. This hormone has been discovered in 1980s and was identified as an incretin hormone (38). The incretin effect is the concept that oral nutrient (glucose) administration induces amplification of insulin secretion from the pancreas compared to a parenteral isoglycemic glucose infusion. This indicates the presence of gut-derived factors that enhance glucose-stimulated insulin secretion from the pancreas. Given the glucose lowering effects of GLP-1, GLP-1-based therapies have been developed for the treatment of diabetes. In addition to glucose lowering effects, treatment with GLP-1 receptor agonists (GLP-1RA) is consistently associated with reduced food intake and appetite and with sustained weight loss in both rodents and humans (39-42).

Discoveries over the last decades from preclinical studies indicate that GLP-1 and GLP-1RA administration have anorectic effects via actions in the CNS (43-49). GLP-1 receptors are present throughout the CNS (43;47) and GLP-1 producing neurons have been found in the nucleus tractus solitarii located in the brainstem (49). The distribution of the GLP-1 receptor has been described in humans (50), but it is unknown whether altered central GLP-1 receptor expression is associated with dysregulation of feeding behaviour and glucose homeostasis, as present in obese T2DM patients.

Currently GLP-1RA's are successfully employed for the treatment of diabetes. Since GLP-1RA treatment is consistently with weight loss (42), GLP-1RA have also been investigated as a treatment option for the treatment of obesity. Recently, treatment with the GLP-1RA liraglutide 3.0 mg has been approved for the treatment of obesity (51). However, this constitutes a higher treatment dosage compared to the indicated dosage for the treatment of diabetes (i.e. 1.8 mg). Although

it was shown that acute administration of GLP-1RA in humans affects CNS activation in response to various food stimuli (52;53), it is not known if endogenous GLP-1 has a physiological role in the central regulation of feeding behaviour in humans. It is also unknown if altered endogenous GLP-1 effects in the CNS may contribute to the development of obesity. In addition, it is unclear if the reported acute effects of GLP-1RA administration on the CNS persist during longer-term treatment.

Roux-en-Y gastric bypass

Among the treatment options for severe obesity, bariatric surgery is currently the most effective therapeutic modality in terms of substantial weight loss and long-term efficacy (54). The most common performed procedure is Roux-en-Y gastric bypass (RYGB) (55). This comprises the formation of a small gastric pouch, resulting in a restrictive component, and the bypass of proximal portion of the small intestine, leading to a malabsorptive component (Figure 2). It is without questioning that these changes in the gastrointestinal tract lead to a reduction in food intake and to some reduction in the absorption of calories. However, it is suggested that the reduction in food intake after RYGB is not only explained by these mechanisms, and that RYGB has additional effects on caloric intake by diminishing appetite via changes in the CNS and endocrine system (56;57). RYGB is associated with favourable postsurgical hormonal changes, such as enhanced postprandial elevations in GLP-1 levels. This increase in GLP-1 levels after RYGB can be explained by the rapid transit of nutrients through the lower gut, which may stimulate a faster and enhanced postprandial release of GLP-1 (Figure 2) (58;59). The favourable hormonal changes after RYGB may contribute to the observed increase in satiety and changes in CNS activation in response to food stimuli (59-62). An important role for endogenous GLP-1 after RYGB is suggested by studies investigating the improved glucose metabolism after RYGB. Improvements in glucose control after RYGB surgery have been observed before noticeable weight loss has occurred (63;64) and it was shown that the exaggerated GLP-1 response is a contributor to the improved glucose tolerance after RYGB in T2DM patient (65). Patient undergoing RYGB can therefore be considered of great interest to investigate the role of the neuroendocrine system in the regulation of food intake and to study how changes in this system, such as elevated levels of endogenous GLP-1, may contribute to improvements in feeding behaviour.

After RYGB nutrients pass through the small gastric pouch, and enter directly the mid-jejunum, by-passing a large part of the stomach and upper small bowel. Only at the common channel the nutrients meet pancreatic enzymes and bile acids. This results in accelerated gastric emptying and rapid entry of undigested food into the jejunum. Consequently, there is enhanced direct contact of nutrients with the surface of L-cells (purple) in this part of the intestine, resulting in (enhanced) Ca^{2+}–dependent stimulation of GLP-1 secretion into intestinal small blood vessels.

The gut-brain axis in the regulation of food intake: The role of GLP-1, from physiology to pharmacotherapy

Aim of thesis

The aim of this thesis was to investigate the effects of endogenous GLP-1 and treatment with GLP-1RA in the CNS regulation of appetite, food intake and consequently body weight in humans.

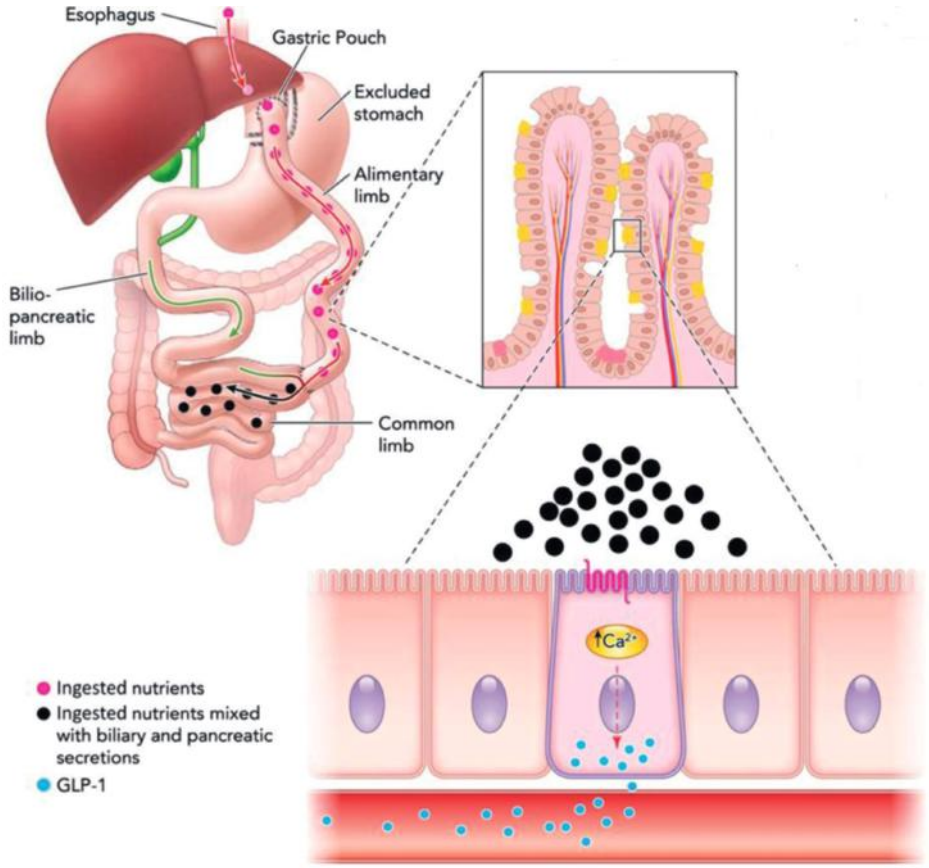

Figure 2: Schematic illustration of the anatomy of RYGB and mechanisms leading to (enhanced) GLP-1 secretion (*Adapted from Manning et al. (59)*)

We hypothesised that endogenous GLP-1 has effects on the CNS, which contribute to the feeling of satiety and that GLP-1 has a physiological role in the regulation of food intake. We also hypothesised that endogenous GLP-1 induced CNS signalling may be altered in obese T2DM patients compared to healthy lean individuals. Furthermore, we hypothesised that after RYGB surgery, the effects of endogenous GLP-1 on the CNS will be increased and contribute to the sustained and large weight reduction observed after this procedure. Finally, we hypothesised that treatment with a GLP-1RA affects the regulation of food intake via effects in the CNS and that this is a mechanism which contributes to the observed weight loss during this treatment.

Outline of the thesis

In **Chapter 2**, we describe the CNS regulation of appetite and food intake and describe the effects of GLP-1 on food intake and body weight. We extensively review the hitherto available literature, mainly from pre-clinical studies, with regard to GLP-1 effects in the CNS regulation of feeding behaviour. Secondly, in **Chapter 3,** we investigated the GLP-1 receptor distribution in the human

post-mortem hypothalamus of normoglycaemic individuals and T2DM patients by means of in situ hybridisation. Next, in **Chapter 4** and **6**, we tested the hypothesis that endogenous GLP-1 has a physiological role in the central regulation of food intake, by modulating activations in the CNS in response to viewing of food pictures and in response to tasting palatable food. Functional magnetic resonance imaging (fMRI) was used to measure CNS activations in response to the different food stimuli and the tests were performed in both healthy lean individuals and obese T2DM patients. In **Chapter 5** and **6** we investigated if treatment with the GLP-1RA liraglutide, in obese T2DM patients affects CNS activations in response to viewing food pictures and in response to tasting palatable food. We also evaluated if these changes are related to the weight loss observed during this treatment and if these effects were maintained after longer-term treatment. In **Chapter 7**, we investigated if the elevated GLP-1 levels after RYGB surgery lead to alterations in the CNS. We studied women before and after RYGB surgery and compared the effects of endogenous GLP-1 on the CNS activation in response to visual and gustatory palatable food stimuli. In **Chapter 8**, in order to evaluate the potential beneficial effects of GLP-1RA treatment on cerebral perfusion, we first evaluated if cerebral perfusion, measured with arterial spin labelling (ASL), in obese T2DM patients is affected compared to healthy lean individuals, both in whole brain volume and/or in areas of the CNS involved in the regulation of feeding behaviour. We then investigated if treatment with GLP-1RA in T2DM patients may improve cerebral blood flow. Finally in **Chapter 9**, we summarise the major findings of this thesis, discuss our data and give directions for future research.

METHODS

Neuroimaging techniques

In this thesis we measured CNS activation in humans in response to viewing food pictures and in response to tasting palatable food. To measure these CNS activation, we used fMRI. This neuroimaging technique is a powerful tool to safely and non-invasively study the CNS processes that underlie human appetitive behaviour.

MRI: In an MR scanner, a magnetic field is generated and magnetic resonance occurs from the interaction of nuclei having a magnetic moment within an external magnetic field. MRI utilises the behaviour of hydrogen nuclei, which consist of single protons that possess angular momentum (spin). Hydrogen makes up 75-80% of the human body, mostly as part of either water or lipids. Within the magnetic field, the protons in tissue tend to align. Generation of MR images requires a radiofrequency pulse at 90 degrees of the main magnetic field, which will lead the protons to align within the radiofrequency pulse, by which they gain energy. After the radiofrequency pulse is switched off, the protons will again realign with the magnetic field, thereby emitting the earlier absorbed energy in the form of a radio wave. This can be measured by a receiver coil and afterwards converted to images. The time needed for the proton to regain their alignment with the magnetic field is the T_1 relaxation time, while the time needed to decay from the radiofrequency pulse is the T_2 relaxation time. T_1 and T_2 vary depending on the proton density and thus the tissue being imaged. With structural MRI, the cerebral anatomy can be visualised with high spatial resolution.

fMRI: fMRI can be used to visualise acute activation within the CNS. It applies the classical MRI technique such that the function or activation of tissues can be analysed. The method makes use of blood oxygen level dependent (BOLD) contrasts, based on the fact that oxygenated and

deoxygenated blood posses different magnetic properties. When neuronal activity occurs in a specific area within the CNS, induced by a stimulus such as viewing or tasting food, it elicits a local haemodynamic response, due to the increased consumption of oxygen by these neurons. The increase in blood flow is greater than necessary for the tissue demands, which results in a locally reduced ratio of deoxyhemoglobin to oxyhaemoglobin concentrations (66). The subsequent local change in magnetic field can be detected using a T_2- weighted imaging sequence (67;68), as the reduced ratio leads to a longer T_2, resulting in an increased image intensity. An increased BOLD signal can be regarded as a marker for increased CNS activation and can be linked to specific stimuli applied or task performed during the imaging.

ASL: We used another MRI technique, i.e. ASL, to measure cerebral perfusion. Series of radiofrequency pulses are applied at the level of the carotid arteries to magnetically label water protons in arterial blood, i.e. before blood reaches the capillary bed. When these protons enter the capillary bed, tissue magnetization is altered which can be measured quantitatively. Therefore, the magnetically labelled protons can be regarded as a diffusible tracer. Two images, the labelled and a (unlabelled) control image are required to generate the perfusion-weighted image by subtraction of these images (69).

fMRI food stimuli paradigms

To investigate effects in the CNS related to the regulation of food intake, we created two food stimuli fMRI paradigms, which were presented to the participants while fMRI was performed. We investigated CNS activation related to visual food stimuli, i.e. viewing food pictures, and we investigated the CNS activation in response to gustatory palatable food stimuli, i.e. tasting chocolate milk. Whilst taste provides an immediate reward for consumed foods which can be a powerful drive for food consumption, the visual characteristics of food are quickly learned and also become powerful secondary reinforcers, capable of influencing subsequent 'food-seeking' behaviour. Thus, showing food images is also a useful way of examining the appetitive reward circuitry within the CNS. This indeed has been confirmed in previous studies, for example investigating the appetitive reward circuitry in obese individuals compared to healthy lean individuals (13;14;70).

Visual food stimuli paradigm: In this task, participants were presented pictures out of three categories: high-caloric food items (ice cream, cakes, chocolate hamburgers pizza, fries), low-caloric food items (apples, oranges, salads, tomatoes, cucumbers) and non-food/neutral items (flowers, trees, bushes, bricks, stones). We analysed the activation in the CNS in response to viewing food pictures in general (i.e. high-caloric food items + low-caloric food items), but also analysed CNS activation in response to only high-caloric food pictures, as this is considered to represent more *hedonic* aspects of food. To evaluate the effects on CNS activation in response to the viewing food pictures *per se*, thus not in response to pictures in general, we subtracted the CNS activation during viewing non-food pictures from the activation during food (or high-caloric food) pictures, thereby creating the contrast for activation during food pictures greater than during non-food pictures (food > non-food). The pictures were presented in a block-design and each block comprised the presentation of seven pictures from one category. In total, each task consisted of the presentation of six blocks per category, which were presented in randomized order.

In our studies, this fMRI paradigm was performed in fasted condition and in postprandial condition, as we were interested in the effects of GLP-1 in these different nutritional states. In addition, this allowed us to study the influence of endogenous GLP-1 and treatment with GLP-1RA on the 'satiating' effect of meal intake in the CNS (i.e. the reduction in CNS activation to visual food stimuli due to meal intake). A standardised liquid meal was chosen, consisting of 450 kcal, carbohydrates 56 gr, fat 17 gr and proteins 18 gr (300ml Nutridrink yoghurt style, Nutricia®, Zoetermeer, The Netherlands). This approximately represents the nutritional value of a (large) breakfast (e.g. two slices of bread, one with cheese and one with jam, and a glas of orange juice) and it eliminates possible differences in the time needed for intake of the meal or the effect of chewing, as this was shown to affect endogenous GLP-1 levels (71;72).

Gustatory food stimuli paradigm: As mentioned above, the taste of palatable food can induce reward related CNS activation, which can be a powerful motivation for food consumption. We therefore investigated the effects of GLP-1 on the CNS activation in response to actual palatable food consumption. We used chocolate milk (Chocomel, FrieslandCampina®, Amersfoort, The Netherlands), as gustatory palatable food stimulus. Chocolate milk is considered a good model for palatable food, as it contains both a high level of sugar and fat, both reinforcers of reward effects of palatable food consumption. Others previously have shown that CNS activation in response to chocolate milkshake is altered in obesity (15;73). We therefore chose a comparable paradigm to investigate the effects of GLP-1. To differentiate the effects from the taste of palatable food solution from the effect of the receipt of a solution in general, we also provided a stimulus with tasteless solution. Thus, we not only investigated the effect of the receipt of chocolate milk, but also created a more narrow contrast evaluating which CNS activation in response to tasting chocolate milk taste was greater compared to the activation in response to tasteless solution receipt (chocolate milk > tasteless solution). The tasteless solution was designed to mimic the natural taste of saliva (consisting of 2.5 mM $NaHCO^3$ and 25 mM KCl (15)) and should provide a superior neutral condition compared to water, which has a taste that activates the gustatory cortex (74;75).

During one fMRI task, the receipt of each solution consisted of 0.4 mL and the participants received per task in total 20 deliveries of chocolate milk and 20 of tasteless solution in randomised order.

Interventions and treatments

In this thesis we studied the effects of endogenous GLP-1 in healthy lean individuals and obese T2DM patients. We also investigated changes in endogenous GLP-1 effects after RYGB surgery. and the effects of treatment with the GLP-1RA. liraglutide.

Endogenous GLP-1: In a placebo-controlled, randomised intervention study we investigated the effects of endogenous GLP-1. We used administration of the synthetic selective GLP-1 receptor antagonist exendin 9-39, which was administered intravenously at a rate of 600 pmol/kg/min. Measurements during exendin 9-39 infusion were compared to measurements during placebo infusion, in order to determine the effect of blocking endogenous GLP-1 signalling. This allowed us to investigate the effect of endogenous GLP-1. In each study, the order of infusion was randomised and the participants were blinded for the type of infusion. To evaluate if the effect of endogenous

GLP-1 may increase due to enhanced GLP-1 secretion, we studied patients undergoing RYGB and compared the endogenous GLP-1 effects in the CNS *before* RYGB and *after* RYGB.

GLP-1RA treatment: To evaluate the effects of treatment with GLP-1RA, we investigated treatment with liraglutide. Liragluitde has 97% amino acid homology to native GLP-1 and is therefore considered a true GLP-1 analogue (76). We investigated treatment with liraglutide 1.8 mg, a dosage indicated for the treatment of diabetes, which was achieved after two weeks dose escalation (0.6 mg per week). Liraglutide is administered once daily by subcutaneous injection. In order to separate the pharmacological effects of GLP-1RA from body weight changes during this treatment, we performed measurements after short-term treatment (i.e. 10 days), before weight changes have occurred, and after longer-term treatment (i.e. 12 weeks), after weight changes have occurred. Because treatment with GLP-1RA lowers glucose levels in T2DM patients, we compared the treatment with an active comparator, i.e. insulin glargine, to achieve an isoglycaemic state during both treatments. Insulin glargine is a long-acting insulin and its administration is comparable to liraglutide (once-daily, subcutaneous injection). Patients were instructed to increase the daily dose based on their fasting self-monitored blood glucose levels according to a predetermined algorithm (77).

We compared the treatments in a randomised, cross-over study. Each treatment period consisted of twelve weeks, with a twelve week wash-out period in between, which was considered long enough to eliminate carry-over effects. To further eliminate possible carry-over effects, effect of order of treatment was modelled in the analyses and investigated.

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