Insulin sensitivity : modulation by the gut-brain axis

Heijboer, A.C.

Chapter 1

General introduction

Contents

1 Introduction
2 Obesity and type II diabetes mellitus
3 Regulation of glucose metabolism
- 1 Glucose homeostasis
- 2 Nutritional status
- 3 Obesity, insulin resistance and type II diabetes
- 4 Therapies for insulin resistance

4 Gut-brain axis
- 1 Brain and food intake
- 2 Brain and glucose metabolism
- 3 Gastrointestinal hormones and food intake

5 Outline of the present thesis

1. Introduction

Maintenance of plasma glucose concentration is highly important for normal body physiology. Glucose is under normal circumstances the only energy source for the brain. The brain is unable to store glucose and is therefore dependent on glucose derived from the circulation. In the control of glucose homeostasis, insulin is an important hormone. Insulin stimulates glucose uptake by tissues like skeletal muscle and adipose tissue, and inhibits glucose production by the liver. The extent of action of insulin on glucose uptake and glucose production is determined by tissue insulin sensitivity. Physiologically, insulin sensitivity can be influenced by many factors, like obesity, FFA concentrations, glucoregulatory hormones, etc. Pathophysiological changes in insulin sensitivity are seen in obesity and type II diabetes mellitus.

The studies in this thesis were performed to investigate the role of feeding status in crosstalk with the gut and the brain in the modulation of insulin sensitivity. In this chapter, a brief review is given of the involved diseases, obesity and type II diabetes mellitus (section 2), and of regulation of glucose metabolism (section 3). In this latter part, glucose homeostasis, nutritional status, insulin resistance and therapies for insulin resistance are discussed. In section 4, the current knowledge of gut-brain interactions and food intake is summarised. This chapter ends with the outline of the present thesis.

2. Obesity and type II diabetes mellitus

Evolution has provided humans with physiological mechanisms to survive times of scarcity of food. The purpose of these mechanisms is to conserve energy, seeking food in times of scarcity and storing energy in times of abundance. Hence, this system leads towards storage of fat and weight gain in conditions of caloric excess. During the last few decades, unique circumstances and lifestyle alterations have developed from an evolutionary perspective in industrialised countries. In contrast to previous eras there is plenty of food and physical activity is reduced. This maladaptive combination of genes to survive periods of scarcity and an environment with abundant dietary calories has led to an increased incidence of overweight and obesity.

Overweight and obesity are commonly assessed by using body mass index (BMI), defined as the quotient of weight in kilograms and the square of height in meters (kg/m^2). A BMI over 25 kg/m^2 is defined as overweight and a BMI over 30 kg/m^2 as obese.

Globally, obesity has reached epidemic proportions, with more than 1 billion overweight adults (more than 300 million are obese among them). Childhood obesity is already epidemic in some areas and on the rise in others. According to the US Surgeon General, in the USA the number of overweight children has doubled and the number of overweight adolescents has trebled since 1980 (1). Recent data show that in the Netherlands, 46.5% of the population is overweight and 10.9% of the population is obese. Dramatically increasing percentages of obese youngsters are seen as well (2).

Obesity is a major risk factor for developing chronic diseases, including cardiovascular disease, hypertension and stroke, certain forms of cancer, and type II diabetes mellitus. Ninety percent of the patients with type II diabetes mellitus are obese or overweight. Type II diabetes mellitus now affects obese children even before puberty. Retinopathy, kidney failure, heart disease, neuropathy and foot diseases are major complications of diabetes. These complications decrease quality of life, and increase the risk for premature death. Diabetes mellitus is the sixth leading cause of death with 3.2 million deaths world-wide every year (1;3).

3. Regulation of glucose metabolism

1. Glucose homeostasis

It is highly important for normal body physiology to keep a constant blood glucose level. As the brain has no endogenous glucose supply and is a major consumer of glucose, it is dependent on glucose derived from the circulation. Plasma glucose concentration is maintained within narrow limits by a fine balance between endogenous (hepatic) glucose production and peripheral glucose utilisation. During fasting, glucose is the obligatory fuel that provides more than 90% of energy needed for brain function (4;5). The liver produces this obligatory amount of glucose by glycogenolysis and gluconeogenesis (6). Glycogenolysis is the process of breakdown of glycogen via glucose-6-phosphate to free glucose, gluconeogenesis is the process of generating new molecules of glucose from intermediates derived from the catabolism of glycerol and some amino acids (7). Glucose is also taken up by peripheral tissues, like skeletal muscle, adipose tissue and heart tissue. A small amount of glucose can be stored in skeletal muscle and the liver, as the polysaccharide glycogen, to provide a reserve supply of energy.

Glucose balance is tightly regulated by the interaction of different regulatory mechanisms, such as the classical glucoregulatory hormones, like insulin, glucagon, catecholamines, cortisol and growth hormone. Insulin inhibits endogenous glucose

production and stimulates glucose uptake in skeletal muscle and adipose tissue. Insulin inhibits gluconeogenesis by inhibiting the transcription of the main gluconeogenic enzyme, phosphoenolpyruvate caboxykinase and by increasing the transcription of the main glycolytic enzyme, pyruvate kinase (8) (9). In addition, insulin decreases hepatic uptake of precursor amino acids and their availability from muscle (10). Insulin stimulates glucose uptake by binding to insulin receptors in the plasma membrane of skeletal muscle or adipose tissue. This binding triggers a variety of signal transduction pathways, which ultimately results in fusion of glucose transporter-4 (GLUT-4) with the plasma membrane. The increased number of plasma-membrane glucose transporters causes a higher rate of glucose movement from the extracellular fluid into the cells (11).

In addition to these effects on peripheral tissues, insulin affects neuropeptides in the hypothalamus involved in regulating food intake and energy expenditure (see also paragraph *'brain and glucose metabolism')*. More than 140 years ago, Claude Bernard (12;13) punctured the fourth ventricle in rabbits, which resulted in glucosuria. Although these striking findings suggested a key role for the brain in glucose homeostasis, its importance was largely neglected after the discovery of insulin in 1922. However, new findings have revived interest in the role played by the brain, in particular the hypothalamus, in both glucose metabolism and the mechanism linking obesity to type II diabetes mellitus (14;15).

2. Nutritional status

With regard to nutritional status, there are two functional states: the absorptive state, during which ingested nutrients are entering the blood from the gastrointestinal tract, and the postabsorptive state, during which the gastrointestinal tract is empty of nutrients and energy must be supplied by the body's own stores.

During the absorptive state, glucose is the major energy source of the body. During this phase, glucose taken up by skeletal muscle is in part oxidised and in part stored as glycogen. In adipocytes, the most important fate of glucose in the absorptive state is the transformation to fat (triglycerides (TG)) for storage. The transformation of glucose to TG is called lipogenesis. The liver takes up glucose as well and stores it either as glycogen or transforms it to TG. Most of this liver-TG is secreted as very low density lipoproteins (VLDL) into the blood. However, *de novo* lipogenesis contributes to only ~5 percent of VLDL-TG, whereas the major part of VLDL-TG is derived from reesterification of fatty acids derived from adipose tissue. VLDL-TG are taken up by peripheral tissues depending on tissue specific activity of lipoprotein lipase. Ingested TG will directly be transported as chylomicrons to

peripheral tissues, especially adipose tissue, for storage and in other tissues, like the heart, for oxidation. During the absorptive state insulin levels are increased, thereby stimulating glucose uptake and glycogen synthesis and inhibiting glucose production. Insulin also stimulates lipogenesis and inhibits lipolysis (catabolism of TG into glycerol and fatty acids) and VLDL production. In this way, insulin lowers plasma glucose levels and promotes the storage of FFA/TG in fat, liver and skeletal muscle. When the absorptive state ends, synthesis of glycogen and fat stops and net catabolism occurs.

In the postabsorptive state and during prolonged fasting, the gastrointestinal tract is empty, resulting in cessation of glucose absorption from the intestine. However, glucose concentrations must be maintained within narrow limits to preserve normal functioning of the body. When glucose concentrations decrease to low values, alterations of neural activity ranging from slight impairment of mental function to coma and even death may occur (16). There are two ways to keep glucose concentrations at a constant level, stimulation of glucose production and inhibition of glucose uptake. During the postabsorptive state glucose is produced by the liver through glycogenolysis and gluconeogenesis (6). The increase in gluconeogenesis is facilitated by low insulin concentrations present during fasting. This also results in a decrease in glucose uptake by insulin dependent tissues such as skeletal muscle and adipose tissue. Consequently, glucose is available for non-insulin dependent tissues such as the brain (4;5). In addition, lipolysis (catabolism of TG into glycerol and fatty acids) increases in adipose tissue, resulting in increased release of fatty acids from adipose tissue, which can be used by muscle and other tissues for energy supply. The liver can transform these fatty acids into ketone bodies by β-oxidation, and release them into the blood or convert them in VLDL-TG (see above). During prolonged fasting, ketone bodies are an important energy source for many tissues, including the brain (5).

3. Obesity, insulin resistance, and type II diabetes

Energy intake, which the body derives from food, is required to match energy expenditure, necessary for physical activity and other body functions. When energy intake exceeds expenditure, energy is stored in the form of adipose tissue to be utilised in conditions of food scarcity or increased energy demand. Overweight and obesity are outcomes of long-term excess of energy intake relative to energy expenditure. Particularly fat and energy intake are strongly and positively associated with body weight gain. A high fat (energy-dense) diet is an independent risk factor for overweight (17). At the metabolic level, the imbalance between energy intake and

energy expenditure, which leads to energy deposition in form of adipose tissue, can be seen as an imbalance between fat deposition and fat oxidation. Fat oxidation occurs predominantly during the postabsorptive state, whereas fat deposition is stimulated during the absorptive state. Obesity is an important determinant of insulin resistance and represents the most important risk factor for the development of type II diabetes mellitus (18-20).

Insulin resistance reflects a condition with reduced biological effects of insulin (21). Different tissues may have different tissue-specific sensitivities to the actions of insulin. As insulin normally inhibits endogenous glucose production, hepatic insulin resistance is characterised by diminished inhibition of glucose production by insulin. In peripheral tissues, especially skeletal muscle and adipose tissue, insulin resistance is characterised by decreased insulin-mediated glucose uptake. With regard to lipid metabolism, the inhibitory effects of insulin on lipolysis and VLDL production are decreased and insulin-mediated lipogenesis is also decreased. Both genetic and environmental factors, such as dietary habits, are involved in tissue-specific insulin sensitivity. Up till now, with the exception of rare monogenic variants, the inherent susceptibility to type II diabetes mellitus is considered to be attributable to complex interacting genetic determinants.

Insulin resistance is a major determinant of the pathophysiology of type II diabetes mellitus. Insulin resistance results in the inability of circulating insulin to properly suppress hepatic glucose production and to stimulate the disposition of glucose (and other metabolic fuels). This leads to progressive hyperglycaemia, and therefore more prolonged stimulation of pancreatic β-cells. When β-cell compensation ultimately fails, glucose levels rise even more, leading to either impaired glucose tolerance or overt diabetes.

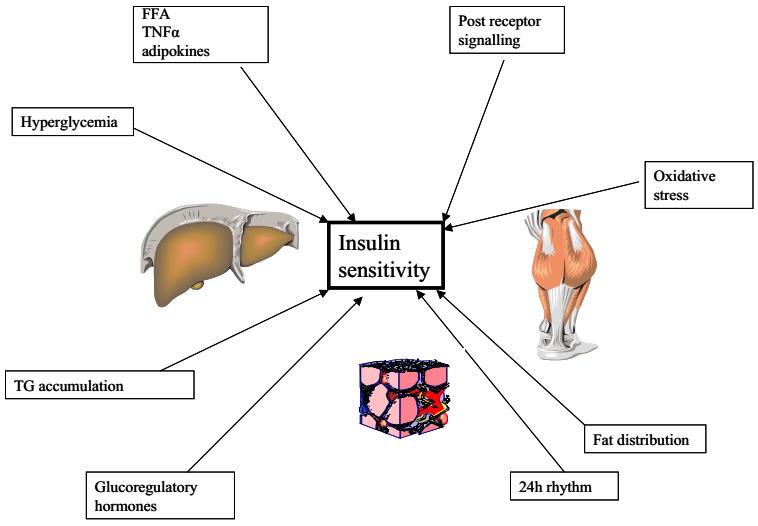

Figure 1. Mechanisms that are involved in regulating insulin sensitivity.

The interaction between overweight and insulin resistance is complex and involves several epidemiological associations (figure 1). Briefly reviewed, these are:

- Fat distribution: Patients with central adiposity have higher insulin levels and are more insulin resistant than subjects with similar weight but with a peripheral type of obesity (22-24).
- Plasma FFA levels: The extent of (direct) exposure of liver and muscle cells to FFA concentrations might be involved in mediating tissue specific insulin resistance. For instance, experimental elevation of FFA induces insulin resistance (25-27). At the cellular level, FFA and their metabolic products can reduce insulin signalling in muscle and liver (27).
- Ectopic triglyceride accumulation: TG content of skeletal muscle and liver correlates directly with insulin resistance (27-31). These observations suggest that accumulation of fat in liver and muscle tissue might (partly) mediate obesity-induced insulin resistance.
- Adipokines: Another major mechanism linking obesity to insulin resistance is a group of peptides, made by fat cells that alter insulin sensitivity. Adiponectin has been shown to reduce insulin resistance and reduced levels of adiponectin are found in progressive obesity (32;33). Tumor necrosis factor-alpha (TNFα), interleukin-6, resistin and leptin increase insulin resistance (34). Elevated levels of these adipocytokines are observed with obesity (34;35). Various adipose tissue beds produce different amounts of these peptides, perhaps adding to the regional differences in the contribution of these adipose depots to insulin resistance.
- Hyperglycaemia: Hyperglycaemia itself is known to induce insulin resistance (36). This partially reversible phenomenon is known as glucose toxicity. In β-cells, oxidative glucose metabolism will always lead to production of reactive oxygen species, normally detoxified by catalase and superoxide dismutase. Because these enzymes are present in low amounts in β-cells, hyperglycaemia can result in the production of large amounts of reactive oxygen species in β-cells, with subsequent damage to cellular components.
- Number of insulin receptors, post-receptor signalling by insulin and synthesis and translocation of GLUT4: It is suspected that alterations in de expression and/or function of these factors underlie insulin resistance in obesity as well as in type II diabetes. Among the many molecules involved in the intracellular processing of the signal provided by insulin, insulin receptor substrate (IRS)-2, the protein kinase B (PKB)-beta isoform and the forkhead transcription factor Foxo1a (FKHR) are of particular interest in this context as recent data

have provided strong evidence that dysfunction of these proteins results in insulin resistance in vivo (37;38).

- Glucoregulatory hormones: Glucocorticoids (39), sex steroids (40), growth hormone (41), and catecholamines (42;43) influence tissue insulin sensitivity.
- Oxidative stress and vascular reactivity: These factors have also been suggested to be involved in the development of insulin resistance (44-46). However, oxidative stress, vascular reactivity, inflammation and insulin resistance seem to be interrelated and more research is needed to elucidate this relationship.
- Diurnal rhythms: It is recently shown in healthy humans that insulin sensitivity changes rhythmically during the day (47).

4. Therapies for insulin resistance

As the mechanisms underlying the development of insulin resistance are not clear, a therapy that directly targets these mechanisms does not exist. A major goal of therapeutic intervention in diabetes is to reduce circulating glucose levels. Lifestyle changes are the first step towards a reduced risk of developing diabetes or better prognosis for diabetes patients. Lifestyle changes are an ideal method of diabetes prevention because of its beneficial effects on cardiovascular risk factors as well as on other benefits related to weight loss and an improved diet (48). Weight loss in obese patients with diabetes can improve survival. In addition, exercise also improves insulin sensitivity by increasing glucose uptake into skeletal muscle (11). However, these interventions require a strong will as lifestyle modification has been difficult to maintain over a long term. Weight loss is not maintained once exercise or diet has been discontinued, and symptoms of diabetes will recur. Therefore pharmacological strategies are required in addition to exercise or diets.

Oral hypoglycemic drugs, such as (combinations of) metformin, acarbose, sulfonylurea's, thiazolidinediones, and anti-obesity agents (like orlistat) are currently used as pharmacological treatment for diabetes. However, none of these treatments is perfect. Recently, a meta-analysis was performed, in which studies were included that have investigated the effects of several different drug classes on type 2 diabetes incidence (48). Oral hypoglycemic medications and orlistat were the only drugs that had been studied in randomised controlled trials with diabetes incidence as the primary end point. The available evidence suggests that oral hypoglycemic drugs may reduce diabetes incidence compared with placebo. The adequately powered studies showed significant decreases in diabetes incidence with metformin, acarbose,

troglitazone, and orlistat. However, they concluded that the data are not definitive and that no single agent can currently be recommended for diabetes prevention (48).

Interestingly, recent reports show that gastrointestinal hormones appear to have effects both on food intake and glucose metabolism (see next paragraph). Therefore, these hormones might be interesting for therapeutic goals in the battle against type II diabetes mellitus.

4. Gut-brain axis

1. Brain and food intake

Food intake is largely regulated by the central nervous system.

Lesion experiments in the 1950's showed that lesions of the ventromedial nucleus resulted in uncontrollable hyperphagia and obesity, whereas lesions of the lateral hypothalamus resulted in anorexia and weight loss (49). These experiments were the basis of the early concepts of hypothalamic appetite regulation. Although these concepts were a gross oversimplification, the hypothalamus is still regarded as an important feeding center of the brain.

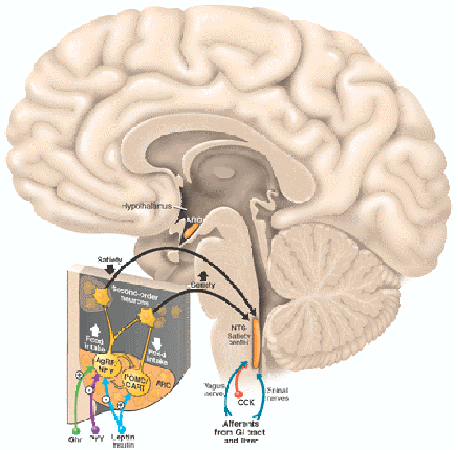

Figure 2. Central command centers. Reprinted with permission from Marx, SCIENCE 299:846 (2003). Illustration: Katharine Cutliff. Copyright 2003 Science.

The hypothalamus consists of several nuclei involved in regulating food intake, including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamic area (LHA), the ventromedial nucleus (VMH), and the dorsomedial nucleus (DMH).

Located at the bottom of the hypothalamus, around the 3rd ventricle, the ARC can be found (see figure 2). ARC neurons are called 'first-order' neurons, because of their 'direct' contact with peripheral satiety factors. The 'second-order' neurons can be found in the PVN, LHA, VMH and DMH. Within the ARC, at least two populations of 'first-order' neurons controlling appetite are characterized: 1) neurons co-expressing Agouti-related peptides (AgRP) and neuropeptide Y (NPY) and 2) neurons co-expressing pro-opiomelanocortin (POMC), the molecular precursor of

alpha-melanocyte stimulating hormone (α-MSH). The first neuronal circuit (AgRP/NPY) stimulates food intake and the other neuronal circuit (POMC/α-MSH) inhibits food intake (50). There is direct interaction between the NPY/AgRP pathway and the POMC/α-MSH pathway (see figure 3). During fasting conditions, the expression of these neuropeptides is altered; fasting results in an increase in NPY and AgRP mRNA expression and a decrease of POMC mRNA expression levels in the hypothalamus (51). Together, during fasting, food intake is stimulated.

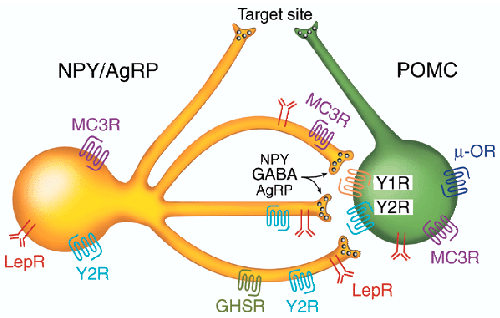

Figure 3. Anatomy and regulation of the NPY and POMC system. Reprinted with permission from Cone, Nat Neuroscience 571-578 (2005). Copyright 2005 Nature Publishing Group

Mutations disrupting these hypothalamic pathways cause obesity in rodents and humans. Examples are obese POMC-/- and MC4R-/- mice (52;53) and humans with POMC, MC4R and CART mutations which are associated with obesity (54-58).

2. Brain and glucose metabolism

The central nervous system is suggested to play a key role in the control of glucose metabolism via brain pathways that overlap with those controlling food intake and body weight (59). The brain is an insulin-sensitive organ. Insulin provides afferent input to the CNS regarding the sufficiency of body fat stores. Receptors for insulin are concentrated in hypothalamic areas. Intracerebroventricular administration of low doses of insulin reduces food intake and body weight (60). Insulin has been shown to increase POMC gene expression, that is normally decreased during fasting, and inhibit the expression of mRNA levels encoding the orexigenic peptide neuropeptide Y (NPY) that are normally increased in the ARC during fasting (61-63).

Brain insulin action nowadays is hypothesised as a requirement for intact glucose homeostasis. Okamoto et al. showed that selective expression of insulin receptors reduces diabetes severity (64). Chronic blockade of hypothalamic insulin receptor signaling was shown to cause hepatic insulin resistance and to increase hepatic glucose production (65;66). In contrast, acute depletion of insulin receptors in the liver impaired downstream insulin signalling, but failed to alter the effect of physiological hyperinsulinemia on the rate of glucose production (67). The

importance of neuronal insulin signalling is further underlined by evidence that mice with neuron-specific insulin receptor deletion are overweight, insulin-resistant, and glucose-intolerant (68).

3. Gastrointestinal hormones and food intake

Hormones secreted from peripheral tissues bind to receptors located in the hypothalamus (see figure 3) (69;70). These hormones are secreted from the pancreas (like insulin), from adipose tissue (like leptin) or from the gastrointestinal tract. In this thesis we focus on the last group of hormones.

Because of permeable blood brain barrier at the bottom of the ARC and the presence of these receptors in the ARC, the AgRP/NPY and POMC/CART neurons can be reached and influenced by these hormones (71). Gastrointestinal hormones may also act indirectly to influence the activity of afferent neuronal pathways and brain stem circuits, which in turn project to the arcuate nucleus (72;73).

Via the hypothalamus these hormones are able to affect food intake (see figure 4). Extensive reviews have been written about gastrointestinal hormones and the regulation of food intake recently (69;74-76). Here, we will give an overview of these hormones and their effects on food intake.

Cholecyctokinin (CCK), which is released from the upper small intestine (duodenal and jejunal mucose) by I cells (77) (78), was the first gastrointestinal hormone shown to decrease food intake. CCK is thought to interact with CCK-1 receptors on vagal sensory fibers, with the signal being relayed to the brainstem. Consistent with this notion, the anorectic effects of CCK can be eliminated by subdiaphragmatic vagotomy or selective damage to vagal afferent nerves. Likewise, lesions of the brainstem area that receives vagal sensory afferents, attenuate CCK-elicited anorexia. Within the brain, recent data suggest that melanocortin-4 receptors (MC4) modulate CCK's action (79-82).

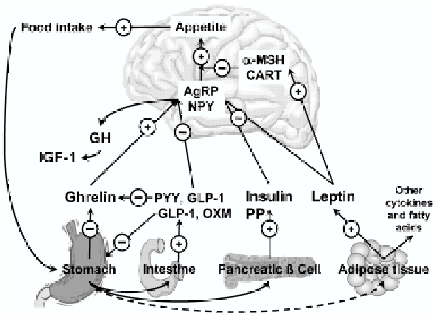

Figure 4. The gut-brain interaction in the regulation of appetite and body weight. Reprinted with permission from Hanusch-Enserer Eur J Clin Invest 35:425-430 (2005). Copyright 2005 Blackwell Publishing Group.

Peptide YY (PYY_{3-36}) is released from L-cells of the distal gut upon feeding. Recently, there has been a lot of discussion about the effects of the gut hormone PYY_{3-36} on food intake (83). However, there is more or less consensus now that PYY_{3-36}

decreases food intake in both rodents (peripheral and central administration) and humans (84-86). To inhibit feeding, PYY_{3-36} may act through the Y2 receptor, a putative inhibitory presynaptic receptor that is highly expressed on NPY neurons in the ARC.

Glucagon Like Peptide 1 (GLP-1) is a proglucagon-derived hormone that is also secreted from the L-cells of the distal gut upon meals and is known to decrease food intake in rodents and humans (87;88). GLP-1 binds to the GLP-1 receptor, that is found in the periphery (gut and endocrine pancreas) and is widespread throughout the central nervous system. The anorectic actions of GLP-1 are probably mediated through both peripheral and central mechanisms. A population of neurons that synthesise GLP-1 is located in the brainstem and projects to hypothalamic and brainstem areas important in the control of energy homeostasis (89;90). GLP-1 is also known to affect glucose metabolism. Numerous studies have shown, that GLP-1 can improve glucose-stimulated insulin secretion and lower fasting and postprandial blood glucose levels in individuals with type 2 diabetes (91;92). Therefore, there is a lot of interest in this peptide for therapeutic goals in type 2 diabetes mellitus. (93;94).

Glucagon Like Peptide 2 (GLP-2) is another product from proglucagon, and is secreted in parallel with GLP-1 from the L-cells of the distal gut. When centrally applied, GLP-2 inhibits food intake (95), which study provides evidence that GLP-2 serves as a neurotransmitter in a distinct ascending pathway linking viscerocepti ve neurons of the brainstem with a hypothalamic target. Recently, a few studies have been performed in which GLP-2 was peripherally administrated in humans. However, these three studies could not find effects of peripheral GLP-2 on appetite, energy intake or satiety (96-98).

Oxyntomodulin (OXM) is a gastrointestinal hormone that is, just like GLP-1 and GLP-2, a product of post-translational processing of preproglucagon and released from the L-cells in response to food ingestion and in proportion to meal calorie content (99;100). OXM inhibits food intake both in rodents and in humans after peripheral administration (101-103). It is currently unclear through which receptor OXM mediates its actions. There is evidence that circulating OXM could mediate its anorectic actions via direct interaction with the hypothalamus, activating POMC neurons within the ARC (103).

Ghrelin, identified in 1999, and released from the X/A-like cells from the stomach, especially just before a meal (104), is, both in rodents and humans, the only peptide hormone found to stimulate appetite when administered peripherally (105-108). To enhance appetite, peripheral produced acylated ghrelin acts in the hypothalamus where it promotes NPY and orexin gene expression and inhibits POMC/α-MSH

expression via activation of the growth hormone secretagogue receptor (GHS-R) (109-115).
Since gastrointestinal hormones influence appetite and food intake in interaction with the brain, especially the ARC, and recent reports point to a central role of the brain in the regulation of insulin sensitivity, we hypothesised that gut-brain interactions might also be involved in the regulation of insulin sensitivity, independently of their effects on food intake and body weight.

5. Outline of the present thesis

The aim of this thesis was to gain more insight in the role of feeding status and gut-brain interaction in the modulation of insulin sensitivity. There is growing evidence that neuropeptides which are situated in the hypothalamus, and gastrointestinal hormones which act on the hypothalamus, and are involved in regulating food intake, seem to be involved in regulating insulin sensitivity as well. Therefore, we first characterized the effects of feeding status itself on insulin sensitivity, and subsequently the effects of some of the signals for feeding status in the gut-brain axis, on insulin sensitivity.

In **chapter 2**, we investigated the effect of fasting on insulin sensitivity in mice. During fasting FFA concentrations and liver TG content are increased. In obesity, increased FFA concentrations and excessive tissue TG storage are associated with tissue insulin resistance. The impact of fasting on tissue insulin sensitivity is unknown. Therefore, we studied the effects of 16 hr of fasting (prolonged fasting) versus 4 hr of fasting (postprandial state) on hepatic and muscle insulin sensitivity in wild-type mice in vivo in relation to tissue TG accumulation and changes in mRNA expression of transcription factors and related proteins involved in glucose and lipid metabolism.

In **chapter 3**, the effects of a 2 week high fat diet on insulin sensitivity in relation to hypothalamic neuropeptides are presented. Studies in rats and dogs on a high fat diet show the induction of hepatic insulin resistance as an early event, followed by muscle insulin resistance later. The question was whether this primacy of hepatic insulin resistance in relation to changes in TG content is also present in mice. Secondly, the aim of this study was to evaluate whether the NPY/POMC circuitry is involved in the induction of insulin resistance during a high fat diet, by measuring

mRNA expression levels of these neuropeptides in the hypothalamus of mice after 2 weeks of high fat diet.

In **chapter 4**, we describe the effect of icv administration of MTII, a synthetic analogue of α-MSH, on insulin sensitivity. It is known that NPY can induce hepatic insulin resistance. However, whether the POMC pathway has effects on insulin sensitivity, independently of changes in food intake and body weight is not investigated. This study was performed to answer that question.

In **chapter 5** we evaluated the effects of acute administration of the gut-hormone PYY_{3-36} on insulin sensitivity. PYY_{3-36} inhibits NPY and activates POMC neuronal activity to inhibit food intake. As both NPY and the POMC pathway affect insulin sensitivity, the aim of this study was to evaluate whether PYY_{3-36} can affect insulin sensitivity independently of its effects on food intake.

In **chapter 6** we focussed on the effects of long-term administration of PYY_{3-36} on insulin sensitivity. A prerequisite for a drug against obesity and insulin resistance is that it has long-term effects. We administered PYY_{3-36} for 7 days, either continuously via subcutaneous mini-pumps or intermittent via daily subcutaneous injections to measure its long-term effects on insulin sensitivity.

In **chapter 7** we investigated whether ghrelin and des-ghrelin, produced by the stomach might affect insulin sensitivity in mice. Ghrelin promotes neuropeptide Y (NPY) gene expression and inhibits pro-opiomelanocortin (POMC)/αMSH expression via activation of the GHS-receptor and thereby stimulates food intake. Our question was whether ghrelin might affect insulin sensitivity. To detect a potential mechanism, we investigated whether GHRP-6, an agonist of the GHS-receptor can also influence insulin sensitivity. Des-ghrelin has not been seen as a bio-active hormone until recently. There are very recent publications that des-ghrelin might affect glucose production in hepatocytes. Therefore, the second aim of this study was to investigate the role of des-ghrelin in the regulation of insulin sensitivity.

In **chapter 8**, the results of the above mentioned studies are summarised and put into perspective.

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