Chapter 1

General introduction
Glucose homeostasis

Blood glucose levels are tightly regulated as a part of metabolic homeostasis. Mean normal blood glucose levels in humans are about 90 mg/dl, equivalent to 5 mmol/l. Glucose levels rise after meals for an hour or two by a few mmol and are usually lowest in the morning, before the first meal of the day. Transported via the bloodstream from the intestines or liver to body cells, glucose is the primary source of energy for body cells, fats and lipids being primarily a compact energy store. Failure to maintain blood glucose in the normal range leads to conditions of persistently low (hypoglycemia) or high (hyperglycemia) blood sugar. Since the brain functions on glucose, hypoglycemia is dangerous and can cause seizures, loss of consciousness, and death. Hyperglycemia, on the other hand, can lead to dehydration and hyper-osmolality, which can eventually result in a coma. Chronic hyperglycemia at levels more than slightly above normal can produce a very wide variety of serious complications over a period of years, including kidney damage, neurological damage, cardiovascular damage, and loss of vision.

Normal glucose homeostasis is regulated by endogenous glucose production and the uptake and utilization of glucose by peripheral tissues. In the fasted state glucose is mainly derived from the liver by 2 pathways, glycogenolysis and gluconeogenesis. Glycogenolysis is the biochemical breakdown of stored glycogen to glucose, while gluconeogenesis is the process of generating new glucose molecules from precursor molecules like amino acids, lactate, and glycerol. Glucose disposal takes place in peripheral tissues like skeletal muscle, adipose tissue, and heart tissue. The initial fate of glucose in tissues is essentially limited to glycolysis (catabolism of glucose) and direct storage either as glycogen or as the glycerol moiety of triglycerides in adipose tissue. The latter, however, accounts for only about 5% of postprandial glucose disposal, whereas liver and muscle are the sites of glycogen formation.

The homeostatic mechanism which keeps the blood value of glucose in a narrow range is composed of several interacting systems, of which hormone regulation is the most important. Two types of mutually antagonistic metabolic hormones affect blood glucose levels; they include the catabolic hormones (such as glucagon), and the anabolic hormone insulin. Insulin secretion by the pancreatic β-cells is stimulated after a meal. Then, insulin inhibits glycogenolysis and gluconeogenesis, and as a result inhibits endogenous glucose production. In addition, insulin stimulates glucose disposal in peripheral tissues. The net result of these actions of insulin is a decrease in plasma glucose levels. In response, glucagon is secreted by the pancreatic α-cells, which stimulates glycogenolysis and glucose production by the liver. Collectively with the interplay of other catabolic hormones (like growth hormone, cortisol, and catecholamine), endogenous glucose production is kept at a relatively constant level.
Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) has become a global epidemic rapidly. Now the disease affects 171 million individuals globally with an estimated mortality of about 4 million deaths each year. Having T2DM increases the risk for complications including heart disease, retinopathy, neuropathy, and nephropathy. T2DM has become one of the major causes of premature illness and death, mainly through the increased risk for cardiovascular disease. The World Health Organization (WHO) predicts that diabetes deaths will increase by more than 50% worldwide in the next 10 years. T2DM is a chronic, multifactorial disease characterized by a combination of impaired insulin secretion by the pancreatic β-cells and insulin resistance of target organs, leading to hyperglycemia. The diagnosis of having diabetes mellitus is based on WHO recommendations from 2006. Criteria include fasting plasma glucose levels ≥ 7.0 mmol/l or 2-h plasma glucose levels ≥ 11.1 mmol/l after a glucose load.

The development of T2DM is the result of both a deficient insulin secretion and insulin resistance. Insulin resistance is a state of reduced sensitivity in peripheral tissues of the body to the action of insulin. Muscle and adipose tissue in particular display a decreased responsiveness to insulin mediated glucose disposal. Resistance of the liver to the effects of insulin will increase endogenous glucose production. In the early stage of the development of T2DM, insulin resistance leads to an increase in insulin secretion by the β-cells to overcome the impairment of glucose disposal and endogenous glucose production and thereby maintaining normal glucose levels. Later in the development β-cells fail to compensate, leading to progressive hyperglycemia and ultimately T2DM.

Consequences of insulin resistance can be viewed in two ways. First, during fasting, the liver represents a major site of insulin resistance, which is reflected by overproduction of glucose despite presence of both fasting hyperinsulinemia and hyperglycemia. This accelerated rate of hepatic glucose output is the primary determinant of elevated fasting plasma glucose concentration in T2DM. Second, with eating, failure of adequate insulin-mediated nutrient disposal into skeletal and adipose tissue combined with an attenuated inhibition of hepatic glucose production cause the raised postprandial glyceremia.

T2DM and lipid metabolism

Besides glucose metabolism, lipid metabolism is also disturbed in T2DM patients. Increased plasma levels of triglycerides (TG) in very low density lipoproteins (VLDL) are characteristic of the dyslipidemia associated with insulin resistance and T2DM. Overproduction of VLDL leads to increased plasma levels of TG which, via an exchange
process, results in low levels of high density lipoprotein cholesterol and the generation of small, dense, cholesterol ester depleted low density lipoproteins. Increased assembly and secretion of VLDL by the liver results from the complex, post-transcriptional regulation of apolipoprotein B (apoB) metabolism in the liver. In the presence of low levels of hepatic TG and cholesterol, much of the constitutively synthesized apoB is degraded by both proteosomal and non-proteosomal pathways. When excess TG, and to a lesser extent, cholesterol, are present, apoB is targeted for secretion. The major sources of TG in the liver: uptake of fatty acids (FA) released by lipolysis of adipose tissue TG, uptake of TG in VLDL and chylomicrons remnants, and hepatic de novo lipogenesis (the synthesis of FA from glucose) are all abnormally increased in insulin resistance.

**Obesity**

Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters \((\text{kg/m}^2)\). Overweight is defined when BMI is ≥ 25 kg/m\(^2\) and obese when ≥ 30 kg/m\(^2\). The prevalence of obesity has reached epidemic proportions for a number of years now. Recent numbers from the WHO show that more than 1 billion adults are overweight worldwide\(^1\). In the Netherlands, 46.9% of the total population is overweight of which 11.1% is obese. A dramatic increase has been found in the percentages of obese youngsters (age 15-25)\(^5\). Overweight and obesity lead to serious health consequences. They are a major risk factor for developing chronic diseases like cardiovascular disease, some type of cancers, musculoskeletal disorders, and T2DM.

Obesity, and in particular visceral adiposity, is negatively correlated with insulin sensitivity. With respect to visceral adiposity, a great deal of evidence suggests two strong links with insulin resistance. First, visceral adipose cells produce significant amounts of proinflammatory cytokines such as tumor necrosis factor-alpha, and interleukins-1 and -6, resistin, and leptin. In numerous experimental models, these proinflammatory cytokines profoundly disrupt normal insulin action in fat and muscle cells, and may be a major factor in causing the whole-body insulin resistance observed in patients with visceral adiposity\(^6\),\(^7\). Second, visceral adiposity is related to an accumulation of hepatic fat stores, a condition known as nonalcoholic fatty liver disease (NAFLD). The result of NAFLD is an excessive release of FFA into the bloodstream (due to increased lipolysis), and an increase in hepatic glucose production, both of which have the effect of exacerbating peripheral insulin resistance and increasing the likelihood of T2DM\(^8\).
General introduction

Therapies for T2DM

T2DM and obesity associated insulin resistance impose a major health risk, due to increased morbidity and mortality. The major goal of therapeutic intervention is to ameliorate hyperglycemia and insulin resistance, but also increased risk factors such as dyslipidemia and hypertension should be taken into account. Without medication, the outcome of the disease can significantly be improved by lifestyle changes. Restricted caloric intake, weight loss, and exercise will significantly improve whole body insulin sensitivity\textsuperscript{9, 10}. Sometimes, these adaptations are not enough to reduce blood glucose levels near the normal range. In addition, lifestyle changes are not easy and require a strong will. Moreover, it has to be continued for a long term, since weight gain and symptoms of T2DM will come back once discontinued. Therefore, the need for pharmacological strategies is required. Sulfonylureas, meglitinides, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors comprise classes of drugs that lower blood glucose levels. Because these drugs work in different ways to accomplish this, they may be used together to get the desired effects. However, taking more than one drug is more costly and can increase the risk of side effects. Recently, the gut hormones have become interesting therapeutic targets in the battle against T2DM, because of their effects on both food intake and glucose metabolism.

Gut-brain axis

The gastrointestinal tract is the largest endocrine organ in the body and releases more than 20 different regulatory peptide hormones that influence a number of physiological processes and act on tissues including exocrine glands, smooth muscle, and the central nervous system. Most of the gut hormones are sensitive to gut nutrient content, and short-term feelings of hunger and satiety are believed to be mediated, in part, by coordinated changes in circulating gut hormone levels. The interaction between the gut hormones and the central nervous system, the gut-brain axis, is regulated via both hormonal and neuronal pathways. At the base of the third ventricle these hormones can cross the semi-permeable blood-brain-barrier to reach the hypothalamus. Alternatively, they act indirectly by altering the activity of afferent neuronal pathways and brain stem circuits, which in turn project to the hypothalamus.

The hypothalamus is regarded as the main feeding center of the brain and consists of several nuclei involved in food intake, including the arcuate nucleus (ARC), the paraventricular nucleus, the lateral hypothalamic area, the ventromedial nucleus, and the dorsomedial nucleus. The ARC located in the hypothalamus is the most important center that regulates food intake. It contains at least two distinct groups of neurons controlling
energy balance: the orexigenic pathway, containing neurons co-expressing neuropeptide Y and agouti-related protein, stimulating food intake, and the anorexigenic pathway, containing neurons co-expressing pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript, inhibiting food intake\textsuperscript{11}.

Over the past decades the pathways of food intake by the gut-brain axis have become increasingly well delineated\textsuperscript{12}. Recently, compelling evidence indicates that the gut-brain axis is also a key partner of glucose homeostasis. This is accomplished via brain pathways that overlap with those controlling food intake and body weight. The gut hormones have emerged as key factors able to convey metabolic information to the brain and also to modulate endogenous glucose production and disposal\textsuperscript{13, 14}. The present thesis deals with a variety of gut hormones, of which their effects on food intake and glucose metabolism will be summarized.

Glucagon-like peptide-1 (GLP-1) is a cleavage product of the proglucagon molecule which is secreted by the intestinal L-cells and in the brain\textsuperscript{15, 16}. It is released in response to food intake in proportion to caloric content to stimulate glucose-dependent insulin secretion\textsuperscript{15, 17}. In addition, GLP-1 exerts multiple other effects, including inhibition of food intake, slowing of gastric emptying, and inhibition of glucagon secretion\textsuperscript{18, 19}. GLP-1 is also known to beneficially affect glucose metabolism in T2DM patients\textsuperscript{19, 20}. However, GLP-1 is easily degraded by the enzyme dipeptidyl-peptidase IV (DPP-IV), limiting its therapeutic efficacy\textsuperscript{21}. Therefore, pharmaceutical companies are developing GLP-1 analogues resistant to inactivation by DPP-IV. Clinical studies have shown that these analogues are very effective in reducing body weight and improving glucose metabolism in patients with T2DM. Exenatide is a synthetic version of exendin-4, a naturally-occurring GLP-1 analogue, which was first isolated from the saliva of the lizard known as the Gila monster. Exenatide, currently on the market (brand name Byetta), works to lower blood glucose levels primarily by increasing insulin secretion. Because it exerts this effect only in the presence of elevated blood glucose levels, it does not tend to increase the risk of hypoglycemia on its own, although hypoglycemia can occur if taken in conjunction with a sulfonylurea. The primary side effect is nausea, which tends to improve over time. Another class of medications called DPP-IV inhibitors work by preventing the breakdown of GLP-1. By interfering in the process that breaks down GLP-1, DPP-IV inhibitors allow it to remain active in the body for a longer duration, lowering blood glucose levels only when they are elevated. DPP-IV inhibitors do not tend to cause weight gain and tend to have a neutral or positive effect on cholesterol levels. Sitagliptin (Januvia) is currently the only DPP-IV inhibitor on the market.
The anorectic effect of GLP-1 is mediated via the GLP-1 receptor in the brain. Recent findings provide evidence linking GLP-1 action in the brain with the peripheral control of glucose flux, insulin sensitivity and insulin secretion. Complementary studies reveal that the arcuate GLP-1 receptors regulate glucose homeostasis but not food intake. In addition, central administration of GLP-1 reduces lipid storage in peripheral adipose tissue.

**Oxyntomodulin (OXM)** is, just like GLP-1, a cleavage product of the proglucagon molecule secreted from the intestinal L-cells in response to food intake in proportion to caloric content. It potently inhibits meal-stimulated gastric acid and enzyme secretion in rodents and man. OXM is insulinotropic, however, as a circulating hormone in humans, its concentration is probably too low to influence insulin secretion under physiological circumstances. OXM is a potential regulator of appetite and body weight, when administered both intracerebroventricular and intraperitoneal in fasted rats. It is suggested that OXM causes these anorexigenic effects by affecting the arcuate nucleus in the brain.

**Peptide YY (PYY)** is produced and released from the L-cells postprandially. Most of the circulating PYY is the truncated form of the full length peptide, PYY\textsubscript{3-36}. PYY\textsubscript{3-36} is transported to the brain via the blood-brain-barrier. There, it binds the Y2 receptor, which is expressed on NPY neurons of the arcuate nucleus, resulting in decreased food intake. Peripheral administration of PYY\textsubscript{3-36} has been shown to regulate long-term body weight reduction. In addition, PYY\textsubscript{3-36} administration to mice shows an improvement of insulin action to stimulate glucose disposal independently of its effects on food intake and body weight.

**Pancreatic polypeptide (PP)** is synthesized in the so-called F-cells, located in the pancreatic islet of Langerhans. PP is the endogenous ligand for the Y4 receptor in the brain. Peripheral administration of PP has been shown to reduce food intake in both rodents and humans. Chronic treatment with PP ameliorates insulin resistance as indicated by decreased plasma glucose accompanied by a moderate decrease in insulin levels.

**Outline of the thesis**

The aim of the present thesis was to gain more insight into the potential of gut hormones or newly developed analogues to impact on insulin sensitivity.
Chapter 2 describes the metabolic effects of peripheral administration of OXM in insulin resistant mice. OXM appears to be an effective regulator of food intake and appetite. OXM probably impacts on food intake via neural routes in the ARC, which are similarly engaged by other gut-derived peptides modulating appetite, like GLP-1, ghrelin, and PYY$_{3-36}$. These peptides affect insulin sensitivity via mechanistic routes that are independent of food intake and body weight. Therefore, we speculated that OXM could also impact on insulin action in insulin resistant mice.

In chapter 3, we evaluated the effects of a novel GLP-1 analogue, CNTO736, on insulin sensitivity of glucose and VLDL metabolism in high-fat-fed mice. GLP-1 appears to be a promising drug target for the treatment of T2DM, however its therapeutic potency is limited by its short half-life. CNTO736 has an increased half-life over GLP-1 and has been shown to retain GLP-1-like biological activity. The aim of our study was to evaluate the potential of this analogue in terms of its metabolic effects.

In chapter 4, we further investigated the potential of the GLP-1 analogue described in chapter 3 to modulate lipid metabolism. Lipid metabolism is also disturbed in patients with T2DM. Increased VLDL-TG concentrations and decreased HDL cholesterol in plasma significantly contribute to the long term risk of macrovascular disease in these patients. We explored the chronic effects of the analogue on lipid metabolism in APOE*3-Leiden transgenic mice, a “human-like” animal model for studies evaluating the impact of drugs on hyperlipidemia.

GLP-1 improves insulin sensitivity in humans and rodents. It is currently unknown to what extent the (metabolic) effects of circulating GLP-1 are mediated by central GLP-1 receptors. Therefore, in chapter 5 we aimed to evaluate the impact of blocking central GLP-1 receptors on the metabolic effects of peripherally administered GLP-1.

Chapter 6 describes the effects of chronic treatment with the novel compounds Obinepitide, a synthetic analogue of PYY$_{3-36}$ and PP, and TM30339, a synthetic analogue of PP. Both compounds, designed as anti-obesity drugs, show reduced food intake and long-term body weight reduction. We administered the compounds for 4 weeks subcutaneously to measure its long-term effects on insulin sensitivity of glucose and VLDL metabolism.
References


Chapter 1


Chapter 2