Chapter 7

Summary and conclusions
Type 2 diabetes mellitus has now become a global epidemic with increasing number of patients every year according to figures of the World Health Organization. It is a disorder characterized by high blood glucose concentrations in the context of insulin resistance and relative insulin deficiency. While it is often initially managed by increasing exercise and dietary modification, medications are typically needed as the disease progresses. The past decade hormones from the gastrointestinal tract have gained much interest as potential new therapeutic drug targets in the battle against type 2 diabetes. Gut peptides play an important role in regulating food intake and energy homeostasis. In addition, via overlapping pathways, they are able to impact on insulin sensitivity. Therefore, and with the increasing incidence of obesity, which can lead to several chronic diseases, including type 2 diabetes, these peptides became even more interesting for therapeutic interventions. Since the physical properties (mainly their short-half lives) of native gut hormones do not ultimately allow for therapy, pharmaceutical companies are now developing peptide analogues with improved qualities. The present thesis focuses on modulation of insulin sensitivity of glucose and lipid metabolism by different gut hormones or their analogues.

In the current thesis all experiments were performed in wild-type male C57Bl/6 mice, except for chapter 4, in which APOE*3-Leiden mice were used. The C57Bl/6 mice are very susceptible to diet-induced insulin resistance, and therefore a suitable model to study modulation of insulin sensitivity by peptide/drug interventions. Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp technique, considered the golden standard method for quantification of insulin action. VLDL production was determined after blocking lipolysis.

Recent findings indicate that gut hormones, like PYY3-36 and GLP-1, target the brain to regulate food intake, and in addition, impact on insulin sensitivity. Another gut hormone, OXM, has also been shown to affect food intake and appetite. Its effects on glucose metabolism are rather unexplored. In chapter 2 we demonstrate that OXM beneficially affects glucose metabolism in diet-induced insulin-resistant C57Bl/6 mice. These effects were found at circulating insulin levels that were more than double during insulin infusion in OXM-treated mice, complicating the interpretation of the data. Indeed, per unit of ambient insulin concentration, glucose uptake and EGP were not different in OXM and placebo-treated animals. The exact mechanism via which OXM affects glucose metabolism remains to be elucidated. OXM is co-released with GLP-1 from intestinal L-cells into the blood in response to food ingestion in amounts proportional to caloric content. After being released, it has to bind its receptor to initiate a reaction; however no unique receptor for OXM has been identified yet. Since the actions of OXM resemble those
described for GLP-1 (it also inhibits meal-stimulated gastric acid secretion and food intake), OXM is believed to act via the GLP-1 receptor, although it also, with a much lower affinity, can bind the glucagon receptor (Gcgr). The anorectic actions of OXM require the GLP-1 receptor only, as centrally administered OXM inhibits food intake in mice that lack the Gcgr, but the anorectic effects of OXM are completely absent in GLP-1R-/- mice. Furthermore, OXM exerts its effects on the islet β-cell in a GLP-1R dependent manner; it stimulates glucose-dependent insulin secretion and activates cell survival pathways leading to reduced β-cell apoptosis. In addition, the amelioration of glucose tolerance we found was confirmed to be mediated via the GLP-1 receptor. Although the precise mechanisms utilized by OXM for transduction of various biological actions remain unclear, a few studies demonstrate a dissociation between actions of OXM and those of GLP-1. Therefore, the importance of OXM as a biologically active peptide would be greatly strengthened by the identification of a separate OXM receptor, or by studies employing specific OXM antagonists or immunoneutralizing antisera that block actions of OXM but not glucagon or GLP-1. However, the fact that OXM can improve glucose tolerance in high fat fed, insulin resistant mice indicates that this peptide (or any novel peptidomimetic) may be useful in the treatment of obesity and type 2 diabetes, like other proglucagon derived peptides. Unexpectedly, OXM enhanced glucose production in the basal condition, probably by virtue of its capacity to activate the glucagon (or a yet unidentified OXM) receptor in the liver. This augmented increase in basal glucose turnover by OXM has not been described before for GLP-1. Indeed, in chapter 3, the GLP-1 analogue CNTO736 even decreases glucose turnover in basal conditions. Obviously, stimulation of glucose production is an undesirable quality of drugs used to treat insulin resistance that requires careful evaluation. At the end of the day, if it turns out to be a consistent metabolic effect of treatment with OXM (mimetics), excessive glucose production clearly limits the clinical applicability of this peptide.

The actions of native GLP-1 (it enhances glucose-stimulated insulin secretion, improves blood glucose profiles of type 2 diabetes patients, reduces body weight and food intake, and slows gastric emptying) were the main reasons for the physiological and pharmacological interest in this peptide. Clinical trials have shown that exendin-4, a potent GLP-1 receptor agonist, is useful in the regulation of glucose homeostasis in people with type 2 diabetes mellitus. A disadvantage of Byetta, the marketing name of Exenatide (the synthetic version of exendin-4), is the twice daily injection within 60 minutes of food intake. Therefore, the development of GLP-1 analogues continues. CNTO736 is one of those novel analogues. It retains many activities of native GLP-1 yet has a significantly enhanced pharmacokinetic profile. Moreover, it has a longer half-life than exendin-4 in mice (15-20h vs. ~2h) and therefore might allow for fewer injections in patients. In
Chapter 3 the effects of this novel GLP-1 analogue were investigated with regards to its impact on insulin sensitivity of glucose and lipid metabolism. Our data corroborate earlier reports indicating that GLP-1 and its analogues in the long run ameliorate whole body insulin resistance in obese animal models and in T2DM patients. They further extend our knowledge of the precise actions of GLP-1 analogues on distinct components of glucose flux in insulin resistant animals, inasmuch as they show that these compounds acutely reinforce insulin’s ability to promote glucose disposal and boost insulin action on both glucose uptake and production in the long term. Since insulin resistance is a complex disease and is associated with obesity and cardiac disease, drug intervention that can handle multiple aspects of the disease are preferable. Therefore, the observations that chronic CNTO736 treatment decreases body weight and inhibits VLDL-TG synthesis are very promising. Since the C57Bl/6 mice are not a suitable model for studying lipid metabolism, we further explored the inhibitory effect of GLP-1M (the latest version of the CNTO molecule) on VLDL-TG production in chapter 4 in APOE*3-Leiden mice. APOE*3-Leiden mice express the human APOE*3-Leiden gene, resulting in a lipoprotein profile reminiscent of that of patients with dysbetalipoproteinemia, marked by elevated plasma cholesterol and triglyceride levels that are mainly confined to the VLDL/LDL-sized lipoprotein fraction. In contrast to other mice, APOE*3-Leiden transgenics respond to various hypolipidemic drugs in a similar way as humans and are therefore considered a “human-like" animal model for studies evaluating the impact of drugs on hyperlipidemia. Chronic administration of GLP-1M in high-fat-fed APOE*3-Leiden mice clearly inhibited VLDL-TG production. Moreover, chronic administration of the drug decreased total cholesterol levels and increased HDL in the same experimental context. Our data suggest that GLP-1M may have powerful anti-atherogenic properties in insulin resistant subjects. Atherosclerosis is driven by increased (VLDL) cholesterol and TG levels, often accompanied by low circulating HDL concentrations. HDL particles are anti-atherogenic, partly because of their role in reverse cholesterol transport, but also because of their anti-oxidative, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties. Therefore, the reduction of plasma cholesterol concentration by GLP-1M as well as its capacity to increase HDL cholesterol levels holds promise as pharmacological tool to prevent atherosclerosis in high risk metabolic conditions. However, the pathophysiological consequence of the capacity of GLP-1M to inhibit VLDL secretion is uncertain, because circulating TG levels were not affected, perhaps as a result of diminished clearance of TG from plasma.

Notably, although exendin-4 did impact on insulin sensitivity to a similar extent as CNTO736 in chapter 3, it did not impact on VLDL-synthesis in our experimental set-up. This suggests that GLP-1M may have compound-specific effects on lipid metabolism that
potentially reinforce its capacity to prevent macrovascular disease in high fat-fed mice (and humans). On the other hand, it might be related to the serum half-life of GLP-1M, which is considerably longer than exendin-4. This issue needs to be clarified before a firm conclusion can be drawn on the effects of GLP-1M on VLDL metabolism.

All peripheral signals from the gastrointestinal tract convey metabolic information to the brain. The main structures involved in the control of glucose homeostasis are the hypothalamus and brainstem. The hypothalamus is the first integration centre of hormonal information. Specifically, the arcuate nucleus (ARC), located in the mediobasal hypothalamus, contains neurons sensitive to both hormones (ghrelin, leptin, GLP-1) and nutrients (glucose, fatty acids). Downstream targets of the ARC include the paraventricular and dorsomedial hypothalamic nuclei. Other projections of the ARC target the medial preoptic area, lateral hypothalamus and ventromedian area. Finally, the structure of the limbic system and cortex also interact with the hypothalamus. It is currently unknown to what extent the (metabolic) effects of circulating GLP-1 are mediated by central GLP-1 receptors. In chapter 5, we show that GLP-1 treatment ameliorates insulin resistance of glucose metabolism in high-fat-fed mice in part via activation of central GLP-1 receptors. The neuronal circuits that are activated by peripheral administered GLP-1 and mediate its metabolic effects remain to be identified.

One possibility is that GLP-1 in the hypothalamus modulates the neuropeptide Y (NPY) pathway. It has been shown previously that intracerebroventricular administration of GLP-1 completely prevents the orexigenic effects of NPY, suggesting that GLP-1 acts by blocking NPY transmission to inhibit food consumption. Intracerebroventricular administration of NPY acutely impairs insulin’s ability to suppress endogenous glucose production. In addition, animal models of obesity and type 2 diabetes (including diet-induced obesity) are marked by elevated NPY expression in hypothalamic nuclei. For these reasons, it is conceivable that these exceedingly active NPY neurons are involved in the pathophysiology of enhanced endogenous glucose production in the face of hyperinsulinemia in these models (and obese humans). GLP-1 has been reported to reduce hypothalamic NPY neuronal activity. Therefore, chronic administration of GLP-1 or any of its analogues may have antagonized NPY-induced insulin resistance to explain the findings presented here.

The hypothalamus is, however, not solely responsible for the integration of peripheral information originating from the gut. The brainstem, in addition to interacting with hypothalamic circuits, also plays a principal role in the regulation of energy homeostasis. Located in the brainstem, the nucleus of the solitary tract (NTS) receives vagal afferents from the gastrointestinal tract. Therefore, GLP-1 (or its analogues) may...
activate neurons in the NTS in the brain stem via afferent vagal inputs to modulate glucose metabolism. GLP-1 evokes vagal afferent nerve activity to initiate a hepatopancreatic reflex that is critically involved in the control of insulin release. It is conceivable that vagal afferent output in this context is not limited to the pancreas also but affects liver and other visceral organs to modulate glucose production. Moreover, vagal ablation attenuates the anorexigenic effects of peripheral GLP-1 administration. In analogy, activation of vagal afferents by GLP-1 may affect glucose metabolism. Besides affecting afferent fibres, peripheral GLP-1 may also impact directly on the brainstem. Like the ARC, the NTS is in close anatomical proximity to a circumventricular organ with an incomplete blood-brain-barrier - the area postrema (AP) - and is therefore in an ideal position to respond to peripheral circulating signals, such as GLP-1. Indeed, administration of GLP-1 results in a significant increase in signal intensity in the AP of fasted mice, reflecting an increase in neuronal activity within the brainstem.

However, despite the compelling evidence of the importance of the gut-brain axis in the control of glucose homeostasis, it remains possible that GLP-1 acts directly on the liver and other peripheral tissues via a structurally and functionally distinct GLP-1R. Indeed, GLP-1 was reported to have insulin-like actions in liver and skeletal muscle, which are not mediated by the classical GLP-1R.

Finally, in chapter 6, the potential of two novel developed analogues, Obinepitide and TM30339, was explored. The results show that chronic treatment with these drugs beneficially impacts on insulin resistance in high-fat-fed mice, with respect to insulin mediated glucose disposal and production. Both compounds, in analogy with its property as anti-obesity drugs, also reduced body weight (by decreasing total fat mass) in our experimental set-up. Since insulin sensitivity and weight loss are strongly correlated, the effect on the later might be the underlying cause for the reduction in insulin resistance. Pair-feeding studies may provide the answer. Regardless of the mechanism, and highly significant, our results indicate that the metabolic effects of Obinepitide and TM30339 do not vanish during chronic treatment, making them valuable drugs to treat both obesity and insulin resistance.

In conclusion, we have shown a variety of gut hormones or its peptidomimetics to beneficially impact on glucose metabolism. However, our animal models still raise questions, which need to be clarified in more detail, before to the drugs can be used in the clinical setting. Long-term treatment may also have side-effects, related to gastric emptying and nausea, which have been reported before for GLP-1 and related molecules. Also, in response to post-marketing reports of acute pancreatitis in patients using
Exenatide, the U.S. Food and Drug Administration (FDA) added a warning to the labeling of Byetta in 2007. In August 2008, four additional deaths from pancreatitis in users of Exenatide were reported to the FDA; while no definite relationship had been established, the FDA was reportedly considering additional changes to the labeling of this drug.

Over the past decade a growing amount of evidence indicates that the gut-brain axis is a key player in the control of glucose homeostasis. Peptides such as GLP-1 have emerged as key factors able to convey metabolic information to the brain and also to modulate endogenous glucose production and disposal. Elucidating more precisely the molecular events in the brain that underlie the effects of these hormones could lead to the identification of new (or improved) therapeutic agents.

References


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Chapter 7