Chapter 9

Summary and conclusion
This final chapter summarizes the main findings of the work presented in this thesis and also provides some methodological considerations and backgrounds. The primary objective of the studies presented in this thesis was to assess and compare the effects of long-term treatment with the GLP-1 receptor agonist exenatide versus insulin glargine on different parameters of pancreatic beta-cell function, in patients with type 2 diabetes who had not achieved an HbA1c of $\leq 6.5\%$ using metformin mono-therapy. In addition, treatment effects on postprandial lipid levels, body composition, circulating cardiovascular risk biomarkers, and safety were assessed.

**Primary study endpoints: beta-cell function parameters**

Type 2 diabetes is characterized by a progressive beta-cell defect in the presence of obesity-related insulin resistance (1, 2). The former is thought to be primarily responsible for the progressive nature of the disease (3, 4). The capacity to secrete insulin is thought to be related to functional beta-cell mass, which is determined by the balance of the rate of beta cell neogenesis and apoptosis (5), and is showed to be reduced in patients with type 2 diabetes (6). Until beta-cell volume can be quantified reliably and non-invasively, we will need to rely on the ability of glucose, with or without other secretagogues, to potentiate insulin release as the best surrogate estimate of the number of beta-cells (7). However, an attempt to estimate beta-cell mass based simply on the use of a functional test as a surrogate measure cannot, by nature, be complete and will therefore be imprecise (7).

A number of different functional indexes have been proposed to estimate beta-cell function in vivo (7-9). Hyperglycemic clamps and experiments in isolated pancreatic islets have demonstrated that glucose administration induces insulin secretion in a biphasic pattern: an initial component (first phase), which develops rapidly but lasts only a few minutes, followed by a sustained component (second phase) (8, 10). Loss of first phase glucose stimulated secretion and a reduction in second phase glucose stimulated, and combined glucose and arginine stimulated insulin secretion are characteristic features of type 2 diabetes mellitus (10, 11). It is known that a decrease in first phase glucose stimulated insulin secretion is found in the early stage of type 2 diabetes and also in patients with impaired glucose tolerance (12, 13). Detailed assessment of beta-cell function will therefore be useful to identify early stages of type 2 diabetes, but also to evaluate (the rate of) disease progression, and to monitor the effects of pharmacological interventions (14).
Evidence supporting the rationale and design of the current study included the demonstration in preclinical models of diabetes, and in *in vitro* assays, that GLP-1 and the GLP-1 receptor agonist exenatide improved beta-cell function, promoted beta-cell health and preserved or increased beta-cell mass (15-19). Initial, often short-term, studies in healthy humans and patients with type 2 diabetes, showed improvement of (surrogate) markers of beta-cell function following exenatide treatment (20-26).

**Beta-cell function measures using the hyperglycemic clamp**

In chapter 2 we studied whether 52 weeks of treatment with exenatide or insulin glargine significantly improved beta-cell function, measured with the hyperglycemic clamp with additional arginine stimulation. Furthermore, durability of treatment effect on beta-cell function was assessed after a 4-week off-drug period. We showed that exenatide administration, relative to insulin glargine, significantly improved first and second phase glucose-stimulated insulin secretion by 53% and 185%, respectively. The combined glucose and arginine stimulated insulin secretion increased by 146%, when compared to insulin glargine, while on active treatment (Table 2.1). This beneficial effect on beta-cell function was accompanied by an improvement in glycemic efficacy (Figure 2.3).

Pancreatic beta-cells contain at least two pools of insulin secretory granules, that differ in release pattern, which account for biphasic insulin secretion. A small pool available for immediate exocytosis and a reserve pool which accounts for the vast majority of granules, and needs to be mobilized to being available for secretion (8, 27). The prevailing hypothesis is that granules from the readily releasable pool account for the first phase of glucose-stimulated insulin secretion, and that mobilization of a subsequent supply of new granules from the reserve pool accounts for the second phase (27-29). GLP-1 receptor agonists, such as exenatide, bind to a specific G-protein coupled receptor resulting in the activation of adenylate cyclase and an increase in cAMP generation (30, 31). In the beta-cell, cAMP binds and modulates activities of both protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factor II (Epac2), thereby increasing intracellular Ca^{2+} concentrations and enhancing glucose-dependent insulin secretion (10, 30, 32). It is postulated that Epac2 signaling increases the size of the readily releasable pool and PKA signaling may increase the size of the reserve granule pool (10). Through these mechanisms exenatide treatment may have contributed to the increase in first and second phase insulin secretion as described in chapter 2.
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Ward and Porte introduced the glucose-dependent arginine stimulation test (33). It is a method which gives thorough information on islet function as it measures both basal and “maximal” alpha-cell and beta-cell secretion (34). Unfortunately, we did not measure glucagon release as a measure of alpha-cell (dys)function in our study. It has been shown that combined glucose and arginine stimulated insulin release increases when arginine is administered at higher clamp glucose concentrations (33). During the design of our study we deliberately chose to test glucose-dependent arginine stimulation at moderately elevated glucose concentrations (15 mmol/L), as used by Ward (33) and Larsson (34). An intravenous glucose infusion rate to achieve higher glucose concentrations, for example 25 mmol/L, during the hyperglycemic clamp was expected not to be feasible while on active exenatide treatment. Therefore, the reported acute insulin responses to arginine (AIRarg) in chapters 2 and 6 cannot be interpreted as the real “maximum acute insulin responses” (AIRmax) in our population.

Following our initial one-year study the improved beta-cell function at 52 weeks was lost 4 weeks after cessation of exenatide and insulin glargine, suggestion an acute pharmacological effect of either treatment (Figure 2.4, Table 2.1). Interestingly, insulin sensitivity remained significantly improved after 4 weeks cessation of exenatide treatment. We did not observe this finding in the insulin glargine group (Figure 2.3F). This observation led to the idea that extra-pancreatic effects of exenatide, such as weight loss and the associated improvement in insulin sensitivity, might last longer. We concluded that the possible effects on preservation of beta-cell function might be dependent on other [additional] factors including: 1) diabetes duration, 2) the amount of functional beta-cells present at the initiation of therapy, 3) overall metabolic control achieved and 4) treatment duration. To study the possible preserving effect of exenatide on beta-cell function, patients were asked to participate in an additional 2-year extension trial, and restart their originally assigned treatment at the end of our original one-year study. The results of this extension study are presented in chapter 6.

At the end of the 3-year treatment period, exenatide and insulin glargine treatment was stopped and patients were only treated with their original metformin dose. As a result of strict regulations by the ethical review board we were not able to measure beta-cell function both on and off active exenatide or insulin glargine treatment. To answer the durability questions, which were raised following the one-year treatment period, we chose to only measure beta-cell function following a 4-week off-drug period. After 3 years of treatment, beta-cell secretory function measured as first and second phase glucose-stimulated insulin secretion showed a statistically significant
reduction in exenatide treated patients as compared to the insulin glargine treated patients (Figure 6.5). Interestingly, insulin sensitivity remained significantly improved during the 4-week off-drug period in the exenatide treated patients. As insulin sensitivity and insulin secretion are closely interrelated this means that the proper assessment and interpretation of beta-cell function parameters requires the incorporation of both insulin sensitivity and the insulin response measures (35). The following paragraph will discuss this relationship in greater detail.

The relation between insulin sensitivity and insulin secretion

The relationship between insulin sensitivity and insulin secretion follows a hyperbolic function, such that the product of the two variables (called the disposition index) remains a constant (35, 36) (Figure 9.1). By acknowledging this hyperbolic relationship, beta-cell function measures should include measures of glucose-stimulated insulin first phase insulin release and insulin sensitivity (37). In response to the decrease in insulin sensitivity as observed in obesity (36), puberty (38) and pregnancy (39), human beta-cells increase insulin release to levels four to fivefold higher than in insulin sensitive individuals (2). As long as this compensatory insulin secretion is sufficient, resulting in a normal disposition index, glucose tolerance remains unaffected. Individuals predisposed to type 2 diabetes show a reduced beta-cell compensatory response to obesity-related insulin resistance and will subsequently develop impaired glucose tolerance, and type 2 diabetes (40).

In chapter 6 we showed that 3-year long treatment with exenatide results in a 7.9 kg reduction in body weight as compared to insulin glargine (Figure 6.4C). Probably as a consequence of this, insulin sensitivity remained improved by 39% in the exenatide treated patients after 4-week cessation of treatment. Insulin secretion responses to glucose and arginine cannot be interpreted without taking the changes in insulin sensitivity into account. Following 4-week cessation of exenatide treatment the disposition index increased significantly. In the insulin glargine treated patients, the disposition index decreased (Figure 6.5E-H).
In people with normal glucose tolerance (1) a non-linear, hyperbolic relation between insulin sensitivity and beta-cell insulin release exists. The product of insulin release and insulin sensitivity is called disposition index (DI), and is assumed to be a constant. Healthy subjects react to a decrease in insulin sensitivity by a reciprocal increase in beta-cell insulin release (2), and vice versa (3). Patients with impaired tolerance (5) and type 2 diabetes (6) are not able to compensate for the decrease in insulin sensitivity. In situation 2, beta-cell insulin release is increased but the DI is normal. In 4, beta-cell insulin release appears normal but the DI is reduced. This hyperbolic relationship means that assessment of beta-cell function requires knowledge of both insulin sensitivity and the insulin response. Impaired glucose tolerance (IGT). Adapted from Kahn (2), Cobelli (8), and Stumvoll (1).

Body weight reduction per se has been shown to improve beta-cell function in subjects with and without type 2 diabetes (41, 42). It is possible that factors secreted from adipose tissue, such as free fatty acids and adipocytokines such as IL-6, TNF-α, or adiponectin, may directly or indirectly impact beta-cell function (43). In chapter 5 we showed exenatide beneficially affected some of these adipocyte-derived proteins. In our 3-year extension study, the improvement in disposition index appears to be driven by the improvement in insulin sensitivity (Figure 6.5G). Interestingly, the improvement in disposition index reported in chapter 6 cannot be fully explained by the reduction in body weight (and subsequent improvement in insulin sensitivity) alone. Most patients treated with exenatide experienced a reduction in body weight. However, about half of the patients treated with exenatide a combined improvement in body weight and disposition index was observed (Figure 6.6). Additionally, there appeared to be no statistical correlation between the body weight reduction and the disposition index improvement in both exenatide and insulin glargine treated patients. A more specific GLP-1 receptor agonist related factor could therefore not be
excluded. Unfortunately, more post-hoc analyses were not possible due to the small sample size.

As acknowledged in chapter 2, residual beta-cell function and mass may be an important determinant of the efficacy of GLP-1 receptor agonist therapy (44). Based on homeostasis model assessment (HOMA) data from the UK Prospective Diabetes Study (UKPDS), it has been suggested that in patients with type 2 diabetes, beta-cell function is already reduced by 50% at the time of diagnosis (45), and that loss of beta-cell function begins 10–12 years before diagnosis (14, 46). The data presented in chapter 2 and chapter 6 may suggest that a temporary improvement in pancreatic beta-cell function may be achieved following prolonged treatment with a GLP-1 receptor agonist. The observed improvement in disposition index was not accompanied by a lasting improvement in glycemic efficacy measures; the HbA1c concentration returned to pre-randomization levels following the 12-week off-drug period (Figure 2.3A and Figure 6.4A). It has been shown that glucose control is closely related to pancreatic beta-cell function in humans (47). It may be that the improvement of the DI was not big enough in these patients with established type 2 diabetes to affect glycemic control. Pharmacological interventions aimed to preserve beta-cell function probably need to be initiated at an earlier stage. Additional studies are needed in patients at high-risk, for example patients with impaired glucose tolerance, to test this hypothesis (48).

**Beta-cell function measured using the mixed-meal test**

As mentioned in chapters 2 and chapter 6, the euglycemic-hyperinsulinemic clamp and the hyperglycemic clamp are currently regarded gold-standard methods for measuring insulin sensitivity and beta-cell function respectively. However, these techniques also have disadvantages. The clamp technique is technically challenging, a burden to patients and time consuming. Additionally, the invasive nature of the clamp may not mirror ‘real life’ situations. Although fasting indices (such as the HOMA (49)) are frequently used as surrogate estimates of insulin sensitivity and insulin secretion, fasting concentrations only reflect a single point on the complex glucose-insulin dose-response curve, and thus cannot provide insight regarding the dynamics of the beta-cell in response to changing glucose concentrations such as typically occur in daily life (8). Therefore, for epidemiologic studies, and for the follow-up of individual changes in insulin sensitivity and insulin secretion, measurements derived from simpler methods such as the oral glucose tolerance test (OGTT) are often used (50, 51). The insulinogenic index, calculated as the ratio of the 30-minute
increment in insulin to glucose concentration (52), is widely used to estimate glucose responsiveness of the beta-cell following the OGTT. Despite its simplicity, this index is able to detect anomalies in beta-cell function in many circumstances (52). Nevertheless, it needs to be acknowledged that correlations between OGTT derived and intravenous derived measures of beta-cell function are not perfect (r values between 0.5-0.7) (53). Being a composite index, it does not reflect specific mechanisms of insulin secretion (for instance, the first phase insulin secretion as assessed with the hyperglycemic clamp) (9). In the interpretation of the OGTT beta-cell indices, it must be kept in mind that giving glucose through the oral route results in a potentiated secretory response due to the activation of the entero-insular axis, which does not occur with the hyperglycemic clamp (9).

By design, the hyperglycemic clamp uses intravenous administration of glucose and other secretagogues (i.e. amino acids or GLP-1) to test beta-cell secretory function (33, 54, 55). Since the primary goal of most studies is to determine how alterations in beta-cell function and insulin action influence human metabolism, ideally these should be assessed under physiologic conditions by using simple tests, i.e. mixed-meals that provide carbohydrates, fats and amino acids (8).

Over recent years, several mathematical models have been developed to quantify the beta-cell response following an OGTT or standardized mixed-meal test (8, 56). A common aspect of these models is a dose-response function representing the relationship between the circulating glucose concentration and subsequent insulin secretion rate (ISR) (8, 53, 56). The simplest models are confined to just the dose response relationship between the glucose and insulin concentration (57), whereas others are more sophisticated (58-62). Insulin and C-peptide are equimolarly secreted from the beta-cell and pass through the liver, where insulin, but not C-peptide, undergoes hepatic extraction (63). Therefore more recently developed models rely on C-peptide deconvolution (64) rather than insulin concentrations per se. Although more models exist, two are now frequently used in the literature (58, 59, 61, 62). In these models insulin secretion is represented as the sum of two main compartments: 1) the glucose/insulin dose-response relations, and 2) one accounting for the observation that rapid changes in glucose concentration enhance insulin release (53) (for review see: (8, 56)). These models share common features, the models mainly differ by the way they interpret the potentiation of insulin secretion (8, 53). For the study presented in chapter 4 we have chosen for the model by Mari et al. (61, 62) (Figure 9.2). Since this model has been used frequently in other studies using GLP-1 (65), exenatide (26), liraglutide (66), and the DPP-4
inhibitor vildagliptin (67, 68), it facilitates the comparison with other incretin-based therapies.

Figure 9.2 Block diagram of the beta-cell model and illustration of the role of the insulin secretion components during an oral glucose tolerance test. The central compartment of the model is the glucose/insulin dose-response relation, which describes the rise and fall of insulin secretion that parallels the rise and fall of the glucose concentration. From the glucose/insulin dose-response relation 2 parameters are calculated: 1) the insulin secretion rate at reference glucose concentration (ISR@Gref) and 2) beta-cell glucose sensitivity which is represented by the slope of the glucose/insulin dose-response relation. The dose-response relation is modulated by the potentiation factor, which is a positive function over time and has been constrained to have a time average of 1 during the experiment. The potentiation factor explains the sustained insulin secretion usually seen at the end of an OGTT or meal test when the circulating glucose concentration already has returned to baseline values. Finally, rate sensitivity is introduced into the model: an early insulin secretion component, which quantifies the sensitivity of the insulin response to the initial rapid glucose rise. The black arrows represent the effects of one-year treatment with exenatide on the specific model-derived measure as compared to insulin glargine. Adapted from Mari (56).

The main findings of the study assessing one-year treatment with exenatide, as compared to insulin glargine, on meal derived measures of beta-cell function are presented in chapter 4. One-year treatment with exenatide resulted in a significantly greater upward shift in the glucose/insulin dose-response relation, and increased potentiation after both breakfast and lunch. Notwithstanding the fact that the potentiation factor has been criticized for being a time-varying correction term that mathematically compensates for
the difference between the observed ISR and the ISR derived from the glucose/insulin dose response relation (8), Ferrannini postulates that potentiation should be considered as an independent parameter of beta-cell function (69). The strong initial increase in potentiation (Figure 4.4C) may represent the strong stimulating effect on insulin secretion following the exenatide injection prior to the breakfast meal. Additionally, both exenatide and insulin glargine treatment equally improved glucose sensitivity, representing the ability of the beta-cell to adjust the ISR to the change in prevailing glucose concentration. Finally, the beneficial effects on the glucose/insulin dose response relationship were sustained after a 5-week off-treatment period in both the exenatide and insulin glargine treated groups. As the improvement in beta-cell glucose sensitivity, and the lasting effect on glucose/insulin dose response relationship after cessation of treatment, was found in both treatment groups a more general mechanism, possibly reduction of glucose toxicity (70) may explain these findings.

Interestingly, the rate sensitivity significantly decreased in exenatide-treated patients when compared to insulin glargine. Rate sensitivity appreciates the relationship between the early, fast rise in plasma glucose concentrations and the subsequent change in insulin secretion. When the time derivative of the glucose concentration is negative, the derivative component equals 0 (61, 62). The observed delta in glucose concentration is negative in the exenatide group (Figure 4.3A). This reduction in postprandial glucose concentration may account for the between-group difference in rate sensitivity, as this parameter is automatically set to be 0 in many exenatide treated patients. Insulin glargine did not have such an effect on the postprandial glucose concentration, and hence on rate sensitivity. These findings are consistent with observations made in similar experiments with GLP-1 infusion (65) and the GLP-1 receptor agonist liraglutide (66).

Secondary study endpoints: cardiovascular disease risk markers
Today, it is well recognized that type 2 diabetic patients have an increased risk of developing micro and macrovascular disease. Mortality from cardiovascular disease (CVD) is 2-4 fold increased in persons with diabetes compared with the general population (71). Although it has been shown that an intensified multifactorial intervention reduces macrovascular and microvascular events by about half, it is still considerably higher than in the background population, leaving room for much improvement (72, 73). Hyperglycemia is associated with cardiovascular risk in patients with type 2 diabetes. However, there is less compelling evidence that glucose lowering
therapy reduces CVD risk. Data from the original United Kingdom Prospective Diabetes Study (UKPDS) cohort did not exhibit a reduction in CVD events, apart from a small subgroup of obese patients treated with metformin that experienced a CVD benefit (74). However, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications trial (DCCT/EDIC) showed that glucose lowering was associated with a long-term cardiovascular complications benefit in patients with type 1 diabetes (75, 76). Over the last few years data from ACCORD (77), ADVANCE (78), and the Veterans Affairs Diabetes Trial (79), did not demonstrate a significant reduction of cardiovascular events in the groups randomized to intensive glucose-lowering therapy as compared to the standard therapy group. More recently the United Kingdom Prospective Diabetes Study Post-Trial (UKPDS post-trial) showed a benefit of glycemic control after 10 years of post-trial follow-up (a so-called ‘legacy effect’) (80). These observations support the concept to treat patients to target as early as possible following diagnosis (81,82).

Effects on postprandial dysmetabolism
Both postprandial hyperglycemia and hypertriglyceridemia lead to mitochondrial free radical production and subsequent oxidative stress, which in turn may contribute to the development of both micro and macrovascular complications (81, 83-85) (Figure 9.3). It has been suggested to include the reduction of postprandial glucose and lipid excursions as treatment targets in the treatment guidelines for type 2 diabetes (86).

To our knowledge, studies on the effects of exenatide on postprandial cardio-metabolic parameters have used a single meal study design (23, 24, 26, 88-92). It is known that postprandial glucose and lipid responses differ following subsequent meals in patients with the metabolic syndrome and type 2 diabetes (82, 84, 93). To simulate the real-life daily food intake we used two consecutive meals in our study.

In chapter 3 the effects of one-year exenatide treatment on postprandial glucose and lipids are presented. One-year exenatide resulted in a significant reduction of prandial glucose, triglycerides, apo-B48, VLDL-C, HDL-C, free fatty acid and MDA excursions (Table 3.2). Additionally, we found a statistically significant correlation between the reduction in postprandial glucose and lipid excursions, and different markers of oxidative stress (Figure 3.3).
Type 2 diabetes is characterized by high circulating levels of atherogenic lipid particles due to an increased supply of fatty acids to the liver and defective hepatic clearance of lipoproteins. In the postprandial state, the lipid abnormalities are further exaggerated, with an additional adverse effect of meal-induced hyperglycemia. These postprandial metabolic derangements increase the production of reactive oxygen species causing oxidative stress. Collectively, postprandial dysmetabolism and the associated oxidative stress may link type 2 diabetes to cardiovascular disease. Adapted from Tushuizen (87).

Gastric emptying is an important determinant of postprandial glucose and lipid excursions in healthy subjects and type 2 diabetes (94, 95). Studies using the acetaminophen technique (96-99) and scintigraphy (100) have shown that exenatide slows gastric emptying in both healthy subjects and subjects with type 2 diabetes. Unfortunately, we did not measure gastric emptying rates in our study. However, it appears to be reasonable to assume that gastric emptying significantly contributed to the reduction in postprandial glucose and lipid excursions. The reduction in postprandial glucose excursions was previously shown to be related to the slowing of gastric emptying during exenatide treatment (100). However, other data suggest that, exenatide may lower postprandial glycemia via a novel mechanism independent of islet hormones and slowing of gastric emptying (101). Although the effects of exenatide on the second meal were attenuated as compared to the first meal, the total glucose and triglyceride excursion remained significantly lower with exenatide compared to insulin glargine during the entire 8-hour mixed-meal test, even after ingestion of an additional lunch meal, 4 hours after the (pre-breakfast) exenatide dose. Combined, our meal test findings may suggest a beneficial effect on the...
cardiovascular risk profile of exenatide as compared to insulin glargine as epidemiological (102) and interventional studies (103) have suggested that postprandial glucose are stronger predictors of CVD than fasting plasma glucose in patients with diabetes.

**Effects on body composition**

Estimates indicate that approximately 60% of all cases of type 2 diabetes can be attributed to weight gain (104). Pharmacological interventions in patients with type 2 diabetes should therefore also target obesity (105). The clustering of cardiovascular risk factors with abdominal obesity is well established (106). The term metabolic syndrome captures a wide spectrum of factors thought to increase the risk of CVD and type 2 diabetes. This syndrome now includes amongst others: abdominal obesity, hypertension, abnormal plasma glucose, microalbuminuria, elevated levels of cytokines, and insulin resistance (107, 108).

Computed tomography (CT) and magnetic resonance imaging (MRI) technologies are regarded the gold standard for measuring body composition in vivo. However, as they are expensive and cumbersome, dual-energy X-ray absorptiometry (DEXA) provides a reliable alternative for regional body composition analysis in large-scale follow-up studies (109). Effects of exenatide and insulin glargine administrations on body composition assessed with DEXA are discussed in chapter 5. We showed that exenatide reduces body weight mainly as a result of a reduction of truncal fat mass and waist circumference. Additionally, circulating concentrations of leptin, adiponectin, and hsCRP were beneficially influenced. Adiponectin and CRP are thought to be independent key molecules in type 2 diabetes related cardiovascular disease (110, 111).

Although the combination of a reduction in truncal fat mass and waist circumference may suggest a reduction in metabolically active visceral fat mass, a recent uncontrolled study did not show an effect of exenatide treatment on visceral fat measured with computed tomography (112). Therefore additional studies to assess the effects of exenatide on body composition and body fat distribution are needed.

Disproportionate accumulation of intra-abdominal and/or hepatic fat may explain variations in serum triglyceride, HDL-cholesterol, and hepatic insulin sensitivity, supporting the assumption that both fat deposits are important determinants of these components of the metabolic syndrome (113). According to the American Association for the Study of Liver Diseases, non-alcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver exceeding 5% to 10% (114). In Chapter 8 we
describe the possible effects of exenatide treatment on hepatic fat content in one patient participating in our study. Following approximately 10 months of exenatide treatment the liver fat content measured by liver MRI-spectroscopy declined from 15.8% to 4.3%.

Diabetic dyslipidemia is a cluster of potentially atherogenic lipid and lipoprotein abnormalities that are metabolically interrelated. Recent evidence suggests that a fundamental defect is an overproduction of large very low-density lipoprotein (VLDL) particles, which initiates a sequence of lipoprotein changes, resulting in higher levels of remnant particles, smaller and denser LDL particles, and lower levels of high-density lipoprotein (HDL) cholesterol (82, 115, 116). In addition, a fatty liver may further contribute to the CVD risk by a higher production of glucose, CRP, and coagulation factors (117). Chapter 3 and chapter 5 present data on the effects of exenatide on metabolic factors that are thought to be related to the amount of liver fat. Hepatic overproduction of apo-B-containing VLDL particles is regarded as the dominant feature of diabetic dyslipidemia driven primarily by liver fat and hyperglycemia (115). Although the reduction in truncal fat mass may play an important role in the in the observed reduction in atherogenic lipid particles and circulating CRP levels, we showed that the reduction in CRP was partially independent of changes in body weight and truncal fat mass changes. This may be attributed to a possible reduction in liver fat content following exenatide treatment.

The combined findings of chapter 3, chapter 5 and chapter 8 may suggest favorable effects on cardiovascular risk in type 2 diabetes treated with exenatide. A recent meta-analysis of exenatide clinical trial data supports this interpretation of our findings (118).

Secondary study endpoints: treatment safety

The exenatide therapy associated adverse event profile as reported in this thesis is similar to earlier published studies. The most common adverse events associated with exenatide treatment were mild-to-moderate gastrointestinal side effects (including nausea, diarrhea, vomiting), which are dose-dependent, more common during drug initiation and decrease over time (119). The risk of hypoglycemia is not increased when exenatide is combined with metformin (88). During our study no major hypoglycemic events occurred in either randomization arm.

In our study one patient randomized to exenatide developed pancreatitis while on active treatment, which resolved after cessation of the study medication. This adverse event is referred to in chapter 2. Cases of acute pancreatitis have been reported in patients treated with exenatide BID (120,
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121), liraglutide (122), and vildagliptin (123). Recently published data showed that exenatide use was not associated with an increased risk of acute pancreatitis (124). Additionally, exenatide does not evoke pancreatitis and attenuates chemically induced pancreatitis in normal and diabetic rodents (125).

Type 2 diabetes is associated with an increased risk of bone fractures (126). Thiazolidinediones have been suggested in increased bone fracture risk in female patients with type 2 diabetes (127). Body weight reduction is advocated in most type 2 diabetes patients to improve their cardiovascular risk profile, however, it may decrease bone density and increase bone turnover (128, 129). Therefore, type 2 diabetes therapies should not only lower cardiovascular risk but also be safe in terms of bone health, also given their chronic use. In chapter 7 we show that exenatide treatment does not affect bone mineral density despite the significant reduction in body weight.

Final conclusion

In addition to an acute pharmacological effect, the studies presented in this thesis showed that exenatide treatment, as compared to insulin glargine, beneficially affects metabolic parameters, which are thought to play an important role in the pathogenesis of type 2 diabetes (Figure 9.4).

Figure 9.4 Model for the effects of body fat content on insulin sensitivity and beta-cell function in the pathogenesis of type 2 diabetes. Adapted from Cnop (130).
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Taken together the following hypothesis could be suggested. Prolonged, multi-year, exenatide treatment leads to:

- a reduction in body weight by lowering truncal fat mass (chapter 5);
- subsequently, circulating adipocytokines (i.e. leptin and adiponectin) and inflammatory biomarkers, and insulin sensitivity will improve (chapter 2, chapter 5 and chapter 6);
- exenatide treatment reduces liver fat content (chapter 8);
- together these metabolic effects lead to a more favorable (prandial) lipid and lipoprotein profile and a reduction in oxidative stress (chapter 3);
- which in turn lead to an amelioration of beta-cell function and glucose tolerance (chapter 2, chapter 4, and chapter 6).

However in our studies, the glucometabolic improvements were not persevered following a 12-week off-drug period suggesting multi-year treatment may be necessary for these effects to happen. Beta-cell preserving pharmacotherapeutic interventions should preferably start early to ensure that sufficient residual beta-cell function is still present. It has been proposed to start early in the disease pharmacological intervention to prevent the further deterioration of beta-cell function using a combination of metformin, thiazolidinediones, and exenatide, preferably in patients with impaired glucose tolerance (i.e. pre-diabetes) (48, 105). Against this background, future long-term pharmacotherapeutic interventions in patients with pre-diabetes are needed to study whether GLP-1 receptor agonists (such as exenatide) might favorably alter the progressive nature of type 2 diabetes.
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82. Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia 2003;46:733-749.


98. Blase E, Taylor K, Gao YH, Wintle ME, Fineman MS. Pharmacokinetics of an oral drug (acetaminophen) administered at various times in relation to


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