CHAPTER 10
Summary and General Discussion of the Thesis

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The main aim of this thesis was to address the hypothesis that incretin-based therapies (i.e. glucagon-like peptide (GLP)-1 receptor agonists and/or dipeptidyl peptidase (DPP)-4 inhibitors) would be suited pharmacological agents to treat or to prevent the diabetogenic side effects associated with glucocorticoid (GC) treatment.

Despite the large number of annual GC prescriptions and the high prevalence of treatment-related diabetes among chronic GC users, there are currently no (evidence-based) guidelines to treat or prevent GC-induced diabetes (also known as steroid diabetes). We and others have previously shown that GC cause diabetes/hyperglycemia by inducing insulin resistance, increasing body weight and causing islet-cell dysfunction [1-4].

Our premise, that incretin-based therapies would be particularly effective to treat or prevent GC-induced diabetes had three main reasons. First, since GC treatment is mainly associated with increases in postload but not fasting glucose levels, the incretin-drug classes, both of which primarily target postprandial glucose excursions, may be most effective [5,6]. Second, incretin-based therapies were shown to improve pancreatic islet-cell function [7-10], which is impaired by GCs, as these agents increase glucagon secretion and reduce insulin secretion [11]. Third, particularly GLP-1 receptor agonists, on top of their blood glucose lowering effect, ameliorate multiple phenotypic defects of GC-induced diabetes, by reducing body weight, and thereby insulin resistance, by lowering blood pressure and lipids, and overall, by improving the cardiovascular risk profile (Table 1).

**SUMMARY OF THE MAJOR FINDINGS**

First, in Chapter 2, we confirmed that a 14-day treatment with low- or high-dose prednisolone (7.5 mg or 30 mg once daily) affected several aspects of islet-cell function in healthy young men leading to a dose-dependent increase in postprandial glucose levels. Prednisolone reduced arginine-stimulated insulin secretion at a glucose level of 10 mM during a hyperglycemic clamp. In addition, several model-derived parameters of beta-cell function, obtained from standardized meal tests, were impaired including decreased glucose-adjusted insulin secretion rates (for both prednisolone dosages), as well as a reduced potentiation factor ratio in the high-dose group. The deleterious effects of GC treatment on these specific beta-cell function parameters confirm and expand previous observations in healthy men where postprandial beta-cell function was shown to be impaired using the same meal-modeling technique [11]. It should be noted that prednisolone increased fasting insulin secretion, post-meal insulin levels and insulin secretion during glucose infusion to compensate for reduced insulin sensitivity, which was apparent in addition to the described impairment of beta-cell function. As such, the M-value obtained during gold standard euglycemic-hyperinsulinemic clamp was dose-dependently reduced following GC treatment. In
addition, GC treatment increased fasting and postprandial glucagon levels in these healthy males, indicating disturbed pancreatic islet-cell balance.

In Chapter 3, we reviewed the additional value of GLP-1 receptor agonists for the treatment of type 2 diabetes. We analyzed clinical trials in which the GLP-1 receptor agonist exenatide was administered to patients with type 2 diabetes and we assessed effects on glycemic control, pancreatic islet function and cardiovascular risk factors such as body weight, waist circumference, lipid metabolism and low-grade inflammation. We concluded that GLP-1 receptor agonists are promising compounds, since they were shown to preserve or even improve beta-cell function in patients with type 2 diabetes, in contrast to the effects of other glucose-lowering drugs. In Chapter 4, we tested the hypothesis that the GLP-1 receptor agonist exenatide would be able to prevent GC-induced diabetogenic effects by means of a proof-of-principle study. In order to create a steady-state condition, with exenatide

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plasma levels in the therapeutic range, we administered exenatide intravenously to healthy men treated with a high dose of prednisolone (80 mg per day, for two consecutive days). Indeed, exenatide was able to prevent GC-induced hyperglycemia during a standardized mixed-meal test. This was achieved by reducing postprandial glucagon levels and slowing down gastric emptying rates - mechanisms by which exenatide has previously been shown to reduce postprandial glucose [12,13]. Probably due to the fact that GLP-1 stimulates insulin secretion in a glucose-dependent manner, we did not observe an increase in postprandial insulin concentrations. This phenomenon is in accordance with a previous study in which exenatide lowered postprandial glucose levels while insulin levels were reduced in patients with type 2 diabetes [14]. Using the hyperglycemic clamp method to assess beta-cell function, we were able to observe that exenatide did prevent GC-induced beta-cell dysfunction and even resulted in increased C-peptide secretion, both during first- and second-phase glucose stimulated C-peptide secretion, and in response to the administration of arginine during hyperglycemia. Thus, GC-treatment induced acute toxic effects on the pancreatic islet cells, which could be reversed by exenatide.

In the following chapters, we explored the potential beneficial effects of DPP-4 inhibition on GC-induced hyperglycemia and islet-cell dysfunction. DPP-4 inhibitors can be orally administered and may therefore be more convenient to use as compared to the injectable GLP-1 receptor agonists. In Chapter 5 we summarized the evidence that DPP-4 inhibitors improve islet-cell function in patients with type 2 diabetes. We reviewed 46 randomized clinical trials (RCTs) that reported static (fasting) or dynamic (after either oral or intravenous glucose administration) parameters of beta-cell function. From these data we concluded that DPP-4 inhibitors have the potential to improve pancreatic islet-cell function, however, long-term trials are needed to assess the durability of these effects. In Chapter 6 we showed that the DPP-4 inhibitor alogliptin in combination with the thiazolidinedione pioglitazone improved glycemic control and beta-cell function in patients with type 2 diabetes. As such, the modeling-derived parameters beta-cell glucose sensitivity and fasting secretory tone as measured during standardized meal tests were improved. The patients who participated in this study were just above glycemic targets as indicated by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) guidelines [15,16] and were on monotherapy with a single agent, mostly metformin. The improvements in beta-cell function, insulin sensitivity and glycemic control were achieved without increased rates of hypoglycemia as compared to placebo. Next, in Chapter 7, we administered the DPP-4 inhibitor sitagliptin (100 mg once daily) concomitantly with prednisolone (30 mg once daily) for two weeks to men with the metabolic syndrome (N=54) to assess the potential of DPP-4 inhibitors to prevent the diabetogenic effects of GCs in a high-risk population. In contrast to the effect of exenatide in the proof-of-principle study (Chapter 4), DPP-4 inhibition by sitagliptin only preserved GC-induced impairment of clamp-measured first and second phase C-peptide secretion, in addition to
a trend towards increased modeling-measured insulin secretion rate at fixed glucose level. Also, a reduction of postprandial glucagon secretion was observed. However, despite these improvements in aspects of islet-cell function, sitagliptin was not able to prevent GC-induced glucose intolerance – i.e. postprandial glucose excursions were not reduced when sitagliptin was added to prednisolone treatment.

GC treatment additionally increases cardiovascular risk by inducing central adiposity, hypertension and dyslipidemia [17-19], all of which factors may be neutralized or ameliorated by incretin-based therapies. In Chapter 8, we summarized current evidence from the literature concerning potential beneficial effects of incretin-based therapies on cardiovascular risk factors. We reviewed 50 RCTs using either GLP-1 receptor agonists (exenatide or liraglutide) or DPP-4 inhibitors (sitagliptin, vildagliptin or saxagliptin) with a minimum duration of 24 weeks, that reported treatment effects on cardiovascular risk factors, including body weight, body composition, blood pressure, lipid profiles and additional bio-markers. We also included small-sized, proof-of-principle studies that investigated direct effects on cardiac or vascular function in humans. In general, treatment with GLP-1 receptor agonists resulted in weight loss while use of DPP-4 inhibitors was weight neutral. Also, GLP-1 receptor agonists, and to a lesser extent DPP-4 inhibitors exerted beneficial actions on cardiovascular risk markers, particularly by reducing blood pressure and (postprandial) lipid levels. Since most cardiovascular risk factors are associated with high body mass index, it is difficult to tease out to what extent the treatment-related beneficial effects occur as a result of (direct) GLP-1 receptor stimulation or secondary to reduction of body weight and/or improved glycemic control. Nonetheless, based on these data we hypothesized that incretin-based therapies could be able to improve the GC-induced cardiovascular risk profile. Interestingly, treatment with sitagliptin in participants with the metabolic syndrome, who received prednisolone 30 mg daily for two weeks in our RCT (reported in chapter 7), did not improve any of the assessed cardiovascular risk factors, including weight, body composition, blood pressure and lipids. Although the study was of short duration, based on these findings, we could not prove this hypothesis.

Finally, Chapter 9 in the Appendix is a proof-of-concept mechanistic study, addressing the role of microvascular function in postprandial hyperglycemia in men with type 2 diabetes and those with the metabolic syndrome, i.e. individuals at risk to develop type 2 diabetes. Insulin is known to mediate (micro)vascular activity [20] and as such, insulin-stimulated glucose uptake has been associated with increased capillary density during hyperinsulinemia [21-23]. Here, we demonstrated that, in spite of postprandial hyperinsulinemia, meal-related glucose was elevated in diabetic patients and men with the metabolic syndrome (relative to matched controls), whereas postprandial capillary recruitment was impaired. Our study is the first to demonstrate video microscopy-measured capillary density in the postprandial state to be associated with glucose tolerance. In the light of this
thesis, these data are of importance, since our group has recently shown that two weeks treatment with prednisolone 30 mg daily in healthy individuals, significantly impaired microvascular function during euglycemic-hyperinsulinemic clamp [24]. Moreover, capillary recruitment was positively correlated with insulin sensitivity (M-value) and negatively with postprandial area under the curve for glucose [24]. Although not studied in this thesis, incretin-based therapies have been shown to improve endothelial function [25-27], however, these studies did not specifically address the link between vascular function, vascular insulin resistance and glucose disposal. We were unable to accommodate the microvascular measurements in our RCT; therefore, future research should investigate whether incretin-based therapies prevent GC-induced glucose intolerance by improving microvascular dysfunction.

DISCUSSION

Glucocorticoid-induced diabetes: potential role for incretin-based therapies

Effects of glucocorticoids on glucose metabolism

In this thesis, we confirmed the diabetogenic effect of GC treatment, which is caused by both the induction of islet-cell dysfunction and insulin resistance. We could also confirm the observation that GCs particularly elevate postprandial glucose levels, whereas fasting glucose levels are only minimally affected. It is well known that the effects of GCs on glucose metabolism are dose-dependent. In Chapter 2, we compared 7.5 mg daily which is equivalent to a low maintenance dose, to 30 mg, which may be considered a high dose, used for so called induction treatment [28]. We decided to administrate the compound for a total of 14 days, since this equals the duration of the induction dose [28] and was previously shown to be safe in healthy individuals [11]. Only recently, our groups showed that prednisolone at dosages compatible with cortisol levels just above physiological range (i.e. 7.5 mg per day equivalent) already affect glucose and lipid metabolism [1], while these were generally considered safe [28]. Thus, this often-prescribed low-maintenance dosage was shown to induce insulin resistance at the level of liver, adipose tissue and skeletal muscle, resulting in increased endogenous glucose production, whole body lipolysis and reduced insulin-stimulated glucose disposal, respectively [1]. In Chapter 2, we confirm and expand these observations by demonstrating that already at this low dose, prednisolone treatment affected beta-cell function parameters in addition to impairment of insulin sensitivity. Collectively, these abnormalities resulted in the development of postprandial hyperglycemia during the standardized meal tests in the healthy volunteers. For the RCTs reported in Chapters 4 and 7, we used a somewhat different design. The RCT described in Chapter 4 was set up as a proof-of-principle study in a cross-over design, with the use of prednisolone 80 mg daily for two consecutive days and intravenously administration of exenatide to test the acute effects. As for Chapter 7, the protocol resembles that of Chapter 2, except that participants received only the higher dose of 30 mg daily, due to the expected stronger effect and the main aim to test the efficacy of additional sitagliptin therapy.
All previous studies that addressed the pathophysiology of GC-related diabetogenic effects were performed in a well-phenotyped population consisting of young (lean) healthy volunteers [17]. In this thesis, we also studied the effects of prednisolone treatment in subjects with the metabolic syndrome. We chose this specific population, since a high proportion of patients with chronic inflammatory diseases (i.e. those who are often prescribed GCs), is characterized by features of the metabolic syndrome, regardless of GC use [29,30]. We observed increased postprandial glucose excursions for GCs (vs. placebo) in both metabolic syndrome participants (~17% increase) and healthy controls (~27% increase). However, at the same exposure (i.e. 30 mg per day for 14 days), prednisolone treatment had differential metabolic effects in men with the metabolic syndrome versus healthy controls. Indeed, in healthy controls, prednisolone 30 mg per day strongly decreased insulin sensitivity, while there was some compensation by enhanced insulin secretion as demonstrated by fasting hyperinsulinemia, increased postmeal insulin levels (Chapter 2) and a tendency towards increased (uncorrected) first- and second phase insulin and C-peptide levels. However, beta-cell dysfunction was demonstrated as well at specific parameters: C-peptide secretion following combined hyperglycemia-arginine stimulation and a number of meal-derived beta-cell parameters were disturbed following prednisolone 30 mg per day treatment. In contrast, in participants with the metabolic syndrome, prednisolone 30 mg daily did not impair insulin sensitivity, while it blunted first- and second-phase glucose-stimulated C-peptide secretion as well as C-peptide secretion following combined hyperglycemia-arginine administration. At present, we have no clear explanation as to why similar GC exposure did not impair insulin sensitivity in participants with the metabolic syndrome as compared to lean healthy men. A potential explanation could be that metabolic syndrome subjects were already insulin resistant at baseline (M-value ~3.5 mg/kg.min in the metabolic syndrome compared to ~8 mg/kg.min in healthy men) and that at this (cumulative) dose, GC treatment was unable to further worsen this.

In addition to beta-cell dysfunction, GCs were also shown to affect alpha-cell function by increasing fasting and postprandial glucagon levels [1,31,32]. In our studies, in both men with the metabolic syndrome as well as in healthy men, prednisolone 30 mg daily increased fasting and postprandial glucagon levels up to ~30% and ~50% respectively, while 7.5 mg prednisolone did not affect glucagon levels in healthy men.

Thus, although GCs induced in both populations a pattern of hyperglycemia, in healthy controls this was caused by combination of insulin resistance and islet-cell dysfunction, whereas in men with the metabolic syndrome islet-cell dysfunction was the predominant contributor to postprandial hyperglycemia.
Treatment of glucocorticoid-induced hyperglycemia/diabetes

Current treatment recommendations of GC-induced hyperglycemia or steroid diabetes are largely based on expert opinion rather than large-sized randomized clinical trials, and official guidelines are lacking [33-37]. As such, the few intervention studies that were performed were of short duration and were designed as mechanistic proof-of-principle investigations in a small number of healthy individuals [38,39]. Indeed, 14-day pretreatment with troglitazone, but not metformin or pioglitazone decreased dexamethasone-induced hyperglycemia during an oral glucose tolerance test after 3 days of dexamethasone treatment at 4 mg daily, in a small-sized cross-over study in healthy individuals (N=5) [38]. Another small-sized uncontrolled study showed effectiveness of the troglitazone in preventing GC-induced glucose intolerance and insulin resistance in healthy participants treated with dexamethasone for 4 days (4 mg daily). However, participants gained 1.7 kilograms of weight during the 4-6 weeks pretreatment with troglitazone [39], a well-known side effect of thiazolidinediones [40]. Moreover, the class of thiazolidinediones cause edema, heart failure and bone fractures [40-42]. Thus, the combination of thiazolidinediones and GCs, which are known to increase the risk for osteoporosis [18], seems unfavorable. Besides, troglitazone was taken off the market in 2000 due to liver toxicity [43], while market authorisation of rosiglitazone was suspended in the EU in 2010, due to the association of its use and ischemic heart disease. At present, given the predominant postmeal rise in glucose during GC treatment, short-acting prandial insulin is currently recommended [33-37]. However, choosing the right dosage of insulin may be challenging due to the fact that GC dosage is often tapered over time making insulin demand variable. Furthermore, insulin therapy may increase the risk for hypoglycemia and induce weight gain, both of which are undesired side effects.

In recent years, incretin-based therapies have become available for the treatment of diabetes. These compounds are promising with regard to their glucose-dependent mechanism of action, with subsequently a low hypoglycemia risk, but also due to the fact that they mainly target postprandial hyperglycemia, improve beta-cell function and reduce body weight with concomitant amelioration of the cardiovascular risk profile [7-10]. However, at the onset of this thesis, there were no trials addressing the effect of incretin therapies, GLP-1 receptor agonists or DPP-4 inhibitors, on GC-induced hyperglycemia. We reasoned that since particularly the GLP-1 receptor agonists ameliorate most of the phenotypic defects of type 2 diabetes, all of which are also mimicked by GC use, these compounds could best be used for the treatment of steroid diabetes.

Incretin-based therapies and glucocorticoid-induced hyperglycemia

GLP-1 receptor agonists. As stated above, to date there are no RCTs investigating the effect of either incretin class on GC-induced hyperglycemia. To the best of our knowledge, the studies described in Chapters 4 and 7 are to date the only studies addressing this research question according to an
RCT-design. However, case studies were previously and also recently reported [44,45]. The potential use of GLP-1 receptor agonists for the treatment of GC-induced diabetes was first proposed in 2007 when Ritzel and colleagues infused GLP-1 (at a dose of 1.2 pmol/kg/min) in 10 patients with diabetes of whom one patient was only later found to have diabetes due to Cushing’s disease [44]. By comparing the one patient with Cushing’s disease to the 9 patients with type 2 diabetes, the investigators studied whether the effects of GLP-1 on glucose metabolism were preserved in hypercortisolism. Their major findings included a similar reduction in (fasted) glucose levels after 4h of GLP-1 infusion in the patient with Cushing’s disease compared to those with type 2 diabetes [44]. More recently, the beneficial effects of the GLP-1 receptor agonist exenatide, administered subcutaneously, on dexethasone-induced glucose intolerance were demonstrated in mice [46]. In addition, 4 cases of patients who were previously diagnosed with diabetes (duration range 1-20 years) but whose glycemic control worsened under GC therapy, were successfully treated with exenatide twice daily [45]. Based on continuous glucose monitoring (CGM) profiles, the timing of exenatide administration was adjusted to match the greatest (postmeal) glucose excursions. The importance of specifically targeting postprandial glucose in GC-treated individuals was illustrated by the fact that one case initially received the long-acting GLP-1 receptor agonist liraglutide. However, under this therapy, HbA1c gradually increased. CGM showed that liraglutide, compatible to all long-acting GLP-1 receptor agonists, did not sufficiently lower postprandial glucose excursions as compared to short-acting exenatide twice daily [47]. This phenomenon is explained by a partial tachyphylaxis of the GLP-1 effects on gastric emptying with continuous (as occurs with long-acting GLP-1 receptor agonist such as liraglutide, exenatide once weekly and albiglutide) versus intermittent GLP-1 receptor agonist exposure (as occurs with short-acting exenatide twice daily and lixisenatide once daily) [48-50]. Interestingly, by finally switching liraglutide once daily to exenatide twice daily, the patient’s glycemic excursions and overall glycemic control improved [45]. In our RCT in Chapter 4, we successfully lowered postprandial GC-induced hyperglycemia by administering exenatide continuously. Due to the fact that we observed participants for only one postprandial period (4h), occurrence of tachyphylaxis could not be studied.

DPP-4 inhibitors. In our studies, the ability to prevent GC-induced postprandial hyperglycemia was different for DPP-4 inhibitors than for GLP-1 receptor agonists. While the GLP-1 receptor agonist exenatide prevented an increase in postprandial glucose levels in healthy humans, the DPP-4 inhibitor sitagliptin was not able to prevent the increase in postprandial glucose levels induced by GC treatment in subjects with the metabolic syndrome.

In spite of this lack of effect on glucose tolerance, the DPP-4 inhibitor sitagliptin improved various aspects of clamp-measured GC-induced beta-cell dysfunction, while the GLP-1 receptor agonist improved both meal-related glucose tolerance and clamp-measured beta-cell function. Since the
secretion of incretin hormones is stimulated by nutrients and their insulinotropic effect is glucose-dependent, higher glucose levels may be needed (than achieved in the metabolic syndrome males) for DPP-4 inhibitors to exert their full potential. Thus, sitagliptin was not able to enhance meal-related insulin secretion, at glucose levels ~8 mM, while clamp-measured insulin-secretion was indeed enhanced, at glucose levels ~15 mM. Previous studies assessing the postprandial effect of DPP-4 inhibitors on beta-cell function have generally been undertaken in patients with type 2 diabetes, who by definition suffer from higher postprandial glycemic levels. However, two studies have investigated the effect of DPP-4 inhibition in subjects with impaired fasting glucose [51] or impaired glucose tolerance [52]. Both studies showed reduced postprandial glycemia and improved beta-cell function. In these studies patients were treated for a longer duration (i.e. 6 weeks [51] or 12 weeks [52]) than our subjects (2 weeks). Although, a single dose of DPP-4 inhibitor lowers postprandial glucose [53], it is unclear whether prolonged dosing is required for these agents to improve GC-induced hyperglycemia. Although the hyperglycemic clamp test [54] is generally considered the gold standard for assessing beta-cell function [55], this methodology does not reflect real-life conditions [56]. In contrast, the standardized mixed-meal test is an elegant method to assess real-life insulin secretion and glucose tolerance. Indeed, after an oral nutrient load, insulin secretion reflects an integrated response to many physiological stimuli, including glucose, other nutrients, such as fatty acids, neurological stimuli and (incretin) hormones. Therefore, the observation that sitagliptin was able to enhance clamp-measured beta-cell function might be of less clinical relevance, since GC-induced glucose intolerance as measured by the more physiological mixed-meal test could not be restored.

Inhibition of the enzyme DPP-4 may result in other biological effects through different mechanisms of action than exogenous GLP-1 administration. This enzyme (also known as CD26) is ubiquitously present in the human body. In addition to presence on various tissues, it is present on the surface of immune cells. As such, it activates lymphocytic T-cells and plays a role in the regulation of the immune response [57]. Diminished activation of the enzyme may lead to immunodeficiency and has been shown to be associated with the human acquired immunodeficiency syndrome (AIDS) [58,59], whereas increased activity of DPP-4 has been associated to several auto-immune diseases such as rheumatoid arthritis, multiple sclerosis or Graves’ thyroid disease [60-62]. Since GCs primarily exert anti-inflammatory effects, by affecting both the innate and the adaptive immune system [18], it is possible that GCs also affect DPP-4 activity or expression of DPP-4 on T-cells. Currently, there is only a small number of – conflicting – experimental studies that investigated GC-effects on DPP-4. In human skin fibroblast cell cultures, 4-day incubation with either dexamethasone or hydrocortisone reduced DPP-4 activity in the fibroblasts [63], whereas in another study that investigated the effect of GC-treatment on T-cell activation in rats, hydrocortisone was shown to increase DPP-4 activity and expression of DPP-4 on the T-cell surface [64]. Based on these limited data GCs may either
increase DPP-4 levels – i.e. antagonizing the effect of DPP-4 inhibitors – or reduce DPP-4 levels – i.e. a potential synergistic effect. However, in this thesis we observed no effect of prednisolone treatment on DPP-4 activity, and co-administration of sitagliptin and prednisolone reduced DPP-4 activity to the same extent as sitagliptin alone.

Taken together, although the differences in design and study population of our two studies should be mentioned, i.e. acute versus two-week drug administration and healthy men vs. those with the metabolic syndrome, the observed differences in outcome may be primarily ascribed to the distinct working mechanisms of the two incretin drug classes. These also include the fact that GLP-1 receptor agonist administration results in supraphysiological levels of (synthetic) active GLP-1 [9], which reduce gastric emptying rate and subsequently result in reduced postprandial glucose levels [65]. Since DPP-4 inhibitors only enhance endogenous incretin levels (by preventing degradation of naturally secreted incretins), gastric emptying rate is not delayed [66]. As is evident from the postprandial glucose curves demonstrated in Chapter 4, the GLP-1 receptor agonist exenatide prevented GC-induced hyperglycemia for a significant part by delayed gastric emptying. Thus, supraphysiological as compared to physiological levels of GLP-1 may additionally be more effective in potentiating beta-cell insulin secretion.

Finally, a similarity in findings was noted between GLP-1 receptor agonists and DPP-4 inhibitors as both compounds were able to prevent the increase in glucagon levels to a similar extent. Thus, the increase in hepatic glucose production that is characteristic of GC therapy [17], may be partially reduced by incretin-based therapies by reducing glucagon secretion. Unfortunately, since we did not employ tracer methods during the meal, we were unable to prove this.

**Mechanism of action: pancreatic islet-cell function.** As described in the introduction of this thesis, little is known about the potential mechanisms by which GCs and incretin hormones interact at the level of the pancreatic islets. However, both GCs and incretins act on the protein kinase A (PKA) pathway that is involved in exocytosis of insulin-containing granules from the beta-cell [67,68]. It might occur through this mechanism that incretins may counteract the deleterious effects of GCs on islet-cell function or vice versa. Moreover, GCs were shown to induce apoptosis of mouse beta cells which could be reversed by the GLP-1 receptor agonist exendin-4, and this effect was also mediated via PKA [69]. In addition, more recently, GCs were shown to induce beta-cell dysfunction by inducing endoplasmic reticulum stress [70]. Since GLP-1 agonists were shown to reduce ER stress, this may be another mechanism by which incretin based therapies may prevent GC-induced beta-cell dysfunction [71].

In humans, a number of clinical studies have investigated the effects of GC treatment on incretin levels and action [32,72-74]. These studies showed that postprandial concentrations of GLP-1 or
glucose-dependent insulinotropic polypeptide (GIP) were not altered (except for elevated GIP in two studies [32,72]) by GC treatment with either 12 days prednisolone 37.5 mg once daily [32,72] or dexamethasone 2 mg twice daily for 7 days [73]. These results are in keeping with the results in this thesis: total GLP-1 or GIP were not decreased by prednisolone (Chapter 2). Interestingly, in two of above-mentioned studies, the investigators also performed isoglycemic clamp tests in order to assess the meal-related incretin effect. The results from the isoglycemic clamps showed that GC treatment did reduce the incretin effect despite normal or even elevated GLP-1 and GIP levels [72,73]. In addition, after pretreatment with prednisolone 37.5 mg for 12 days, the insulin response to (physiological levels of) GLP-1 administration during a hyperglycemic clamp test was reduced compared to placebo [74]. Therefore, we may conclude that GCs do not affect incretin release per se but may hamper their effectiveness e.g. by interference with downstream signaling pathways in the pancreatic beta cells. The fact that in this thesis sitagliptin and to a greater extent exenatide, were both able to restore clamp-measured GC-induced beta-cell dysfunction, might indicate that GCs do not fully block the GLP-1 mediated insulin secretory pathway and, moreover, that the GC-impaired incretin effect may best be prevented by supraphysiological levels of GLP-1.

Not only beta-cell function, but also pancreatic alpha-cell function plays an important role in glucose metabolism [75]. As such, both increased fasting glucagon concentrations and a lack of suppression of glucagon secretion in the postprandial state have been demonstrated to be important features in the development of type 2 diabetes [76,77]. Very few studies have investigated the effects of GCs on glucagon secretion before [1,31,32], however the results are in keeping with our findings: increased fasting glucagon concentrations and lack of suppression of glucagon secretion in the postprandial state. As for incretin hormones, the effect of either GLP-1 or GIP may differ: GLP-1 has been shown to suppress glucagon secretion when hyperglycemia or euglycemia occurs [78,79], whereas GIP was shown to enhance glucagon secretion in the euglycemic state [80]. In the above-mentioned study by Hansen and colleagues, 12 days of prednisolone 37.5 mg in healthy subjects increased both glucagon and GIP in the postprandial state; the authors hypothesized that GCs may increase glucagon via increased levels of GIP [32]. Our results however, do not support this hypothesis since we observed GC-induced hyperglucagonemia despite unaltered levels of GIP (Chapter 2).

Glucocorticoid-induced increased cardiovascular risk: potential benefit of incretin-based therapies

GCs not only hamper glucose metabolism but also affect several other metabolic pathways or organ systems, thus resulting in an increased appetite and significant weight gain, altered body composition with increased visceral fat mass, elevated blood pressure, atherogenic lipid profile, prothrombotic state and increased vascular resistance [17,18]. Through these mechanisms, GC-treatment increase the risk to develop cardiovascular disease [19]. Indeed, we showed that in healthy young men, two-
week prednisolone 30 mg daily increased blood pressure and induced insulin resistance. In men with the metabolic syndrome however, we did not observe alterations in any cardiovascular risk factor, i.e. blood pressure, body weight or lipid profile, after two weeks of prednisolone treatment. Possibly because these study subjects were already metabolically compromised, in contrast to the healthy young men, prednisolone did not further aggravate blood pressure or body weight or body composition. Another explanation might be that the effects on cardiovascular risk might only become apparent after prolonged treatment, and thus could not be observed after merely two weeks of treatment. Due to the absence of GC-induced alterations in cardiovascular risk factors, the potential beneficial effect of sitagliptin could not be assessed. Future, longer-term studies may be needed to test this specific hypothesis. However, we did review the potential beneficial effects of incretin-based therapies on several cardiovascular risk factors, such as blood pressure, body weight and lipid profiles (Chapter 8). Beneficial effects were observed for GLP-1 receptor agonists as well as for DPP-4 inhibitors, however, the effects were more pronounced for GLP-1 receptor agonists. In general, GLP-1 receptor agonists reduced body weight and total body fat and visceral fat, lowered blood pressure, improved both fasting and postprandial dyslipidemia, and improved several markers of inflammation and oxidative stress, while DPP-4 inhibitors were weight neutral, and - from the limited data available - did not significantly alter blood pressure, lipid profiles or cardiovascular biomarkers [81,82]. Taken together, current evidence suggests that GLP-1 receptor agonists, rather than DPP-4 inhibitors, may be more effective with regard to ameliorating GC-induced diabetogenic effects (as described above) and with regard to reducing (GC-induced) cardiovascular risk. However, large-scaled trials are underway to evaluate the effects of both incretin drug classes on cardiovascular and other safety and efficacy measures in high-risk patients (reviewed in [47], also refer to Chapter 8, Table 2).

Limitations
In general, one of the limitations to the results reported in this thesis might be, that we only investigated healthy individuals whereas in clinical practice GCs are mostly prescribed to patients with chronic inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) [83] or rheumatoid arthritis [84]. Those patients are generally characterized by low to high grade inflammation which may affect glucose metabolism, since (low grade) inflammation is associated with the development of insulin resistance and beta-cell dysfunction [85,86]. Furthermore, the duration of treatment (2 weeks) might have been too short to fully unveil the metabolic side effects of GCs, since GCs - in a low maintenance dose - may be prescribed for years, although the present studies are of the longest duration up to date in a healthy population. In addition, the RCTs reported in this thesis are of relative small size, however, not only did previous mechanistic studies (by using similar methodology) demonstrate meaningful effects in comparably sized protocols, also, the study populations were relatively homogenous (males, defined age-groups, either healthy or metabolic
syndrome criteria, randomized) allowing detection of small differences even in a relatively small number of participants. However, the use of respectively either healthy men or men with the metabolic syndrome precluded a direct comparison between the GLP-1 receptor agonist exenatide (Chapter 4) and the DPP-4 inhibitor sitagliptin (Chapter 7). Additionally, treatment-related changes in oral glucose uptake and hepatic glucose production could not be quantified due to the absence of stable isotope-use in the meal-tests. Also, postprandial plasma levels of incretin hormones could not be determined in Chapters 4 and 7, nonetheless in Chapter 2 there were no alterations in incretin levels observed. Off note, for all protocols we deliberately chose to include white men, as ethnicity and gender may be effect modifiers of several treatment-related study variables [87].

With regard to the use of incretin-based therapies, one major limitation is the relative short clinical experience with these compounds. Since type 2 diabetes is a chronic disease, full potential of (early) treatment to prevent disease-related complications may only become apparent after years [88]. Yet available long-term trials have a maximum duration of three years, and are mostly open-labeled, lacking an appropriate control group. However, even a three-year study might not be long enough to truly assess the efficacy (and safety) of a new drug with regard to glycemic control. That is, from long-term trials like the ‘United Kingdom Prospective Diabetes Study’ (UKPDS) or the ‘A Diabetes Outcome Progression Trial’ (ADOPT) study [89-91] we have learned that one should be cautious when interpreting short-term trial results. For instance, in the ADOPT trial, after one year the sulfonylurea drug glyburide was superior with regard to glycemic control and beta-cell function compared to metformin or rosiglitazone. However, after a follow-up period of four years, it appeared that the patients treated with glyburide, after the initial improvements, had rapidly progressed to worsened glycemic control over years [89]. Besides, only through the longer-term exposure, safety issues, such as an elevated fracture risk for rosiglitazone, became apparent [89]. Therefore, longer-term clinical trials should be awaited for to fully appreciate the clinical efficacy and safety of incretin-based therapies.

Although little experience yet exists, over years concern has risen about the safety of incretin-based therapies with regard to increased risk of pancreatitis or even pancreatic cancer. In particular, DPP-4 inhibitors are of concern due to the potential role of DPP-4 in systemic immunity, although in large safety analyses there seems to be no increased infection rate [92,93]. Recently however, a large-numbered data base study revealed an increased risk for pancreatitis with either exenatide or sitagliptin treatment [94]. Moreover, a small-numbered post mortem human pancreas study observed an increased prevalence of pancreatic hyperplasia compared to age-matched healthy controls [95]. However, others did not find an increased risk for pancreatitis with either exenatide or sitagliptin [96-98]. The relevance of these data for clinical practice and whether the benefits outweigh the risk, must be determined by longer-term studies [99]. Large-scaled trials are underway.
to evaluate efficacy and most importantly safety of both incretin drug classes in high-risk patients (refer to Chapter 8, Table 2 for an overview).

CONCLUSIONS AND FUTURE PERSPECTIVES

Novel GC compounds that are designed to generate less glucometabolic side effects are currently under development. These so-called dissociated GC-receptor compounds have been developed based on the observations that GCs exert their anti-inflammatory effects mostly by inhibiting transcription of pro-inflammatory genes (‘transrepression’), while the metabolic side effects predominantly occur through stimulatory effects on gene transcription (‘transactivation’) [100]. These compounds are designed such that they mostly induce transrepression with reduced transactivation activity [100]. However the development of these compounds will take some more years, thus in the meanwhile it may be necessary to develop effective treatment strategies to treat GC-induced hyperglycemia. Based on the findings of this thesis, GLP-1 receptor agonists, rather than DPP-4 inhibitors, may be suited to treat or prevent GC-induced hyperglycemia. Both strategies improved GC-induced islet-cell dysfunction, nevertheless only the GLP-1 receptor agonist exenatide was able to restore GC-induced glucose intolerance during the meal test. However, GLP-1 receptor agonists – as compared to DPP-4 inhibitors – induce more severe gastro-intestinal side effects (mainly nausea) and have to be administrated by means of subcutaneous injections, which both may hamper patient tolerance and acceptance. This may even be aggravated by the fact that patients treated with GCs may already suffer from reduced quality of life due to the underlying chronic inflammatory illness.

As mentioned above, we only investigated healthy individuals instead of patients with chronic inflammatory diseases, while inflammation may affect both insulin resistance and beta-cell dysfunction [85,86]. To what extent the use of GCs improves this inflammatory component and thus may improve the patients’ glucose metabolism – thereby preventing hyperglycemia instead of inducing it – remains uncertain. Possibly GC treatment may worsen glucose metabolism in patients suffering from systemic low-grade inflammation (such as COPD), while in patients suffering from higher-grade inflammation (such as rheumatoid arthritis) the detrimental effects of systemic inflammation on insulin resistance and beta-cell function will be abolished by GCs, resulting in a neto beneficial effect on glucose metabolism. As such, one week prednisolone treatment in newly-diagnosed rheumatoid arthritis patients showed a large inter-individual difference in glucometabolic side effects resulting in either improved or deteriorated glucose tolerance status [101]. It would be of interest to test the effect of GLP-1 receptor agonists on GC-induced diabetogenic side effects in patients with chronic inflammatory diseases that develop hyperglycemia. Therefore, ideally, in future studies patients with rheumatoid arthritis who have developed hyperglycemia during GC-treatment, would be treated with a GLP-1 receptor agonists (e.g. exenatide twice daily) or placebo. Prospective observation of these patients for at least 12 months would be needed in order to assess
whether the development of GC-induced diabetes could be halted or even prevented by GLP-1 receptor agonist treatment. In addition, another interesting study would be a randomized trial in which patients that already have developed GC-induced diabetes would be included and randomized to different glucose-lowering agents: metformin, a DPP-4 inhibitor, a GLP-1 receptor agonist or short-acting insulin; and to assess efficacy and compliance of either treatment. A number of current trials are ongoing that investigate either the effect of metformin on metabolic side effects of GCs (NCT01319994; NCT01187849) or the effect of distinct insulin regimens on (post-transplant) GC-diabetes (NCT01648218; NCT01810952), in patients using GCs for several inflammatory conditions. However, to date, no incretin-based interventions to be combined with GC exposure in relevant patient populations have been registered on clinicaltrials.gov.

To conclude, in this thesis we demonstrated that pancreatic islet-cell dysfunction is an important feature of GC-induced diabetogenic effects. We showed that, in healthy subjects, GLP-1 receptor agonists were able to treat or even prevent GC-induced hyperglycemia, as demonstrated by improvements in glucose tolerance and pancreatic islet-cell function. In contrast, DPP-4 inhibitors were not able to prevent GC-induced glucose intolerance in metabolic syndrome, despite improvements in several aspects of islet-cell function. Future studies should investigate to what extent either GLP-1 receptor agonists or DPP-4 inhibitors might be useful to treat or to prevent GC-induced diabetes in relevant populations in clinical practice.
Concluding Key Messages

- Glucocorticoids mainly induce postprandial hyperglycemia rather than fasting hyperglycemia, this has important consequences for treatment
- Glucocorticoid-induced diabetes not only occurs as a result of insulin resistance, but also due to pancreatic islet-cell dysfunction
- Glucocorticoid diabetogenic effects occur dose-dependently, but side effects may already be present at a low dosage of prednisolone 7.5 mg dose equivalent
- Glucocorticoid-induced diabetes should ideally be treated with an agent that both targets postprandial hyperglycemia and preserves pancreatic islet-cell function
- Incretin-based therapies are promising compounds with regard to preservation of pancreatic islet-cell function, although the durability of the effect is yet unknown
- Both the GLP-1 receptor agonist exenatide and the DPP-4 inhibitor sitagliptin were shown to restore glucocorticoid-induced pancreatic islet-cell dysfunction
- Exenatide, but not sitagliptin, was able to restore glucocorticoid-induced (postprandial) glucose intolerance
- GLP-1 receptor agonists, rather than DPP-4 inhibitors, are suited to target glucocorticoid-induced diabetes
- Since incretin-based therapies have beneficial effects on cardiovascular risk, these compounds may also reduce the increased cardiovascular risk associated with glucocorticoid treatment
- Future research should investigate whether incretin-based therapies will be effective in the treatment or prevention of glucocorticoid-induced diabetes in patients with a chronic inflammatory disease
REFERENCE LIST


79. Vilsboll T., Krapur T., Madisbad S., Holst J.J. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept 2003; 114:115-121.


