Chapter 15

General Discussion:
"Diabetogenic Effects of Glucocorticoid Drugs: The Knowns and The Unknowns"

D.H. van Raalte and M. Diamant
Glucocorticoids: efficacious anti-inflammatory agents limited by their side effects

For decades, glucocorticoids (GCs) are the most commonly prescribed anti-inflammatory and immunosuppressive agents. GCs reduce the activity of the innate immune system by decreasing phagocytic activity and by attenuating the production of pro-inflammatory mediators [1]. Additionally, GCs affect the acquired immune system by impairing a variety of T-cell functions [1]. Because of these combined immunomodulatory actions, GCs are the cornerstone in the treatment of numerous inflammatory diseases, including rheumatic diseases (rheumatoid arthritis (RA) [2] and polymyalgia rheumatica [3]), systemic lupus erythematosus [4], small and large vessel arteritis [5], inflammatory bowel disease [6], chronic obstructive pulmonary disease [7] and various other conditions of inflammatory origin. Already in 1949, the beneficial effects of systemic cortisone treatment, the first available GC, on some of these conditions were recognized and the published results were received with great enthusiasm. Pioneers in the discovery of adrenal gland hormones and their clinical effects, Tadeusz Reichstein, Philip Showalter Hench and Edward Calvin Kendall were awarded the Nobel Prize in 1950.

In the following decade, however, the initial enthusiasm for GC treatment was tempered when its detrimental side effect profile became increasingly apparent. The earliest side effects reported, included salt and water retention, increased gastric acidity and psychosis [8]. Soon the full scope of side effects induced by GC treatment became evident and is now known to include weight gain, fluid retention, hypertension, impaired glucose metabolism, diabetes, skeletal muscle atrophy, osteoporosis, gastric ulcer disease, glaucoma, neuropsychiatric symptoms and increased risk to develop cardiovascular disease. And this summary of GC-related side effects may even not be complete [9, 10]. Despite the formulation of newer synthetic compounds, such as prednisolone and dexamethasone, the side-effect profile remained largely unchanged. This unfavorable side effect profile of systemic GC treatment has several important clinical consequences. First, physicians are often forced to lower GC dosages compromising efficacy of treatment of several disease entities. Secondly, resistance among physicians to prescribe and among patients to take GCs due to their infamous side effect profile is a well-characterized phenomenon, resulting in inadequate treatment of several disease entities [11]. Finally, in clinical practice, additional therapies are often initiated to mitigate the side effects induced by GC treatment, e.g. bisphosphonates to prevent osteoporosis [12] and proton pump inhibitors to prevent gastric ulcer disease [13].

Glucocorticoids diabetogenic effects: focus of this thesis

In this thesis, we have focused on the unfavorable effects of GCs on glucose metabolism, and to a lesser extent on lipid and protein metabolism. Additionally, we have performed pioneering studies to further detail the underlying mechanisms of the metabolic side effects. Specifically,
the effects of GCs on insulin production (beta-cell function) and insulin sensitivity were assessed. We have concentrated on these glucometabolic side effects of GC treatment since GC-induced glucose intolerance and overt diabetes are frequently occurring adverse events of GC treatment [14-16]. However, these effects, in particular the mechanisms contributing to their development, are still only partly understood. Additionally, no guidelines currently exist how to treat or prevent this adverse effect, as has been the case for some of the forementioned side effects of systemic GC treatment.

Glucocorticoids diabetogenic effects: importance of understanding the pathophysiology

Understanding both the mechanisms of GC action and the mechanisms underlying GCs diabetogenic effects is relevant for several reasons. First, at a molecular level, increased insight into the (nuclear) actions of GCs has led to the current development of dissociated glucocorticoid receptor (GR) agonists or selective GR modulators (as extensively reviewed in chapter 2). These compounds are designed to specifically induce transrepression, but not transactivation, thus aiming to reduce metabolic side effects with unchanged anti-inflammatory capacities, potentially leading to an enhanced therapeutic index [17-19]. At present, several pharmaceutical companies are developing such compounds, including Merck Sharp Dome (former NV Organon), our partner in this Top Institute Pharma consortium (see general introduction). In recent studies, they were able to show that their compound ORG214007-0 \( (C_{24}H_{23}N_{5}OS) \) was able to reduce inflammation both in vitro and in vivo in collagen-induced arthritis mice to the same extent as prednisolone, while demonstrating reduced transactivation. As such, the expression of key enzymes in gluconeogenesis phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (GPPase) was decreased, resulting in unchanged fasting glucose plasma levels in mice treated with ORG214007-0, while prednisolone induced hyperglycemia [20]. However, to enable further development of these compounds for use in humans, insight into the mechanisms of GC-induced side effects is necessary. More specifically, there is need for the identification of biomarkers that in a dose-dependent manner reflect or predict the occurrence of side effects of the classic GR agonists, in order to compare these adverse effects with novel GR modulators. Secondly, increased understanding of the mechanisms underlying GCs diabetogenic effects may improve strategies to treat or even prevent these glucometabolic abnormalities, since the novel dissociated GR activators are not likely to become available for clinical use within a short period of time. Thus, identification of the organs and pathways involved may provide an evidence base to develop therapeutic and preventive strategies in the context of chronic GC treatment and its associated diabetogenic side effects. Additionally, these data will be critical to expand our knowledge that can be harnessed for the development of targeted dissociated GR agonists.
Studies conducted in this thesis

In order to study the mechanisms underlying the adverse metabolic effects of GCs, a number of studies were performed in this thesis. Most of the data in this thesis were obtained from the PANTHEON (I + II) studies: the Peripheral effects of prednisolone on glucose metabolism, metabolic Hormones, insulin sensitivity and insulin secretion in healthy young males: two randomized, placebo-controlled, double blind, dose-response intervention studies. In both PANTHEON-I and -II study, 32 healthy male volunteers were randomized to a two-week treatment with prednisolone 30 mg daily (so called induction dosage; n=12), prednisolone 7.5 mg daily (so called maintenance dosage; n=12) or placebo (n=8) (chapters 5, 8, 9, 10, 11 and 12). These dosages were chosen since they represent the commonly prescribed high- and low dosage in clinical practice. In both studies, participants were treated for a period of two weeks in order to enable studying past the acute effects of prednisolone treatment. However, for ethical reasons, it was not feasible to administer healthy volunteers high-dose prednisolone for a more prolonged period of time.

In the PANTHEON studies, the dose-dependent effects of GCs on glucose tolerance was assessed by standardized meal challenge tests (chapter 5). We studied the effects of GC treatment on insulin sensitivity of liver, skeletal muscle and adipose tissue (chapter 5 and 8) by using hyperinsulinemic-euglycemic clamps with stable isotopes. To gain insight into mechanisms of GC-induced skeletal muscle insulin resistance, insulin signaling was studied in skeletal muscle biopsies before and during GC treatment (chapter 10). Additionally, the potential role for both accumulation of glycosphingolipids and the ganglioside GM-3, and mitochondrial dysfunction in GC-induced skeletal muscle insulin resistance was addressed in chapter 9. The contribution of microvascular dysfunction to the GC-induced attenuation of insulin-stimulated glucose uptake in skeletal muscle is reported in chapter 10.

To characterize the effects of GC on adipose tissue function at the molecular level, subcutaneous adipose tissue biopsies were collected before and during prednisolone treatment and were analyzed for insulin signaling, lipolytic enzymes, markers of differentiation and secretion of adipocytokines (chapter 11). In addition, the role of the recently discovered protein angiopeptin-like protein (Angptl)-4 in GC-induced changes in lipid metabolism particularly in relation to insulin resistance was studied in chapter 12.

In addition to the effects of GCs on insulin sensitivity, the effects of GCs on beta-cell function was assessed, both following acute (chapters 4 and 7) and more chronic exposure (chapter 5). In chapter 3, we report that GC-induced beta-cell dysfunction is characterized by endoplasmic reticulum (ER) stress resulting in impaired insulin production and secretion and beta-cell apoptosis.
In chapter 7, we have described a proof-of-concept study demonstrating the potential of glucagon-like peptide-1 receptor agonists (GLP-1 RA) to prevent GC-induced glucose intolerance and beta-cell dysfunction. In this study, called the PREDEX (PREDnisolone-induced beta-cell Dysfunction prevented by EXenatide) study, 8 healthy men were treated with a single gift of prednisolone 80 mg (or placebo) and received the GLP-1 RA exenatide (or saline) intravenously.

Finally, we investigated the effects of GCs on glucose tolerance, insulin sensitivity, and insulin secretion in patients with RA. In these studies, the metabolic effects of chronic inflammation and reduction thereof by prednisolone treatment could be added into the equation. To this extent, a single blind, randomized controlled trial in forty-one patients with early active RA was conducted with two high dosages of prednisolone (30 mg daily and 60 mg daily) dosed for a single week, as a sub study of the COBRA-light trial (COmbinatie Behandeling Reumatoide Artritis) (chapter 13). Of note, the COBRA-light study was designed to compare two different treatment schedules for early RA (COBRA schedule according to BeSt compared to a modified COBRA schedule (‘light’) with lower prednisolone dosages).

Moreover, a cross-sectional study in chronic RA patients was performed. In this study, glucose metabolism was compared among RA patients chronically treated with GCs and RA patients that had never used GCs, allowing studying the metabolic effects of GC treatment while correcting for the influence of the underlying disease (chapter 14).

In the following section, the key organs involved in insulin action and insulin secretion that are affected by GC treatment are summarized in the section below, based both on the results obtained from our studies and on previous data or data recently obtained by other research groups.

Glucocorticoids diabetogenic effects: involved organs and pathways

1. Liver

The liver is a key regulator of metabolism, within a complex regulatory network of hormonal, autonomic nervous and metabolic stimuli. Under fasting conditions, the liver maintains euglycemia by producing glucose through both gluconeogenesis and glycogenolysis. Insulin, which is secreted in response to a meal or carbohydrate load is the most important hormone that suppresses endogenous glucose production (EGP). On the other hands, the so-called contra-regulatory hormones cortisol and glucagon increase EGP under hypoglycemic conditions. The endogenous hormone cortisol as well as exogenously administered GCs act
Figure 1. Currently known mechanisms by which glucocorticoids (GCs) induce hyperglycemia in healthy humans. GCs impair beta-cell function by inducing endoplasmic reticulum (ER) stress and by reducing calcium signaling in beta cells, resulting in impaired insulin secretion. GCs furthermore impair the effects of a number of non-glucose insulin secretagogues, including arginine, acetylcholine and the gut hormones glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP). In addition, GC impair alpha-cell function, since glucagon levels are inappropriately elevated both under fasting conditions and in the postprandial state. Increased glucagon levels may stimulate endogenous glucose production (EGP) by the liver. In addition, GCs induce liver insulin resistance, thus further contributing to GC-induced increment of EGP, especially in the hyperinsulinemic state. GCs also induce adipose tissue insulin resistance, resulting in impaired glucose uptake and increased lipolysis in the postprandial period. Moreover, plasma levels of adipokines are altered towards a more diabetogenic profile. Finally, GCs impair glucose uptake in skeletal muscle by impairing insulin signaling. This reduction in insulin-stimulated glucose uptake may in part be explained by impaired insulin-stimulated capillary recruitment, since GCs also induce vascular insulin resistance. By these combined actions, GCs induce hyperglycemia in healthy men. (See page 367 for full color figure)
affect EGP in the fasted state. As reviewed in chapter 2, GCs may increase EGP and particular gluconeogenesis by several mechanisms. First, GCs increase the expression and activity of rate limiting enzymes of gluconeogenesis including PEPCK and G6Pase [29-31]. Indeed, the PEPCK gene contains a glucocorticoid response element (GRE) in its promoter region and is considered a key player in GC-induced hyperglycemia [32], but several other genes in liver glucose metabolism were identified as GC target genes in gene expression profiling studies [33]. Interestingly, it was recently demonstrated that other nuclear receptors, including peroxisome-proliferator activated receptor (PPAR)-alpha and liver X receptors (LXRs), important regulators of lipid and cholesterol metabolism respectively are potentiating factors in GC-induction of PEPCK and G6Pase, since PPAR and LXR knock-out mice models were shown to be in part resistant to the metabolic effects of GC treatment by reducing the activation of these enzymes [34, 35]. In addition, GC treatment promotes breakdown of protein and fat stores [36], thus providing increased supply of substrates for gluconeogenesis, such as alanine and glycerol, to the liver [37, 38]. As such, we could demonstrate in chapter 8 that during hyperinsulinemic conditions, high-dose prednisolone treatment impaired the insulin-mediated suppression of lipolysis and proteolysis, whereas low-dose treatment only increased lipolysis [28]. Finally, GCs may potentiate the effect of other glucoregulatory hormones, such as glucagon and epinephrine, thus further enhancing fasting EGP [35, 39].

The strongest effects of GCs on liver glucose metabolism in our studies, however, were observed under hyperinsulinemic conditions. In healthy volunteers, insulin infusion at 200 mU/m².min decreased EGP by approximately 80%. This insulin-effect was markedly and dose-dependently reduced by prednisolone treatment, by 17±6 and 46±7 % respectively (chapter 8) [28]. The observation that glucocorticoids blunt the suppressive effects of insulin on EGP was shown previously during a 24-hr high-dose continuous cortisol infusion [21, 22]. We could demonstrate for the first time that this effect is already present at prednisolone dosages just above physiological levels (7.5 mg daily). The mechanisms underlying GC-induced liver insulin resistance are at present not clarified. In rats, dexamethasone was shown to impair insulin-induced phosphorylation of insulin receptor substrate (IRS)-1 and phosphatidylinositol 3-kinase (PI-3K) in liver cells indicating impaired insulin signaling [40]. We conclude that the liver is a key player in the diabetogenic effects induced by GC treatment through increased EGP, and more particular, by inappropriate suppression of glucose production in the hyperinsulinemic state (Figure 1). Thus, several experimental approaches have been undertaken to further characterize the involved mechanisms and to assess strategies to reduce these hepatic metabolic effects of excess GC levels. First, treatment of rats with a liver-selective GR antagonist resulted in reduced fasting plasma glucose levels and decreased EGP during a hyperinsulinemic-euglycemic clamp [41]. In addition, also in rats, activation of
AMP-activated protein kinase (AMPK) was shown to suppress GC-induced hyperglycemia by reducing PEPCK and G6Pase expression [42]. Interestingly, the most commonly prescribed blood-glucose lowering agent metformin has been shown to exert its effects by activating AMPK, which potentially renders this drug capable to prevent or counteract GC-induced diabetogenic effects [43].

2. Skeletal Muscle
Skeletal muscle tissue is the most important site for insulin-stimulated glucose uptake in the postprandial period and plays a crucial role in glucose metabolism [44]. Already in studies performed several decades ago, GCs were shown to reduce insulin-stimulated glucose disposal in a dose-dependent matter [21, 45, 46], which was confirmed in our studies (chapters 5, 9, 10 and 11). In agreement with other studies [47, 48] we could demonstrate that particularly nonoxidative glucose disposal (reflecting glycogen synthesis) is impaired by GC treatment, whereas glucose oxidation rates remained intact (chapter 9) [28]. Despite the fact that GC-induced skeletal muscle insulin resistance is a well-known observation, the pathways in skeletal muscle tissue responsible for this impairment presently remain incompletely detailed. From rodent studies, it has become evident that insulin signaling is impaired by GC treatment. As such, the phosphorylation of several proteins in the insulin-signaling cascade including IRS-1, PI-3K, Akt and glycogen synthase kinase (GSK) was shown to be reduced [40, 49-52]. We could for the first time confirm this finding in humans, where high-dose prednisolone treatment impaired insulin-induced phosphorylation of its downstream targets in skeletal muscle (chapter 10) [53]. However, to date, it is unclear which molecular mechanisms are targeted by GC treatment that could interfere with insulin signaling.

It has been postulated that GC-induced changes in protein metabolism may be involved. Indeed, GC treatment is frequently associated with skeletal muscle wasting due to proteolysis [54-59]. GCs not only reduce skeletal muscle mass, but may also increase plasma levels of amino acids which were shown to impair insulin signaling in skeletal muscle [60, 61]. As shown in chapter 8, and in line with previous reports [57], we observed that high-dose prednisolone treatment impaired the suppressive effects of insulin on whole-body valine turnover, but that it did not affect whole-body valine turnover under fasting conditions. Given the small effect size that was observed and the absence of a correlation with measures of glucose metabolism, we feel that altered amino acid metabolism is unlikely to be responsible for the dramatic reduction in insulin-stimulated glucose uptake following short-term GC treatment.

Additionally, GC-induced changes in lipid metabolism could play a role in skeletal muscle insulin resistance. Glucocorticoids were shown to impair the suppressive effects of insulin
on whole-body lipolysis, resulting in increased levels of nonesterified fatty acids (NEFA) (chapter 8) [28]. NEFA spillover may enhance the accumulation of intramyocellular lipids (IMCL) [62], including triacylglycerol, but also more toxic intermediates such as fatty acyl CoA, diacylglycerol, sphingolipids such as ceramide and gangliosides such as GM-3, which have been shown to impair insulin signal transduction and have been associated with reduced skeletal muscle insulin sensitivity [63-66]. We were particularly interested in the role of sphingolipids, since attenuation of ceramide synthesis either by pharmacological treatment with myriocin or through genetic ablation of dihydroceramide desaturase (des1) was shown to prevent GC-induced insulin resistance in rodents [67]. However, in the presence of marked insulin resistance, skeletal muscle concentrations of ceramide, glucosykeramide and GM-3 were not affected by prednisolone treatment (chapter 9) [28]. An explanation for this discrepant finding as compared to the rodent study may possibly be explained by the different tissue examined. Although GC treatment was shown to attenuate insulin-mediated glucose uptake in skeletal muscle, increased ceramide levels were particularly demonstrated in liver, and not in skeletal muscle. In addition, several other factors, including different treatment duration and differences in lipid metabolism between rodents and humans, may contribute to the observed difference.

Recently, impaired mitochondrial function has received much interest given its association with reduced peripheral insulin sensitivity in subjects with T2DM [68]. It was hypothesized that impaired mitochondrial function results in accumulation of toxic lipid intermediates, which affect insulin signaling and glucose metabolism. Since GCs induce myopathy, we hypothesized that mitochondrial dysfunction could be involved in GC-induced insulin resistance. Thus, we measured ex vivo mitochondrial respirometry capacity in human skeletal muscle, adjusted for mitochondrial copy number, before and after 14-days prednisolone treatment. However, despite marked insulin resistance, mitochondrial function was not altered by prednisolone treatment (chapter 9). It should be noted that several measures of mitochondrial function exist in literature, where the gold standard measure remains to be established [68]. Therefore, we cannot completely rule out a potential role of mitochondrial dysfunction in GC-induced insulin resistance [69, 70].

Other factors may contribute to GC-induced impairment in insulin signaling and insulin-stimulated glucose disposal. First, GCs were shown, despite its general anti-inflammatory properties, to induce the acute phase response in skeletal muscle tissue, which could reduce insulin sensitivity [71]. Moreover, the endoplasmic reticulum (ER) has been identified as a major regulator of metabolic homeostasis and inflammatory responses and ER stress was shown to have unbenefficial effects on insulin sensitivity [72]. Whereas GCs were recently
shown to induce ER stress in beta cells [73], it is uncertain whether GCs may also alter ER function in skeletal muscle tissue. Finally, GCs may impair insulin sensitivity by impairing the delivery of insulin and glucose to skeletal muscle by reducing capillary recruitment through the induction of vascular insulin resistance as detailed below [53].

We conclude that skeletal muscle tissue is a key player in GC-mediated insulin resistance resulting in a dose-dependent reduction in insulin-stimulated glucose uptake (Figure 1). However, a single causative pathway could not be identified in our studies.

3. Adipose tissue

Although the adverse metabolic effects of elevated GC levels on glucose [74] and protein metabolism [75] are reasonably well defined, the effects on lipid metabolism are presently less clear [76]. In addition, the involvement of a number of organs in the development of GCs diabetogenic effects, including pancreatic islet cells, skeletal muscle and liver are understood to a greater extent than the role of adipose tissue [9]. However, several observations indicate that GCs exert unfavorable effects on adipose tissue and lipid metabolism.

As such, chronic GC excess in Cushing’s syndrome or during GC treatment increases fat deposition in the visceral compartment [77] and promotes liver fat accumulation [78], at the cost of subcutaneous fat deposition. This increment in visceral adipose tissue (VAT) by GCs may be induced by several factors, including increased intake of high-caloric “comfort food” [79], increased lipoprotein lipase (LPL) activity particularly in VAT [80, 81], increased VAT adipogenesis through stimulation of differentiation of pre-adipocytes into mature adipocytes [82] and possibly enhanced de novo lipogenesis [83]. Although we observed no significant changes in body weight, body composition, body fat distribution and liver fat content following two-week treatment with low- or high-dose prednisolone (chapter 8) [28], in subcutaneous adipose tissue (SAT) biopsies decreased protein expression of adipogenesis markers (peroxisome-proliferator activated receptor (PPAR)-γ and Kruppel-like factor (KLF)-4) was observed (chapter 11). Although our study did not allow to examine VAT, on extrapolation, these findings are in line with and may ultimately lead to the classical body fat distribution observed following chronic GC treatment as indicated above. The GC-induced reduction in SAT with concomitant increase in VAT is clinically relevant since VAT is well known to be associated with an untoward metabolic profile as opposed to SAT and is associated with increased cardiovascular risk [84].

In addition to altering adipose tissue distribution, GCs may also affect adipose tissue function. We were able to show that GC treatment impaired insulin signaling in SAT in vivo in healthy
humans. The insulin-stimulated phosphorylation of well-known insulin target proteins Akt/PKB and the downstream target PRAS-40 was impaired by high-dose prednisolone treatment (chapter 11). These data confirm and expand in vitro and in vivo rodent studies, where GC treatment impaired insulin signaling [85]. Impaired insulin action in adipose tissue may adversely affect both glucose and lipid metabolism. Indeed, an intact insulin-signaling pathway is of pivotal importance for insulin-stimulated glucose uptake through recruitment of the glucose transporter (GLUT)-4 to the plasma membrane enabling glucose transport into the cell [86]. Although the overall contribution of glucose uptake by adipose tissue in the postprandial state is thought to be relatively modest [44], it does contribute to glucose tolerance as was elegantly demonstrated in adipose tissue-specific glut4-null mice [87]. These mice developed hyperglycemia and were characterized by liver and skeletal muscle insulin resistance, most likely due to altered secretion of adipokines [87]. In our study, prednisolone 30 mg altered the circulating levels of various adipokines towards a more diabetogenic profile, as was previously observed in in vitro studies [88]. Thus, plasma concentrations of adiponectin, which are generally positively associated with insulin sensitivity, were reduced following high-dose prednisolone treatment, whereas adiponectin protein expression in SAT was decreased by both prednisolone dosages. The adipokines resistin and leptin, which are usually negatively associated with insulin sensitivity were both increased by prednisolone 30 mg (chapter 11). Although increased leptin levels have been shown to inhibit food intake in the central nervous system (CNS), leptin also acts as pro-inflammatory factor in adipose tissue by promoting the release of pro-inflammatory adipokines. Similarly, resistin has also been demonstrated to promote circulating pro-inflammatory adipokine levels [89].

In addition to promoting glucose uptake in adipose tissue, insulin is a key hormone in the regulation of lipid metabolism, amongst others by inhibiting adipose tissue lipolysis and thus decreasing plasma NEFA levels [90]. In contrast, GCs were shown, despite their lipogenic actions in VAT, to acutely increase fasting lipolysis rates in a number of in vitro studies [76]. GC-induced induction of lipolysis could be explained by increased activity of key lipolytic enzymes adipose triglyceride lipase (ATGL) [91] and hormone sensitive lipase (HSL) [92] and possibly by augmented beta-adrenergic signaling following GC treatment [76]. It should be noted that differences were observed between various adipose tissue depots where GC-induced augmentation in lipolysis rates was particularly observed in VAT and less pronounced in SAT [93]. In addition, antilipolytic effects of GC treatment have also observed in vitro [76]. In line with most of these in vitro observations, acute GC administration was shown to increase fasting whole-body lipolysis in healthy humans [83, 94, 95]. During more prolonged GC exposure, however, this lipolytic effect was no longer observed most likely due to compensatory hyperinsulinemia [25, 96, 97]. In our study, high-dose prednisolone treatment even lowered fasting lipolysis (chapter 8). In line with these findings, we observed
decreased fasting ATGL expression, the rate-limiting enzyme in adipose tissue lipolysis, during both low- and high-dose prednisolone treatments, while fasting HSL expression was unchanged (chapter 11).

In the same study, however, we observed for the first time that prednisolone treatment dose-dependently impaired insulin-stimulated suppression of lipolysis and increased plasma NEFA levels during hyperinsulinemia, indicating adipose tissue insulin resistance. Since both ATGL expression and phosphorylation of HSL in SAT during insulin infusion were unaltered by prednisolone treatment, it is unclear through which mechanisms this effect occurs. Possibly, the inhibiting effects of insulin on protein kinase A and perilipin expression, a lipid droplet-associating protein, are reduced by prednisolone treatment, thereby promoting triglyceride lipolysis. An alternative hypothesis is that the increment in whole-body lipolysis is mostly derived from VAT, from which no biopsies could be obtained in our study design. Increased lipolysis and the ensuing consequently enhanced plasma NEFA levels have been associated with impaired glucose metabolism via their inhibiting effects on muscle and liver insulin signaling [98].

In conclusion, based on our findings in healthy volunteers, GCs diabetogenic effects also involve actions on adipose tissue, i.e. induction of adipose tissue insulin resistance, resulting increased lipolysis, as well as induction of untoward plasma adipokine levels, and therefore it is likely that adipose tissue is involved in GCs diabetogenic effects.

4. Pancreatic beta cells

The pancreatic beta-cell plays a crucial role in glucose metabolism and in the past decades, beta-cell dysfunction has been acknowledged as the key defect underlying the development of T2DM [99]. Under physiological conditions, insulin secretion is directly related to insulin sensitivity through a hyperbolic relation [100]. The product of these parameters, known as the disposition index, remains constant. Thus, when the workload on the beta cell increases (by factors such as obesity, insulin resistance or low-grade inflammation), healthy beta cells can adapt by augmenting insulin secretion to meet this increased demand, thus maintaining euglycemia [101]. Before addressing the various aspects of beta-cell function that are altered by GC treatment, it is important to state that beta-cell function cannot be quantified by one single measure. Fasting measures include homeostatic model assessment of beta-cell function (HOMA-B) [102] and pro-insulin/insulin ratio (PI/I ratio), the latter giving an impression of insulin processing in the beta cell [103]. Dynamic parameters, including the insulinogenic index (IGI) and the total C-peptide response in relation to prevalent glucose levels (AUC_{CPEP}/AUC_{GLUC}) may be obtained from oral and intravenous glucose challenge tests. In addition,
mathematical models have been developed to calculate various parameters of beta-cell function from dynamic tests. In the model developed by Mari, a dose-response curve relating insulin secretion and glucose levels is calculated, from which glucose sensitivity of the beta cell is derived [104]. This dose-response curve is modulated by two additional factors. First, the insulin response to the rate of change of glucose concentrations is calculated as rate sensitivity. Secondly, the effects of non-glucose insulin secretagogues, including non-glucose substrates, gastrointestinal hormones and neurotransmitters are expressed as a potentiation factor [104]. The gold standard of beta-cell function measurements is the disposition index, where insulin secretion is adjusted for insulin sensitivity, both determined by clamp method. Thus, insulin secretion is assessed by the hyperglycemic clamp, whereas insulin sensitivity is obtained from the hyperinsulinemic-euglycemic clamp [105]. Arginine may additionally be added during the hyperglycemic clamp to obtain maximal secretory capacity at this level of hyperglycemia [106].

Direct effects of glucocorticoids on beta-cell function: in vitro studies
As reviewed extensively in chapter 2, GCs were shown to directly impair insulin secretion in vitro in insulinoma cell lines and in rodent-derived islets [9]. GCs reduced glucose-stimulated insulin secretion (GSIS) by reducing glucose uptake and oxidation, resulting in reduced ATP synthesis and calcium influx. However, GCs also impaired more distal pathways in the insulin secretory process. As such, glucocorticoid treatment reduced the activity of protein kinase A (PKA) and protein kinase C (PKC), two key enzymes involved in insulin exocytosis. These distal sites of interference by GCs in the insulin secretory process may explain the wide range of non-glucose insulin secretagogues that are also inhibited by GC treatment, including the PKA ligand ketoisocaproate, the amino acid arginine, the sulfonylurea drugs tobutamide and glibizide and the phorbol ester PMA, which activates PKC [9]. Interestingly, GCs may also impair insulin secretion by disturbing autonomous nervous system (ANS) function. As such, GCs inhibited the phospholipase C (PLC)-PKC pathway, which is used by acetylcholine to induce insulin secretion [107]. In addition, GCs increased the expression of α2 adrenergic receptors, thus enhancing sympathetic activity, leading to reduced PKA activity and subsequent decreased insulin release [108, 109].

In addition to reducing insulin release, GCs may display other detrimental effects on beta-cell function by several different mechanisms. First, GC treatment was shown to reduce insulin biosynthesis by reducing ATP/ADP ratio and pancreatic and duodenal homeobox (PDX)-1 expression [73, 110, 111]. Second, in vitro studies have indicated that GCs induce beta-cell apoptosis, which may ultimately result in reduced beta-cell mass [112].
Interestingly, endoplasmic reticulum (ER) stress may be an important factor in these GC-induced beta-cell effects [73]. The ER is a highly dynamic cell organelle, which is responsible for the synthesis of all secreted proteins. In the ER, proteins are translated, folded and assessed for quality before they are released. In the beta cell, (pro)-insulin is the most abundant produced protein, and in the glycemia-stimulated state, it may account for up to 50% of total protein production. Sustained increased demand for insulin due to e.g. hyperglycemia, however, may impose burden or ‘stress’ on the ER. ER stress is characterized by an accumulation of misfolded proteins inside the organelle. In response, the so-called unfolded protein response (UPR) is initiated, which aims to restore ER homeostasis by decreasing ER protein load and by increasing folding capacity. When the UPR fails to alleviate ER stress, however, the UPR triggers apoptosis [113]. We observed that prednisolone induced ER stress and initiated the UPR as demonstrated by increased expression of activating transcription factor (ATF) 6 and inositol-requiring enzyme (IRE)-1/X-box binding protein (XBP)-1 pathways. These modulations of ER stress pathways were accompanied by upregulation of calpain 10 and increased cleaved caspase 3, confirming that exposure to prednisolone may promote apoptosis (chapter 3) [73].

Acute effects of glucocorticoids on beta-cell function in humans

Nearly four decades ago, the acute effects of GCs on glucose metabolism were studied for the first time. Cortisol infusion induced fasting hyperglycemia in the absence of an appropriate insulin response [114], suggesting impaired beta-cell function. Moreover, 24-hr treatment with prednisolone 60 mg was shown to impair insulin secretion during low-dose glucose infusion [115]. We could confirm and extend these acute effects of prednisolone treatment on glucose metabolism and insulin secretion. Prednisolone 80 mg acutely impaired first-phase GSIS, arginine-induced insulin secretion and beta-cell disposition index as measured by hyperglycemic clamps in healthy males (chapter 7). In addition, treatment with a similar prednisolone dosage acutely increased postprandial glucose levels by impairing various beta-cell function parameters. These included empirical measures of beta-cell function such as IGI and AUC<sub>CPEP</sub>/AUC<sub>gluc</sub>, but also model-derived parameters including glucose sensitivity and the potentiation factor (chapter 4). Thus, high-dose GC treatment acutely impairs beta-cell function resulting in postprandial glucose excursions.

Subacute glucocorticoid treatment in healthy humans

A number of studies have addressed the subacute effects of GCs on beta-cell function, typically with treatment duration of 3 days. We have extended these studies by prolonging the treatment duration to a two-week period as this more closely mimics clinical treatment schedules and by studying dose-dependency (chapter 5 and 6). The difficulty of studying the subacute
effects of GCs on insulin secretion, however, is the fact that GCs also induce insulin resistance, which influences the response of the beta cell. As such, short-term treatment with high doses of dexamethasone or prednisolone in healthy subjects resulted in increased fasting insulin levels [23, 45, 46, 116-122] and increased insulin secretion during oral glucose tolerance tests (OGTT) [23, 118, 119], standardized meal tolerance tests [121, 122], intravenous glucose tolerance tests (IVGTT) [45, 120] and hyperglycemic clamp studies [23, 46, 116, 117, 121]. This increased beta-cell response does not indicate improved beta-cell function, but likely compensation for GC-induced insulin resistance. As such, we observed that the increase in first- and second-phase GSIS was no longer significant when we adjusted for insulin sensitivity (M-value obtained by hyperinsulinemic-euglycemic clamp), i.e. that the disposition index remained unchanged following prednisolone treatment. Other studies calculating different variants of the disposition index, similarly reported adequate compensation in healthy volunteers. However, in susceptible populations, including normoglycemic insulin resistant individuals [46] or low GSIS [23, 117] prior to treatment with GCs, healthy, first-degree relatives of patients with T2DM [45] and obese women [123], GSIS was not enhanced to such extent to compensate for GC-induced impairment of insulin sensitivity, and thus, the disposition index decreased following GC treatment.

In addition to first- and second-phase GSIS, we additionally studied other parameters of beta-cell function during the two-week treatment. On top of the hyperglycemic clamp, 5 g of arginine was administered to assess maximal insulin secretion capacity at the level of 10 mM glucose. Interestingly, arginine-stimulated insulin secretion was dose-dependently reduced by prednisolone treatment. In addition, various aspects of beta-cell function were measured during standardized meal tests using the model developed by Mari as described. We observed that prednisolone reduced fasting insulin secretion rates when adjusted for prevailing glucose levels. In addition, the potentiation factor, which comprises insulin secretion induced by non-glucose stimuli, was reduced by both low- and high-dose prednisolone treatment (Figure 1). From both the combined hyperglycemic clamp and the meal test data we conclude that GC treatment induces beta-cell dysfunction in healthy individuals. This effect is detectable on specific beta-cell function parameters that seem to involve potentiation phenomena [104, 106, 124]. On the other hand, the enhancement of first- and second phase GSIS as well as postprandial insulin release is mainly mediated by an increase in glucose levels and a compensatory response to GC-induced insulin resistance. Although it appeared that prednisolone already at a low dose impaired several aspects of beta-cell function, our study was limited in its power to detect these changes.
Mechanisms of glucocorticoid-induced beta-cell function

The mechanisms underlying GC-induced beta-cell dysfunction following short-term treatment presently remain unclear. It is unlikely that chronic processes such as glucotoxicity, lipotoxicity, deposition of amyloid or loss of beta-cell volume due to extensive apoptosis are involved. Given GC-induced insulin resistance, ER stress, on the other hand, could be a mechanism limiting the production of a bioactive insulin molecule. In addition, we found evidence that impaired sympathovagal balance may contribute to beta-cell dysfunction. As detailed previously, whereas the parasympathetic nervous system (PNS; neurotransmitter: acetylcholine) promotes insulin secretion, this is inhibited by the sympathetic nervous system (SNS; neurotransmitter: adrenaline). *In vitro*, dexamethasone was found to reduce insulin secretion by increasing α2 adrenergic receptors [108, 109] and by impairing acetylcholine-induced insulin secretion [125]. In our healthy participants, we observed a tendency towards increased SNS over PNS activity as assessed by cardiovascular ANS measurements derived from continuous beat-to-beat finger blood pressure recordings, which was associated with fasting glucose levels and arginine-stimulated insulin secretion (chapter 5). Further experimental data are needed to clarify the role of ANS in prednisolone-induced beta-cell effects.

Chronic glucocorticoid treatment in healthy humans

At present, there are no data available from studies addressing the chronic effects of GCs on beta-cell function in healthy individuals, due to the obvious ethical reasons. However, the chronic effects of GCs on beta-cell function have been addressed in patients with RA, as detailed below. However, it is difficult to compare these data to the results obtained in healthy volunteers, since chronic inflammation also compromises both beta-cell function [126] and insulin sensitivity [127].

Glucocorticoid receptor polymorphisms and beta-cell function

GCs exert many effects by binding to its cytosolic GR, following which the ligand-activated GR translocates to the nucleus where it regulates target gene transcriptional activity [128-130]. Functional single nucleotide polymorphisms (SNPs) in the GR gene (*NR3C1*), which demonstrated altered GC sensitivity *in vitro*, were linked to various metabolic parameters *in vivo* [128-130]. Since pancreatic GR overexpression in mice resulted in the development of beta-cell dysfunction and diabetes [131], we hypothesized that alterations in the GR gene could be related to measures of beta-cell function. We addressed this hypothesis in 449 subjects with normal- or impaired glucose tolerance, who underwent a hyperglycemic clamp.

We observed that the N363S and ER22/23EK polymorphisms were negatively associated with first-phase GSIS and disposition index in women, but not in men [132]. The N363S
SNP displays increased GC sensitivity in vitro [133] and is associated with features of a Cushingoid phenotype including increased BMI, waist-to-hip ratio, dyslipidemia and fasting hyperinsulinemia [130, 134]. The ER22/23EK SNP on the other hand, demonstrated reduced GR activation in vitro, and relative GC resistance in vivo. As such, in men, the ER22/23EK was associated with a beneficial metabolic phenotype, including increased muscle mass and strength, lower LDL cholesterol and insulin levels [135, 136]. On the other hand, female carriers of the ER22/23EK SNP were at increased risk to develop cardiovascular disease (CVD) [137]. In another cohort, carriers of the ER22/23EK had higher HbA1c levels as compared to noncarriers, thus raising doubt on the hypothesis that this SNP may induce a more favourable metabolic profile, especially in women [138]. Although we only observed the negative association between these NR3C1 SNPs and beta-cell function parameters in women, the outcome of this study strengthens the evidence for a key role of pancreatic beta-cell dysfunction in the development of GCs diabetogenic effects.

5. Pancreatic Alpha Cells

By secreting glucagon, the pancreatic alpha cell has an important role in glucose metabolism [139]. As previously mentioned, glucagon stimulates hepatic glucose production by promoting glycogenolysis and gluconeogenesis [140]. In many patients with T2DM, glucagon levels are increased in the fasted state and are incompletely suppressed in the postprandial state. Thus, elevated glucagon levels were shown to contribute importantly to both fasting and postprandial hyperglycemia [141].

Already in 1971, GCs were shown to augment glucagon levels. This was demonstrated both in healthy persons treated with dexamethasone 2 mg daily for 3 days and in patients with Cushing’s syndrome. In both groups, fasting glucagon levels were increased and glucagon concentrations were incompletely suppressed following ingestion of a protein meal or following alanine infusion [142]. After this single study, the effects of GCs on glucagon levels were not studied for another 4 decades. Here, we studied the dose-dependent effects of GCs on glucagon levels and observed that a two-week treatment with low-dose prednisolone treatment (7.5 mg daily) did not alter fasting or postprandial glucagon levels, however, that high-dose prednisolone treatment (30 mg daily) administered for this time period increased fasting glucagon levels and impaired the suppression of glucagon levels following a standardized meal test and during insulin infusion (chapter 5 and 8) [28, 121]. These effects of GC treatment were already observed in the acute setting, since a single gift of 80 mg prednisolone increased postprandial glucagon levels, as described in chapter 7 [143]. Similar results were obtained by Hansen and colleagues, who observed increased glucagon levels following a 12-day combined treatment with prednisolone 37.5 mg daily, physical inactivity
and a hypercaloric diet. Glucagon levels were increased in the fasted state and incompletely suppressed during an OGTT and isoglycemic intravenous glucose infusion [118, 119]. We conclude that both fasting and postprandial hyperglucagonemia are present in GC-induced hyperglucagonemia (Figure 1).

6. The Gut

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) are hormones secreted by the gut that are released following nutrient ingestion [144]. GLP-1 lowers (postprandial) blood glucose through several mechanisms, including stimulation of meal-related insulin response and suppression of glucagon secretion in a glucose-dependent manner. In addition, GLP-1 slows down gastric emptying, promotes satiety, decreases appetite and reduces body weight. In human beta cells and in vivo in animals and in humans, exogenous GLP-1 administration, in the presence of elevated blood glucose, acutely induces insulin-secretion while more prolonged GLP-1 exposure may result in increased insulin production. Interestingly, in rodents, chronic GLP-1 treatment was shown to increase beta-cell mass by promoting beta-cell regeneration and proliferation, and inhibiting apoptosis [145, 146]. In addition, GLP-1 treatment may protect beta cells against several toxic processes, including ER stress [147].

When a similar level of glycemia is induced by glucose ingested by the oral versus the intravenous route in healthy individuals, the oral glucose yields an insulin response that is approximately 50-70% higher than the intravenous administered glucose. In patients with T2DM, this so-called incretin effect is substantially diminished [148]. Although post-load GLP-1 secretion was found to be reduced in some studies, but not in others, the reduced incretin effect in T2DM seems rather attributed to a decreased sensitivity of beta cells to endogenous GLP-1 and GIP [149]. In T2DM patients, a vast body of research [144] indicates that an impaired incretin effect contributes substantially to (postprandial) hyperglycemia.

Up to recently, the effects of GCs on both GLP-1 and GIP secretion and their insulinotropic actions have been little addressed. At the onset of our studies, we speculated that GC treatment would reduce secretion of GLP-1 and GIP based on data obtained in rodents, where GC treatment resulted in reduced mRNA stability of the preproglucagon gene, a precursor of GLP-1 [150]. However, during our standardized meal tests, GLP-1 and GIP levels were not reduced by low- or high-dose prednisolone treatment (chapter 5) [23]. In contrast, a trend towards increased levels of GIP was observed in the prednisolone 30 mg daily group. A similar observation was made in two recent studies by another research group, who found that high-dose prednisolone in combination with physical inactivity and high calorie diet did
not affect GLP-1 secretion but increased GIP levels during an OGTT and liquid meal test [118, 119]. Interestingly, the insulinotropic effects of GLP-1 and/or GIP were shown to be reduced, since the incretin effect was significantly decreased [118]. Indeed, the potentiation of GSIS by incretin hormones was shown to be reduced in a recent study [151]. It is unclear whether this impaired incretin effect is a specific defect induced by GC treatment or whether it may be secondary to general GC-induced beta-cell dysfunction, as detailed previously. Thus, an impaired gut-islet axis characterized by impaired insulinotropic effects of GLP-1 and GIP is present in GC-induced hyperglycemia (Figure 1).

7. Microcirculation
As discussed to extent previously, GCs dose-dependently induce insulin resistance at the level of liver, adipose tissue and skeletal muscle, resulting in impaired suppression of EGP and reduced insulin-stimulated glucose uptake [28]. However, insulin action is not restricted to these metabolically active tissues. It is now evident that vascular tissue, and particularly endothelial cells, represents an important physiological target for insulin. Insulin exerts a vasodilatory action by promoting nitric oxide (NO) release from the endothelial cells. This involves the phosphorylation of endothelial NO synthase (eNOS), which on its turn is mediated by the PI3K/Akt pathway [152]. By increasing blood flow and by recruiting capillaries to expand the endothelial transporting surface available for nutrient exchange, the vascular actions of insulin significantly contribute to overall insulin-stimulated glucose uptake [153-155]. In addition, the local actions of insulin on (micro)vascular tissue may be important in the regulation of blood pressure and the pathogenesis of hypertension [7, 156, 157]. Prior to our experiments (chapter 10), the effects of GCs on microvascular function, in particular in the presence of insulin, in vivo in humans had not been investigated. In addition, it was unclear whether impairments in microvascular function could contribute to GC-induced hyperglycemia. An indication that GCs may impair microvascular function came from observations in resistance arterioles isolated from C57BL/6 mice. Treatment of these mice with dexamethasone suppressed endothelial eNOS protein levels and impaired endothelium-dependent vasodilation [158].

We assessed the effects of a two-week treatment with low- and high-dose prednisolone on microvascular function, as measured by capillary microscopy, in healthy humans both in the fasted state and during insulin infusion. Prednisolone dose-dependently impaired capillary recruitment during insulin infusion, but not in the fasted state, when prednisolone induced fasting hyperglycemia. Moreover, prednisolone-induced impairment in insulin-stimulated capillary recruitment was related to insulin-stimulated glucose disposal during the hyperinsulinemic-euglycemic clamp, as well as to postprandial glucose levels
and postprandial insulin sensitivity, suggesting that capillary recruitment may also be an important factor contributing to postprandial glucose metabolism. In addition, the decrease in insulin-stimulated capillary recruitment was associated with the prednisolone-induced increase in systolic blood pressure. In isolated cremaster resistance arteries of prednisolone-treated male Wistar rats, the vasodilatory actions of insulin were similarly attenuated. At the molecular level, prednisolone impaired the vascular effects of insulin by reducing phosphorylation of the Akt pathway, thus providing a mechanism for the vascular insulin resistance observed \textit{in vivo} [53]. In summary, prednisolone impairs the vasodilatory actions of insulin at the level of the microcirculation by attenuating insulin signaling through the Akt pathway, resulting in impaired glucose disposal, impaired glucose tolerance and increments in systolic blood pressure. Thus, impaired microvascular function may be regarded among the mechanisms by which GC act to exert their diabetogenic effects. Figure 1 summarizes the adverse effects of GCs on glucose metabolism.

8. Other potential glucocorticoid-target organs

A number of organs that were not addressed in the sections above may also be involved in GC-associated adverse metabolic effects. The brain most likely represents a very important contributor to GC-induced hyperglycemia, but this area was not studied in our conducted trials, which focused on the peripheral effects of GC treatment. Indeed, GRs are expressed in various part of the brains, including the hypothalamus [159]. In rodents, intracerebroventricular infusion of dexamethasone increased food intake [160] and decreased peripheral glucose uptake [161]. In addition, it was recently shown that dexamethasone administration in the arcuate nucleus of the hypothalamus resulted in hepatic insulin resistance through activation of sympathetic pathways to the liver [162].

In addition, the kidney has been shown to play a role in glucose homeostasis. Glucose is freely filtered by the glomerulus and is reabsorbed in the proximal tubule mainly by the sodium glucose transport protein (SGLT)2. Increased SGLT2 activity, particularly in the presence of hyperglycemia, stimulates tubular glucose absorption and may sustain hyperglycemia [163]. Interestingly, in a mouse sepsis model, GCs were shown to decrease glucose excretion by upregulating SGLT2 [163]. The role of the kidney in GC-induced hyperglycemia in humans, however, remains to be established.

Finally, GCs were shown to alter cardiac glucose and lipid metabolism, possibly by altering cardiac insulin sensitivity. Dexamethasone treatment was shown to reduce glucose oxidation in myocytes in rodents. In addition, GC treatment increased NEFA uptake and oxidation, but was also shown to promote myocardial NEFA overload [164]. Importantly, the latter has been
linked to the development of cardiomyopathy [164]. Although the effects of GCs on cardiac substrate metabolism could in part explain the GC-associated increased risk to develop heart failure or cardiovascular disease [165], it is presently unclear whether changes in cardiac metabolism may contribute to the systemic diabetogenic effects of GC treatment. Although the brain, kidneys and heart seem important GC target organs, they were beyond the scope of our studies.

**Glucocorticoids diabetogenic effects and interaction with inflammation: studies in rheumatoid arthritis patients**

Addressing glucocorticoids metabolic effects in RA patients is interesting for several reasons. First, in RA patients GCs are used on large scale [2], thus rendering these patients into a very clinically relevant population. In addition, in RA patients, it is possible to examine the interaction of GC treatment and systemic inflammation, of which the latter also affects both beta-cell function [126] and insulin sensitivity [127]. Moreover, some RA patients are treated chronically with GCs, thus providing data that extend the short-term studies conducted in healthy individuals.

Irrespective of treatment given, RA patients are at increased risk to develop impaired glucose metabolism and eventually T2DM likely due to chronic inflammation [166]. As such, correlations were found between insulin resistance, glucose tolerance and various markers of systemic inflammation in several studies [167-171]. The metabolic effects GC treatment in RA population, however, are a topic of extensive discussion.

**Short-term intervention studies**

Reduction of inflammation by GC treatment was shown to improve glucose tolerance in two short-term studies [172, 173]. We similarly studied the short-term effects of high-dose (60 mg or 30 mg once daily administered for 1 week) prednisolone treatment in recently-diagnosed, drug-naive RA patients with active disease (chapter 13) [170]. Glucose tolerance, beta-cell function and insulin sensitivity were measured during frequently-sampled OGTTs. Following treatment, we observed no deterioration of glucose tolerance in the entire group, however there was considerable between-subjects variability. The incidence of T2DM increased from 3% to 10%, but a significant number of patients also improved their glucose tolerance state. The duration of disease was the most important predictor to determine whether patients progressed to T2DM or improved their glucometabolic state. Fasting insulin sensitivity (HOMA-IR) was reduced by treatment, but this was compensated for by enhanced beta-cell response during the OGTT. To conclude, it remains unclear whether the reduction of inflammation may completely offset the adverse metabolic effects induced by GCs, however,
short-term treatment in RA patients does not seem to be complicated by marked glucose intolerance as is characterized in healthy subjects, although inter-individual variability is considerable (chapter 13) (Figure 2).

Figure 2. Hypothetic representation of the balance between systemic inflammation severity and (high)-dose glucocorticoid (GC) treatment in relation to glucose tolerance. In healthy volunteers, the diabetogenic effects of GCs are well described. However, in patients with inflammation, GCs may improve glucose tolerance by reducing systemic inflammation. In patients treated with GCs for conditions that are usually characterized by low-grade systemic inflammation, such as chronic obstructive pulmonary disease (COPD), the diabetogenic effects of GC treatment may dominate its anti-inflammatory actions with respect to glucose metabolism. However, in patients with high-grade inflammation, such as recently diagnosed rheumatoid arthritis (RA) patients, GCs anti-inflammatory actions may outweigh their diabetogenic effects. (See page 367 for full color figure)

Cross-sectional studies in chronic rheumatoid arthritis patients

In a number of small cross-sectional studies, chronic GC exposure was related to impaired fasting insulin sensitivity as measured by HOMA-IR [174] and tended to predict T2DM, although in these studies the presence of a chronic disease (i.e. RA) affecting these parameters was poorly addressed [175, 176]. In order to further characterize the effects of GCs in chronic RA patients, we recruited a large cohort of chronic RA patients treated with GCs for over 3 months (RA+GC; n=58) and assessed glucose tolerance, beta-cell function and insulin sensitivity by frequently-sampled OGTTs. This group was compared to chronic
RA patients who were GC-naïve (RA-GC; n=82) (chapter 14). We observed no differences in glucose tolerance and static or dynamic measures of beta-cell function and insulin sensitivity between groups, however, cumulative GC dosage was independently associated with HOMA-IR and T2DM prevalence, also after adjusting for disease activity and patient characteristics. However, in this cross-sectional study, confounding by indication should be kept in mind. This indicates that cumulative GC use might be a proxy for long-term disease activity which itself influences glucose metabolism, since GCs are mostly given to the patients with refractory disease activity [171]. We concluded that chronic GC use may be related to T2DM as compared to other RA medications; however, since these findings were not substantiated in randomized controlled trials, no definitive conclusions can be drawn.

From the studies in RA patients it is evident that the metabolic effects of GCs are much less pronounced than in healthy controls, as GC may simultaneously alleviate the diabetogenic effects of the inflammatory disease. The amount of systemic inflammation when GC therapy is initiated may be an important determinant of the net effects of GC treatment on glucose tolerance (Figure 2).

Glucocorticoids diabetogenic effects: implications for the development of dissociated glucocorticoid receptor agonists

A number of lessons may be appreciated from the above-summarized studies for the current development of dissociated GR agonists. First, it is clear that various organ systems and different pathways contribute to the diabetogenic effects of GCs. This should be kept in mind when assessing the metabolic effects of novel GR modulators. In addition, we have shown that several unfavorable effects (i.e. increased postprandial glucose levels, impaired suppression of EGP and lipolysis by insulin) may already develop during treatment with dosages as low as 7.5 mg prednisolone daily (chapters 5 and 8) [28, 121] (Table 1), which were previously considered to be ‘safe’. Thus, ideally, the novel GR agonists should display anti-inflammatory properties similar to high-dose prednisolone treatment, but should have a side-effect profile of an equivalent prednisolone dose lower than 7.5 mg daily. Finally, since many metabolic adverse effects become apparent in the postprandial state or during hyperinsulinemia (detailed below), the metabolic effects of classic GR agonists and dissociated GR agonists, when compared in future head-to-head studies in humans, should be assessed using dynamic tests such as OGTTs and clamp tests, since fasting measures may underestimate the GC-induced effects.
Table 1. Adverse metabolic effects induced by low-dose glucocorticoid treatment in healthy men as observed in our studies.

<table>
<thead>
<tr>
<th>Glucose levels</th>
<th>Elevated fasting plasma glucose levels*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postprandial hyperglycemia</td>
</tr>
</tbody>
</table>

**Potentially involved mechanisms**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Impaired suppression of EGP by insulin</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Reduced insulin-stimulated glucose uptake*</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Impaired suppression of lipolysis by insulin</td>
</tr>
<tr>
<td></td>
<td>Increased NEFA levels during hyperinsulinemia</td>
</tr>
<tr>
<td></td>
<td>Decreased markers of adipogenesis in SAT</td>
</tr>
<tr>
<td></td>
<td>Decreased adiponectin expression in SAT</td>
</tr>
<tr>
<td>Beta cells</td>
<td>Reduced glucose-adjusted basal insulin secretion*</td>
</tr>
<tr>
<td></td>
<td>Impaired potentiation of insulin secretion by non-glucose stimuli*</td>
</tr>
<tr>
<td></td>
<td>Postprandial midday hyperinsulinemia</td>
</tr>
</tbody>
</table>

* Indicates trend. EGP: endogenous glucose production; NEFA: non-esterified fatty acids; SAT: subcutaneous adipose tissue.

**Glucocorticoids diabetogenic effects: from mechanism to treatment?**

Despite the vast number of documented observations clearly showing that glucocorticoids induce glucose intolerance and diabetes in the clinic [14-16], remarkably, there are no studies that have investigated how GC-induced diabetes may best be treated, or preferentially, could be prevented. It has become increasingly clear that the most pronounced effects of GC treatment are in the postprandial period. We could demonstrate increased postprandial glucose levels, increased EGP, lipolysis and proteolysis, and impaired capillary recruitment during hyperinsulinemic conditions, whereas all these parameters were not affected in the fasted state when prednisolone treatment induced fasting hyperinsulinemia (chapters 4, 5, 7, 8 and 10) [28, 53, 121, 122, 143]. In line with these data, patients with Cushing’s syndrome have predominantly increased postprandial glucose levels as opposed to near normal fasting glucose levels [177]. And more recently, similar observations have also been observed in clinical studies in patients treated with medium-to-high dose GCs [16, 178, 179]. Patients with chronic obstructive pulmonary disease (COPD) that were treated with GCs at 08.00 in the morning displayed significant glucose elevation resulting in afternoon and evening, but not overnight, hyperglycemia [179]. In another study, this phenomenon was explained by an early inhibition of insulin secretion followed by decreased insulin action later during the day. All metabolic parameters were completely restored by the next morning [178].
These observations have important implications for treatment of GC-induced hyperglycemia. Glucose-lowering therapy should be predominantly directed at the time period between midday and midnight, and caution should be exercised with the use of long-acting basal insulin, because it may precipitate nocturnal hypoglycemia when the effect of prednisolone wanes. Given this specific period of time, short-acting insulin analogues seem a reasonable choice to treat the typical pattern of hyperglycemia.

In this thesis, we have also explored the potential of GLP-1 based treatment to treat GC-induced hyperglycemia [143]. Since GLP-1 treatment stimulates insulin secretion [180, 181], reduces glucagon secretion [182] and improves postprandial insulin sensitivity [183, 184], it addresses at least three important pathophysiological features of GC-induced hyperglycemia. Indeed, in a proof-of-concept study in healthy volunteers, infusion of the GLP-1 receptor agonist (GLP-1 RA) exenatide prevented glucose intolerance and islet-cell dysfunction induced by acute prednisolone treatment (chapter 7) [143]. In addition, in the long term, GLP-1 RA reduce appetite [185], stimulate weight loss [186], decrease visceral fat tissue [187] and hepatic steatosis [188], improve postprandial lipid profiles [189] and favorably change adipose tissue biology [187], effects that could counteract the features associated with chronic GC treatment. Currently, studies are ongoing in which the effects of more prolonged treatment with the dipeptidyl-peptidase (DPP)-4 inhibitor sitagliptin on glucose metabolism are assessed in men with the metabolic syndrome concomitantly treated with high-dose prednisolone [190]. It will be interesting to see whether also in this population, incretin-based therapies may be useful in the treatment of GC-induced hyperglycemia. Thus, expanding knowledge of the pathophysiology of the diabetogenic effects of GC treatment should in due time result in a more tailored therapy to treat the associated hyperglycemia.
REFERENCES


118. Hansen KB, Vilsboll T, Bagger J, Holst JJ, Knop FK. Reduced glucose tolerance and insulin resistance induced by steroid treatment, relative physical inactivity, and high-calorie diet impairs the incretin effect in healthy subjects. J Clin Endocrinol Metab. 2010;95(7):3309-17.


