Chapter 2

Novel Insights into Glucocorticoid-Mediated Diabetogenic Effects: Towards Expansion of Therapeutic Options?

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ABSTRACT

At pharmacological concentrations, glucocorticoids (GCs) display potent anti-inflammatory effects, and are therefore frequently prescribed by physicians to treat a wide variety of diseases. Despite excellent efficacy, GC therapy is hampered by their notorious metabolic side-effect profile. Chronic exposure to increased levels of circulating GCs is associated with central adiposity, dyslipidemia, skeletal muscle wasting, insulin resistance, glucose intolerance and overt diabetes. Remarkably, many of these side effects of GC treatment resemble the various components of the metabolic syndrome (MetS), in which indeed subtle disturbances in the hypothalamic-pituitary-adrenal (HPA) axis and/or increased tissue sensitivity to GCs have been reported. Recent developments have led to renewed interest in the mechanisms of GCs diabetogenic effects. First, 'selective dissociating glucocorticoid receptor (GR) ligands', which aim to segregate GCs anti-inflammatory and metabolic actions, are currently being developed. Second, at present, selective 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors, which may reduce local GC concentrations by inhibiting cortisone to cortisol conversion, are evaluated in clinical trials as a novel treatment modality for the MetS.

In this review, we provide an update of the current knowledge on the mechanisms that underlie GC-induced dysmetabolic effects. In particular, recent progress in research into the role of GCs in the pathogenesis of insulin resistance and beta-cell dysfunction will be discussed.
1. INTRODUCTION

Glucocorticoid (GC) hormones are secreted by the cortex of the adrenal gland, under control of the hypothalamic-pituitary-adrenal (HPA) axis. They play essential roles in glucose, lipid and protein metabolism and contribute to energy homeostasis. In the postabsorptive state, GCs provide substrate for oxidative metabolism by increasing lipolysis, proteolysis and hepatic glucose production [1].

At supraphysiological concentrations, GCs display strong anti-inflammatory and immunosuppressive actions. Hence, GCs are, since decades, the cornerstone in the treatment of numerous inflammatory conditions. Annually, approximately 10 million new prescriptions for oral corticosteroids are issued in the United States. Despite their excellent efficacy, GC use is limited by their side-effect profile, which is dependent of the administered dose and duration of treatment [2]. Among these side effects are metabolic derangements, including the development of central adiposity [3], hepatic steatosis [4], dyslipidemia characterized by increased plasma levels of triglyceride rich lipoproteins (TRL) and nonesterified fatty acids (NEFA) [5,6], increased breakdown of skeletal muscle mass [7], insulin resistance, glucose intolerance and overt diabetes in susceptible individuals [2]. In addition, GC-induced beta-cell dysfunction, which may determine the progression from insulin resistance to hyperglycemia, has been the topic of extensive research more recently [8,9].

A similar cluster of metabolic abnormalities is also present in subjects with the metabolic syndrome (MetS) [10]. Although in one study glucose intolerant subjects were shown to have higher cortisol levels during an oral glucose tolerance test (OGTT) than healthy controls [11], at present, no clear association was established between excess plasma levels of GCs and "idiopathic" obesity and/or the MetS [12]. In the last decade, however, the importance of factors that modulate the biological effects of GCs in target tissues has been recognized [13]. In this respect, the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, which interconvert cortisol and its inactive metabolite cortisone, are of particular interest. 11β-HSD type 1, which is predominantly expressed in liver and adipose tissue, amplifies local GC action by conversion of cortisone to cortisol, whereas 11β-HSD type 2, which is mainly expressed in the kidney, reduces GC-induced effects by converting cortisol to cortisone [14]. Indeed, in several studies in both rodents and humans, 11β-HSD1 expression and activity in subcutaneous adipose tissue (SCAT) were shown to be positively associated with insulin resistance and obesity [15].
Figure 1. Molecular mechanisms of GR action. GCs regulate protein activity both by genomic and nongenomic pathways. In addition, various modes of transcription regulation by the GR-ligand complex have been described. Positive regulation of genes (transactivation) is mediated by binding of a ligand-activated GR homodimer to a GC response element (GRE), which is usually located in the promoter region of the target gene. An example of such regulation is the gene encoding for the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK). Ligand-activated GR homodimers may also bind to negative GREs, leading to regression of gene transcription. Moreover, inhibition of target genes may also be achieved by interaction of GR monomers with other transcription factors via protein-protein interaction (transrepression). An example of the latter includes binding of GC monomer to the p65 subunit of nuclear factor κB (NF-κB), thereby preventing gene transcription. The transactivation pathway seems mainly responsible for GC-induced side effects, whereas the transrepression pathway is thought to primarily induce GC's anti-inflammatory effects. Abbreviations: DNA: deoxyribonucleic acid; GC: glucocorticoid; GR: glucocorticoid receptor; GRE: glucocorticoid response element; mRNA: messenger ribonucleic acid; P: phosphorylated.

1.1 Pharmacological modulation of glucocorticoid action
Due to increased insight into the mechanisms of GC-induced dysmetabolic effects in recent years, two novel therapeutic classes are currently being explored. First, improved understanding of the molecular and cellular actions of GCs has lead to the development of compounds that may display a reduced metabolic side-effect profile compared to classic glucocorticoid receptor (GR) ligands, while preserving GC-induced anti-inflammatory effects. These so-called ‘dissociating (GR) activators’ are based on the finding, that the mechanisms by
which GCs enhance gene transcription ('transactivation') differ from the modes by which GCs inhibit gene transcription ('transrepression') (detailed in Figure 1). GC-induced mechanisms of transrepression are considered to be responsible for GCs anti-inflammatory actions, whereas the transactivation pathways are associated with most of GC-induced side effects. Clearly, new compounds with a ‘dissociated profile’, i.e. that mainly induce transrepression with reduced transactivation activity, will have an increased therapeutic index compared to the currently available GCs. Currently, a battery of these ligands are being developed by pharmaceutical companies [16,17].

Second, since in rodent models of obesity and type 2 diabetes (T2DM) pharmacological inhibition of 11βHSD1 improved glucose tolerance, insulin sensitivity and lipid profiles [18], 11β-HSD type 1 inhibitors are currently being evaluated in early clinical trials. These agents are expected to provide a new treatment modality for subjects with the MetS and/or T2DM.

In this review, we will describe the tissue-specific mechanisms underlying the metabolic derangements associated with GC excess, in particular GC-induced insulin resistance and beta-cell dysfunction. In addition, the role of GC metabolism in the MetS will be discussed.

2. GLUCOCORTICOID ACTIONS ON SKELETAL MUSCLE GLUCOSE AND PROTEIN METABOLISM

Skeletal muscle tissue plays a crucial role in glucose metabolism. It is responsible for 80% of postprandial glucose uptake from the circulation [19] and contains the body’s largest glycogen stores. The uptake of glucose in skeletal muscle is dependent on the presence of insulin, and is therefore referred to as insulin-mediated glucose uptake (IMGU). Since impaired glucose tolerance is a very consistent finding in clinical studies after GC exposure [9,20,21], it has been assumed that GC-induced changes in metabolic functions of skeletal muscle tissue may contribute to the development of GC-induced glucometabolic abnormalities. A number of potential mechanisms through which GCs decrease IMGU in skeletal muscle tissue have been identified. GCs may induce insulin resistance by directly interfering with the insulin signaling cascade in skeletal muscle. In addition, GC-induced changes in protein [22] and lipid metabolism [23,24] may reduce insulin sensitivity of skeletal muscle tissue. The above-mentioned mechanisms are further detailed below.
Glucocorticoids directly inhibit insulin signaling in skeletal muscle

GCs impair IMGU by directly interfering with several components of the insulin signaling cascade (Figure 2), as was extensively demonstrated both in vitro and in vivo in animals [25-29]. Since studies into the effects of GCs on the expression of the insulin receptor (IR) in skeletal muscle have rendered contradictory results [1,28], the markedly reduced IMGU seen
after GC-treatment has been proposed as a postreceptor defect [21,30]. Accordingly, in both L6 skeletal muscle cells and in rat skeletal muscle, dexamethasone treatment decreased the expression and phosphorylation of insulin receptor substrate (IRS)-1, phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (PKB)/Akt [25,26,28]. In rats, this was indeed shown to result in reduced glucose transporter (GLUT4) migration to the cell surface [29]. In addition to reducing glucose uptake, GCs decreased glycogen synthesis rate in Wistar rats by lowering PKB/Akt and glycogen synthesis kinase (GSK)-3 phosphorylation [27].

To date, few data exist regarding the effects of GCs on insulin signaling in humans. In healthy volunteers, treated for 5 days with 4 mg dexamethasone daily [31], and in patients who were exposed to long-term, high-dose GCs following renal transplantation [32], reduced glycogen synthesis rates with concomitant decreased glycogen synthase (GS) concentration and activity in skeletal muscle biopsies were reported. Based on studies in cell lines and animals, a schematic overview of the insulin signaling pathway in skeletal muscle and the proposed sites of modulating action by GCs is depicted in Figure 2.

2.2 Glucocorticoid-induced protein catabolism is linked to insulin resistance
Marked skeletal muscle atrophy is a key feature of prolonged exposure to increased plasma levels of GCs, as seen in Cushing’s syndrome and during steroid therapy [7]. Also following short-term high-dose GC treatment, enhanced protein degradation, as measured by stable isotope dilution methodology, with concomitant elevating circulating amino acid levels, was reported [33]. Amino acids interfere with insulin signaling by inhibiting insulin-stimulated IRS phosphorylation and activation of PI3-K in cultured hepatoma cells and myocytes [34], and were shown to reduce IMGU and glycogen synthesis in humans [22]. Thus, the atrophy-related decrease in total muscle area as well as the elevated circulating amino acid levels contribute to reduced IMGU in GC excess state.

GCs reduce skeletal muscle mass both by decreasing the rate of protein synthesis and by increasing the rate of protein breakdown [35]. GCs inhibit protein synthesis by reducing transport of amino acids into the muscle and by abolishing the anabolic effects of insulin and insulin-like growth factor (IGF)-1. Specifically, the activation of two key proteins in the protein synthesis machinery, eIF4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1), is reduced through inhibition of PKB/Akt and mammalian target of rapamycin (mTOR) phosphorylation. GCs induce muscle proteolysis by activating a number of proteolytic systems. Most notably, various proteins of the ubiquitin-proteasome system are activated by GCs, such as Muscle Ring Finger (MuRF)-1 and Atrogin-1. The transcriptional factors FOXO 1 and 3, the gene expression of which are upregulated by GCs, are thought to play a pivotal role in this pathway [35] (Figure 2).
2.3 Glucocorticoid-induced dyslipidemia reduces insulin sensitivity

Glucocorticoids (GCs) increase whole-body lipolysis [36], resulting in increased plasma levels of NEFA and TG [5,6] (further discussed under the section 'GCs enhance whole-body lipolysis'). Augmented plasma levels of NEFA may enhance the accumulation of intramyocellular lipids (IMCL), such as fatty acyl CoA, diacylglycerol (DAG) and ceramide, which reduce glucose uptake and disposal [37]. It was proposed by Randle in 1963 [38], that intracellular lipids decrease IMGU by competing with glucose for oxidation, but more recent studies have demonstrated that IMCL rather reduce IMGU by interfering with insulin signaling. Thus, intracellular lipids may activate various serine kinases, such as c-Jun amino-terminal kinase (JNK) and IkB kinase-β (IKK-β), which phosphorylate serine sites on IRS-1, resulting in decreased insulin signaling [24].

To summarize, GCs reduce insulin sensitivity and consequently IMGU in skeletal muscle tissue, not only by directly perturbing insulin signaling, but also secondary to unfavourable changes in protein and lipid metabolism.

3. GLUCOCORTICOIDS AND HEPATIC GLUCOSE AND LIPID METABOLISM

The liver plays a key role in controlling glucose and lipid homeostasis, within a complex regulatory network of hormonal, autonomic nervous and metabolic stimuli. In the fasting state, the liver maintains euglycemia by producing glucose through both gluconeogenesis (GNG) and glycogenolysis (GGL). Insulin, in the presence of abundant glucose, is the most important hormone that suppresses endogenous glucose production (EGP) [39], whereas GCs and glucagon increase hepatic glucose output.

3.1 Glucocorticoids increase endogenous glucose production

Excess plasma levels of GCs may increase EGP both directly and indirectly, the latter by antagonizing insulin's metabolic actions [1,40]. GC-induced hepatic insulin resistance results in impaired suppression of hepatic glucose production by insulin, especially in the postprandial state, as was extensively demonstrated in healthy subjects following short-term GC exposure [21,41,42]. In addition, GCs directly increase EGP by activating a large number of genes involved in hepatic carbohydrate metabolism [43]. Accordingly, GCs were shown to augment EGP in the fasting state in healthy humans [21,42,44], although this could not be confirmed in other studies [41,45-48]. Possibly the use of different GC compounds (cortisol versus dexamethasone), different modes of administration (intravenous versus
The vulnerability of the population exposed [44] and the different stable isotope dilution protocols that were employed to quantify EGP, may explain these seemingly contrasting results. GC-induced increments in EGP in the basal state seem to be driven by increased GNG, through a number of proposed mechanisms. First, GCs were shown to induce the expression of rate limiting enzymes of GNG, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), both in vitro and in vivo in rats [49-51]. Indeed, the PEPCK gene contains a glucocorticoid response element (GRE) in its promoter region and is considered a key player in GC-induced hyperglycemia [43]. Second, long-term GC-exposure promotes breakdown of protein and fat stores [52], thus increasing the supply of substrates for GNG, such as alanine and glycerol, to the liver [53]. As such, increased proteolysis was shown to contribute to augmented GNG in obese nondiabetic subjects, in whom enhanced skeletal muscle GC sensitivity has been reported [54], as compared to lean controls [55].

Third, GCs facilitate metabolite transport across the mitochondrial membranes in rat liver, which enhances GNG [1,53]. Fourth, GCs might potentiate the effects of other glucoregulatory hormones, such as glucagon and epinephrine, thus further enhancing EGP [52,56]. Finally, a role for the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-alpha in GC-induced hepatic insulin resistance and hyperglycemia has recently been confirmed [57,58]. PPAR-α, which is predominantly expressed in the liver and is the receptor for the hypolipidemic fibrates, acts as a fatty acid sensor and is a major regulator of energy homeostasis by promoting fatty acid oxidation, GNG and ketogenesis [59]. It was elegantly demonstrated in PPAR-α knock out mice that PPAR-α expression is necessary for GC-induced increases in EGP [57], and that the autonomous nervous system plays a key role [58].

The importance of the hepatic GC-GR axis in regulating EGP was additionally demonstrated in transgenic mice with a liver-specific inactivation of the GR. These mice developed hypoglycemia after prolonged fasting due to impaired activity of gluconeogenic enzymes [60].

3.2. Glucocorticoids alter hepatic lipid metabolism
In addition to regulating hepatic carbohydrate metabolism, insulin plays a key role in hepatic lipid metabolism. In the postprandial state, portal insulin stimulates lipogenesis and lipoprotein synthesis, whereas it suppresses very low-density lipoprotein (VLDL) secretion [61]. GCs induce hepatic insulin resistance, both by direct interference with insulin signaling and indirectly, by elevating plasma NEFA and TG supply to the liver [61]. GC-induced changes in hepatic lipid metabolism may induce hepatic steatosis and dyslipidemia in humans, although the currently available data are not conclusive.
Both in vitro and in vivo in rats, GC treatment was shown to induce accumulation of intrahepatic lipids [62,63], both through increased TG synthesis and through decreased fatty acid oxidation [63]. In line with these results, mice with a liver-specific disruption of the GR had diminished hepatic TG levels [64]. In humans, one study indeed reported a prevalence of hepatic steatosis of up to 20% in patients with Cushing’s disease [65], although additional reports linking fatty liver to GC excess are limited to case reports in present literature [66,67]. In addition to changing hepatic fat content, GCs may also augment hepatic VLDL secretion, resulting in increased plasma levels of TG. In studies in rodents, systemic GC excess induced an atherogenic plasma lipid profile [63]. In patients with Cushing’s disease, enhanced VLDL secretion by the liver was reported, which was normalized after correction of cortisol levels [5]. In addition, short-term treatment with prednisolone also increased VLDL concentration in healthy subjects [68], although the published results are not unequivocal [69].

To summarize, increased levels of GCs in the liver contribute to GC-related dysmetabolic effects, including insulin resistance, hyperglycemia and dyslipidemia. The effects of GCs on the development of hepatic steatosis in humans warrants further investigation.

4. GLUCOCORTICOIDS, BODY FAT DISTRIBUTION AND ADIPOSE TISSUE BIOLOGY

4.1 Glucocorticoids increase body fat content and alter body fat distribution

GCs play a critical role both in the regulation of adipose tissue metabolism and in the differentiation of pre-adipocytes into mature adipocytes [70]. Excess plasma levels of GCs, as seen in Cushing’s disease or during prolonged GC therapy, increase body fat mass. Remarkably, GCs specifically enhance fat deposition in the visceral compartment, while reducing peripheral fat stores [70]. It has been raised that, in peripheral fat tissue, GCs induce hormone-sensitive lipase (HSL) and reduce intravascular lipoprotein lipase (LPL) activity, resulting in augmented fat mobilization. In contrast, in visceral adipose tissue (VAT), GCs may enhance pre-adipocyte differentiation, LPL activity and TG synthesis, leading to increased adipose tissue mass [70]. However, the molecular mechanisms underlying the distinct regulation of central and peripheral adipose tissue depots by GCs remain currently largely unknown. Off note, recent research demonstrating the role of endogenous GC dysmetabolism in the development of central adiposity in ‘idiopathic’ obesity and the MetS will be addressed in a separate section.

Inasmuch as increased VAT is well associated with risk for metabolic and cardiovascular disease [71], the GC-related increase in VAT may indeed translate into clinical disease [72].
4.2. Glucocorticoids modulate adipose tissue biology

4.2.1. Glucocorticoids alter the secretion of adipokines
In addition to changing adipose tissue distribution, GCs also affect adipose tissue biology. In the past decades, adipose tissue has been identified as an endocrine organ, secreting numerous metabolically active hormones and peptides, collectively referred to as adipokines [73]. GCs were shown to change the secretion of a number of these adipokines in vitro and in vivo in rodents [74]. Whether the expression of the encoding genes are directly altered by GCs, or whether the changed expression may be secondary to GC-induced adipose tissue insulin resistance [75-77], is currently not clear. In 3T3-L1 adipocytes and in white adipose tissue from dexamethasone-treated mice, GCs increased mRNA and protein expression levels of resistin, an adipocytokine that impairs glucose tolerance. Furthermore, in 3T3-L1 and human adipocytes, dexamethasone decreased the expression of adiponectin, which is associated with enhanced insulin sensitivity. Conversely, GCs suppressed the pro-inflammatory adipokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α in vitro, making these proteins unlikely candidates for mediating GC-induced insulin resistance. Finally, also in vitro, GCs stimulated leptin expression and secretion [78].

In humans, both a two and five-day treatment with dexamethasone had no effects on plasma levels of adiponectin, resistin, TNF-α or leptin in healthy subjects, while insulin sensitivity was markedly reduced [79,80]. In addition, surgical treatment of Cushing’s disease did not alter serum adiponectin levels, despite a decrease in cortisol plasma levels and increase in insulin sensitivity [81]. Whether the expression levels of these adipokines were modulated in adipose tissue, was not determined. In contrast, a seven-day treatment with the GC prednisolone did increase the expression of leptin in a group of overweight, postmenopausal women [82].

4.2.2 Glucocorticoids enhance whole-body lipolysis
In addition to modifying the secretion of various adipokines, GCs also alter lipid metabolism in adipose tissue. GC-induced insulin resistance in adipose tissue may contribute, but GCs also directly alter the expression of key proteins in lipid metabolism. Thus, GCs were shown to induce the expression and activity of HSL, the enzyme responsible for the hydrolysis of TG in adipocytes [83]. Indeed, acute cortisol infusion in healthy humans lead to increased whole-body lipolysis in several studies, as measured with stable isotopes dilution techniques [36,84-86]. Although it remains to be determined which adipose tissue compartment particularly contributes to increased NEFA fluxes, one of these studies reported a decreased FFA efflux from subcutaneous abdominal tissue concurrently with increased whole-body lipolysis [85], an observation compatible with the adipose tissue distribution that is seen in patients with Cushing’s syndrome. In addition, GCs were shown to augment LPL activity in the presence
of insulin [87]. Increased LPL activity and intravascular lipolysis, stimulates the uptake of NEFA and glycerol into adipose tissue. It has been proposed that GCs regulate LPL activity in a site-specific manner, i.e. increase LPL activity in VAT, but reduce LPL activity in peripheral fat stores [6,88,89], thus contributing to the Cushing phenotype. Robust evidence for this hypothesis, however, is lacking in humans.

5. GLUCOCORTICOIDS AND INSULIN SECRETION

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 [90]. Beta cells can adapt to changes in insulin sensitivity by varying insulin secretion, thus maintaining euglycemia. During prolonged periods of reduced insulin sensitivity, e.g. in conditions such as pregnancy and obesity, beta cells may additionally meet this increased demand by expanding their volume through both hypertrophy and hyperplasia [91]. From the above, it is clear that if beta-cell function is assessed by measuring insulin secretion, this value needs to be corrected for prevailing glucose levels and insulin sensitivity.

Since GCs induce occasional hyperglycemia, it was speculated that GCs, in addition to inducing insulin resistance, might exert an inhibitory effect on beta cells. From present literature it is apparent that the effects of GCs on beta-cell function are highly dependent of duration of exposure, dosage, and susceptibility of the population exposed.

5.1 Glucocorticoids impair insulin secretion in vitro

Several studies have assessed the effects of GCs on insulin secretion in vitro. Rodent-derived islets decreased insulin release in response to various GC compounds, both following acute exposure (minutes) [92,93] and following more prolonged exposure (hours to days) [8,94-99]. GCs may impair the pathways of both nonmetabolizable and metabolizable insulin secretagogues, most notably glucose.

Glucose uptake and its oxidation in mitochondria of beta cells results in an increase in the adenosine triphosphate (ATP)/adenosine monophosphate (ADP) ratio, closure of ATP-sensitive potassium channels, plasma membrane depolarization, increased cytoplasmatic calcium concentrations, and finally exocytosis of insulin-containing granules, through yet incompletely understood pathways. In addition, calcium fluxes activate a number of potentiating signal pathways, including protein kinase A (PKA) en protein kinase C (PKC), which amplify insulin secretion [100]. GCs were shown to impair beta-cell glucose
metabolism by reducing the expression levels of GLUT2 [95,101] and glucokinase (GK) [102], and to increase futile glucose cycling by enhancing G6Pase activity [102,103]. Through these mechanisms, GCs may reduce glucose uptake and phosphorylation, and thereby decrease ATP synthesis and calcium influx.

Figure 3. Insulin secretory process in pancreatic beta cells and the proposed modes of interference by glucocorticoids. GCs induce beta-cell dysfunction by inhibiting several pathways. Most notably, GCs impair beta-cell glucose uptake and oxidation, decrease protein kinase A and C activation, and reduce calcium fluxes by permitting repolarizing potassium currents. Abbreviations: AC: adenyl cyclase; Ach: acetylcholine; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; DAG: diacylglycerol; G6P: glucose-6-phosphatase; G: G-coupled inhibitory protein; GC: glucocorticoid; GK: glucokinase; GLUT2: glucose transporter 2; HK: hexokinase; IP3: inositol triphosphate; K,1,5: voltage-dependent K channel; PIP2: phosphatidylinositol biphosphate; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; SGK-1: serum- and glucocorticoid inducible kinase-1.

Other than impairing β-oxidation of glucose in beta-cells, additional GC-induced effects may contribute to beta-cell dysfunction. Dexamethasone was shown to augment inward, repolarizing potassium currents by upregulating K, ion channels, through activation of the serum- and glucocorticoid inducible kinase (SGK)-1 [98]. These repolarizing currents may limit calcium influx and insulin secretion. Furthermore, GCs inhibited more downstream steps in the insulin secretory pathway. In isolated rat islets, dexamethasone decreased the activation...
of PKC through inhibition of the DAG-phospholipase C (PLC) pathway [99]. Moreover, GCs increased the expression of \( \alpha_2 \) adrenergic receptors [99,104], leading to reduced cyclic adenosine monophosphate (cAMP) levels, PKA activity and subsequent decreased insulin release. 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 GCs, including ketoisocaproate [8,93], the amino acid arginine [8], the sulfonylurea drugs tolbutamide [8] and gliclazide [93], and the phorbolester PMA, which activates PKC [93,99]. In line with these results, cAMP, a potent activator of PKA, was shown to prevent GC-induced effects on insulin secretion [8]. In addition to perturbing the process of insulin secretion, GCs may also decrease insulin biosynthesis by reducing the ATP/ADP ratio [96,105] and may reduce beta-cell mass by inducing apoptosis [106].

To summarize, GCs interfere with the signaling pathways of various insulin secretagogues, but the exact mechanisms through which this occurs, remain largely unknown, despite major research efforts. Figure 3 shows a schematic overview of the here described insulin secretion process and the proposed modes of interference by GCs.

5.2 Glucocorticoids effects on insulin secretion ex vivo and in vivo in rodents

The effects of GCs on insulin secretion have also been assessed ex vivo and in vivo in rodents, however, establishing direct effects of GCs on beta cells under these conditions may be more challenging, since systemic metabolic consequences of GC treatment (e.g. changes in levels of glucose, NEFA and other metabolites) will interfere with GC-mediated changes in beta-cell function as well.

Hydrocortisone treatment acutely inhibited insulin secretion in mice, resulting in hyperglycemia following an intravenous glucose challenge [107]. However, pancreatic islets isolated from healthy rats after more prolonged treatment with dexamethasone or hydrocortisone (several days), showed both unchanged and increased glucose-stimulated insulin secretion (GSIS) [101,108], most likely to compensate for GC-induced insulin resistance. The increases in GSIS were accompanied by expansion of beta-cell volume [101,109]. However, if dexamethasone treatment induced fasting hyperglycemia in these animals, GSIS was impaired [101]. Similar results were obtained in rodent models of obesity and insulin resistance. In Zucker fatty rats (fa/fa) [101,110] and ob/ob mice [103] dexamethasone readily induced hyperglycemia and markedly reduced or completely abrogated GSIS. In rats with streptozocin-induced diabetes, dexamethasone further increased fasting hyperglycemia and diminished GSIS [111]. Transgenic mice overexpressing the GR in beta cells under control of the insulin promoter were glucose intolerant, due to blunted insulin secretion as compared to wildtype mice [112].
At older age, these mice developed manifest diabetes, which was attributed to progressive loss of beta-cell function due to higher expression of $\alpha_2$-adrenergic receptors, while insulin sensitivity remained intact [113].

5.3 Assessment of glucocorticoid-induced beta-cell dysfunction in humans

5.3.1 Acute inhibitory effects of glucocorticoids on insulin secretion in vivo
Both cortisol infusion [47] and acute treatment with high-doses of oral prednisolone [114,115] acutely impaired insulin secretion in healthy volunteers. In these studies, fasting insulin levels remained unchanged following GC treatment, despite the increments in fasting glucose. Furthermore, insulin release during glucose infusion [114] and during a meal test [115] were decreased, suggesting an acute inhibitory effect on the beta-cell.

5.3.2 Glucocorticoids increase insulin secretion after short-term treatment
More prolonged exposure (2 to 5 days) of healthy subjects to high doses of dexamethasone or prednisolone, resulted in fasting hyperinsulinemia and increased insulin secretion during hyperglycemic clamp studies or intravenous glucose tolerance tests [9,20,44,116-118]. In these studies, healthy subjects were able to compensate for GC-induced insulin resistance. Consequently, the product of insulin secretion and insulin sensitivity, also called the disposition index, remained constant. However, in susceptible populations, including normoglycemic individuals with a reduced insulin sensitivity [9] or a low GSIS [44,117] before treatment with GCs, healthy, first-degree relatives of patients with T2DM [20] and obese women [119], this compensation failed, resulting in hyperglycemia. These studies were limited by the fact that beta-cell function was assessed by intravenous glucose tolerance tests. Although the hyperglycemic clamp is regarded as the gold standard for the quantification of insulin secretion, it represents a less physiological condition relative to tests using orally administered insulin secretagogues, since insulin secretion also depends on, among others, non-glucose metabolic stimuli, incretins, and neuronal inputs [120]. Recently, new methods have been validated to determine various aspects of beta-cell function from a frequently-sampled OGTT or from standardized mixed-meal tests, by using modeling analysis [121]. Using this approach, GCs were recently shown to induce beta-cell dysfunction in completely healthy males, indicating that the full scope of GC-induced effects on beta-cell function may as yet not be fully detailed [115].

5.3.3 Chronic effects of glucocorticoid-treatment on beta-cell function
The most important limitation of most studies performed in humans, however, is the short exposure (maximally up to 14 days) to GCs. In daily practice, many patients are treated with...
GCs for prolonged time periods, even years. However, due to ethical issues, it is not viable to chronically expose healthy volunteers to GCs in order to study the long-term effects on beta-cell function. Conversely, it is not feasible to study the prolonged effects of GCs on beta-cell function in patients using GCs in clinical practice. Since the indication for GC treatment often comprises diseases with a state of systemic inflammation, inflammation-induced beta-cell effects will interfere with GC-modulation of beta-cell function [122].

As chronic GC treatment is associated with the development of diabetes, GCs most likely exert inhibitory effects on the beta cell after prolonged exposure, although strong (direct) evidence is currently lacking. As described previously, GC-induced pro-apoptotic effects and reductions in insulin biosynthesis may contribute to beta-cell failure in time. In addition, chronic GC-treatment may induce beta-cell failure indirectly through elevation of plasma concentrations of TG and NEFA [5,6], which contribute to beta-cell dysfunction by mechanisms related to lipotoxicity, a process which is characterised by accumulation of NEFA in the pancreas, resulting in beta-cell dysfunction [123].

Taken together, GCs acutely inhibit insulin secretion, both in vitro and in vivo. Prolonged exposure however, induces hyperinsulinemia, due to compensation for GC-induced insulin resistance. Chronic exposure likely results in progressive loss of beta-cell function in susceptible individuals.

6. ENDOGENOUS GLUCOCORTICOID METABOLISM IN THE METABOLIC SYNDROME

Since conditions with systemic GC excess shear great similarities with the MetS, the role of (tissue-specific) GC dysmetabolism in the latter condition has been the topic of intense research in the past decade. Most notably, the 11β-HSD enzymes and their relation with the development of central adiposity and hepatic steatosis have been investigated [12,14,70,89].

6.1 Altered 11β-HSD activity in adipose tissue induces central adiposity

In several studies in rodent obesity models, strong evidence for disturbed GC metabolism by an altered activity of 11β-HSD1 was observed. Transgenic mice selectively overexpressing 11β-HSD1 in white adipose tissue under control of the AP2 promoter, thereby increasing local cortisol concentrations, developed visceral obesity, dyslipidemia and insulin resistance [124]. In contrast, 11β-HSD1 (-/-) mice gained significantly less weight in response to a high-fat diet and preferentially stored adipose tissue in peripheral fat depots [125]. In addition, transgenic
mice with adipose tissue-specific 11β-HSD2 overexpression, resisted weight gain on high-fat diet, and displayed improved insulin sensitivity compared to wildtype mice [15]. These data support the concept that reducing GC activity in adipose tissue has favourable effects on body composition and metabolic profile. Translating these promising data to humans, however, has proven to be difficult. In obese subjects, increased 11β-HSD1 mRNA and activity were shown in SCAT [126,127], rather than in VAT [128], and increased cortisol metabolite excretion was associated with total body fat content, but not specifically with VAT area [67].

6.2 Glucocorticoid dysmetabolism in the liver is linked with hepatic steatosis

Hepatic steatosis represents a pathophysiological hallmark of the MetS, and altered hepatic cortisol metabolism has been linked to this condition [40]. Again, particularly the enzyme 11β-HSD1 has received much attention. In transgenic mice, it was elegantly demonstrated that liver-specific overexpression of 11β-HSD1 induced fatty liver by increasing the lipogenesis/lipid oxidation ratio, without increasing abdominal fat depots [129]. However, studies in humans linking 11β-HSD1 activity to hepatic fat content were less successful [67].

In conclusion, involvement of GC metabolism in obesity and its metabolic consequences seems evident, although translating data from animal studies in humans has proven to be difficult. Nevertheless, given its important role in GC metabolism and disturbed activity of this enzyme in other insulin resistant states, such as polycystic ovary syndrome [130], HIV-associated lipodystrophy [131] or cortisone reductase deficiency [132], 11β-HSD1 remains a promising target for novel therapeutics, as will be discussed below.

7. NOVEL THERAPEUTICS RELATED TO GLUCOCORTICOID METABOLISM AND ACTION

Advances in research regarding GC metabolism and mode of action, has led to the development of two novel classes of agents, albeit it for different indications, i.e. the dissociated GR agonists and the 11β-HSD1 inhibitors.

7.1 Dissociated glucocorticoid receptor agonists

This new class of synthetic GR ligands is designed to specifically induce GC-induced transrepression pathways, while minimizing transactivation activity. As described earlier, and as demonstrated in figure 1, these compounds are thus expected to display the anti-inflammatory properties of classic GR agonists, such as prednisolone and dexamethasone, but without inducing dysmetabolic side effects [16,17]. Both pharmaceutical companies and university groups have made great efforts to identify dissociated GR ligands with an increased
therapeutic index (reviewed in [133]).

The first compounds, which still had a steroidal structure, showed good dissociation in vitro, however, their beneficial profile was not sustained in vivo. Since then, several non-steroidal GR-ligands have been identified. A number of these compounds, such as AL-438, ZK 216.348 and BI115, indeed demonstrated an increased therapeutic index in vivo in different disease- and side effect animal models. These GR ligands were shown to be as efficient as prednisolone and dexamethasone to suppress inflammation, but they did not elevate blood glucose levels [133]. Although all dissociated GR ligands are currently still at the preclinical level of development, and many aspects of the molecular pathways that underlie GC's actions remain to be elucidated, it is evident that these compounds may become of great value for patients who require treatment with the classic GR agonists. It will be exciting to see whether the promising results derived from in vitro assays and animal models will hold in studies in humans that are expected to be conducted in the coming years.

7.2 11β-HSD type 1 inhibitors

Given the role of increased GC activity in liver and adipose tissue in the development of obesity and the MetS, 11β-HSD1 inhibition has been proposed as an interesting strategy for the treatment of these conditions. Indeed, in obese rodents, pharmacological inhibition of 11β-HSD1 improved glucose tolerance, insulin sensitivity and lipid profiles [15]. In humans, the first studies were performed with carbenoxolone, a nonselective 11β-HSD1 inhibitor. Although carbenoxolone decreased hepatic glucose production during a hyperinsulinemic-euglycemic clamp, it did not augment glucose disposal [70]. Several new classes of selective 11β-HSD1 inhibitors are currently in development, including arylsulfonamidothiazoles [134] and perhydroquinolylbenzamides [135]. Although these agents were shown to lower blood glucose concentrations by increasing hepatic insulin sensitivity, clamp studies in obese mice demonstrated no beneficial effects on glucose disposal [136]. Most recently, new compounds with benzamide [137] and thiazole [138] structures have been published. The important challenge now is to assess whether these compounds (and other future 11β-HSD1 inhibitors), in addition to improving hepatic insulin sensitivity, exert beneficial effects on adipose tissue and whole-body insulin sensitivity in humans. Despite the limitations of the current available 11β-HSD1 inhibitors, 11β-HSD1 remains an attractive target for the development of novel drugs that may be added to the present arsenal of therapeutics in the combat against the MetS pandemic.

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