Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes.

Running title: Glucocorticoids and pancreatic endocrine cells

Alex Rafacho¹,*, Henrik Ortsäter², Angel Nadal³ and Ivan Quesada³,*

¹Department of Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil.
²Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden.
³Institute of Bioengineering and the Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM), Miguel Hernández University, Elche, Spain.

*Corresponding authors:

A. Rafacho, Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas (CCB), Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil. Tel.: +55 48 37212290. E-mail: alex.rafacho@ufsc.br

I. Quesada, Instituto de Bioingeniería, Universidad Miguel Hernández, Avenida de la Universidad s/n, 03202 Elche, Spain. E-mail: ivanq@umh.es

Word count: 214 words (abstract); 5917 words (main text).

Number of Figures: 3 figures.
**Keywords:** glucocorticoids, insulin resistance, insulin sensitivity, insulin secretion, glucagon secretion, glucose tolerance, diabetes.

**Abbreviations:** GCs, glucocorticoids; GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase; IR, insulin resistance; GSIS, glucose-stimulated insulin secretion; IMGU, insulin-mediated glucose uptake; LXR, liver X receptor.
Abstract

Glucocorticoids (GCs) are broadly prescribed for numerous pathological conditions due to their anti-inflammatory, antiallergic and immunosuppressive effects, among other actions. Nevertheless, GCs can produce undesired diabetogenic side effects through interactions with the regulation of glucose homeostasis. Under conditions of excess and/or long-term treatment, GCs can induce peripheral insulin resistance (IR) by impairing insulin signalling, which results in reduced glucose disposal and augmented endogenous glucose production. Additionally, GCs can promote abdominal obesity, elevate plasma fatty acids and triglycerides and suppress osteocalcin synthesis in bone tissue. In response to GC-induced peripheral IR and in an attempt to maintain normoglycaemia, pancreatic beta-cells undergo several morphofunctional adaptations that result in hyperinsulinaemia. Failure of beta-cells to compensate for this situation favours glucose homeostasis disruption, which can result in hyperglycaemia, particularly in susceptible individuals. GC treatment does not only alter pancreatic beta-cell function; pancreatic alpha-cells are also affected by GC actions that can lead to hyperglucagonaemia, further contributing to glucose homeostasis imbalance and hyperglycaemia. Additionally, the release of other islet hormones, such as somatostatin, amylin and ghrelin, are also affected by GC administration. These undesired GC actions merit further consideration for the design of improved GC therapies without diabetogenic effects. In summary, in this review, we consider the impact of GC treatment on peripheral IR, islet function and glucose homeostasis.
1. Introduction.

Glucocorticoids (GC), such as cortisol in humans and corticosterone in rodents, are produced in the adrenal cortex and play a key role in regulating glucose homeostasis and nutrient metabolism. Synthetic GCs, which include dexamethasone and prednisolone, are used in medical practice because of their anti-inflammatory, antiallergic and immunosuppressive effects. Although synthetic GCs are broadly prescribed in numerous pathological conditions, they have important adverse metabolic effects, including peripheral insulin resistance (IR) and glucose intolerance as well as overt hyperglycaemia and diabetes. These side effects are observed particularly in susceptible individuals such as pregnant women, obese subjects, insulin-resistant individuals or first-degree relatives of diabetic patients (Van Raalte et al. 2009). The ability of GCs to produce peripheral IR is central to explain their impact on glucose homeostasis. It is well known that any reduction in peripheral insulin sensitivity, e.g., when GCs are administered, is adaptively compensated by augmented pancreatic beta-cell function (Beard et al. 1984, Nicod et al. 2003, Ahrén 2008, Rafacho et al. 2008).

This islet compensation meets the principle of the disposition index, the product of insulin secretion and peripheral insulin sensitivity. When beta-cells fail to adjust to the insulin demand imposed by the GC treatment, fasting and/or postprandial hyperglycaemia may arise. The severity and progression of these alterations depend on several parameters including dosage, period and previous individual susceptibility among others (Novelli et al. 1999, Rafacho et al. 2008, Jensen et al. 2012). In addition to the islet’s compensatory responses to IR, GCs directly affect beta-cell function, which may further complicate adequate glycaemia regulation. Although less explored than insulin release, these steroids also affect the secretion of other islet hormones with important roles in glucose homeostasis, such as glucagon, somatostatin and amylin. All
these alterations in islet hormonal secretion can exacerbate GCs’ diabetogenic actions.

In the next sections, we review the main effects of GCs on peripheral tissues and the endocrine pancreas and also consider the risks and limitations of their therapeutic use.

1.1. Cellular mechanisms of glucocorticoid action. Ninety-five percent of circulating cortisol is bound to corticosteroid-binding globulins and albumin (Andrews & Walker 1999). The plasma levels of the inactive form, cortisone, are approximately 50-100 nM, and the hormone is largely unbound to plasma proteins (Walker et al. 1999). Local conversion between active and inactive forms is catalysed by 11beta-hydroxysteroid dehydrogenase (11beta-HSD). 11beta-HSD type 1 is a reductase that converts inactive cortisone (in humans) and 11-dehydrocorticosterone (in rodents) to active cortisol and corticosterone, respectively (Low et al. 1994, Voice et al. 1996). The type 2 isoform works as a dehydrogenase that catalyses the opposite reaction (Brown et al. 1993). The actions of 11beta-HSD1 and 11beta-HSD2 serve as a pre-receptor control of GC action and determine local GC concentrations.

GC action at the site of cells is activated by the steroid hormone binding to its receptor. The classical GC receptor (GR), a ligand-regulated transcription factor that belongs to the superfamily of nuclear receptors, binds GCs and regulates transcription of target genes by activation or repression (Mangelsdorf et al. 1995). The GR is expressed in virtually all tissues; however, GR is able to regulate genes in a cell-specific manner, indicating that the response to GCs is regulated by factors beyond receptor expression. The GR is guided from the moment of synthesis to decay through signal transduction and by a variety of molecular chaperones such as HSP70 (Nelson et al. 2004) and HSP90 (Pratt et al. 2006), which facilitate folding, maturation and ligand binding. In addition, GR-mediated transcriptional activation is modulated both
positively and negatively by phosphorylation (Ismaili & Garabedian 2004) performed
by kinases and phosphatases. Although the activity of the GR is often thought in terms
of direct gene transactivation, considerable cross-talk also occurs between the GR and a
cohort of molecules to mediate their function as transcriptional factors, including
coctamer transcription factors Oct1 and Oct2, CREB (cAMP response element binding
protein) and STAT5 (signal transducers and activators of transcription-5) (Chen et al.
co-activators is an important determinant of the fate of the cross-talk between the GR
and other transcription factors. In addition to these genomic GC actions, the steroid
hormone can induce effects on a minute time scale, which is difficult to explain by
mechanisms involving gene expression changes (Long et al. 2005). Localised cell
membrane receptors with GC affinity have recently been identified (Strehl & Buttgereit,
2014).

1.2. Glucocorticoid therapy in clinical practice. Drugs based on GCs were
introduced in the 1950s and have been an important therapeutic strategy to treat
rheumatic and inflammatory diseases ever since. In this regard, the relevant properties
are the immunosuppressive, anti-inflammatory and anti-allergic effects that GCs exert
on primary and secondary immune cells, tissues and organs (Stahn & Buttgereit 2008).
Estimates suggest that between 1 and 2% of the adult population in the Western world is
receiving some form of long-term GC treatment, with a clear higher usage among the
geriatric patient group (Van Staa et al. 2000). In dermatology, GCs are the most widely
used therapy, for example, to treat atopic eczema. Inhaled GCs are used to treat allergic
reactions in airways and to dampen bronchial hyperreactivity in asthma. Systemically,
GCs are used to combat connective tissue inflammation, rheumatoid arthritis, bowel diseases and in allotransplantation (Thiele et al. 2005).

2. Diabetogenic actions of glucocorticoids in skeletal muscle and adipose, hepatic and bone tissues. There are a myriad of risks associated with excessive GC use; these risks have been recognised since GCs came into clinical use (Schäcke et al. 2002). Given GCs’ strong capacity to counteract the action exerted by insulin and raise blood sugar levels, it is not surprising that IR and glucose intolerance is a concern in patients with Cushing’s syndrome and disease (endogenous GC overproduction) and in patients prescribed GC-based therapy for immunomodulatory purposes (Raúl Ariza-Andraca et al. 1998). In addition, hypercortisolaemic conditions share many characteristics with metabolic syndrome, a cluster of abnormalities including hyperglycaemia, abdominal obesity, dyslipidaemia and hypertension (Anagnostis et al. 2009). Low-dose GC therapy is considered when the daily dose is less than 7.5 mg prednisolone or equivalent (van der Goes et al. 2010). When such a dose is administrated orally, plasma prednisolone levels peak 2-4 hours after intake at about 400-500 nM (~150-200 ng/ml) and return to baseline within 12 hours after steroid administration (Wilson et al. 1977, Tauber et al. 1984). These values are in the same range as normal endogenous cortisol levels: reference values for samples taken between 4:00 am and 8:00 am are 250-750 nM and for samples taken between 8:00 pm and 12:00 pm are 50-300 nM. This indicates that the absolute cortisol values are not as important for developing adverse effects during low-dose GC therapy as is the diurnal variation. Current knowledge gives at hand that developing diabetes after starting low-dose GC treatment seems rare but progression of already impaired glucose tolerance to overt diabetes is possible (van der Goes et al. 2010). Therefore, clinical recommendation states that baseline fasting glucose should be
monitored before initiating therapy and during following up according to standard patient care. Certainly, the adverse effects are more pronounced during high-dose GC therapies (>30 mg prednisolone or equivalent daily). In a retrospective study of hemoglobin A1c (HbA1c) levels in patients with rheumatic diseases subjected to prednisolone treatment, it was found that around 82% had HbA1c levels higher than 48 mmol/mol (given in IFCC standard, corresponding to 6.7% in DCCT standard). Serum HbA1c levels higher than 52 mmol/mol (7.1%), were seen in 46% of the patients and 23% of the patients had HbA1c levels as high as 57 mmol/mol (7.6%), which should be considered as a high risk factor for diabetes. Taken together, it was found that the cumulative prednisolone dose was the only factor significantly associated with the development of steroid-induced diabetes among rheumatic patients (Origuchi et al. 2011).

2.1. Adipose tissue. GCs regulate the maturation of pre-adipose cells into differentiated adipose cells as well as metabolism in adipose tissue (Rebuffé-scrive et al. 1992). Because the GR is predominantly expressed in adipose cells located in intra-abdominal fat, GCs are more highly activated in these fat deposits (Pedersen et al. 1994). A striking feature observed under conditions of GC excess is enhanced accumulation of visceral fat and loss of peripheral fat deposits in the arms and legs (Reynolds et al. 2012) (Figure 1). In the peripheral fat deposits, GCs promote expression of the key lipolytic enzyme hormone-sensitive lipase (Slavin et al. 1994) and, thus, acute infusion of cortisol in healthy humans induces triglyceride hydrolysis and the release of fatty acids and glycerol to the systemic circulation (Divertie et al. 1991). On the contrary, it has been suggested that GCs promote increased fat mass and triglyceride synthesis in visceral fat. Hence, GCs and insulin work in concert to activate lipoprotein lipase (Ottosson et al. 1994), which leads to relocation of fat deposits from
arms and legs to abdominal sites. Furthermore, GC treatment was shown to inhibit AMPK (5′ AMP-activated protein kinase) activity specifically in rat visceral but not subcutaneous adipose tissue (Christ-Crain et al. 2008), which may explain the redistribution of fat deposits that occurs during GC excess. This hypothesis remains to be proven in humans but is supported by the observation that patients with Cushing's syndrome exhibited a 70% lower AMPK activity in visceral adipose tissue (Kola et al. 2008). Additionally, GC-induced attenuation of insulin signalling in the adipose tissue has been associated with reduced glucose uptake (Ortsäter et al. 2012). In summary, GCs exposure leads to impaired insulin signalling and a systemic elevation of fatty acids and triglycerides which contributes to IR. Furthermore, GCs induce abdominal obesity.

2.2. Skeletal muscle. Skeletal muscle accounts for approximately 80% of insulin-mediated glucose uptake (IMGU) and is the largest glycogen store. GCs interfere directly with insulin signalling in skeletal muscle cells. Studies have shown that administration of dexamethasone reduces expression and activity of IRS1 (insulin substrate-1) and PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase) in rodent skeletal muscle cells (Saad et al. 1993, Morgan et al. 2005), which would presumably lead to a reduction in IMGU and abrogation of glycogen synthesis (Figure 1). Indeed, in a study with healthy human volunteers, prednisolone treatment for 6 days (0.8 mg·kg⁻¹ day⁻¹) reduced insulin-induced leg glucose uptake by 65% compared to placebo treatment (Short et al. 2009). In support, rats treated with GCs were shown to have reduced insulin-stimulated glucose uptake, caused by attenuated insulin-induced GLUT4 (glucose transporter type 4) translocation to the cell membrane in myotubes (Dimitriadis et al. 1997). The condition is worsened by the accumulation of ectopic fat deposition in skeletal muscle (Fransson et al. 2013) (Figure 1), which originates from
the systemic GC-induced fatty acid elevation as discussed above. Taken together, these
data show that GCs directly interfere with insulin signalling in skeletal muscle cells
leading to reduced IMGU.

2.3. Hepatic tissue. Hepatic tissue plays a key role in controlling glucose and
lipid homeostasis. Although insulin does not directly stimulate glucose uptake in liver
cells, the hormone is responsible for hepatic glycogen synthesis and gluconeogenesis
suppression. These insulin actions are mediated via insulin receptor signalling. As in
skeletal muscle, GC excess also interferes with the insulin signalling cascade in hepatic
tissue. In one study, dexamethasone-treated rats (1.5 mg/kg body weight for 6
consecutive days) exhibited an approximately 50-70% reduction in insulin receptor
binding in hepatocytes (Olefsky et al. 1975). A significant reduction in insulin receptor
density was also observed in hepatocytes from rats chronically treated with
dexamethasone (Caro & Amatruda 1982). Diminished tyrosine phosphorylation in
either insulin receptor or IRS1 was observed in liver from rats treated with
dexamethasone for 5 consecutive days (Saad et al. 1993). In addition, GCs were shown
to augment endogenous glucose production in several (Rizza et al. 1982, Pagano et al.
1983, Rooney et al. 1993) but not all (Wajngot et al. 1990) studies conducted in healthy
humans. GC-driven glucose production may be caused by enhanced gluconeogenesis, as
GCs induce rate limiting enzymes for gluconeogenesis, e.g., phosphoenolpyruvate
carboxylase and glucose-6-phosphatase (Lange et al 1994, Cassuto et al. 2005) (Figure
1). GC-mediated expression of gluconeogenic enzymes appears to be dependent on liver
X receptor (LXR) expression (Patel et al. 2011). Indeed, mice lacking LXRbeta (but not
LXRalpha) were demonstrated to be resistant to dexamethasone-induced
hyperglycaemia, hyperinsulinaemia, and hepatic steatosis but remained sensitive to
dexamethasone-dependent immune system repression (Patel et al. 2011). Moreover,
since GCs promote muscle wasting and lipolysis, they also increase the bioavailability of substrates for gluconeogenesis (Divertie et al. 1991, Kim et al. 2012) (Figure 1). Finally, fat accumulation leads to hepatic steatosis (Fransson et al. 2013), which, by itself, attenuates insulin sensitivity (Kim et al. 2012). To summarise, elevated GC levels promote gluconeogenesis in hepatic tissue leading to fasting hyperglycaemia.

2.4. Bone tissue. Osteoporosis is a common side effect observed in patients on GC-based therapy (Hoes et al. 2010). GCs also suppress osteoblast function, including osteocalcin synthesis (Prummel et al. 1991) (Figure 1). Osteocalcin is an osteoblast-specific peptide that is reported to be involved in normal murine fuel metabolism (Ferron et al. 2008). In pioneering work by Lee et al (Lee et al. 2007), it was demonstrated, both in cell culture and in mice, that osteocalcin increased pancreatic beta-cell proliferation as well as insulin expression and release, resulting in improved glucose tolerance. In addition, uncarboxylated osteocalcin increased adiponectin expression and secretion in adipose tissue, which in turn enhanced insulin sensitivity (Lee et al. 2007). In human type 2 diabetes, serum osteocalcin concentrations are positively correlated with improved glucose control (Bao et al. 2011). In another study, osteoblast-targeted disruption of GC signalling significantly attenuated the suppression of osteocalcin synthesis and prevented the development of insulin resistance, glucose intolerance, and abnormal weight gain in corticosterone-treated mice (Brennan-Speranza et al. 2012). Nearly identical effects were observed in GC-treated animals following hepatic expression of both carboxylated and uncarboxylated osteocalcin. These data suggest a link between GC effects on the skeleton and the steroid hormone effects on glucose homeostasis.
3. Effects of glucocorticoid treatment on pancreatic beta-cells and insulin secretion.

Pancreatic beta-cells respond to increasing plasma glucose levels by secreting insulin, which maintains glycaemia within narrow physiological ranges. This key function can be altered by GCs through direct and indirect actions and may also depend on whether GCs act as acute or chronic stimuli. In the next sections, we consider the different aspects of GCs’ effects on beta-cells.

3.1. Acute effects of glucocorticoids. The direct in vitro effects of GCs on glucose-stimulated insulin secretion (GSIS) are generally inhibitory and occur within a few minutes, as demonstrated in isolated rat islets exposed to corticosterone (0.02-20 mg/L) (Billaudel & Sutter 1979) (Figure 2A, left). This inhibitory action involves alpha-adrenergic signalling because phentolamine (a non-selective alpha-adrenergic antagonist) blocks GCs’ effect (Barseghian & Levine 1980). This rapid impact of GCs is not reproduced by synthetic steroids. GSIS inhibition in mouse (Lambillotte et al. 1997) and rat islets (Zawalich et al. 2006) is apparent only after the third hour of exposure to 1 µM dexamethasone.

GCs may also exert a negative in vivo effect during acute administration. A single oral dose of prednisolone (75 mg) (van Raalte et al. 2010) or dexamethasone (1 mg) (Schneiter & Tappy 1998) in healthy volunteers resulted in decreased insulin secretion and/or a reduced insulinogenic index (the ratio between Δinsulinaemia and Δglycaemia) during a meal or an oral glucose tolerance test (oGTT), respectively. In contrast, other studies did not demonstrate this acute GC effect in healthy men (Vila et al. 2010) or normal adult rats (Stojanovska et al. 1990) during an intravenous or oGTT, respectively. Similar to the in vitro observations mentioned above, increased sympathetic drive may be involved in GCs’ inhibition of in vivo insulin secretion.
(Longano & Fletcher 1983). This hypothesis is based on a study conducted with adult
Swiss mice treated with hydrocortisone (300 mg/kg body weight) 1 hour prior to
determining fed blood glucose and plasma insulin values. The insulinogenic index was
reduced 1 hour after steroid administration in fed mice but unaltered when
chlorisondamine (a ganglionic blocker) or phentolamine were given 10 minutes before
GC administration (Longano & Fletcher 1983) (Figure 2B, left). Overall, acute
exposure or administration of GCs appears to cause a decline in the insulinogenic index
in humans and rodents, and this effect may be mediated by sympathetic activation of
alpha-adrenergic receptors. It is important to highlight that 24 hours after interrupting
GC administration, all beta-cell function parameters return to normal values (van Raalte
et al. 2010).

3.2. Chronic effects of glucocorticoids. As observed in acute in vitro
experiments, chronic incubation (hours to days) with synthetic GCs in in vitro
conditions leads to decreased GSIS in rodent isolated islets, dispersed beta-cells and
insulin-secreting cell lines (Lambillotte et al. 1997, Zawalich et al. 2006, Shao et al.
2004, Ullrich et al. 2005). GCs’ deleterious effects on GSIS involve impaired glucose
oxidative metabolism (Shao et al. 2004), activation of repolarising K+ channels (Ullrich
et al. 2005), generation of reactive oxygen species (Roma et al. 2011), endoplasmic
reticulum dyshomeostasis (Linssen et al. 2011), activation of 11β-HSD1 (Davani et al.
2000) and decreased efficiency of intracellular Ca2+ on the secretory response
(Lambillotte et al. 1997, Zawalich et al. 2006, Shao et al. 2004) (Figure 2A, right).

However, in contrast to the above-mentioned inhibitory effects observed in both
acute and long-term GC incubation, chronic in vivo administration of these steroids
leads to up-regulated beta-cell function as a result of the compensatory adaptation to
GC-induced IR. Administration of high doses of prednisolone (30 mg) or dexamethasone (2 to 15 mg) to healthy individuals for prolonged periods (up to 15 days and up to 4 days, respectively) resulted in normoglycaemia or a modest increase of fasting glycaemia (Beard et al. 1984, Schneiter & Tappy 1998, Hollindgal et al. 2002, Willi et al. 2002, Nicod et al. 2003, Ahrén 2008, van Raalte et al. 2010, Petersons et al. 2013). Importantly, in most of these studies, volunteers developed hyperinsulinaemia. In fact, during glucose challenging with a hyperglycaemic-clamp (Beard et al. 1984, Nicod et al. 2003) or an oGTT (Schneiter & Tappy 1998, Hollindgal et al. 2002, Willi et al. 2002) insulin release was significantly higher in GC-treated individuals compared to control groups. Plasma C-peptide values were also elevated after prednisolone treatment in healthy men at basal conditions (Hollindgal et al. 2002) and during a meal tolerance test (van Raalte et al. 2010). This enhanced beta-cell function was also observed in adult rats treated for up to 13 consecutive days with dexamethasone (0.125-2.0 mg/kg) based on basal hyperinsulinaemia (Novelli et al. 1999, Karlsson et al. 2001, Rafacho et al. 2008) or in vivo glucose challenging (Rafacho et al. 2008, 2011). This augmented beta-cell function occurred in a dose- (Rafacho et al. 2008) and time-dependent manner (Rafacho et al. 2011). In normal adult mice, administration of dexamethasone for 10 days or corticosterone from the first consecutive week also resulted in basal hyperinsulinaemia (Thomas et al. 1998, Fransson et al. 2013).

This hyperinsulinaemia is consistent with insulin hypersecretion observed in pancreatic islets isolated from GC-treated rats (Novelli et al. 1999, Karlsson et al. 2001, Rafacho et al. 2008, 2010a, 2010b). This enhanced beta-cell secretion involves an improvement in glucose responsiveness (Karlsson et al. 2001, Rafacho et al. 2008), sensitivity (Rafacho et al. 2008) and oxidative metabolism (Rafacho et al. 2010a) as well as augmented Ca\textsuperscript{2+} handling (Rafacho et al. 2010a) and an improved response to
cholinergic signals (Angelini et al. 2010, Rafacho et al. 2010,) (Figure 2B, right). The islet compensatory response is also accompanied by structural changes. It has been demonstrated that, beta-cell mass increases in a time- (Rafacho et al. 2011) and dose-dependent manner (Rafacho et al. 2009) with GC administration, according to the correspondent degree of insulin insensitivity. Taken together, these results show that when humans or animal models are exposed to prolonged steroid treatment, they develop augmented beta-cell function and mass to counteract the IR resulting from GC administration.

3.3. Glucocorticoid treatment, beta-cell dysfunction and glucose intolerance.

Depending on the GC regimen, glucose homeostasis is maintained at normal or near normal physiological conditions by adaptive beta-cell compensations. However, these adaptations do not always guarantee an adequate glucose homeostasis. Although insulin hypersecretion observed after prolonged steroid treatment appears to be consistent in most experiments performed with healthy volunteers (Beard et al. 1984, Schneiter & Tappy 1998, Ahrén 2008, van Raalte et al. 2010) and normal adult rats (Karlsson et al. 2001, Rafacho et al. 2008, 2009, 2011), glucose intolerance is also present. In these studies, hyperinsulinaemia is normally associated with normoglycaemia or modest increases in blood glucose values, but the insulin (Rafacho et al. 2008, 2011, Schneiter & Tappy 1998) and c-peptide hypersecretion (van Raalte et al. 2010) during glucose or meal challenges, respectively, do not prevent elevation in postprandial blood glucose levels. Therefore, the insulinogenic index may not necessarily match the peripheral insulin demand imposed by GCs.

The negative impact of GCs on glucose homeostasis is more apparent in individuals with any degree of susceptibility to glucose intolerance, such as those with
low insulin sensitivity (Larsson & Ahrén 1999), low insulin response to glucose (Wajngot et al. 1992), first-degree relatives of patients with type 2 diabetes (Jensen et al. 2012), obesity (Besse et al. 2005) and those who are older (Novelli et al. 1999). In these individuals, beta-cell function does not correspond to the peripheral insulin demand, and the deregulation of glucose homeostasis becomes more pronounced, reinforcing that individual background is a critical factor. Indeed, this susceptibility to beta-cell failure after treatment with dexamethasone has also been observed in animal models with an obesity background, such as fa/fa rats (Ogawa et al. 1992) and ob/ob mice (Khan et al. 1992).

In an attempt to analyse whether GCs have any direct effects on beta-cells in vivo independent of peripheral GC actions, a transgenic mouse model that specifically over-expresses GR in these cells was generated (Delaunay et al. 1997, Davani et al. 2004). These mice were normoglycaemic but displayed glucose intolerance associated with reduced insulin secretion during a glucose load (Delaunay et al. 1997). When these transgenic mice aged, hyperglycaemia developed together with marked glucose intolerance and reduced in vivo and ex vivo GSIS. Remarkably, no change in beta-cell apoptosis was observed in these mice (Davani et al. 2004). This deterioration in GSIS was prevented by incubating islets with benextramine (a selective α2-adrenergic receptor antagonist), suggesting the involvement of adrenergic signals. In any case, the analysis of direct GC effects on beta-cells in vivo is difficult because the systemic metabolic consequences of GC treatment most likely mask the GC-mediated changes in beta-cell function. Of note, almost all the morphofunctional beta-cell changes elicited by GC administration are transitory and reversible after 10 days of discontinuation of steroid treatment in rats, suggesting an unacknowledged plasticity in the regulation of beta-cell function and growth (Rafacho et al. 2010b).
4. Effects of glucocorticoids on glucagon release and other islet hormones.

Glucagon secretion by pancreatic alpha-cells plays a key role in glucose homeostasis. Glucagon’s release is enhanced at low plasma glucose levels but decreases under hyperglycaemic conditions (Quesada et al. 2008; Marroqui et al. 2014). Glucagon is one of the most important hyperglycaemic hormones and acts as insulin’s counterpart, opposing numerous anabolic insulin-mediated actions. The hyperglycaemic effect is mainly produced by activating hepatic glycogenolysis and gluconeogenesis, which results in the release of endogenous glucose into the bloodstream. This process restores normoglycaemia under hypoglycaemic conditions (Quesada et al. 2008; Marroqui et al. 2014). Hyperglucagonaemia may be present in diabetes. Additionally, inhibition of glucagon release at high glucose levels may be impaired in this metabolic condition. This impaired alpha-cell function can lead to higher hepatic glucose output, further contributing to hyperglycaemia in diabetic patients (Quesada et al. 2008; Marroqui et al. 2014). As in the case of beta-cells, in the next section we summarise the acute and chronic effects of GCs on alpha-cell function.

4.1. Acute effects of glucocorticoids on alpha-cell function and glucagon release. One study reported that corticosterone (10^{-7} M) potentiated glucagon release induced by a glucose-free medium or arginine in isolated perfused rat pancreas (Barseghian & Levine 1980). In contrast, incubation of mouse pancreatic islets with dexamethasone (0.5-50 nM), corticosterone (50 nM) or 11-dehydrocorticosterone (50 nM) for 2 hours reduced glucagon secretion induced by low glucose levels, effects that were reversed by a GR antagonist (Swali et al. 2008). The inhibitory action of 11-dehydrocorticosterone was partially reversed by a selective 11beta-HSD1 inhibitor. This
fact, along with the co-localisation of this enzyme with human and rodent islet alpha-cells, indicates that this islet cell type serves an important local function in pancreatic GC metabolism (Swali et al. 2008). This situation may be different in other species, for example in rats, where this enzyme is expressed in non-alpha-cells (Rafacho et al. 2014). In contrast with the above-mentioned results, prednisolone (10^{-5} M) failed to modify glucagon secretion in mouse pancreatic islets (Marco et al. 1976). Likewise, incubation of rat pancreatic islets with dexamethasone (1 \mu M) for 3 hours did not modify glucagon secretion (Rafacho et al. 2014). Thus, in vitro experiments with acute GC exposure have reported divergent effects on glucagon secretion. These divergences may depend on different factors, including the preparation and species studied as well as the specificity and potency of the different GCs used.

4.2. Chronic effects of glucocorticoids on alpha-cell function and glucagon release. Alpha-cell growth regulation by long-term GC exposure has been explored during development. Alpha-cell mass was decreased in 21-day-old foetuses obtained from pregnant rats that received dexamethasone in drinking water (1 \mu g/ml) either during the last week of pregnancy or throughout gestation (Dumortier et al. 2011). In contrast, GR inactivation in the pancreatic beta-cell (rat insulin promoter-Cre transgene) or in cells expressing pancreatic and duodenal homeobox-1 (PDX-1), which is involved in pancreas development, did not modify alpha-cell mass in adult mice (Gesina et al. 2004). Adult rats treated with dexamethasone (1 mg/kg) for 5 consecutive days exhibited a 50\% increase in alpha-cell mass (Rafacho et al. 2014). Similarly, administration of corticosterone to adult rats fed a high-fat diet promoted a synergistic positive effect on alpha-cell mass (Beaudry et al. 2013). In general, GC administration
in adults appears to up-regulate alpha-cell mass, while the opposite effect is observed during development.

Glucagon release is also modulated by GCs. Rats treated with dexamethasone (1 mg/kg) for 5 consecutive days showed hyperglucagonaemia (Rafacho et al. 2014). In this model, isolated pancreatic islets exhibited impaired inhibition of glucagon release at high glucose levels. Similarly, dexamethasone (0.25 mg/kg) administered for 7 days in rhesus macaques induced fasting hyperglucagonaemia (Cummings et al. 2013), and prednisolone (0.2-0.3 mg daily) administered for 4 days increased basal and arginine-induced glucagon secretion in isolated mouse islets (Marco et al. 1976). In contrast to the above-mentioned results obtained for in vivo GC treatment, glucagon release was suppressed in isolated rat islet cells incubated for 18 hours with dexamethasone at $10^{-9}$ and $10^{-10}$ M, but was without effect at higher steroid concentrations (Papachristou et al. 1994). Thus, most in vivo and ex vivo chronic studies point to enhanced alpha-cell secretion after GC administration. The resulting hyperglucagonaemia may aggravate GC-induced hyperglycaemia by stimulating hepatic glucose release and opposing insulin actions (Quesada et al. 2008) (Figure 3).

Clinical studies have also examined GCs’ effects on human alpha-cell function. Administration of prednisolone (40-100 mg daily) for up to 4 days induced fasting hyperglucagonaemia and glucagon hypersecretion in response to arginine (Marco et al. 1973). Similarly, daily dexamethasone treatment (2 mg) for 3 days led to increased basal plasma glucagon levels and enhanced alanine-induced glucagon release in non-obese subjects (Wise et al. 1973). Both responses were even more pronounced in obese individuals and patients with Cushing’s syndrome. Moreover, administration of dexamethasone (3 mg twice daily for 2 days) and prednisolone (30 mg for 2 consecutive weeks) led to increased fasting and postprandial glucagon levels (Beard et al. 1984, van
raalte et al. 2013). In contrast, in a few studies, fasting glucagon concentrations were
found to be unchanged by dexamethasone (3 mg twice daily for 2 and ½ days) (Larsson
& Ahrén 1999). Thus, the majority of clinical studies show that GC treatment may up-
regulate alpha-cell function, which may enhance GCs’ diabetogenic actions (Figure 3).

4.3. Effects of glucocorticoids on somatostatin, amylin and ghrelin release.

Pancreatic delta-cells secrete somatostatin, which indirectly affects glucose
homeostasis, suppressing both insulin and glucagon release (Quesada et al. 2008). In
vivo experiments showed that dexamethasone administration (0.5 mg/kg) for 3 or 8 days
in rats increased somatostatin gene expression and protein content in the pancreas
(Papachristou et al. 1994). However, plasma somatostatin levels were not measured in
these conditions. In in vitro experiments, incubation of isolated islet-cells with
dexamethasone for 18 hours produced a biphasic effect: while low doses (10^{-10} M)
stimulated the somatostatin gene and protein expression, high doses (10^{-8}-10^{-5} M)
produced the opposite effect (Papachristou et al. 1994). At this chronic exposure, the
high doses reduced somatostatin release into the medium. When foetal pancreatic islets
were cultured for 8 days with corticosterone (0.1 µg/ml), both the somatostatin
concentration in the medium and the islet somatostatin content were increased (McEvoy
et al. 1981). Thus, few experiments indicate that GC may regulate directly or indirectly
delta-cell function (Figure 3). Elevation in plasma somatostatin concentrations should
inhibit alpha and beta-cell function under normal physiological conditions. However,
this appears not to be the case during GC administration, given that GC treatment
results in hyperglucagonaemia and hyperinsulinaemia.

The islet amyloid polypeptide (IAPP), also called amylin, is co-secreted with
insulin by pancreatic beta-cells in response to food intake, most likely via the same
mechanisms that allow for insulin release. This hormone decreases postprandial
glycaemia by inhibiting gastric emptying and suppressing glucagon secretion
(Westermark et al. 2011). However, type 2 diabetes has also been related to the
formation of toxic amyloid aggregates that can induce beta-cell apoptosis (Westermark
et al. 2011). This aggregation might be associated with IR and insulin (and amylin)
hypersecretion (Westermark et al. 2011), which also result from GC treatment. With
this enhanced hormonal release, impaired intracellular IAPP processing may initiate the
amyloid aggregation process. For instance, dexamethasone treatment for up to 12 days
led to increased levels of both proinsulin and IAPP mRNA in rat islets (Bretherton-Watt
et al. 1989, Koranyi et al. 1992). Similarly, both enhanced plasma amylin levels and
amylin secretion from isolated pancreata were found in dexamethasone-induced insulin-
resistant rats (Pieber et al. 1993, Mulder et al. 1995). Similar findings in amylin
changes have been reported in humans after dexamethasone treatment (Ludvik et al.
1993), indicating that GC administration may enhance IAPP release (Figure 3).

Ghrelin is released by P/D1 cells from the stomach but also by epsilon-cells
from the pancreas (Wierup et al. 2013). Only few epsilon-cells are present in each islet.
Ghrelin inhibits insulin and somatostatin secretion but increases glucagon release
(Chuang et al. 2011, Wierup et al. 2013). Additionally, this hormone potently stimulates
growth hormone release from the anterior pituitary gland and stimulates appetite (Malik
et al. 2008). In hypercortisolemic patients with Cushing's disease, plasma ghrelin
concentrations increased after successful surgery, while prednisolone administration (30
mg/day) for five days decreased plasma ghrelin levels in healthy individuals (Otto et al.
2004). However, no changes were observed in response to a unique bolus of
hydrocortisone (0.6 mg/kg) in healthy men (Vila et al. 2010). In a neonatal rat model,
dexamethasone (0.5-0.05 mg/kg) administrated for four consecutive days led to
augmented plasma ghrelin levels in newborns (Bruder et al. 2005). However, any of the above-mentioned studies discriminated the ghrelin source, either the stomach or the pancreas. Thus, much research is necessary to address whether GCs can affect the function of epsilon islet-cells.


The diabetogenic effects of GCs are a limiting factor to their clinical use, particularly in individuals with diabetes risk factors. These side effects include unfavourable actions on peripheral tissues, such as skeletal muscle, liver, bone and adipose tissue, which mainly result, among other effects, in decreased insulin sensitivity, augmenting insulin needs. In response to this GC-induced IR, the endocrine pancreas undergoes compensatory beta-cell changes in function and mass, which lead to hyperinsulinaemia and enhanced stimulated insulin release, to maintain normoglycaemia. Despite the fact that most of these adaptations are observed in healthy subjects and animal models under GC treatment, the adaptations do not necessarily guarantee an adequate insulinogenic index to prevent glucose intolerance. These beta-cell adaptations are less efficient in susceptible individuals, increasing the risk of impaired glucose homeostasis during GC treatment. Up-regulated beta-cell function resulting from steroid treatment contrasts with the direct inhibitory actions observed in both acute and long-term in vitro GC exposure. Thus, the effects derived from in vivo GC treatment may prevail over the potential direct GC actions on beta-cells. In any case, further research is necessary to unravel the molecular mechanisms of both direct and indirect GC actions on the endocrine pancreas.

Several studies have also documented acute and chronic GC effects on non-beta pancreatic cells. The mechanisms implicated are not clear but may involve multiple
factors, including direct actions on islet cells as well as effects derived from adaptations to IR, hyperglycaemia, hyperinsulinaemia or other conditions. Remarkably, the majority of *in vivo* animal studies and clinical reports show that, in addition to hyperinsulinaemia, GC treatment induces higher plasma levels of glucagon and amylin and may probably affect somatostatin. The increased plasma amylin levels might also be considered diabetogenic because enhanced IAPP concentrations may lead to increased rates of toxic amylin aggregation (Couce *et al.* 1996). Additionally, the hyperglucagonaemia observed with GC treatment opposes insulin actions and may aggravate steroid-induced hyperglycaemia by increasing hepatic glucose output, as indicated in diabetes (Quesada *et al.* 2008). Thus, the impaired release of the different islet hormones may increase the diabetogenic effects of GCs.

The majority of studies about GC actions involve the use of murine models, and thus, prudence is required when translating this experimental data to humans. However, it is also important to mention that the prolonged duration of several GC therapies in clinical practice may exceed the safe period proposed in experimental approaches in human studies, which generally do not surpass 2-15 days of GC treatment (van Raalte *et al.* 2009). Thus, experimental data from human, although of great relevance, fail to totally mimic the conditions of clinical practice (i.e. duration). Elaboration of protocols to investigate GC actions in human volunteers is not feasible, considering the risk of irreversible negative effects, ethical issues, as well as the nature of *ex vivo* and *in vitro* techniques available for the mechanistic studies (van Raalte *et al.* 2009). In this regard, animal models are valuable tools, since part of the above-mentioned limitations can be resolved.

Improved knowledge of GCs’ intracellular signalling mechanisms and effects will help to design better GC therapies. In this regard, it has been suggested that gene
transrepression accounts for the majority of therapeutic GC effects, while transactivation of metabolic target genes is mainly responsible for the side effects (Strehl & Buttgereit 2013). Using this concept, several GR agonists dissociating transrepression from transactivation were developed (Löwenberg et al. 2008). Some of these agonists have proven useful for maintaining GCs’ anti-inflammatory and immunosuppressive effects, while reducing side effects like hyperglycaemia. However, the above-mentioned concept may be over-simplistic, and side effects may not only be explained by transactivation but also by non-genomic actions (Vandevyver et al. 2013). Thus, a great deal of research is still necessary to develop GR agonists with reduced drawbacks for glucose homeostasis. Moreover, the combination of GC-based therapies with glucose-lowering drugs could also be an interesting alternative to explore to minimise the disadvantages of GC treatment.

Declaration of interest.

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding. This study was supported by grants from the Brazilian foundation Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 471397/2011-3), the Spanish foundations Ministerio de Ciencia e Innovación (BFU2013-42789; BFU2011-28358), and the European Foundation for the Study of Diabetes (EFSD/BI Basic Programme and the Albert Renold Fellowship). CIBERDEM is an initiative of the Instituto de Salud Carlos III.
Author contributions: AR, HO, AN and IQ discussed and wrote the manuscript. All authors approved the final version of the manuscript to be published.

Acknowledgments. The authors give special thanks to the members of their laboratories.
REFERENCES.

Ahrén B 2008 Evidence that autonomic mechanisms contribute to the adaptive increase in insulin secretion during dexamethasone-induced insulin resistance in humans. *Diabetologia* **51** 1018-1024.


Besse C, Nicod N & Tappy L 2005 Changes in insulin secretion and glucose metabolism
induced by dexamethasone in lean and obese females. *Obesity Research* **13** 306-311.


Chen DY, Bambah-Mukku D & Pollonini G 2012 Glucocorticoid receptors recruit the CaMKIIα-BDNF-CREB pathways to mediate memory consolidation. *Nature*
Neuroscience 15 1707-1714.


Delaunay F, Khan A, Cintra A, Davani B, Ling ZC, Andersson A, Ostenson CG,
Gustafsson J, Efendic S & Okret S 1997 Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *Journal of Clinical Investigation* **100** 2094-2098.


Gesina E, Tronche F, Herrera P, Duchene B, Tales W, Czernichow P & Breant B 2004 Dissecting the role of glucocorticoids on pancreas development. *Diabetes* **53** 2322-


Olefsky JM, Johnson J, Liu F, Jen P & Reaven GM 1975 The effects of acute and chronic dexamethasone administration on insulin binding to isolated rat hepatocytes and adipocytes. Metabolism 24 517-527.


Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM,


Vila G, Krebs M, Riedl M, Baumgartner-Parzer SM, Clodi M, Maier C, Pacini G & Luger


**FIGURE LEGENDS**

**Figure 1. Effects of glucocorticoids on peripheral tissues involved in the control of glucose homeostasis.** Excess or prolonged GC treatment may disrupt glucose homeostasis by interfering with several metabolic-related tissues. In visceral adipose tissue, GCs elevate LPL activity, leading to fat accumulation at this fat site. Fat in the limbs appears to respond to GCs with increased HSL activity, resulting in increased lipid (FFA and glycerol) release, supplying substrates for hepatic TG synthesis and gluconeogenesis, and also in intramuscular fat accumulation. These steroids may also affect insulin signalling in adipose tissue. GCs impair insulin-stimulated glucose uptake in skeletal muscles and induce muscle wasting, which, in turn, provides gluconeogenesis substrates. In the liver, GCs have a negative effect on rate-limiting enzymes controlled by insulin. Finally, GC in excess may also alter osteocalcin synthesis in osteoblast cells leading to reduced osteocalcinaemia. Abbreviations: FFA, free fatty acids; GCs, glucocorticoids; G6Pase, glucose-6-phosphatase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PEPCK, phosphoenolpyruvate carboxykinase; TG, triacylglycerol.

**Figure 2. Sites of the insulin secretory process affected by in vitro or in vivo (ex vivo) exposure to glucocorticoids.** In (A), the known components involved in the acute or chronic in vitro effects of GCs on the beta-cell insulin secretory process are highlighted with a positive signal (indicates GCs stimulate/increase that action/function) or a negative signal (indicates GCs inhibit/diminish that action/function). Most notably, GCs impair beta-cell glucose metabolism, favour repolarising $K_v^+$ currents, decrease PKA and PKC activation, induce ER dyshomeostasis, increase 11beta-HSD1 activity and ROS generation and impair calcium handling. Together, these effects inhibit insulin
secretion. In (B), the known components involved in beta-cell function which are
affected by acute or long-term in vivo GC treatment are highlighted with a positive
signal, which indicates increased content or activity. Most notably, augmented glucose
metabolism and cholinergic pathway activity cause increased calcium influx and insulin
secretion. In this context, a positive GC effect on K\(^+\) and VDCC channels could not be
excluded. Abbreviations: AC, adenylyl cyclase; Ach, acetylcholine; alphaAR, alpha
adrenergic receptor; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol;
ER, endoplasmic reticulum; Gi, G-coupled inhibitory protein; GLUT2, glucose
transporter 2; IP\(_3\), inositol triphosphate; K\(^+\), ATP-dependent K\(^+\) channel; K\(_v\)\(^+\), voltage-
dependent K\(^+\) channel; M3R, muscarinic receptor type 3; PIP\(_2\), phosphatidylinositol
bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C;
ROS, reactive oxygen species; VDCC, voltage-dependent Ca\(^{2+}\) channel; 11beta-HSD-1,
11beta-hydroxysteroid dehydrogenase type 1.

**Figure 3. Diabetogenic effects of GC treatment: implication of islet hormones.** GC
treatment can induce IR in peripheral tissues. As a compensatory adaptive process, the
endocrine pancreas increases insulin release, leading to hyperinsulinaemia. An adequate
compensatory response to the insulin requirements imposed by IR allows for
normoglycaemia. However, an insufficient beta-cell response could lead to impaired
glucose tolerance, which can progress to overt hyperglycaemia and type 2 diabetes. GC
treatment also induces high plasma levels of glucagon and amylin, and may affect
somatostatin concentrations. Although somatostatin inhibits alpha and beta-cells, the
potential changes in this hormone induced by GCs do not appear to produce a
significant negative effect in these conditions. Hyperglucagonaemia increases hepatic
glucose output, which exacerbates hyperglycaemia and glucose intolerance and further
opposes insulin action, decreasing the insulin effect. High amylin levels have been related to increased predisposition to amyloid formation in decreased insulin sensitivity conditions, like those generated by GCs. Amyloid aggregation is related to increased beta-cell death and malfunction. The molecular mechanisms underlying the high plasma levels of glucagon and amylin induced by GC treatment are still unknown.
Figure 1

- Increased fat accumulation (↑ LPL activity)
- Increased lipids release (↑ HSL activity)
- Increased TG synthesis
- Increased Gluconeogenesis
- Increased [GCs]
- Increased PEPCK / G6Pase

Decreased osteocalcin synthesis

Increased muscle wasting

Increased fat accumulation (↑ LPL activity)

Decreased insulin signalling (↓ Glucose uptake)

Increased gluconeogenesis

Increased skeletal muscle

Increased fat accumulation
Figure 2

**A** *In vitro*

- (nor)adrenaline
- Ach
- glucose
- blood capillary

**Acute**

- alphaAR
- Gi/AC
- cAMP
- PKA
- K⁺ channel
- repolarizing
- insulin release
- blood capillary

**Chronic**

- alphaAR
- Gi/AC
- cAMP
- PKA
- PKC
- GLUT2
- oxidative metabolism
- [Ca²⁺]
- 11beta-HSD1
- ROS
- [Ca²⁺]
- insulin release

**B** *In vivo*

- (nor)adrenaline
- Ach
- glucose
- blood capillary

**Acute**

- alphaAR
- Gi/AC
- cAMP
- PKA
- K⁺ channel
- repolarizing
- insulin release
- blood capillary

**Chronic**

- alphaAR
- Gi/AC
- cAMP
- PKA
- PKC
- GLUT2
- oxidative metabolism
- [Ca²⁺]
- 11beta-HSD1
- ROS
- [Ca²⁺]
- insulin release
- VDCC channel
- depolarizing
Figure 3