Glucocorticoids and Metabolic Control

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Contents

Abstract

In response to stress, the central nervous system initiates a signaling cascade, which leads to the production of glucocorticoids (GCs). GCs act through the glucocorticoid receptor (GR) to coordinate the appropriate cellular response with the primary goal of mobilizing the storage forms of carbon precursors to generate a continuous glucose supply for the brain. Although GCs are critical for maintaining energy homeostasis, excessive GC stimulation leads to a number of undesirable side effects, including hyperglycemia, insulin resistance, fatty liver, obesity, and muscle wasting leading to severe metabolic dysfunction. Summarized below are the diverse metabolic roles of glucocorticoids in energy

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homeostasis and dysregulation, focusing specifically on glucose, lipid, and protein metabolism.

Keywords
Adipose tissue · Glucocorticoid hormones · Glucocorticoid receptor · Gluconeogenesis · Lipid metabolism · Liver · Muscle · Protein metabolism

1 Introduction

Glucocorticoids (GCs) are steroid hormones, essential for survival under stress. The physiologic stress response is mediated by the release of ACTH from the pituitary that acts on the adrenal gland to increase the production and release of cortisol (in humans) or corticosterone (in rodents) into the circulation. The GC hormone then acts through the GC receptor (GR) to coordinate the appropriate cellular response to stress with the primary outcome of increasing blood glucose levels. The mechanisms by which GCs achieve this effect involve the interplay primarily between liver, muscle, and adipose tissue. This adaptive response to stress, however, is meant to be of short duration and is regulated by negative feedback at the level of the hypothalamus and pituitary gland. Prolonged, elevated GC exposure, as observed with therapeutic use of GCs or in Cushing's syndrome, leads to increased insulin secretion eventually resulting in severe metabolic dysfunction and insulin resistance.

2 Glucose Metabolism

Under stressful stimuli, GCs coordinate a number of physiological processes with the end goal of generating a sustained glucose supply for the brain. GCs affect whole-body glucose metabolism by decreasing peripheral glucose uptake and inducing hepatic gluconeogenesis by mechanisms described below (Fig. 1).

2.1 Liver

The most well-studied effects of GCs are by far those related to hepatic gluconeogenesis. Glucose is the primary energy source for the brain, renal medulla, and erythrocytes, and the liver is the main organ responsible for de novo glucose production under fasting conditions. Not surprisingly, therefore, hepatic gluconeogenesis is under very tight hormonal regulation. In the fed state, insulin facilitates glucose uptake and utilization, whereas in the fasted state, glucagon, catecholamines, and GCs stimulate glucose production and release. In fact, mice

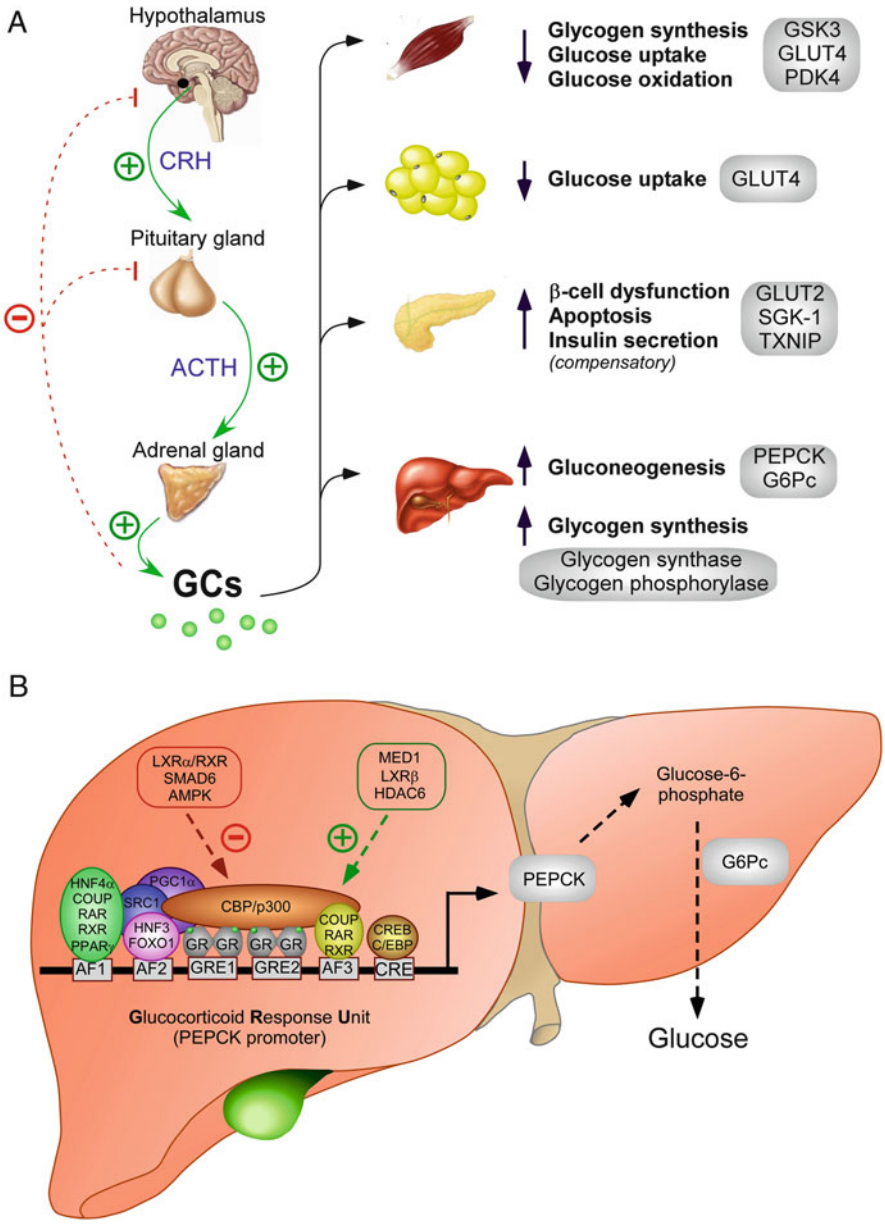

Fig. 1 Mechanisms by which GCs regulate whole-body glucose homeostasis. (**a**) Schematic representation of the HPA axis and the effects of GCs/GR on glucose metabolism in the liver, adipose tissue, muscle, and pancreas. Genes/proteins that are involved (either directly or indirectly) in the mentioned events are in *shaded boxes*. (**b**) Representation of the PEPCK glucocorticoid response unit in the liver, together with the location of some of the accessory factors necessary to initiate transcription. Depicted in the *square boxes* are some of the known positive

lacking GR in the hepatocytes fail to appropriately respond to prolonged fasting, resulting in severe hypoglycemia (Opherk et al. 2004).

Ligand-bound GR directly activates the transcription of two key enzymes involved in gluconeogenesis: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pc). PEPCK is the rate-limiting enzyme required to generate glucose-6-phosphate, whereas G6Pc is the enzyme that cleaves the phosphate allowing for glucose release into the circulation. PEPCK regulation is complex and requires a myriad of accessory proteins and transcription factors to ensure a maximal gluconeogenic response. Through extensive promoter mapping, it was found that the *Pepck* promoter harbors a GR response unit (GRU), which has two GR response elements (GREs) as well as binding sites for forkhead transcription factor (FOXO1), retinoid X receptor (RXR), chicken ovalbumin upstream promoter-transcription factor (COUP-TF), CCAAT/enhancer-binding protein β (C/EBPβ), hepatocyte nuclear factors 3 and 4 (HNF-3 and HNF-4), peroxisome proliferator-activated receptor γ (PPARγ2), and retinoic acid receptor (RAR) [reviewed in Chakravarty et al. (2005)]. Similarly, three functional GREs have been identified in the proximal *G6Pc* promoter, and similar to *Pepck* regulation, GCs act in cooperation with HNF-1, HNF-4, and FOXO1 to fully induce *G6Pc* transcription (Lin et al. 1998; Nakae et al. 2001; Vander Kooi et al. 2005).

Interestingly, cholesterol-sensing liver X receptors (LXRα and LXRβ) can also influence the recruitment of GR to gluconeogenic gene promoters (Nader et al. 2012; Patel et al. 2011). Specifically, rats treated with GW3965 (a dual LXRα/β agonist) were found to be refractory to the GC-induced hyperglycemia (Nader et al. 2012). This is believed to be due to direct competition for DNA binding, where the LXRα/RXRα dimer was found to displace GR from its GRE on the *G6Pc* promoter. Making matters more complex, it was found that LXR's effects on GC-mediated induction of gluconeogenesis are isoform specific. In fact, LXRβ is necessary for GR binding to the *Pepck* promoter, and LXRβ knockout mice are protected from dexamethasone (Dex)-induced hyperglycemia (Patel et al. 2011).

Adding another layer of control to the systemic regulation of energy homeostasis, the transcriptional activity of GR can also be modified through the recruitment of various coactivator and corepressor complexes. Coactivators including SRC1,

Fig. 1 (continued) (+) and negative (−) regulators of GC signaling. Also shown are the steps leading to the release of glucose into circulation. *ACTH* adrenocorticotropin hormone, *AMPK* AMP kinase, *CBP* CREB-binding protein, *C/EBP* CCAAT/enhancer-binding protein, *COUP* chicken ovalbumin upstream promoter-transcription factor, *CREB* cAMP-response element binding protein, *CRH* corticotropin-releasing hormone, *GLUT2* glucose transporter 2, *GLUT4* glucose transporter 4, *FOXO1* foxhead box protein O1, *G6Pc* glucose-6-phosphatase, *GR* glucocorticoid receptor, *GRE* glucocorticoid response element, *GSK3* glycogen synthase kinase 3, *HDAC6* histone deacetylase 6, *HNF* hepatic nuclear factor, *LXR* liver X receptor, *MED1* mediator complex subunit 1, *PDK4* pyruvate dehydrogenase kinase 4, *PEPCK* PEP carboxykinase, *PGC1α* PPAR-γ coactivator-1, *PPARγ* peroxisome proliferator-activated receptor γ, *RAR* retinoic acid receptor, *RXR* retinoid X receptor, *SGK-1* serum- and glucocorticoid-regulated kinase 1, *SMAD6* SMAD family member 6, *SRC-1* steroid receptor coactivator 1, *TXNIP* thioredoxin-interacting protein

CBP/p300, and PGC1α have all been shown to be involved in *Pepck* transactivation (Sommerfeld et al. 2011). Under fasting conditions, the expression of *Pgc1α* is induced synergistically by glucagon and GCs (Yoon et al. 2001). PGC1α then binds and coactivates GR as well as HNF-4 and FOXO1 to induce a coordinated gluconeogenic response on both *Pepck* and *G6pc* promoters (Puigserver et al. 2003; Rhee et al. 2003).

GCs also recruit chromatin-modifying enzymes, p300 and CBP, to the *Pepck* promoter in order to maintain the surrounding chromatin in an open conformation, whereas insulin opposes these actions partly by displacing p300/CBP, leading to chromatin condensation (Hall et al. 2007; Wang et al. 2004). In addition, AMPK, which acts as a "low-energy sensor" within the cells, also counteracts GC-induced expression of *Pepck* by phosphorylating GR at serine 211 leading to the release of p300 and the SWF/SNF chromatin remodeling complex from the promoters of *Pepck* and *G6pc* (Nader et al. 2010). In fact, rats treated with the AMPK activator, AICAR, were refractory to Dex-induced hepatic gluconeogenesis. Moreover, SMAD6, a member of the transforming growth factor β family, was identified as a GR corepressor protein, which recruits histone deacetylase 3 (HDAC3) and opposes histone H3 and H4 acetylation mediated by the coactivator SRC1 (Ichijo et al. 2005). Finally, HDAC6 was found to affect GC signaling by deacetylating the heat shock protein 90 (HSP90) (Kovacs et al. 2005). Inhibition of HDAC6 activity results in hyper-acetylation of HSP90 leading to an impaired GR nuclear translocation and activation (Kovacs et al. 2005). In agreement, HDAC6 knockout animals were protected from GC-induced hyperglycemia and insulin intolerance (Rhee et al. 2003).

Another mechanism by which GCs can affect liver glucose homeostasis is by directly antagonizing the actions of insulin. For example, the expression of a pseudo kinase, *Trb3*, is increased by GC treatment leading to the inhibition of AKT phosphorylation and development of hyperglycemia and insulin resistance (Du et al. 2003). Similarly, ceramides, which are lipid-derived signaling molecules, can also mediate GC-induced hepatic insulin resistance by blocking AKT activation (Holland et al. 2007). This mechanism will be discussed in further detail below (see: lipid metabolism/liver).

Paradoxically, GC-treatment results in an increase in glycogen synthesis. This represents one of the few anabolic actions of this otherwise catabolic hormone. Our understanding of the mechanism by which GCs increase glycogen synthesis is derived largely from long-standing biochemical studies. Regulation of glycogen synthesis requires the reciprocal action of two key enzymes: glycogen synthase and glycogen phosphorylase. Both enzymes exist in active and inactive states regulated by phosphorylation and dephosphorylation events. Interestingly, studies found that GCs lead to inactivation of glycogen phosphorylase (glycogen-mobilizing enzyme) and a concomitant activation of glycogen synthase, resulting in an overall increase in hepatic glycogen content (de Wulf and Hers 1968; Laloux et al. 1983).

2.2 Muscle and Adipose Tissue

Muscle is the organ that makes the largest contribution to glucose utilization in the body, with more than 80% of circulating glucose being taken up by muscle in an insulin-dependent fashion. Insulin is an anabolic hormone, whose actions in the muscle are to stimulate glucose uptake, utilization, and storage. Most of the catabolic actions of GCs in muscle arise through antagonizing the actions of insulin. The main mechanism by which GCs decrease muscle glucose uptake is by inhibiting the translocation of the glucose transporter, GLUT4, to the plasma membrane (Haber and Weinstein 1992; Weinstein et al. 1995, 1998). Suppression of insulin-stimulated glycogen synthesis by GCs is mediated by decreasing the phosphorylation of GSK3, leading to the repression of glycogen synthase (Ruzzin et al. 2005). Both GLUT4 and GSK3 are downstream targets of AKT in the insulin-signaling cascade, highlighting the antagonistic interaction between insulin and GCs. The mechanism of this crosstalk between GCs and insulin has been extensively studied. The ability of GCs to inhibit AKT phosphorylation has been observed in vitro (C2C12 myotubes) and in vivo (rat skeletal muscle) (Long et al. 2003; Sandri et al. 2004). In rat skeletal muscle, GC excess decreases insulin receptor tyrosine phosphorylation (Giorgino et al. 1993). Dex treatment in rats has also been shown to reduce muscle PI3 kinase activity (Saad et al. 1993).

Inhibition of glucose oxidation is another mechanism by which GCs decrease glucose utilization in the muscle. GCs strongly upregulate the expression of the pyruvate dehydrogenase kinase 4 (*Pdk4*) (Sugden and Holness 2003). PDK4 inhibits the activity of the pyruvate dehydrogenase complex, thus inhibiting glucose oxidation to acetyl-CoA, resulting in decreased glucose utilization. *Pdk4* is a direct target gene of GR. Interestingly, the *Pdk4* GRE overlaps with the FOXO binding site, which is in turn required for insulin-mediated suppression of *Pdk4* expression (Connaughton et al. 2010; Kwon et al. 2004).

Similar to their effects in muscle, GCs also antagonize insulin signaling in adipose tissue, leading to decreased localization of GLUT4 transporters to the plasma membrane (Sakoda et al. 2000). Moreover, Dex treatment in rats was shown to decrease insulin-induced IRS-1 and IRS-2 phosphorylation with a concomitant decrease in AKT phosphorylation (Caperuto et al. 2006).

3 Lipid Metabolism

GCs are important regulators of whole-body lipid homeostasis. When fasting, or under starvation conditions, elevated systemic GC levels stimulate adipose tissue lipolysis, resulting in the generation of free fatty acids and glycerol. Muscle and liver both utilize the energy (ATP) derived from the oxidation of FFAs, whereas glycerol is used primarily by the liver as a precursor for gluconeogenesis. Given these effects, it is not surprising that elevated GC levels can lead to central obesity, dyslipidemia, and fatty liver. Summarized below are some of the complex effects of GCs on adipose tissue and liver lipid metabolism (Fig. 2).

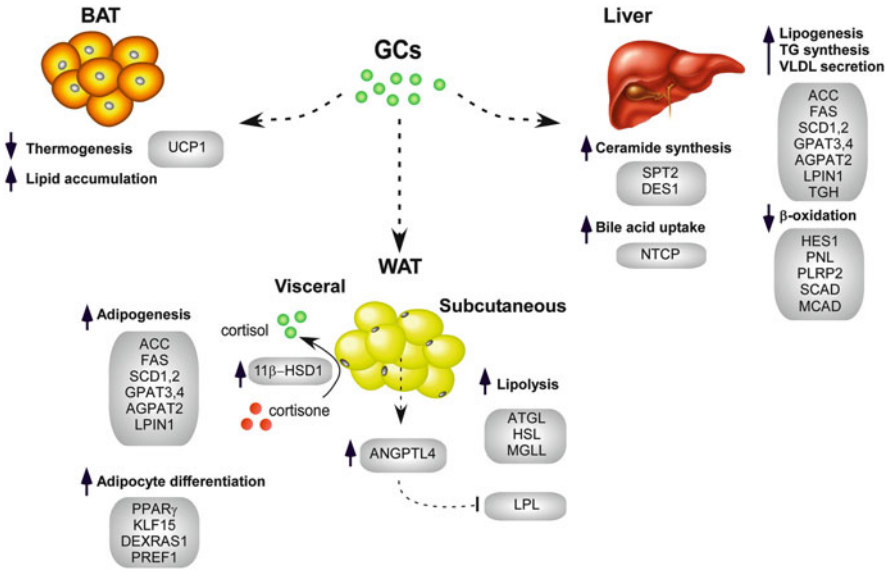

Fig. 2 Schematic view of the role of GCs in lipid metabolism in the liver, white adipose tissue (WAT) and brown adipose tissue (BAT). Proteins that are involved (either directly or indirectly) in the depicted metabolic processes are in *shaded boxes*. *11β-HSD* 11β-hydroxysteroid dehydrogenase, *ACC* acyl-CoA carboxylase, *AGPAT2* acylglycerolphosphate acyltransferase 2, *ANGPTL4* angiopoietin-like 4, *ATGL* adipose triglyceride lipase, *DES1* dihydroceramide synthase, *DEXRAS1* dexamethasone-induced Ras 1, *FAS* fatty acid synthase, *GPAT* glycerophosphate acyltransferase, *HES1* hairy and enhancer of split-1, *HSL* hormone-sensitive lipase, *KLF15* Kruppel-like factor 15, *LPIN1* lipin 1, *LPL* lipoprotein lipase, *MCAD* medium-chain acyl-CoA dehydrogenase, *MGLL* monoacyl glycerol lipase (MGLL), *NTCP* Na^+-taurocholate cotransporting polypeptide, *PPARγ* peroxisome proliferator-activated receptor γ, *PREF1* pre-adipogenic factor 1, *PNL* pancreatic lipase, *PLRP2* pancreatic lipase-related protein-2, *SCAD* short-chain acyl-CoA dehydrogenase, *SCD1* stearoyl-CoA desaturase, *SPT2* serine palmitoyltransferase 2, *TGH* triacylglycerol hydrolase, *UCP1* uncoupling protein 1

3.1 Adipose Tissue

GCs exhibit pleiotropic effects on lipid metabolism by causing both increased lipolysis and increased adipogenesis [reviewed recently by Peckett et al. (2011)]. Under fasting conditions, when GC levels are elevated, increased adipose tissue lipolysis occurs due to increased expression of adipose triglyceride lipase (*Atgl*) and hormone-sensitive lipase (*Hsl* or *Lipe*) (Slavin et al. 1994; Villena et al. 2004; Xu et al. 2009). Monoacyl glycerol lipase (MGLL), which converts monoacyl glycerol to glycerol, is also known to be induced by GCs (Yu et al. 2010). GC regulation of *Hsl* and *Mgll* appears to be direct through a functional GR binding site, whereas no GRE has been identified to date in *Atgl* (Yu et al. 2010).

Recently, GCs were found to directly upregulate the expression of angiopoietin-like 4 (*Angptl4*), a secreted protein synthesized in WAT and liver in response to

fasting. ANGPTL4 inhibits the activity of extracellular lipoprotein lipase (LPL) (Shan et al. 2009), important for FFA uptake, and, at the same time, induces intracellular adipocyte lipolysis (Gray et al. 2012), resulting in an overall increase in plasma triglyceride (TG) levels. In vitro and in vivo studies have shown that GCs regulate *Angptl4* expression though a GRE located in the 3′ untranslated region of the gene (Koliwad et al. 2009). *Angptl4*-/- mice were protected from Dex-induced hypertriglyceridemia and hepatosteatosis (Koliwad et al. 2009). In agreement, treatment of mice with a synthetic GC antagonist, RU486, also attenuated fasting-induced expression of *Angptl4* (Gray et al. 2012). It should be noted that although GCs are believed to be in general "lipolytic," there is mounting evidence suggesting that they also have anti-lipolytic actions (Peckett et al. 2011). In fact, studies in 3T3-L1 adipocytes showed that both dose and duration of GC stimulation dictate the net outcome of increased or decreased lipolysis (Campbell et al. 2011).

In the fed state, when insulin levels are elevated, GCs may act synergistically with insulin to promote de novo lipogenesis by directly upregulating (via a functional GRE) the expression of two key enzymes involved in fatty acid synthesis: acyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Diamant and Shafrir 1975; Volpe and Marasa 1975). Studies in cultured adipocytes showed that corticosterone in combination with insulin was able to increase lipogenesis by 66% when compared to insulin alone (Minshull and Strong 1985). Involvement of GCs in TG synthesis was also demonstrated in a genome-wide analysis of 3T3-L1 adipocytes and in vivo studies of mice treated with Dex, where a large number of GR target genes were identified in the TG synthetic pathway: *Scd1*, *Scd2*, *Gpat3*, *Gpat4*, *Agpat2*, and *Lpin1* (Yu et al. 2010). Most of these genes, with the exception of *Agpat2*, have at least one functional GR binding site (Yu et al. 2010).

Excessive GC stimulation has been shown to be instrumental for the development of central obesity and its associated metabolic disorders. Although there is some controversy surrounding the correlation of plasma GCs with obesity (Abraham et al. 2013; Hautanen et al. 1997; Kjolhede et al. 2014; Praveen et al. 2011), positive correlations between elevated GC activity and the development of metabolic syndrome have been observed in humans (Phillips et al. 1998; Reynolds et al. 2001; Stolk et al. 1996; Walker et al. 1998). HPA axis hyperactivity has similarly been linked to the development of insulin resistance and hypertension. Studies in Zucker rats showed that both adrenalectomy and GR antagonist treatment were able to improve the metabolic phenotype in these animals, directly implicating GCs in the development of obesity (Langley and York 1990; Yukimura et al. 1978). In fact, patients with Cushing's syndrome exhibit a characteristic redistribution of adipose tissue from the periphery to the abdominal depots. This fat-mass redistribution is believed to arise from the differential activity of GCs in various fat depots. In the periphery, GCs induce the activity of HSL and ATGL leading to increased lipolysis (Slavin et al. 1994), whereas, in central fat depots, GCs promote lipogenesis (Chimin et al. 2014; Rebuffe-Scrive et al. 1988; Seckl et al. 2004).

Pre-receptor metabolism has also been implicated in the depot-specific actions of GCs. 11β-Hydroxysteroid dehydrogenase (11β-HSD1) is an enzyme that

catalyzes the conversion of inactive cortisone to cortisol (in humans), thus increasing the intra-tissue levels of active GCs (Seckl and Walker 2001). Interestingly, the activity of 11β-HSD1 in omental adipocytes was found to be higher than that in subcutaneous depots, suggesting that GCs might have a greater impact in the abdominal depots (Bujalska et al. 1997). Indeed, mice overexpressing 11β-HSD1 have higher intra-abdominal GC levels and exhibit central adipocyte hypertrophy (Masuzaki et al. 2001).

Adding more complexity to our understanding of GC-regulated lipid metabolism is a recent study using a stable isotope (heavy water) labeling technique which showed that GCs can, in fact, simultaneously increase TG synthesis and lipolysis in inguinal fat pads of wild-type mice treated with Dex and in subcutaneous and visceral depots of CRH-Tg mice (Yu et al. 2010). It was found that 4-day Dex treatment of wild-type mice was able to induce the expression of genes involved in TG synthesis (*Scd2*, *Gpat3*, *Gpat4*, *Agpat2*, and *Lpin1*), lipolysis (*Lipe* and *Mgll*), lipid storage (*S3-12*), and lipid transport (*Cd36*, *Lrp1*, *Slc27a2*, *Vldlr*) (Yu et al. 2010). Most of these genes had at least one functional GR binding site, hinting at the direct regulation by GCs. Several unanswered questions remain: (1) why do GCs stimulate lipolysis and lipogenesis simultaneously resulting in futile cycling, and (2) what dictates the fat redistribution in Cushing's patients or in patients following chronic GC treatment? One possibility is that other hormones participate in the regulation of lipid metabolism by tipping the scale from TG synthesis to lipolysis or vice versa leading to a depot-specific adiposity.

Another mechanism by which GCs can increase adipose tissue mass is by stimulating pre-adipocyte differentiation. In vitro, GCs are required to fully induce adipocyte differentiation and as such they represent a key component of the adipogenic differentiation cocktail (Steger et al. 2010). In 3T3-L1 cells, activated GR transiently induces the expression of a key adipogenic transcription factor *Pparγ* (a master regulator of adipogenesis) and suppresses the expression of pre-adipogenic factor 1 (*Pref1*) (Steger et al. 2010). Interestingly, two direct target genes of GR, *Klf15* and *Dexras1*, have been recently implicated in GC-induced adipogenesis. MEFs and 3T3-L1 cells lacking KLF15 or DEXRAS1, respectively, were unable to stimulate adipocyte differentiation in vitro and animals lacking DEXRAS1 were protected against Dex-induced obesity. In vivo, depot-specific actions of GCs on adipocyte differentiation have also been observed, where treatment of rats for 10 days with corticosterone was able to increase adipocyte differentiation in visceral adipose tissue but not in subcutaneous depots (Campbell et al. 2011). However, the relative contribution of adipocyte hypertrophy vs. hyperplasia in the development of central obesity still needs to be examined.

Interestingly, GCs are also reported to induce the differentiation of brown preadipocytes (Shima et al. 1994) while inhibiting uncoupling protein 1 (*Ucp1*) expression and activity (Soumano et al. 2000). In fact, GC treatment in rats resulted in decreased thermogenesis and increased lipid accumulation in both BAT and WAT (Strack et al. 1995). In rodents, BAT plays an important role in regulating insulin sensitivity and glucose homeostasis by regulating thermogenesis (Stanford et al. 2013). With the recent discovery of metabolically active BAT in adult humans, it will be

exciting to investigate the role of GCs at this site to determine the relative contribution of BAT to GC-mediated glucose and lipid dysregulation (Cypess et al. 2014).

3.2 Liver

GC excess can lead to the ectopic accumulation of fat in the liver, causing the formation of "fatty liver" also known as hepatic steatosis, which is implicated in the development of insulin resistance and metabolic syndrome. Indeed, increased liver fat content has been observed in patients with Cushing's syndrome (Shibli-Rahhal et al. 2006) and in patients undergoing chronic GC treatment (Schacke et al. 2002). Unlike the extensive literature describing the role of GCs in adipose tissue lipid metabolism, the role of GR signaling in hepatic lipid metabolism is not well defined. A number of in vitro and in vivo studies have shown that GCs act in the liver to increase fatty acid synthesis (Diamant and Shafrir 1975; Altman et al. 1951), decrease fatty acid oxidation (Letteron et al. 1997), and increase VLDL secretion (Cole et al. 1982), although the latter is controversial (Dolinsky et al. 2004). Similar to adipose tissue, GCs in the liver can regulate de novo lipogenesis by directly upregulating the expression of *Fas* and *Acc*, and these effects are synergistic with insulin (Diamant and Shafrir 1975; Altman et al. 1951). In addition, acyl-CoA dehydrogenase enzymes involved in fatty acid β-oxidation are decreased by GC treatment in mice (Letteron et al. 1997). Similar observations have been made in primary hepatocytes suggesting that these effects are at least partially cell autonomous (Amatruda et al. 1983; Mangiapane and Brindley 1986). Moreover, downstream genes encoding enzymes in TG synthetic pathways, such as DGAT1 and DGAT2, were found to be upregulated by GCs, but whether this regulation is direct requires further investigation (Dolinsky et al. 2004). The combined effect of increasing lipogenesis and decreasing β-oxidation is thought to contribute to the observed hepatic steatosis. The effects of GCs on VLDL secretion are not well defined. Studies looking at patients with Cushing's syndrome are inconclusive, showing either elevated (Taskinen et al. 1983) or normal (Tiryakioglu et al. 2010) plasma VLDL levels. Numerous in vitro studies in both mouse and rat primary hepatocytes and isolated livers found an increase in VLDL secretion following Dex treatment; however, Dolinsky et al. found that VLDL secretion rates were not affected in vivo or in primary hepatocytes (Dolinsky et al. 2004). Interestingly, the stability of triacylglycerol hydrolase (TGH/Ces3), a lipase involved in intracellular TG hydrolyses prior to incorporation into VLDL, was found to be decreased by Dex treatment (Dolinsky et al. 2004).

A recent study performed by de Guia et al. 2015 has implicated microRNAs in the regulation of hepatic triglyceride metabolism by GCs (de Guia et al. 2015). miR-379/410 cluster was found to be a direct target of GR in the liver, and miR-379 levels were shown to be positively correlated with serum GCs and triglyceride levels in humans (de Guia et al. 2015). Moreover, knockdown of miR-379 in wild-type mice as well as obese animals decreased plasma TG and VLDL levels (de Guia et al. 2015). It was discovered that miR-379 acts by decreasing the levels of LDLR

and the lipolysis stimulated lipoprotein receptor (LSR), leading to decreased hepatic TG uptake and increased circulating lipids (de Guia et al. 2015).

The ability of GR to orchestrate these complex events relies on its interaction with a number of accessory proteins. For example, LXRβ was recently identified as a critical player in GC-induced hepatosteatosis (Patel et al. 2011). Mice lacking LXRβ were refractory to developing fatty liver following Dex treatment, although the exact molecular mechanism of GR-LXRβ interaction is not known. Furthermore, liver-specific knockouts of MED1, a GR coactivator, are protected from Dex-induced TG accumulation (Jia et al. 2009). In MED1-null livers, Dex fails to inhibit fatty acid β-oxidation leading to reduced TG accumulation.

GR can also elicit its control over hepatic dyslipidemia via the repression of *Hes1* gene expression (Lemke et al. 2008). GCs were found to reduce *Hes1* mRNA and protein levels in vitro (U2OS-GR cells and rat primary hepatocytes) and in livers of adrenalectomized mice (Revollo et al. 2013). In accordance, shRNA-mediated knockdown of GR in the liver of *db*/*db* mice was found to induce the expression of *Hes1* with a concomitant reduction in hepatosteatosis, suggesting a direct role of GR in the regulation of *Hes1* expression. Overexpression of HES1 in the liver of *db*/*db* mice was shown to be protective against GC-induced hepatosteatosis. Beneficial effects of HES1 overexpression are believed to be due to its ability to upregulate the expression of pancreatic lipases, *Pnl* and *Pnlrp2*, both of which contribute to TG hydrolysis. Chromatin immunoprecipitation analyses and luciferase-reporter assays revealed that *Hes1* is a direct target gene of GR in vivo (Lemke et al. 2008; Revollo et al. 2013). However, the exact molecular mechanism of*Hes1* regulation by GR is controversial, with studies hinting at the involvement of HDAC and NFκB proteins (Lemke et al. 2008; Revollo et al. 2013). In conclusion, GCs were found to stimulate hepatic TG accumulation via the repression of *Hes1*, thus blocking the induction of pancreatic lipase gene expression.

GCs can also regulate the production and accumulation of ceramides in the liver by stimulating the expression of genes involved in ceramide synthesis (serine palmitoyltransferase 2, SPT2, and dihydroceramide synthase, DES1) (Holland et al. 2007). Ceramides are sphingolipids composed of a fatty acid and sphingosine moiety (Hannun 1994), which act as important signaling molecules that generally promote catabolic processes. Ceramide levels are markedly elevated in rodent models of insulin resistance induced by GC excess, whereas mice heterozygous for *Des1* are protected from Dex-induced insulin resistance (Holland et al. 2007). This represents a mechanism by which GCs can indirectly antagonize insulin signaling.

With respect to regulation of cholesterol metabolism, studies have revealed that liver-specific GR deficiency results in dysregulation of cholesterol and bile acid homeostasis (Lemke et al. 2008; Rose et al. 2011). Hepatocyte-specific GR knockout mice exhibit reduced serum cholesterol levels, increased cholesterol accumulation in the liver, and elevated fasting bile acid levels. Moreover, mice lacking liver-specific GR had lower gallbladder bile acid concentrations and were more prone to developing cholesterol gallstones when placed on a cholesterol-rich diet (Rose et al. 2011). It was then found that liver GR deficiency impaired hepatic bile acid

uptake due to decreased expression of the basolateral bile acid transporter, *Ntcp* (*Slc10a1*) (Rose et al. 2011).

4 Protein Metabolism

4.1 Muscle

It is known that GCs both increase skeletal muscle catabolism and decrease muscle synthesis. The result of these combined processes is an increased rate of muscle breakdown, which is observed in patients with Cushing's disease. In vitro studies have shown that GCs can elicit their catabolic actions in a cell autonomous manner. For example, Dex treatment resulted in decreased cell diameters in C2C12 and L6 myotubes compared to vehicle treatment (Menconi et al. 2008). In vivo, animals treated with GCs exhibit a decrease in skeletal muscle size (Baehr et al. 2011), whereas muscle-specific GR knockout animals are resistant to Dex-induced muscle atrophy (Watson et al. 2012). GC control of muscle breakdown comes from its ability to upregulate two muscle-specific E3 ubiquitin ligases: muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) (Bodine et al. 2001). MuRF1 and MAFbx are induced in many catabolic states including starvation, diabetes, and GC treatment. Through ubiquitination, MAFbx and MuRF1 mark distinct protein targets for proteosomal degradation. MuRF1 has been shown to target primarily myofibrillar proteins such as myosin heavy chain (MYHC), whereas MAFbx was found to interact with regulatory proteins including MyoD and eIF3-f (Clarke et al. 2007; Csibi et al. 2009; Lagirand-Cantaloube et al. 2009). Interestingly, mice lacking MuRF1 were spared from Dex-induced muscle wasting, while *Mafbx-/-* animals were not (Baehr et al. 2011). Even more surprising is the fact that sparing of the *Murf1-/-* muscle mass was found to be primarily due to maintenance of protein synthesis rather than changes in proteolytic pathways (Baehr et al. 2011). These findings suggest that MuRF1 can regulate muscle atrophy through yet unknown non-proteolytic pathways, and this regulation is distinct from that of MAFbx. It should be noted than unlike skeletal muscle, cardiac muscle responds to GCs by cardiomyocyte hypertrophy suggesting that the catabolic actions of GCs on protein turnover are also tissue specific (Ren et al. 2012).

GCs can also directly increase the expression of myostatin, which in turn negatively regulates muscle growth (Ma et al. 2003). Mice lacking myostatin are resistant to developing Dex-induced muscle atrophy (Gilson et al. 2007). The expression levels of *Murf1* and *Mafbx* are also decreased in myostatin-null mice, implicating myostatin as an important mediator of GC-induced muscle atrophy (Ma et al. 2003). More recently, it was found that Dex was able to suppress muscle satellite cell function through the upregulation of myostatin and a resultant suppression of *Akirin1* (promyogenic gene) (Dong et al. 2013).

In addition to increased proteolysis, GCs can induce muscle atrophy by decreasing protein synthesis. GCs achieve this via the inhibition of mTOR, a kinase that phosphorylates S6K1 and 4E-BP1, two proteins involved in mRNA translation

initiation (Schakman et al. 2008). Recent studies identified *Klf15* and *Ddit4* (*Redd1*) as two direct target genes of GCs involved in mTOR inhibition (Shimizu et al. 2011). KLF15 has been shown to induce the expression of *Bcat2*, a mitochondrial enzyme that decreases mTOR activity (Shimizu et al. 2011). DDIT4, on the other hand, was found to increase the activity of the regulatory TSC1/TSC2 protein complex leading to mTOR inhibition (Shimizu et al. 2011; Wang et al. 2006). Interestingly, KLF15 was also found to regulate the atrophy genes, *Murf1* and *Mafbx*, and is also regulated by GCs in adipose tissue to promote adipocyte differentiation. Several other GR target genes, *Sesn1*, *Depdc6*, and *Mknk2*, have also been shown to interact and inhibit mTOR activity or signaling (Kuo et al. 2012, 2013). Finally, GR was found to upregulate the expression of *p85*α through a GRE (Kuo et al. 2012). Studies utilizing shRNA to knockdown p85α in C2C12 myotubes found that Dex failed to inhibit AKT activity and atrophy gene expression. Interestingly, studies by Hu et al. found that activated GR is able to directly bind p85α (regulatory subunit of PI3 kinase) and prevent its association with IRS-1, thus inhibiting insulin signaling (Hu et al. 2009). Overall, these data suggest that GCs may suppress insulin signaling via p85α through genomic (direct DNA binding) and non-genomic mechanisms.

5 Glucocorticoids and Other Target Organs

5.1 Pancreas

The endocrine pancreas is a major sensor of circulating glucose levels. Pancreatic β-cells respond to elevated blood glucose by secreting insulin to promote glucose uptake and utilization in peripheral tissues. The role of GCs on insulin secretion is complex and a detailed review was published recently (Rafacho et al. 2012). GCs impact pancreatic β-cell function early during embryonic development. Studies in $Gr^{lox/lox}$ and Gr^{PdxCre} mice have shown that maternal food restriction during late pregnancy (which causes elevated fetal corticosterone levels) irreversibly decreases the β-cell mass of newborn mice (Valtat et al. 2011). Moreover, there is evidence suggesting that GCs may shift the fate of pancreatic progenitor cells from an endocrine to an exocrine lineage, thus compromising β-cell expansion later in life (Valtat et al. 2011). Interestingly, excessive GC signaling in mature β-cells does not affect cell numbers but instead leads to impaired insulin secretion (Blondeau et al. 2012). Studies performed in vitro on isolated islets and cultured β-cells also showed that GCs inhibit insulin secretion and promote apoptosis (Lambillotte et al. 1997; Ranta et al. 2006; Reich et al. 2012). Mechanistically, GCs impair pancreatic cell function via several distinct mechanisms. Dex treatment of isolated pancreatic β-cells decreases the stability and protein levels of the GLUT2 glucose transporter leading to impaired insulin secretion (Gremlich et al. 1997). Moreover, GC-mediated induction of serum-/glucocorticoid-regulated kinase 1 (*Sgk-1*) in INS-1 cells led to increased activity of voltage-gated K^+ channels, leading to reduced insulin release (Ullrich et al. 2005). Furthermore, recent studies found

that Dex can induce the expression of *Txnip*, a negative regulator of the antioxidant thioredoxin in β-cells of mice and human islets, resulting in apoptosis (Reich et al. 2012). Lastly, the unfolded protein response was also recently implicated in β-cell dysfunction, where prednisolone administration to β-cells resulted in the activation of ATF6 and IRE1/XBP1 pathways and increased caspase-3 activity leading to apoptosis (Linssen et al. 2011).

Intriguingly, oral glucose tolerance tests performed in normal subjects immediately after receiving a single i.v. bolus of hydrocortisone showed an increase in insulin secretion compared to vehicle treatment (Vila et al. 2010). Similarly, Dex administration in healthy individuals was shown to cause hyperinsulinemia (Nicod et al. 2003). Higher insulin levels were able to compensate for Dex-mediated insulin resistance in skeletal muscle and adipose but not in the liver since hepatic glucose production remained elevated during the clamp (Nicod et al. 2003). It is believed that hyperinsulinemia, which arises following acute GC treatment, is a result of compensatory actions by pancreatic β-cells to respond to hyperglycemia. Chronic GC stimulation, on the other hand, leads to a decrease in insulin signaling due to β-cell dysfunction and apoptosis.

5.2 CNS

A well-known role of GCs in the brain is the classical negative feedback of the HPA axis, where circulating GCs inhibit the expression of the hormones CRH (hypothalamus) and ACTH (pituitary gland) leading to inhibition of GC synthesis from the adrenal cortex. A number of recent studies have shown that GC signaling in the brain can also regulate peripheral metabolic responses. GR is highly expressed in the paraventricular (PVN) and arcuate (ARC) nuclei in the brain where it was discovered to regulate feeding behavior and glucose homeostasis by regulating the expression of the orexigenic peptide neuropeptide Y (NPY). Local administration of Dex (via retrodialysis) into the ARC, but not the PVN, was able to induce hepatic insulin resistance during a hyperinsulinemic-euglycemic clamp (Yi et al. 2012). In agreement, intracerebroventricular coadministration of the NPY1 receptor antagonist BIBP3226, or hepatic sympathetic denervation, was able to block this effect (Yi et al. 2012). In summary, GCs seem to be able to regulate peripheral insulin responsiveness via hypothalamic signaling and the sympathetic nervous system.

Interestingly, hepatic vagal innervation is also required for GC-induced insulin resistance, hyperglycemia, and hypertension. Studies by Bernal-Mizrachi et al. revealed that selective hepatic afferent vagotomy, as well as central afferent vagal nerve sectioning, decrease Dex-induced *Ppar*α and *Pepck* expression and reverse insulin resistance in wild-type mice (Bernal-Mizrachi et al. 2007). PPARα's involvement in GC-induced insulin resistance and hyperglycemia has been previously established, and animals lacking hepatic PPARα are protected against Dex-mediated effects (Bernal-Mizrachi et al. 2003; Lemberger et al. 1994). Intriguingly, adenoviral reconstitution of hepatic PPARα in normoglycemic Dex-treated *Pparα*-/- animals increased PEPCK activity, blood glucose, and blood pressure in

sham-operated mice but not after vagotomy, suggesting that both hepatic vagal innervation and intact PPARα signaling are necessary for GC-induced metabolic effects (Bernal-Mizrachi et al. 2007).

6 Concluding Remarks

The metabolic actions of glucocorticoids are highly coordinated between multiple tissues, facilitating the rapid catabolic actions of GCs that have the overall effect of increasing circulating glucose levels. While many of the biochemical processes mediating these effects are now understood, the individual genes responsible for these effects and the molecular mechanisms regulating their expression are still being elucidated. Further understanding the complex feedback responses mediated by hormones and the sympathetic nervous system will provide new insight into possible mechanisms of inhibiting the detrimental metabolic consequences of chronic GC exposure.

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