# Chapter 5
# Regulation of Glucose Homeostasis by Glucocorticoids

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**Abstract** Glucocorticoids are steroid hormones that regulate multiple aspects of glucose homeostasis. Glucocorticoids promote gluconeogenesis in liver, whereas in skeletal muscle and white adipose tissue they decrease glucose uptake and utilization by antagonizing insulin response. Therefore, excess glucocorticoid exposure causes hyperglycemia and insulin resistance. Glucocorticoids also regulate glycogen metabolism. In liver, glucocorticoids increase glycogen storage, whereas in skeletal muscle they play a permissive role for catecholamine-induced glycogenolysis and/or inhibit insulin-stimulated glycogen synthesis. Moreover, glucocorticoids modulate the function of pancreatic α and β cells to regulate the secretion of glucagon and insulin, two hormones that play a pivotal role in the regulation of blood glucose levels. Overall, the major glucocorticoid effect on glucose homeostasis is to preserve plasma glucose for brain during stress, as transiently raising blood glucose is important to promote maximal brain function. In this chapter we will discuss the current understanding of the mechanisms underlying different aspects of glucocorticoid-regulated mammalian glucose homeostasis.

**Keywords** Glucocorticoids • Glucocorticoid receptor • Gluconeogenesis • Insulin • Glucose utilization • Glycogen • Pancreas • Glucose metabolism

## Introduction

Glucocorticoids (GC) are stress hormones that play a key role in the regulation of mammalian glucose homeostasis. The name "glucocorticoids" originates from their profound effects on plasma glucose levels. GC regulate multiple aspects of glucose

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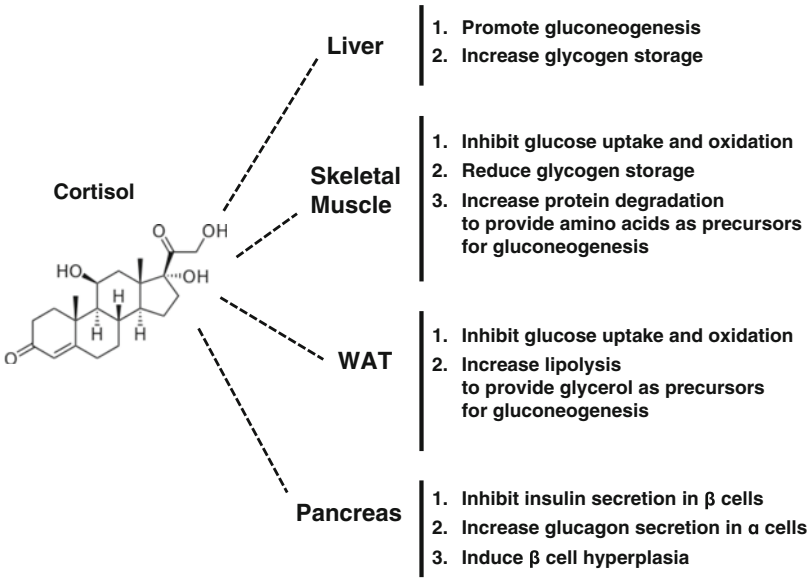


**Fig. 5.1** Glucocorticoid effects on glucose homeostasis. The effects of cortisol on glucose homeostasis in peripheral tissues

homeostasis (Fig. 5.1). First, GC promote hepatic gluconeogenesis [1, 2] and reduce glucose uptake and utilization in skeletal muscle and white adipose tissue (WAT) [3, 4]. These effects are critical for metabolic adaptation during stress, such as fasting/starvation, when plasma glucose needs to be preserved because it is the brains' primary energy source, and transiently raising blood glucose is important to promoting maximal brain functions [5]. Insulin, a hormone secreted from pancreatic β cells, exerts opposite effects on these physiological processes by inhibiting hepatic gluconeogenesis and promoting glucose utilization in skeletal muscle and WAT. Thus, to exert their responses, GC need to antagonize insulin actions. These effects are critical during stress, which in short term does not affect or even enhances glucose tolerance. However, chronic GC exposure results in hyperglycemia and insulin resistance [3, 4, 6]. Second, GC exert tissue-specific effects on glycogen metabolism. In liver, GC increase glycogen storage, whereas in skeletal muscle GC play a permissive role for catecholamine-induced glycogenolysis or inhibit insulin-stimulated glycogen synthesis [7–9]. Third, GC modulate insulin and glucagon secretion from pancreas. GC treatment increases plasma glucagon levels [10, 11], whereas the effects of GC on insulin secretion are complex [12–16]. GC induce pancreas islet hyperplasia *in vivo* that leads to hyperinsulinemia [12, 17–19] and have been shown to exert cytotoxic effects on β cells [20, 21]. Overall, in this chapter we will discuss the current understanding of the mechanisms underlying these distinct aspects of GC-regulated glucose homeostasis.

## Gluconeogenesis

The gluconeogenic pathway generates glucose from non-carbohydrate substrates (Fig. 5.2) [22, 23]. Gluconeogenesis mainly occurs in liver, though kidney and intestine are also contributors. The major gluconeogenic precursors are lactate, glycerol and gluconeogenic amino acids, such as alanine. Both lactate and alanine can be converted to pyruvate, which is then carboxylated to oxaloacetate (OAA) by pyruvate carboxylase (PC) [24, 25], a step that occurs in mitochondria. OAA needs to be converted to malate to be shuttled to the cytoplasm where it is converted back to OAA. Cytosolic phosphoenolpyruvate carboxykinase (PCK1) [26, 27] then catalyzes OAA to phosphoenolpyruvate (PEP), which then enters the gluconeogenic pathway. There is also a mitochondrial form of PCK1 (m-PCK1) that can directly convert OAA to PEP in mitochondria, which is then transported to cytoplasm and participates in gluconeogenesis. Recent studies suggest that m-PCK1 could also play a role in gluconeogenesis [28–31].

Conversion of PEP to fructose-1,6-phosphate (F1,6BP) requires five enzymatic steps that are essentially the reverse of glycolysis (Fig. 5.2). The "bifunctional" enzyme, phosphofructokinase 2 (PFK2)/fructose bisphosphatase 2 (FBPase2) (a.k.a. PFKFB1) [32, 33], plays a critical role in the switch between gluconeogenesis and glycolysis. PFKFB1 regulates the production of fructose 2,6 bisphosphate (F2,6BP), which is an allosteric activator of phosphofructokinase 1 (PFK1), an enzyme in the glycolytic pathway. When circulating glucose levels are low, such as during fasting and starvation, glucagon inactivates PFK2, which allows FBPase2 activity to be favored. This results in decreased production of F2,6BP, and reduced glycolysis and enhanced gluconeogenesis. Fructose 1,6-bisphosphatase (FBP1) converts F1,6BP to fructose-6-phosphate (F6P) [34], which is then converted to glucose-6-phosphate (G6P). G6P enters the endoplasmic reticulum (ER), where the enzyme glucose-6-phosphatase (G6PC) [35–37] converts G6P to glucose (Fig. 5.2). Notably, distinct gluconeogenic amino acids can be converted to specific intermediates in the tricarboxylic acid (TCA) cycle. These TCA cycle intermediates are converted to OAA to enter the gluconeogenic cycle. Glycerol enters gluconeogenesis through conversion to dihydroxyacetone phosphate (DHAP), which can then be metabolized to glycerol-3-phosphate (G3P) or directly to F1,6BP (Fig. 5.2) thus entering the gluconeogenic cycle above PCK1.

The positive effect of GC on hepatic gluconeogenesis is well established and has been under extensive study for several decades. Injecting GC into humans [38, 39] and rodents increases hepatic gluconeogenesis. In addition, GC are important for other hormones to activate gluconeogenesis. Although glucagon is regarded as a major hormone that activates gluconeogenesis during fasting, fasting-induced gluconeogenesis is reduced in adrenalectomized mice, an effect that is restored by treating mice with GC [40–42]. In fact, glucagon-, epinephrine-, or cyclic AMP (cAMP)-induced gluconeogenesis are all attenuated in adrenalectomized mice. Giving GC to adrenalectomized mice restores the ability of these hormones to induce gluconeogenesis. Thus, GC play a "permissive" role promoting the optimal ability of

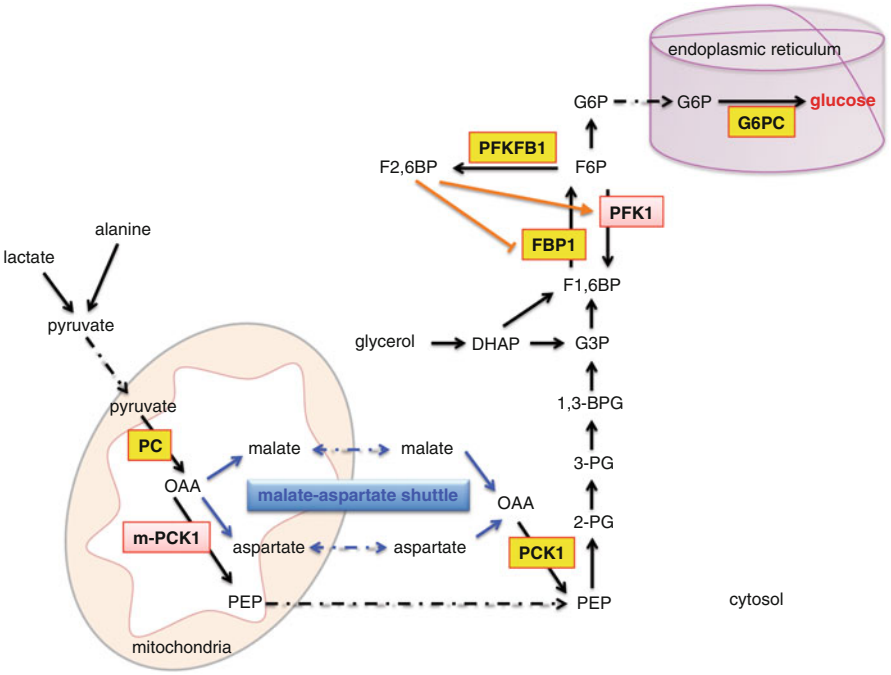


**Fig. 5.2** Gluconeogenic pathway in hepatocytes. Lactate and alanine are converted to pyruvate, which enters the mitochondria and is then converted to OAA by enzyme PC. Through malate-aspartate shuttle, OAA exits the mitochondria to form PEP. OAA can also be converted to PEP directly within the mitochondria. PEP then feeds into the gluconeogenic pathway. In addition, glycerol is metabolized to DHAP, which is then converted directly or indirectly through G3P to F1,6BP. The final product, glucose, is produced in the ER by enzyme G6PC. The key enzymes are boxed, with GR primary targets shown in *yellow*. *Abbreviation*: *OAA* oxaloacetate, *PEP* phosphoenolpyruvate, *DHAP* dihydroxyacetone phosphate, *G3P* glyceraldehyde-3-phosphate, *F1,6BP* fructose-1,6-bisphosphate, *2-PG* 2-phosphoglycerate, *3-PG* 3-phosphoglycerate, *1,3-BPG* 1,3-bisphosphoglycerate, *G3P* glyceraldehyde-3-phosphate, *F1,6BP* fructose-1,6-bisphosphate, *F2,6BP* fructose-2,6-bisphosphate, *F6P* fructose-6-phosphate, and *G6P* glucose-6-phosphate. *Enzyme abbreviation*: *PC* pyruvate carboxylase, *m-PCK1* mitochondrial phosphoenolpyruvate carboxykinase, *PCK1* cytosolic phosphoenolpyruvate carboxykinase, *FBP1* fructose-1,6-bisphosphatase 1, *PFK1* phosphofructokinase 1, *PFKFB1* phosphofructokinase 2/fructose bisphosphatase 2, *G6PC* glucose-6-phosphatase catalytic subunit

these hormones in gluconeogenesis. GC also provide gluconeogenic precursors by promoting protein degradation in skeletal muscle to generate gluconeogenic amino acids [1, 4]. They also enhance lipolysis in WAT. This releases glycerol and fatty acids. Glycerol is used as a gluconeogenic precursor, whereas fatty acids provide energy to drive the gluconeogenic pathway [8, 43, 44].

GC promote gluconeogenesis mainly through activation of the transcription of genes encoding enzymes in the gluconeogenic pathway. The transcription of *PC, PCK1, FBP1, PFKFB1, G6PC* and *G6P transporter* (*SLC37A4*) are all stimulated by GC (Fig. 5.2). GC convey their signals mainly through an intracellular receptor, the glucocorticoid receptor (GR). Before binding to GC, GR resides in cytoplasm and is associated with Hsp90 chaperone complex. Upon binding to GC, GR dissociates from Hsp90 chaperone complex and enters the nucleus, where the GR is recruited to specific genomic sequences, called glucocorticoid response elements (GREs). The GR:GRE association could be a direct GR:DNA interaction or an indirect association through other DNA-binding transcription factors. In any case, once GR associates with a specific GRE, it recruits a number of transcriptional coregulators. While they do not bind to DNA directly, these coregulators are able to assist the GR in modulation of the transcriptional rate of nearby genes through distinct mechanisms: altering chromatin structure, inducing histone modifications; recruiting RNA polymerase II containing basal transcription machinery; and modulating transcriptional elongation. Notably, most genomic GREs are "composite" GREs (also called glucocorticoid response units, GRUs) that consist of multiple cis-acting elements, which include binding sites for GR and other transcription factors (called accessory elements and accessory factors, respectively), to mediate a complete GC response. Because different DNA binding transcription factors other than GR are involved in the regulation of distinct GR primary target genes, the multi-protein transcriptional regulatory complex assembled on each GRE is likely distinct. Composite GREs could allow GC to differentially regulate distinct target genes in different cell types depending on the presence of cell type specific accessory factors and transcriptional coregulators. Another advantage of employing composite GREs is to allow specific cross-talk between GC and other signaling pathways at the accessory elements and their respective accessory factors. Thus, cross-talk does not need to occur directly through GR.

The GREs of *Pck1, Pfkfb1*, and *G6pc* genes have been identified. In particular, the mechanism of GR-regulated *Pck1* gene transcription has been extensively studied. By contrast, the mechanisms governing GC-activated *Pc* and *Fbp1* gene are unclear. Below we will discuss the mechanisms of GR-stimulated *Pck1, Pfkfb1*, and *G6pc* gene transcription.

## *PCK1*

Rat *Pck1* gene contains two GREs, GRE1 and GRE2 (Fig. 5.3) [45]. GRE1 is located between −388 and −374 (relative to transcription start site, TSS), whereas GRE2 is located between and −367 and −354 in the *Pck1* gene promoter. When GRE1 or GRE2 is placed in front of TATA box in a synthetic reporter gene, neither mediates a GC response [46]. The combination of GRE1 and GRE2 also fails to confer GC-induced transcription [46]. In fact, both GRE1 and GRE2 bind GR very weakly *in vitro* [46]. However, in cooperation with other accessory elements on the

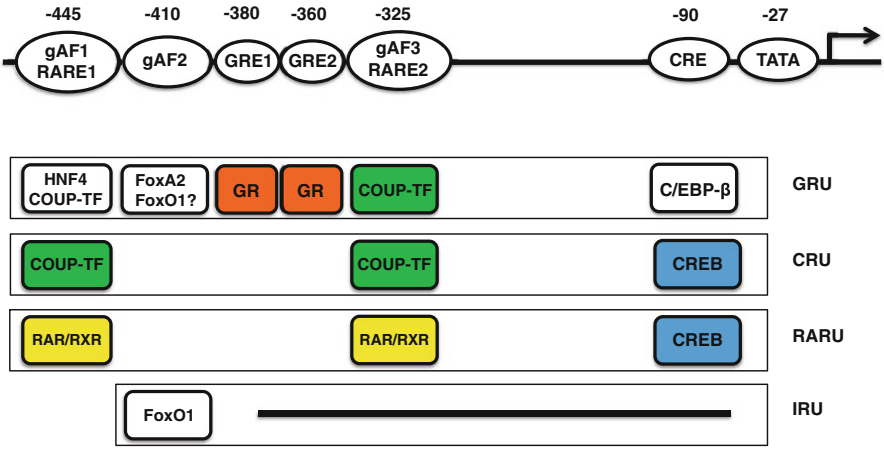


**Fig. 5.3** Hormone response units in the PEPCK gene. Binding sites for various regulatory and transcription factors are shown in the *top row*, with the number indicating the center nucleotide position of each element with respect to the transcription start site. Four hormone-specific response units are drawn: proximal glucocorticoid response unit (GRU), cyclic AMP response unit (CRU), retinoic acid response unite (RARU), and insulin response unit (IRU). In the absence of the other hormones, the components of each response unit are depicted. These units interact functionally, cooperating or competing, to comprise the PEPCK promoter. Except for gAF2, DNA elements involved in IRU are not yet identified

*Pck1* promoter, they confer a robust GC response. These accessory elements include gAF1 [45], gAF2 [45], gAF3 [47] and the cAMP response element (CRE) [48]. Both gAF1 (between −451 and −434 of rat *PCK1* promoter) and gAF2 (−416 and −407) are located 5′ from GRE1 and GRE2, whereas gAF3 and CRE are located in 3′ of these GREs (Fig. 5.3). Hepatic nuclear factor 4 (HNF4, NR2A1) and chicken ovalbumin upstream transcription factor (COUP-TF, NR2F2) bind to gAF1 and serve as accessory factors for a complete GC response [49]. The gAF1 element also serves as a retinoic acid response element (RARE). An all-trans retinoic acid receptor (RAR) and 9-cis retinoic acid receptor (RXR) heterodimer binds to gAF1 and confers retinoic acid (RA)-activated *Pck1* gene transcription [50]. RA has been shown to synergize with GC to stimulate *Pck1* gene transcription [51].

The gAF2 element binds to members of the forkhead box transcription factor family that include FoxA1 (also called hepatic nuclear factor 3 α, HNF3α), FoxA2 (hepatic nuclear factor 3 β, HNF3β), FoxO1 (FKHR) and FoxO3A (FKHRL1). FoxA2 have been shown to act as accessory factors for GR-regulated *Pck1* gene transcription *in vitro* [52]. Liver specific deletion of FoxO1 but not FoxO3A significantly reduces fasting-induced *Pck1* gene expression [53]. Because GC play an important role in fasting-induced *Pck1* gene transcription, these results suggest that FoxO1 may serve as an accessory factor for GR *in vivo*. The gAF2 element also serves as an insulin response sequence (IRS) that confers at least part of repressive effect of insulin on *Pck1* gene transcription [54, 55]. The ability of insulin to suppress *Pck1* gene expression is compromised in liver specific *FoxO1* knockout mice [53] or mice overexpressed dominant negative FoxO1 [56]. Notably, the ability of insulin to reduce *Pck1*

gene expression is not affected in *FoxO3A* deletion mice [53]. These results support the key role of FoxO1 in the regulation of *Pck1* gene expression *in vivo*. The potential role of FoxA2 inhibiting insulin effects has been reported [57], although more studies are needed to confirm the importance of FoxA2 in insulin-suppressed *Pck1* gene expression.

Streptozotocin is a relatively specific pancreas β cell cytotoxin. Treatment of mice with streptozotocin induces a state that mimics type 1 diabetes. Circulating GC levels are increased in such animals and *Pck1* gene expression is augmented. In mice bearing a reporter gene containing a construct containing −2 kb rat *Pck1* gene promoter with a mutation at gAF2, streptozotocin-induced reporter gene expression is markedly reduced [58]. These results confirm the importance of the gAF2 element in GC-activated *Pck1* gene *in vivo*.

The gAF3 element (−337 and −321) binds to COUP-TF [47] and, like gAF1, also serves as a RARE that binds to RAR/RXR heterodimer [59]. The cAMP response element (CRE, between −90 and −82) is also required for a complete GC-stimulated *Pck1* gene transcription [48]. CCAAT enhancer binding protein β (C/EBPβ) binds to the CRE to mediate the accessory activity for the GC response [60].

Chromatin immunoprecipitation (ChIP) was used to monitor the recruitment of GR and various accessory factors to their respective binding sites in rat H4IIE hepatoma cells. GC treatment increases the recruitment of GR, FoxO1, FoxO3A and RNA polymerase II (Pol II) to the *Pck1* promoter [61]. FoxA2, C/EBPβ, HNF4 and COUP-TF occupy the *Pck1* promoter before GC treatment and their occupancy is not altered after GC treatment [61]. The recruitment of transcriptional coregulators, SRC-1, p300 and CREB binding protein (CBP), to the *Pck1* GRU is markedly increased by GC treatment [61]. This suggests that GC treatment initiates the assembly of multiprotein transcriptional regulatory complex on the *Pck1* promoter. Insulin treatment for just 3 min markedly decreases the GC-induced recruitment of GR, FoxO1, FoxO3A, FoxA2, SRC-1, p300, CBP, and Pol II, and only the occupancy of C/EBPβ, HNF-4 and COUP-TF remains unchanged [61]. Thus, insulin treatment rapidly disrupts the assembly of GC-induced transcriptional complex on the *Pck1* GRU. Analyzing epigenetic marks showed that the most significant change is the methylation at histone H3 arginine residue 17, which is significantly increased upon GC treatment and is abolished by insulin [61]. CARM1/PRMT4, is the histone methyltransferase that methylates histone H3 tail arginine 17 residue (H3R17) [62, 63]. CARM1 has been shown to serve as a transcriptional coactivator for GR. However, the occupancy of CARM1 on the *Pck1* promoter is not significantly changed upon GC or insulin treatment. One way to explain these results is that CARM1 is present on the *Pck1* GRU before GC treatment and the activity of CARM1 is modulated by insulin treatment. The activity of CARM1 is regulated by post-translational modifications [64]. Alternatively, it is possible that an unknown histone methyltransferase is involved in the elevation of H3R17 methylation on the *Pck1* GRU.

An analysis of the rat *Pck1* promoter in human hepatoma HepG2 cells identified two additional accessory elements (dAF1 and dAF2) that are involved in GC-stimulated *Pck1* gene transcription. The dAF1 element is located at −993 and has sequence similarity to the gAF1 element, whereas the dAF2 element, located at −1365, resembles more proximal gAF2 [65]. HNF4 and peroxisome proliferator

activated receptor α (PPARα) and RXR heterodimer (PPARα/RXR) bind to dAF1, whereas FoxA1, FoxA2 and FoxO1 bind to dAF2 [65]. ChIP experiments showed that GC treatment increases FoxO1 and PPARα recruitment to the gAF2 and the dAF2, and HNF4 to the dAF1 in mouse liver [65]. Overexpressing PPARα or HNF4 synergizes with GR to activate a reporter gene harboring 2 kb of rat *Pck1* promoter [65]. A role for PPARα in GC-induced *Pck1* expression *in vivo* is consistent with the observation that GC-induced *Pck1* gene expression is markedly reduced in *Pparα* null mice [66]. Notably, streptozotocin-induced *Pck1* gene expression and blood glucose levels are reduced in transgenic mice that harbor a targeted ablation of the dAF1 in the *Pck1* gene promoter. These results support the importance of the dAF1 in GC-activated *Pck1* gene expression *in vivo* [65].

Overall, GC activate *Pck1* gene transcription through a complex GRU. Intriguingly, all accessory elements in the *Pck1* GRU are involved in the responses of other hormones that provide potential cross-talk between GC and other hormones, including glucagon, retinoic acid and insulin (Fig. 5.3). The complexity of GRU also allows various signaling pathways to fine tune the transcription levels of *Pck1* gene. Interestingly, GC repress the transcription of *Pck1* gene in adipocytes [67, 68] where the major metabolic role of *Pck1* is glyceroneogenesis [69, 70]. It is not entirely clear why *Pck1* GRU is not functional in adipocytes, although it is proposed that GR inhibits *Pck1* gene transcription through antagonizing C/EBP family of transcription factors in adipocytes [67, 68]. Nonetheless, the requirement of accessory factors to act with GR on "weak" GREs provides the flexibility for GC to regulate *Pck1* gene transcription in a tissue specific manner. While it is unknown how accessory factors participate in GR-regulated *Pck1* gene transcription, there are two potential mechanisms. First, accessory factors may aid in recruitment of transcriptional coregulators to the GRU to stimulate the transcription. Previous studies have shown that the transactivation domain of HNF4 and FoxA2 are required for their accessory activities [71]. When gAF1, gAF2 or gAF3 is replaced by the binding site of a yeast transcription factor Gal4, a fusion protein that consists of GAL4 DNA binding domain and a transcriptional coregulator, SRC1, is able to provide accessory activity [72]. Moreover, another transcriptional coregulator, peroxisome proliferator activated receptor γ coactivator-1α (PGC1α), has been shown to interact with HNF4 to participate in GC-activated *Pck1* gene transcription. PGC1α also interacts with and coactivates FoxO1 [73] that binds to the gAF2 element. But the role of PGC1α-FoxO1 interaction in GC-stimulated *Pck1* gene transcription has not been examined. Second, accessory factors can potentiate GR:GRE association. Using quantitative, real time equilibrium and stopped-flow fluorescence anisotropy measurements of nuclear protein-DNA interactions it was shown that GR binds to the *Pck1* GREs poorly. However, the presence of the gAF1 and the gAF2 elements markedly enhanced the association between GR and the *Pck1* GREs [74]. It is possible that the assembly of a multi-protein complex that includes GR, accessory factors and transcriptional coregulators enhances the association between GR and the two *Pck1* GREs.

## *G6PC*

*G6PC* gene transcription is induced by GC, whereas insulin suppresses both basal and GC-activated *G6PC* gene transcription. Mouse *G6pc* also contains a complex GRU in the proximal promoter region. This GRU consists of three GREs, a CRE, a HNF4 binding site, a hepatic nuclear factor 1 (HNF1) binding site, and multiple FoxO/FoxA binding sites (FREs) [75]. GC significantly activate the expression of a reporter gene that contains the *G6pc* GRU. Mutation at any of these accessory elements in the *G6pc* GRU reporter gene reduces the GC response [75]. Mutations at FREs that bind FoxO1 and FoxO3A also reduce both the inhibitory effect of insulin and basal expression of *G6pc* gene [76]. In liver specific FoxO1 deletion mice the expression of *G6pc* in 18 h fasted mice is markedly lower than that of 18 h fasted wild type mice [53]. Moreover, the ability of insulin to suppress the expression of *G6pc* is abolished in liver specific FoxO1 deletion mice [53] or mice overexpressed dominant negative FoxO1 [56]. In contrast, liver specific FoxO3A deletion does not affect basal expression of *G6pc* and the ability of insulin to inhibit *G6pc* remains intact [53]. These results are reminiscent of the regulation of *Pck1* gene and suggests that FoxO1 plays a key role in the regulation of gluconeogenic genes *in vivo*. Also, similar to the regulation of *Pck1* gene, transcription coregulator PGC1α positively regulates basal *G6pc* gene transcription and enhances GC-stimulated *G6pc* gene transcription through interaction with HNF4 [77, 78]. FoxO1 and PGC1α appear to synergistically activate *G6pc* gene transcription [73, 79].

GC also activate the transcription of the G6P transporter (*SLC37A4*) gene, which encodes a protein that is responsible for shuttling G6P from the cytoplasm to the ER lumen. The mouse *Slc37a4* gene promoter contains a GRE [80, 81] and a FoxO1 binding site is identified nearby the GRE [81]. GC increase the activity of a luciferase reporter gene under the control of the *Slc37a4* gene promoter in 293 cells, whereas the mutation at the FoxO1 binding site reduces the ability of GC to potentiate this reporter gene activity [81]. Overexpression of FoxO1 in 293 cells potentiates the ability of GC to activate the reporter gene activity [81]. These results suggest that GC stimulate the *Slc37a4* gene through a GRU that contains at least a GRE and a FoxO1 binding site.

## *PFKFB1*

Hepatic rat *Pfkfb1* gene transcription is stimulated by GC and a GRU has been identified in the intronic region of this gene. In addition to the GRE, this GRU consists of binding sites for FoxA2, hepatic nuclear factor 6 (HNF6, a.k.a. Onecut1), C/EBP and Nuclear factor 1 (NF1) [82]. Insulin antagonizes the stimulatory effect of GC on *Pfkfb1* gene [82, 83]. While insulin acts through PI3K and Akt to inhibit *Pck1* and *G6pc* gene expression [84, 85], this pathway is apparently not involved in the suppressive effect of insulin on GC-induced *Pfkfb1* gene. Instead, insulin activates the Jun N-terminal Kinase (JNK) pathway to inhibit GC-induced *Pfkfb1* gene expression [86].

### Factors Regulating GC-Stimulated Hepatic Gluconeogenesis

Many other factors regulate gluconeogenesis by modulating GC signaling. Liver X receptor β (LXRβ) is involved in GC-activated *Pck1* gene expression. In *Lxrβ* null mice (*Lxrβ*$^{-/-}$) GC-induced *Pck1* gene expression and glucose production are reduced, and GC-induced recruitment of GR to the *Pck1* GRE is impaired [87]. In contrast, in *Lxrα* null mice (*Lxra*$^{-/-}$) GC-regulated *Pck1* gene expression is not affected. Moreover, GC-stimulated expression of *tyrosine aminotransferase* (*Tat*) gene, which encodes the enzyme that converts tyrosine to 4-hydroxyphenolpyruvate, is not affected in *Lxrb*$^{-/-}$ mice. Thus, the role of LXRβ in GC action is relatively specific to the *Pck1* gene. The mechanisms governing LXRβ action in GC-activated *Pck1* gene transcription are unclear, especially in view of another study, which showed that treating hepatoma cells with LXR ligands suppresses GC-stimulated *Pck1* and *G6pc* gene expression [88]. Microarray analyses showed that LXR ligands affect only a subset of GC-regulated genes. Both gel shift and ChIP experiments suggest that an LXRα/RXRα heterodimer competes with GR for binding at rat *G6pc* GRE [88]. In agreement with its effects on GC-induced gluconeogenic gene expression is the observation that treating rats with an LXR ligand attenuates GC-augmented plasma glucose levels [88]. In summary, unliganded LXRβ is required for maximal GC-induced *Pck1* gene transcription and is necessary for GC-induced recruitment of GR to the *Pck1* GRE. By contrast, LXR ligands suppress GC-activated gluconeogenic gene transcription by inhibiting the recruitment of GR to the GREs of these genes.

The expression of transcription factor ying yang 1 (YY1) is increased upon fasting and in insulin resistant state [89]. Overexpression of YY1 in mouse liver increases gluconeogenesis [89]. In contrast, the deletion of YY1 in mouse liver results in hypoglycemia. YY1 potentiates gluconeogenesis through the increase of hepatic GR expression, which in turn augments the expression of gluconeogenic genes [89].

The expression of farnesoid x receptor (FXR, NR1H4), a bile acid receptor, is also increased during fasting [90]. *Fxr* null mice become hypoglycemic during fasting and have a reduced glucose production after a pyruvate challenge and a decreased expression of *Pck1* and *G6pc* [90]. The treatment of fasted mice with an FXR ligand, 6α-ethylchenodeoxycholic acid (6E-CDCA), increases hepatic glucose production and *Pck1* and *G6pc* gene expression. These effects are not observed in *Fxr* null mice, nor are they seen in fed mice [90]. 6E-CDCA elevates the expression of GR. By contrast, the expression of GR is decreased in *Fxr* null mice. Reducing GR expression in liver abolishes 6E-CDCA-induced glucose production and the expression of *Pck1* and *G6pc* [90]. Thus, FXR activation elevates GR expression, which in turn results in enhanced gluconeogenesis.

Ubiquitin-specific protease 2 (USP2) expression is induced by fasting by both GC and glucagon, and by PGC1α overexpression [91]. Overexpression of Usp2 in mouse liver increases glucose production and exacerbates high fat diet-induced glucose intolerance, whereas the reduction of Usp2 expression in mouse liver improves systemic glycemic control [91]. Usp2 induces the expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme that converts the inactive GC,

11-DHC (rodents) or cortisone (humans), to active corticosterone (rodents) or cortisol (humans) in liver. Thus, Usp2 increases hepatic gluconeogenesis by increasing the active GC levels in hepatocytes. Usp2 is a ubiquitin specific protease. How Usp2 increases the expression of 11β-HSD1 is unclear.

Transforming growth factor β (TGFβ) decreases hepatic gluconeogenesis. TGFβ increases the expression of SMAD6, which directly associates with the N-terminus of GR [92]. When SMAD6 associates with GRE-bound GR, it recruits histone deacetylase 3 (HDAC3) which antagonizes the acetylation of histone H3 and H4 on genomic regions near the GREs [92]. This results in an inhibition of the transactivation activity of GR. Overexpression of SMAD6 in liver reduces GC-induced *Pck1* transcription and blood glucose levels, which mimics TGFβ effects [92].

GR associates with the Hsp90-containing chaperone complex in the cytoplasm, an interaction that induces a GR conformation favorable for binding GC. The Hsp90-containing chaperone complex also plays a role in the translocation of GR into the nucleus. Acetylation of the lysine 294 residue of Hsp90 reduces its interaction with GR [93]. Therefore, keeping lysine 294 in a deacetylated state is critical for the GR-Hsp90 interaction. Histone deacetylase 6 (HDAC6) deacetylates lysine 294 of Hsp90. Ablation of *Hdac6* results in a decreased GR response due to defective GR translocation into the nucleus [93]. In *Hdac6* knockout mice, GC-induced hepatic *Pck1* and *G6pc* gene expression and glucose production are reduced [93]. GC-induced glucose production in primary hepatocytes isolated from *Hdac6* knockout mice is markedly lower than in primary hepatocytes isolated from wild type mice [93]. Notably, *Hdac6* ablation generally reduces GR signaling. Not surprisingly, other GR-regulated processes, including GC-induced insulin resistance and lipolysis in adipocytes, are also affected when *Hdac6* is deleted.

## Glucose Utilization

GC inhibit glucose utilization by reducing both glucose uptake and glucose oxidation in skeletal muscle and WAT, two major tissues involved in insulin-responsive glucose utilization [94, 95]. These GC effects counteract those of insulin, which promote glucose uptake, glycolysis and glucose oxidation. These result in a transient increase of circulating glucose, which is considered beneficial during stress [5]. In both mouse and human myotubes GC reduce insulin-stimulated glucose uptake [96–98] by attenuating insulin-induced GLUT4 translocation to the cell membrane. By contrast, reduced GC signaling improves insulin sensitivity and glucose utilization in mouse and human skeletal muscle. Circulating GC levels are increased in genetically obese *ob/ob*, *db/db* and lipotrophic *A-ZIP/F-1* [99] mice as compared to wild type. These mice are insulin resistant, but adrenalectomy improves insulin-stimulated muscle glucose disposal [100, 101]. In high fat diet-induced obese mice, adrenalectomy or treatment with the GR antagonist, RU-486, improves insulin sensitivity and increases glucose utilization in skeletal muscle. Moreover,

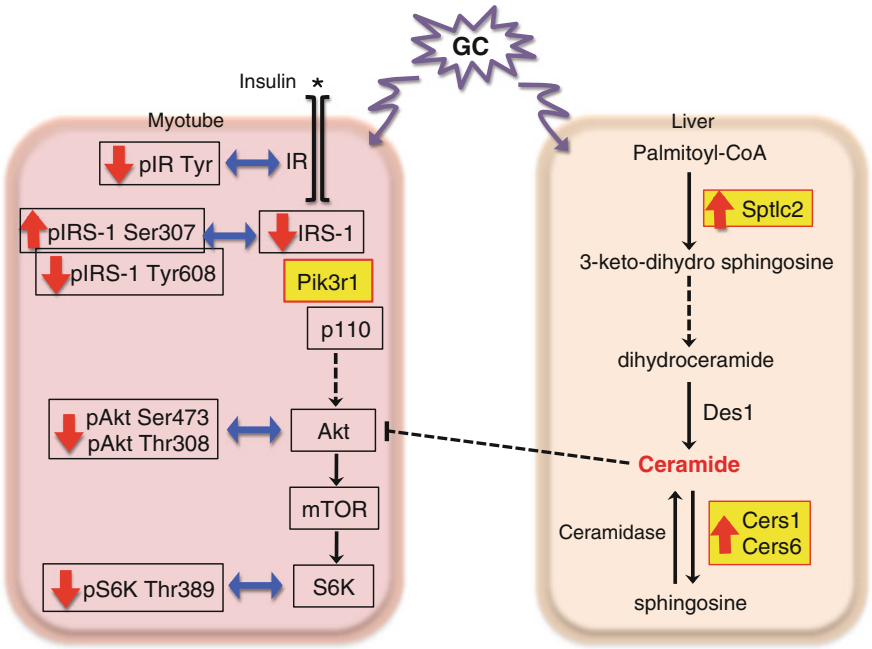


**Fig. 5.4** Models of glucocorticoid-regulated insulin action. Mechanisms of glucocorticoid-induced insulin resistance are depicted. In myotubes, glucocorticoids (GC) decrease the tyrosine phosphorylation of insulin receptor (IR) and the expression of IRS1. They increase the serine 307 phosphorylation while decrease the tyrosine 608 phosphorylation of IRS1. GC also increase the expression of Pik3r1, which results in decreased activity of Akt and p70 S6 kinase (S6K). In the liver, GC increase the gene expression of enzymes involved in ceramide synthesis, including Sptlc2, Cers1 and Cers6, which results in increased levels of ceramides. These ceramides then interfere with insulin signaling. The GC-regulated genes are shown in *yellow*

11β-HSD1-specific inhibitors, which reduce corticosterone levels in various tissues, improve insulin sensitivity and skeletal muscle glucose utilization in animal models of diabetes or insulin resistance [96].

The ability of GC to inhibit glucose uptake and glucose oxidation in skeletal muscle is due to, at least in part, a direct effect of GCs on myotubes. Treating cultured myotubes with GC inhibits insulin-stimulated glucose utilization [96, 102]. One mechanism by which GC reduce glucose utilization is the inhibition of insulin signaling [96, 103, 104]. Insulin binds to and activates the cell-surface insulin receptor (IR) tyrosine kinase, which in turn phosphorylates the members of insulin receptor substrate (IRS) protein family [105] (Fig. 5.4). Tyrosine-phosphorylated IRS proteins associate with the IR and initiate downstream signaling events [105] (Fig. 5.4). Mice treated with GCs have reduced levels of tyrosine-phosphorylated IR and total IRS-1 proteins in skeletal muscle [96], and the activities of phosphoinositide-3-kinase (PI3K) and Akt, two signaling molecules downstream of IRS-1, are decreased [96, 106, 107] (Fig. 5.4).

The ability of GC to inhibit glucose utilization in myotubes requires protein synthesis. A list of potential GR primary target genes that can suppress insulin action has been identified in mouse C2C12 myotubes [108]. Among these genes, the role of *Pik3r1* (a.k.a. *p85α*) in the GC response was examined *in vitro*. *Pik3r1* encodes the regulatory subunit of PI3K, which binds to activate IRS1 (Fig. 5.4) through its SRC homology 2 (SH2) domain to bring the catalytic subunit of PI3K, Pik3ca (a.k.a. p110), to the plasma membrane [109, 110]. Pik3ca then catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-bisphosphate (PIP3) [109, 110]. PIP3 anchors the protein kinase Akt protein kinase family to the plasma membrane and thus initiates downstream signaling events [110]. Pik3r1 is a key component in the insulin signaling pathway. But monomeric Pik3r1 is thought to compete with the Pik3r1/Pik3ca heterodimer to interact with IRS-1 to suppress insulin action [111, 112]. In addition, Pik3r1 is required for the maximal activity of phosphatase and tensin homolog (PTEN) [113], which antagonizes PI3K activity. In C2C12 mouse myotubes, GC treatment reduces the activity of several components of the insulin signaling pathway. These GC effects are markedly decreased in C2C12 myotubes that have reduced Pik3r1 expression [108]. Thus, *Pik3r1* is a potential GR primary target gene in the mediation of the suppressive effect of GC on glucose utilization in skeletal muscle, though this notion needs to be confirmed *in vivo*. Interestingly, global heterozygous deletion of *Pik3r1* gene in mice has improved whole body insulin sensitivity [114, 115]. Elevated expression of *PIK3R1* is found in patients with insulin resistance [116]. Notably, *Pik3r1* is likely not the only GR target gene that mediating suppressive effects of GC in insulin response, and additional GR primary target genes could also participate in this process.

GC also modulate insulin sensitivity and glucose utilization through the generation of specific lipid mediators. GC treatment increases ceramide levels in mouse liver and portal circulation [117]. These ceramides cause hepatic insulin resistance [118]. They are also delivered to skeletal muscle so one might expect systemic effects. In fact, hyperinsulinemic-euglycemic clamp studies in mice show that GC decrease the glucose infusion rate required to maintain euglycemia, prevent insulin-inhibited hepatic glucose output, and inhibit 2-deoxyglucose uptake into skeletal muscle, all evidence of reduced insulin sensitivity, whereas mice pretreat with myriocin, an inhibitor of serine palmitoyaltransferase (Sptlc1 and Sptlc2), an enzyme in the ceramide synthetic pathway, have reduced GC-induced insulin resistance in skeletal muscle and hepatic glucose output (Fig. 5.4) [117]. Notably, GC augment the expression of enzymes in the ceramide synthetic and metabolic pathway in liver, such as Sptlc2, ceramide synthase 1 (Cers1, a.k.a. Lass1), and ceramide synthase 6 (Cers6, a.k.a. Lass6) (Fig. 5.4) [117]. However, it is not yet clear whether the genes encoding these enzymes are primary targets of GR signaling.

In addition to increased ceramide levels in liver, GC-promoted lipolysis in WAT could impair whole body glucose homeostasis. Acipimox, an inhibitor of lipolysis in adipocytes, improves whole body glucose homeostasis in human subjects treated with GC [119]. Although it is not clear how GC-induced lipolysis affects glucose homeostasis, it is likely that the fatty acids generated from lipolysis are mobilized to skeletal muscle and liver and converted to lipid mediators, such as diacylglycerol (DAG) and ceramides, that cause insulin resistance.

Notably, Brennan-Speranza et. al. also reported that GC inhibit the expression of osteocalcin, a secreted protein from bone that reduce adiposity and hepatic steatosis, to decrease insulin sensitivity.

GC also inhibit glucose oxidation by stimulating the expression of several members of the pyruvate dehydrogenase kinase family (PDK). PDK regulates glucose oxidation by inhibiting the pyruvate dehydrogenase complex that converts pyruvate to acetyl-CoA [120]. Among the PDK family, *PDK4* is a GR primary target gene; GREs have been identified in human *PDK4* and rat *Pdk4* genes [121, 122]. FoxO1 binding sites have been identified near the human *PDK4* gene GRE and they are required for the maximal induction of *PDK4* gene transcription by GC. These FREs also mediate the inhibitory response of insulin on *PDK4* gene transcription [122]. For the rat *Pdk4* gene, an FRE located approximately 6 kb away from the GREs is thought to participate in both the insulin and GC responses [121]. Thus, the mechanisms governing the transcriptional regulation of the *PDK4* gene by GC appear to be similar to GC-activated *Pck1* and *G6pc* gene transcription discussed above.

In addition to skeletal muscle, GC reduce glucose uptake and glucose oxidation in many other tissues. GC inhibit insulin-stimulated glucose uptake in both mouse 3T3-L1 and primary adipocytes. The mechanisms governing these GC effects are mostly unknown. Overexpression of *dual specificity protein phosphatase 1* (*Dusp1*, a.k.a. MAP kinase phosphatase 1, *Mkp1*), a primary GR target gene, inhibits insulin-stimulated glucose uptake in 3T3-L1 adipocytes [123]. However, the exact role of Dusp1 in GC-induced insulin resistance in adipocytes has not been established. Most reports indicate that GC inhibit glucose uptake by antagonizing the insulin response, though the direct inhibition of glucose transporter 4 (Glut4) trafficking process by GC in 3T3-L1 adipocytes has also been reported [124]. *Pik3r1* expression is also increased by GC in adipocytes [125]. Thus, Pik3r1 may also participate in the GC-inhibited insulin response in adipocytes. Studies of human adipocytes found that GC inhibit insulin-stimulated glucose uptake and signaling in omental but not subcutaneous adipocytes [126]. In fact, studies in human primary subcutaneous adipocytes show that GC pre-treatment potentiates insulin-stimulated glucose uptake [102, 127]. This suggests that GC affect insulin signaling in a depot-specific manner in humans.

The ability of GC to inhibit glucose oxidation has been linked to GC-induced apoptosis in leukemia cells. GC inhibit the expression of glucose transporter 1 (GLUT1) that results in a decreased glucose uptake into leukemia cells [128]. GC also suppress glucose uptake and oxidation in certain regions of brain, such as the hypothalamus and hippocampus [129–131]. The exact mechanisms of these GC effects on these cell types are mostly unclear.

## Glycogen Metabolism

GC regulate glycogen metabolism in a tissue-specific manner. In liver the administration of GC to fasted mice increases liver glycogen content [40, 132]. GC induce the activity of glycogen synthase [7, 133, 134]. Glycogen synthase activity is

regulated by post-translational modification. Protein kinase A (PKA) and glycogen synthase kinase 3 (GSK3) phosphorylates and inactivates glycogen synthase [135–137]. In contrast, protein phosphatase 1 (PP1) dephosphorylates glycogen synthase, which potentiates its activity [138, 139]. The active form of glycogen phosphorylase, glycogen phosphorylase *a*, breaks down glycogen to glucose units. Glycogen phosphorylase *a* also inhibits the dephosphorylation of glycogen synthase by PP1. PKA can also inhibit PP1 activity. Data suggest that the activation of glycogen synthase phosphatase, PP1, by GC [140, 141], though the exact the mechanism of this activation is unclear.

Epinephrine plays a key role in skeletal muscle glycogenolysis. This effect is blunted in adrenalectomized rats [142]. The stimulatory effects of epinephrine on muscle glycogen phosphorylase and phosphorylase kinase are all attenuated in the skeletal muscle of adrenalectomized rats. PP1 dephosphorylates glycogen phosphorylase and phosphorylase kinase and suppresses their activities. PP1 activity is increased in skeletal muscle of adrenalectomized rats and the ability of epinephrine to inhibit PP1 is reduced in the skeletal muscle of adrenalectomized rats. Cortisol treatment in adrenalectomized rats restores normal epinephrine effects through the activation of phosphorylase kinase and glycogen phosphorylase and the inhibition of PP1. The induction of *PDK4* gene transcription by GR may contribute to GC-regulated glycogen metabolism in myotubes. One report shows that a reduction of the expression of PDK4 in human primary myotubes diminishes GC-repressed glycogen synthesis [143]. Notably, GC also inhibits insulin-stimulated glycogen synthesis and the activity of glycogen synthase [9]. These GC effects are mainly due to their ability to reduce insulin signaling in skeletal muscle. Interestingly, one report shows that glycogen storage is actually increased in soleus muscle by dexamethasone treatment despite a decrease of glycogen synthesis [9]. The mechanisms underlying these phenotypes are not clear.

In cardiac muscle, adrenalectomy also blocks epinephrine-induced glycogenolysis [144]. GC treatment, however, has been shown to facilitate glycogen storage in cardiac muscle [145]. GC increase AMP-activated protein kinase (AMPK) activity that leads to the elevation of glucose uptake, the induction of glycogen synthase, and the reduction of glycogen phosphorylase. These contribute to an augmentation of glycogen content in cardiac muscle. Brain stores certain amounts of glycogen, though the levels are lower than in skeletal muscle or liver. Astrocytes store most of the brain glycogen, whose levels are increased by norepinephrine. In primary astrocyte culture norepinephrine induces glycogen synthesis, an effect which is suppressed by GC [146].

## GC Effects on Pancreas

As an endocrine organ, the pancreas secretes several hormones that include insulin (from β cells in the islets of Langerhans), glucagon (from α cells), and somatostatin (from δ cells). Insulin secreted during the fed state promotes glucose uptake and

utilization, and inhibits gluconeogenesis. In contrast, glucagon secreted during fasting stimulates gluconeogenesis and glycogenolysis. Somatostatin suppresses both insulin and glucagon secretion.

The effect of GC on insulin secretion is an area of vigorous research; however, the direct mechanistic relationship between GC and β cell function *in vivo* remains elusive. This is largely due to the difficulty of separating the indirect effects of GC-induced peripheral insulin resistance from the direct actions of GC on pancreatic β cells. Hypercortisolism induces insulin resistance in tissues such as liver, adipose and skeletal muscle, as discussed above. In murine models and human studies, this GC-induced insulin resistance can lead to compensatory β cell hyperplasia and hyperinsulinemia, and consequent normoglycemia. However, long-term GC treatment that exceeds the β cell compensatory capacity leads to insufficient insulin secretion, hyperglycemia, and eventually Type 2 diabetes [15, 17, 147–150]. Overall, the effects of GC on β cell function are dependent on the length and dosage of the treatment, the experimental animal model under investigation, as well as the susceptibility of the strain exposed [151]. It is important to note that short-term effects of GC on β cells are likely reversible [152]. To begin to dissect the direct *in vivo* effects of GC on β cells, a mouse model of insulin promoter-driven GR overexpression was generated [14]. These mice show glucose intolerance due to blunted insulin secretion.

Insulin secretion is mainly triggered by a postprandial increase in the concentration of plasma glucose, which enters pancreatic β cells through GLUT2 transporters. Glucokinase phosphorylates glucose to produce glucose-6-phosphate, which enters the glycolytic cycle. Through mitochondrial β-oxidation, the ratio of ATP to ADP increases, which leads to the closure of ATP-sensitive potassium channels and plasma membrane depolarization. The increase in cellular electrical conductivity drives the opening of voltage-sensitive calcium ion channels. The consequent influx of calcium ions eventually leads to exocytosis of insulin-containing granules.

Compared to islets isolated from GC-treated animals, isolated islets directly treated with GC present opposite results. First, synthetic GC dexamethasone promotes posttranslational degradation of GLUT2 and decreases glucose-stimulated insulin secretion (GSIS) in isolated rat pancreatic islets [153]. Second, dexamethasone inhibits glucokinase mRNA expression in RIN cells, a rat pancreatic islet tumor cell line [154]. GC have also been reported to suppress the gene expression of insulin [155] and pancreatic and duodenal homeobox 1 (Pdx1) in β cells [156]. Pdx1 is a transcription factor essential for pancreatic development and β cell maturation. Third, in *ob/ob* mice, GC enhance the activity of glucose-6-phosphatase, which dephosphorylates glucose-6-phosphate to produce glucose, resulting in a futile cycle [157]. Fourth, in the INS-1 rat pancreatic β cell line, the transcription of voltage-gated potassium channel Kv1.5 is upregulated by dexamethasone treatment [158]. This results in an increase in the repolarizing outward current and reduces the influx of calcium ions, which compromises GSIS. On the contrary, islets purified from GC-treated rats display enhanced insulin secretion, likely due to compensatory hyperinsulinemia [159–161], and have improved glucose sensitivity and oxidative metabolism [162]. Furthermore, transmission electron microscopy shows an increased amount of docked secretory granules in GC-treated β cells [148].

GC also regulates the expression of genes that modulate insulin secretion. The blunting of GSIS by dexamethasone is significantly restored in islets isolated from *serum and glucocorticoid-induced kinase (Sgk1)* knockout mice compared to wild type [158]. *Sgk1* is a well-established GR primary target gene. Moreover, GC treatment of MIN6 mouse insulinoma cells and mouse islets augments the expression of corticotropin-releasing factor receptor type 2α (CRFR2α) and inhibits the expression of CRFR type 1 (CRFR1) [163]. CRFR1 potentiates both glucose-induced insulin secretion *in vitro* and *in vivo* and the proliferation of neonatal rat β cells [164].

GC can stimulate β cell proliferation in vivo; however, GC exposure could also have cytotoxic effects on mouse isolated pancreatic islets of Langerhans and MIN6 cells [165]. Interestingly, increased intracellular cAMP levels have previously been shown to attenuate dexamethasone-induced β cell death using exendin-4 [20] and forskolin [166]. Co-treatment with RU486, an antagonist of GC, abolished these GC effects. Mitogen-activated protein kinases (MAPK) such as p38 MAPK and JNK regulate apoptosis. In MIN6 cells, inhibition of p38 MAPK reduces glucocorticoid-induced apoptosis [167]. This may be due to decreased phosphorylation of mouse GR at serine residue 220 by p38 MAPK. Phosphorylation of GR at serine 220 is positively associated with GR transcriptional activity [168, 169]. Moreover, in isolated islets, inhibition of p38 MAPK decreases glucocorticoid-induced formation of cleaved caspase 3, which plays a key role in the execution phase of apoptosis [167]. Thus, p38 MAPK is required for glucocorticoid-induced cytotoxicity. In contrast, JNK signaling dampens glucocorticoid-induced cytotoxicity, as inhibition of JNK potentiates the cytotoxic effect of GC in MIN6 cells [167]. A plausible explanation is that JNK is responsible for phosphorylating GR at serine 234, which is negatively associated with the transcriptional activity of GR [170].

Reactive oxygen species (ROS) may also mediate glucocorticoid-induced cytotoxicity [171]. Thioredoxin-interacting protein (Txnip) could exert its pro-apoptotic effect by blocking the activity of thioredoxin, which is involved in a major pro-survival thiol-reducing pathway in cells. In *db/db* mice, overexpression of thioredoxin suppresses the progression of hyperglycemia, likely through the prevention of the reduction of Pdx1 and V-maf musculoaponeurotic fibrosarcoma oncogene homologue A (MafA) transcription factors [172]. GC induces Txnip expression in human and mouse islets, and in MIN6 cells [165]. Overexpressing Txnip mimicked pro-apoptotic effect of GC, while knocking down Txnip partially rescued this phenotype in MIN6 cells. Furthermore, thioredoxin overexpression protected MIN6 cells from GC-induced cytotoxicity [165]. Interestingly, GC-induced Txnip is dependent on the presence of p38 MAPK. Studies in leukemia and other cell types have shown that Txnip is a primary GR target gene [173].

In addition to insulin secretion, GC has been shown to modulate glucagon secretion. GC treatment causes both hyperinsulinemia and hyperglucagonemia. Rodents treated with GC have unaltered insulin/glucagon ratio from the fasted state to the fed state. They tend to have increased α cell mass, and show impaired high glucose-suppressed glucagon secretion. This hyperglucagonemia leads to hyperglycemia through the activation of hepatic glycogenolysis and gluconeogenesis [174]. Notably, co-treatment of GC and a glucagon receptor antagonist on rats resulted in

normoglycemia [11]. The mechanisms underlying the effects of GC on glucagon secretion, however, are unclear.

## Conclusion and Future Directions

The physiological responses of GC that modulate glucose homeostasis have been well documented. Significant progress has been made to understand these GC actions in the last three decades. However, the precise manner by which GC regulates glucose homeostasis is still unclear. The key to understanding the mechanisms of GC-regulated glucose homeostasis is the identification of GR primary target genes that mediate GC actions, and understanding how these primary target genes are transcriptionally regulated by GR in various processes, including gluconeogenesis, glucose uptake and utilization, glycogen metabolism, and pancreatic endocrine secretions. For transcriptional regulation, GC-activated *Pck1* gene transcription has received the most attention. Nevertheless, further studies are needed to understand how different accessory factors coordinate with GR and transcriptional coregulators to activate *Pck1* gene transcription. Analyzing the transcriptional regulatory mechanisms of these GC-regulated gluconeogenic genes should allow the identification of common molecular features in GR-stimulated gluconeogenesis. *In vivo*, GC actions are affected by other hormones and environmental cues. Understanding the crosstalk and integration of these signals should be the focus of future work.

The emergence of advanced techniques and technologies during the last decade have facilitated the characterization of molecular features of GC-regulated glucose homeostasis. Using high throughput DNA sequencing technology, genomic GR binding regions, transcriptional coregulator occupancy sites, and global chromatin structure changes were identified. The discovery of GC-regulated non-coding RNA and enhancer RNA, using genomic run-on sequencing (GRO-seq) and RNA sequencing (RNA-seq), unveiled another layer of glucose homeostasis regulation [175–178]. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing technology can now be used to efficiently ablate a GR primary target gene, and modify a particular residue of GRE to study its role in the natural chromosomal context [179–183]. These new approaches in combination with refined genetic, physiological and biochemical tools will play a critical role in improving our understanding of GC-regulated biology in metabolic tissues.

Ultimately, the goal of the studying the basic mechanisms of GC-regulated glucose homeostasis is to develop a pharmacotherapy targeting GC signaling for treating metabolic diseases. Reducing GC signaling *in vivo* improves insulin sensitivity and decreases plasma glucose levels, which would largely benefit type 2 diabetes patients. However, reducing GC actions globally is not ideal. For example, the body's inflammatory status would be elevated with reduced GC signaling. In fact, as inflammation promotes insulin resistance, the anti-inflammatory activity of GR could also improve insulin sensitivity. Overall, identifying approaches to dissociate metabolic and anti-inflammatory actions of GR could provide novel ways to treat metabolic disease.

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