How much we know about the attenuation of insulin signaling in the adipose tissue caused by glucocorticoid treatment?

Alex Rafacho¹, Everson A Nunes¹, Silvana Bordin²

¹Department of Physiological Sciences and Multicenter Graduate Program in Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, SC, 88040-900, Brazil
²Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, SP, 05508-900, Brazil

Correspondence: Alex Rafacho
E-mail: alex.rafacho@ufsc.br
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Glucocorticoid (GC) hormone exerts numerous physiological roles that include modulation of immune, nervous, cardiovascular and metabolic systems. Synthetic GCs such as dexamethasone and prednisone/prednisolone are widely prescribed in the clinical context to the treatment of inflammatory-related diseases. In spite of its positive therapeutic effect, GC-based therapies may cause several adverse effects including glucose intolerance and peripheral insulin resistance. Reduction of insulin sensitivity in the adipocytes and adipose tissue caused by GC treatment is associated with increased lipolysis and abnormal Ser phosphorylation of insulin substrate receptor (IRS)-1 and protein kinase B (PKB). However, there is no consensus about the precise mechanisms whereby GC treatment promotes such attenuation in the insulin signaling pathway. In this paper, we will briefly discuss and present some molecular evidences that might be involved with this negative impact of GC in the insulin signaling in the adipose tissue.

Keywords: adipocytes; adipose tissue; dexamethasone; glucocorticoid; insulin signaling; 3T3-L1 cells

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Many studies have emerged with the aim to elucidate the role of elevated and/or chronic exposure to glucocorticoids (GCs) on glucose, lipid and protein metabolism. GC hormones (mainly cortisol in humans and corticosterone in rodents) are released by the adrenal glands at the zona fasciculate [1]. GCs are synthetized and secreted under dynamic control by the hypothalamic-pituitary-adrenal (HPA) axis and exhibit an availability and effectiveness that depends on serum corticosteroid-binding globulin, intracellular abundance of 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes and/or glucocorticoid-receptor (GR) [2], GR affinity, and post-translational modifications of GR protein (e.g., phosphorylation, ubiquitination, SUMOylation, acetylation, nitrosylation and oxidation) [2,3].

The main biological role of the natural GCs is to supply enough blood glucose into circulation to guarantee the central nervous system functions and compensatory responses of the organism during conditions of acute stress or reduced food intake [4]. For this, GCs promotes several systemic actions that include i) increase in the hepatic glucose production, ii) reduction in the peripheral glucose uptake into adipose tissue and skeletal muscles, iii) increase in the fat lipolysis and muscle proteolysis to provide enough substrates for gluconeogenesis, and iv) attenuation of the insulin secretion from pancreatic β cells [4].
Synthetic GCs are drugs that mimic natural GCs and dexamethasone and prednisone/prednisolone are among the most prescribed synthetic GCs. These drugs retain higher potency and lower metabolic clearance in relation to the natural GCs and are resistant to the inactivation by the 11β-HSD-2 (the isoenzyme that inactivates active GCs), which favor an increase of local GC availability [5]. Dexamethasone and prednisone/prednisolone show potent immunos suppressive, anti-inflammatory and antiallergic effects, which justify their wide prescription for the treatment of chronic autoimmune diseases, inflammatory diseases, organ transplant rejection, among others [6]. However, chronic exposure to the GCs impairs the normal metabolic homeostasis and causes deleterious adverse effects as demonstrated clinically and experimentally including hyperglycemia, glucose intolerance, dyslipidemia, peripheral insulin resistance, muscle wasting, hepatosteatosis, infertility, growth retardation, cognitive dysfunction, glaucoma, cataracts, topical skin thinning, osteoporosis and diabetes, among others [6-8].

GC actions vary in a tissue- and cell-specific manner, and depend on the dosage, length of administration (acute or chronic), source (endogenous or synthetic), the species investigated (e.g., human, rodents), individual susceptibility (e.g., insulin-resistant, glucose-intolerant), among other questions (e.g., direct vs. indirect actions) that make difficult to obtain a general consensus about their actions [7].

GCs modulate several processes in the adipose tissue such as adipocyte differentiation/adipogenesis [9-13], and exert both anabolic (e.g., lipogenesis) and catabolic (e.g., lipolysis) functions, depending on the fat depot evaluated and stage of adipocyte differentiation/maturation. It is consensually accepted that GCs promote adipogenesis and/or lipogenesis (visceral adipose tissue) and lipolysis (subcutaneous adipose tissues), which correspond with clinical features of abdominal obesity and limited subcutaneous fat on the extremities in patients with Cushing’s syndrome [4]. GCs also promote lipolysis in differentiated adipocytes and/or adipose tissue fragments explanted from visceral depots [10, 14, 15]. In accordance, the ability of insulin to inhibit 8-bromo cAMP-stimulated glycerol release in adipocytes is significantly reduced in rats treated with dexamethasone for 11 consecutive days [14]. Furthermore, rats treated with dexamethasone [15] and corticosterone [10] for 5 or 10 consecutive days, respectively, also develop augmented lipolysis rate preferentially under basal condition. This dual effect of GCs on visceral fat (induction of adipogenesis and lipolysis) corroborates to the expansion of abdominal fat and to the increase of hepatic flux of lipids that may lead to hepatosteatosis [10, 17]. For comprehensive reviews about the effects of GCs in the adipose tissue biology and the development of central obesity refers to [12, 13].

Rats treated with GCs exhibit reduced insulin response in the epididymal fat/adipocytes that is associated with diminished insulin receptor substrate (IRS)-1 and IRS-2 Tyr phosphorylation, decreased IRS-1 and IRS-2 association with the phosphatidylinositol 3-kinase (PI3K), decreased protein kinase B (PKB) Ser and Thr phosphorylation, and reduced atypical protein kinase C (PKC)ζ phosphorylation [10, 14, 15, 18]. These rats also exhibit increased mRNA and protein content of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) in the epididymal tissue [10]. In differentiated 3T3L-1 cells, IRS-1 Tyr phosphorylation and PKB activity are also impaired by prednisolone [19] and dexamethasone [20] treatment. The exact mechanisms by which GCs attenuate the main insulin signaling components (e.g., IRSs and PKB proteins) are not well established. It was recently demonstrated that rats treated with dexamethasone for 5 consecutive days exhibit reduced PKB Ser phosphorylation in the epididymal fat after an oral bolus of glucose, indicating that a physiological peak of insulin does not result in proper insulin cascade activation [15].

It is well known that insulin resistance induced by obesity is associated with impairment of insulin signaling in parallel with increased c-Jun-N-terminal kinase (JNK) and inhibitor of nuclear factor kappa-B (IKKα/β) protein phosphorylation [21-24]. JNK and IKKα/β are serine protein kinases activated upon ligands commonly upregulated during obesity [e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1], saturated fatty acids] that act through proinflammatory pathways and exert a negative crosstalk with the insulin signaling, not only in the adipose tissue but also in muscle and liver [21-24]. By an apparent obvious sense, GC-induced insulin resistance in the adipose tissue should not be associated with increased JNK and IKKα/β activation, since GC exert anti-inflammatory actions in the human and mice adipose tissue [25, 26]. By interacting with proinflammatory transcription factors such as nuclear factor kappa-B (NF-κB) and activator protein (AP)-1, GC-GR complex represses NF-κB and AP-1 transactivation activity leading to suppression of gene transcription of key inflammatory components, favoring for an attenuation of the proinflammatory milieu [2, 3]. In fact, an obese mice model induced by high-fat diet had prevented accumulation of adipose tissue macrophages (ATM) after a treatment with dexamethasone, showing the beneficial effects of GC treatment in this context [25]. However, emerging evidences show both proinflammatory and anti-inflammatory actions of GCs, depending on the duration and magnitude of the GC signaling and proinflammatory stimulus [25]. Thus, it is likely
that the impact of GCs in cell response is much broader than the interplay between GR and NF-κB or AP-1.

The role of GCs in fat biology is complex and under continuous investigation. In models of visceral obesity by prolonged exposure to corticosterone [10, 16] or diet-induced obesity [11, 28], it is common to observe an increase of GC availability in the visceral adipose tissue as a consequence of 11β-HSD-1 upregulation (the isoform that catalyzes the intracellular activation of GCs) [16, 28]. These studies demonstrated that systemic and/or local GC excess is associated with reduced adipose tissue insulin signaling and significant glucose and lipid homeostasis imbalance [10, 11, 16, 28]. The alterations in insulin signaling pathway include reduced adipose tissue IRS-1 Tyr and PKB Ser/Thr phosphorylation [10, 11, 16]. By blocking/reducing the 11β-HSD-1 enzyme activity with pharmacological [28] genetic (knockout) [11] or molecular (knockdown) [16] tools, obese mice made by prolonged GC exposure or high-fat diet become partially prevented from the glucose and lipid imbalance, suggesting a negative impact of elevated local GC abundance for adequate adipose tissue biology. There are similar impairments of insulin signaling in the adipose tissue in a non-obese rat model of insulin resistance made by 5-day dexamethasone treatment that include increased IRS-1/PKB phosphorylation and reduced PKB Ser phosphorylation [15]. In spite of a pronounced reduction in the GR epididymal adipose tissue (EAT) content, these rats treated with dexamethasone does exhibit reduced JNK and IKKβ phosphorylation in EAT as well as reduced circulating levels of IL-1β and TNF-α, indicating the suppressive effects of dexamethasone in the proinflammatory signaling pathway [15]. Whether the well-known negative crosstalk of JNK and IKKβ with insulin signaling seems not to be occurring in this GC-treated rat model, as judged by reduced JNK and IKKβ phosphorylation, what could be the candidates for such insulin signaling impairment made by GCs? The answer for that is not simple and several candidates could be implicated on that matter.

Studies with 3T3-L1 cells demonstrated that dexamethasone exerts non-genomic impacts on insulin signaling pathway that was GR-dependent and transcription-independent [29]. The phosphorylation of insulin receptor (IR), IRS-1, PKB, phosphoinositide-dependent kinase (PDK) and the proto-oncogene tyrosine-protein kinase (Fyn) were all reduced in 3T3-L1 cells after 30 min incubation with dexamethasone. These results were associated with increased JNK phosphorylation, although IKKα/β phosphorylation was not altered [29]. This non-genomic effect in the JNK phosphorylation in the 3T3-L1 cells does not reproduce those observed in the adipose tissue of the 5-day dexamethasone treated rats; which exhibit impaired IRS-1/PKB phosphorylation concomitant to reduced JNK/IKKβ phosphorylation [15]. Thus, different kinases and/or phosphatases could be involved in the negative regulation exerted by dexamethasone treatment in vivo on insulin signaling.

PKCs may also be candidates for dexamethasone-induced insulin resistance in adipocytes. These proteins are activated by different lipid or lipid-derived mediators that have been linked to insulin resistance (e.g., free fatty acids as palmitic and stearic acid, diacylglycerol, ceramide, etc.) [30]. The importance of free fatty acids and ceramides in the mediation of dexamethasone effects related to glucose homeostasis in vivo was previously demonstrated by its pronounced attenuation or abolishment using antilipolytic agents (e.g., nicotinic acid) [31] or directly inhibiting ceramide synthesis [32]. Since fatty acids, diacylglycerol and ceramides can differentially activate conventional (α, β1, β2 and γ), novel (δ, ε, η and θ), and atypical (ζ and λ/τ) PKCs [30], there are numerous different signaling pathways that can be target for the investigation of mechanistic dexamethasone effects focusing on PKCs. The dexamethasone-induced reduction of insulin-stimulated glucose uptake in isolated adipocytes is abolished in the presence of conventional PKC inhibitors (Go6976 and LY379196) or myristoylated PKC pseudosubstrate (inhibitor of Ca2+) and phospholipid-dependent PKCs) [33]. These authors also showed that there were redistribution of PKCβ and PKCζ isoforms from cytosol to the membrane and that Ser/Thr phosphorylation of IRS-1 induced by dexamethasone seems to be completely blocked by PKC inhibitor Go6976 [33]. However, the insulin resistance caused through activation of PKCs may be both mediated by JNK/IKKβ crosstalk with the insulin-related signaling proteins or by direct Ser phosphorylation of IRS-1 and/or reduction of PKB phosphorylation after insulin stimuli [30]. As an example, PKC0 activation in soleus muscle is associated with Ser phosphorylation of IRS-1 [34]. Such event, if reproduced in the adipose tissue, might corroborate the increment in Ser phosphorylation of IRS-1 observed in rats treated with dexamethasone in vivo [15], a context associated with decreased JNK and IKKβ phosphorylation. In accordance, it was shown that, in 3T3-L1 cells, PKCζ mediates the negative effect of ceramide in the insulin-stimulated PKB phosphorylation [35]. Since ceramide production seems to be necessary for dexamethasone effects on peripheral insulin action [32], PKCζ can be a promising mediator of dexamethasone effects on PKB functionality, independently of JNK and IKKβ. Therefore, further studies could target the participation of the PKCζ action on PKB phosphorylation to test whether such mechanism is also involved in the in vivo adipose tissue insulin resistance caused by dexamethasone treatment.
Other candidates for GCs adverse effects upon insulin signaling are Src homology 2-containing inositol 5-phosphatase (SHIP)-2 and phosphatase and tensin homolog (PTEN), two phosphoinositide phosphatases known to negatively regulate insulin signaling pathway [36, 37]. PTEN, but not SHIP-2, suppressed the insulin signaling (e.g., PKB Ser/Thr phosphorylation) in 3T3-L1 cells [38]. In accordance, cortisone or dexamethasone induces an elevation in the PTEN protein content and a reduction in the PKB Ser phosphorylation in mature 3T3-L1 cells treated with lipopolysaccharide. This dexamethasone effect was reverted by emodin (an 11β-HSD-1 inhibitor), thus suggesting the involvement of 11β-HSD-1 in this negative regulation [39]. Tribbles protein (TRB)-3, a protein kinase involved with endoplasmic reticulum stress-induced cell death [40], is considered another candidate, since TRB-3 exert a negative impact on PKB phosphorylation and activity. Incubation of 3T3-L1 cells with dexamethasone results in increased TRB-3 mRNA expression that was paralleled with significant reduction in the PKB Ser phosphorylation, indicating a relationship between both proteins [41]. These protein kinases and protein phosphatases are some of candidate components that can negatively impact on insulin signaling and exemplify how much clues need to be addressed. Thus, further studies merit investigation to define whether these and other kinases/phosphatases are or are not involved with the in vivo adverse effects of GC excess upon insulin action.

Studies performed in vitro (hepatocytes) and in vivo (lean mice) revealed that mitogen-activated protein (MAP) kinase phosphatase (MKP)-3 is a downstream component of dexamethasone effects [17]. In this study the authors demonstrated that several metabolic adverse effects caused by chronic GC treatment can be attributed to induction of MKP-3 expression and it was dependent on forkhead box protein O1 (FOXO1). MKP-3 dephosphorylates FOXO1 and promotes its nuclear translocation [42]. Since FOXO1 upregulation is associated with reduced insulin signaling in adipocytes treated with TNF-α [43], a molecular feature similar to those caused by GCs, in vivo studies addressing this question might contribute for elucidation of the possible involvement of MKP-3 and FOXO1 in the GC-induced impairment of insulin signaling in the adipose tissue. Finally, recent studies demonstrated the involvement of two proteins - progranulin [44] and plasminogen activator inhibitor (PAI)-1 [45] - on glucose and lipid abnormalities caused by high-fat diet and GC, respectively, reinforcing the diversity and complexity of signals that may modulate peripheral insulin response.

Conclusions

Chronic GC treatment commonly results in decreased insulin action in the peripheral tissues including adipose tissue. Despite GCs actions in the adipose tissue seem to be depot- and/or cell-specific, adipose tissue lipolysis is associated with diminished insulin-suppressive effect and impairment of IRS-1 and PKB phosphorylation. Although much is known about the effects of GCs on adipose tissue/adipocytes, there is no consensus about the precise mechanisms whereby GC impairs IRS-1 and PKB activities. The answer to this question will lead to the development of new and effective strategies for the attenuation of this common side effect that subsist with metabolic disturbances caused by GC excess.

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Conflict of interest

The authors declare that there are no conflicts of interests.

List of abbreviations


Authors contribution

AR conceived and designed the paper. AR, EAN and SB wrote and edited the paper.

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