Glucocorticoids produce whole body insulin resistance with changes in cardiac metabolism

Dake Qi and Brian Rodrigues
Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences,
The University of British Columbia, Vancouver, British Columbia, Canada

Qi D, Rodrigues B. Glucocorticoids produce whole body insulin resistance with changes in cardiac metabolism. Am J Physiol Endocrinol Metab 292: E654–E667, 2007. First published October 31, 2006; doi:10.1152/ajpendo.00453.2006.—Insulin resistance is viewed as an insufficiency in insulin action, with glucocorticoids being recognized to play a key role in its pathogenesis. With insulin resistance, metabolism in multiple organ systems such as skeletal muscle, liver, and adipose tissue is altered. These metabolic alterations are widely believed to be important factors in the morbidity and mortality of cardiovascular disease. More importantly, clinical and experimental studies have established that metabolic abnormalities in the heart per se also play a crucial role in the development of heart failure. Following glucocorticoids, glucose utilization is compromised in the heart. This attenuated glucose metabolism is associated with altered fatty acid supply, composition, and utilization. In the heart, elevated fatty acid use has been implicated in a number of metabolic, morphological, and mechanical changes and, more recently, in “lipotoxicity”. In the present article, we review the action of glucocorticoids, their role in insulin resistance, and their influence in modulating peripheral and cardiac metabolism and heart disease.

dexamethasone; fatty acid; glucose; lipoprotein lipase; pyruvate dehydrogenase kinase

IN THE HUMAN BODY, insulin resistance is viewed as an insufficiency in insulin action. This disorder is hard to identify, and a number of patients remain undiagnosed. Insulin resistance is often associated with conditions like obesity, hypertension, and dyslipidemia. The cluster of these abnormalities has been defined as the “metabolic” or “insulin resistance” syndrome (140). Insulin resistance leads to a cardiac pathology. In the San Antonio Heart Study (77), patients with insulin resistance had a 2.5-times increased risk of dying of cardiovascular disease (CVD) than those without insulin resistance. In addition, ~30% of patients who have insulin resistance eventually develop type 2 diabetes. Diabetes itself promotes vascular diseases and nonvascular cardiac injury (55).

Increasing evidence from clinical and experimental studies has established that metabolic abnormalities play a crucial role in the development of heart diseases (96, 145). Under physiological conditions, heart acquires most of its energy from metabolism of glucose and fatty acid (FA), with the latter being the major substrate consumed by cardiac tissue (145). During insulin resistance and diabetes, characterized by inadequate glucose utilization, cardiac FA consumption supersedes glucose oxidation. In the heart, elevated FA use has been implicated in a number of metabolic, morphological, and mechanical changes and, more recently, in “lipotoxicity” (148). During lipotoxicity, when the capacity to oxidize FA is saturated, FA accumulates and can, either by itself or via production of second messengers such as ceramides, provoke cell death (148).

Both endogenous hormones and anti-inflammatory and immunosuppressive drugs characterize glucocorticoids. Through their effects on inflammation and cellular proliferation, glucocorticoids have beneficial effects on heart disease (174). However, excessive endogenous (129, 150) and exogenous glucocorticoids (163, 169) are linked to insulin resistance. In addition, epidemiologic studies indicate that atherosclerosis and myocardial infarction occur in patients with long-term glucocorticoid treatment or Cushing’s syndrome (33, 109, 187). In the present article, we will briefly review the mechanisms by which glucocorticoids induce insulin resistance; highlight their role in modulating metabolism, specifically cardiac substrate utilization; and illustrate how they may promote cardiac disease.

Glucocorticoids

The term “glucocorticoid” represents both secreted hormones and anti-inflammatory and immunosuppressive agents. As a family of therapeutic drugs, glucocorticoids have widespread use in nonendocrine and endocrine diseases (159). Today, glucocorticoids are used in a broad spectrum of anti-inflammatory and immunosuppressive therapies, which include allergic and hematological disorders, and renal, intestinal, liver, eye, and skin diseases. Rheumatic diseases and bronchial asthma are the main indications of long-term therapy with these hormones (162). In addition, glucocorticoids are also used in the suppression of the host-vs.-graft or graft-vs.-host reactions following organ transplantation surgery.
Secretion, regulation and metabolism. Historically, glucocorticoids were defined as a group of hormones released from the cortex of the adrenal gland. Secretion into the peripheral circulation occurred in a circadian fashion (~800 nM in the morning and ~200 nM at midnight) (188). In the human body, the main endogenous glucocorticoid is cortisol, and its basal daily secretion is ~6–8 mg/m². In response to stress, cortisol release is increased up to 10-fold of the basal value (38). Endogenous glucocorticoid synthesis and release are regulated by the pituitary and hypothalamus. This regulatory system is termed the hypothalomo-pituitary-adrenal (HPA) axis (38). Under physiological conditions, the neuroendocrine neurons in the hypothalamus synthesize and secrete corticotropin-releasing hormone (CRH), which subsequently acts on the pituitary gland, causing release of adrenocorticotropic hormone (ACTH). ACTH is transported to the adrenal gland, where it stimulates secretion of glucocorticoids (Fig. 1). Increased glucocorticoids can negatively feed back and inhibit the hypothalamus and pituitary (38).

Following HPA-mediated release, glucocorticoids undergo a further intracellular conversion in peripheral tissues (161) (Fig. 1). In this process, active cortisol is converted to its inactive form, cortisone; cortisone can also be converted to cortisol (Fig. 1). This conversion mediates the tissue-specific actions of glucocorticoids. 11β-Hydroxysteroid dehydrogenase (11β-HSD), a family of microsomal enzymes, plays a crucial role in this transformation (167). Two separate isoforms of 11β-HSD have been identified in mammalian tissues (161). 11β-HSD2 is highly expressed in classical aldosterone-selective target tissues, such as the kidney. This enzyme shows a high NAD-dependent dehydrogenase activity and rapidly inactivates glucocorticoids, allowing aldosterone access to mineralocorticoid receptors (92, 198). Unlike 11β-HSD2, 11β-HSD1 is widely expressed in insulin target tissues, such as liver, adipose tissue, and central nervous system (142). Although 11β-HSD1 shows both dehydrogenase and reductase activities in vitro (110), in vivo experiments demonstrate only its reductase action (80), which converts intracellular cortisone to cortisol in the human body and 11-dehydrocorticosterone to active corticosterone in rodents (161).

The biological action of cortisol occurs when it is in the free form. However, the majority of cortisol in the circulation is bound with corticosteroid-binding globulin (CBG, 90%) and albumin (6%) (44, 195) (Fig. 1). CBG is a 383-amino acid glycoprotein, present not only in the blood but also in tissues (17). Intracellular CBG can be localized in the cells through transmembrane uptake by a membrane CBG receptor, or it can be synthesized in extrahepatic organs, like lung, ovary, and endometrium (17). The binding of cortisol to CBG restricts the access of this glucocorticoid to target cells (plasma CBG) or serves to mediate intracellular cortisol action (intracellular CBG) (17). Both cortisol and its inactive form cortisone are metabolized by hepatic A-ring reductases and eventually form 5α- and 5β-tetrahydrocortisol (5α- and 5β-THF) and 5β-tetrahydrocortisone (THE) (2) (Fig. 1). The kidney excretes 95% of these metabolites, and the gut eliminates the remainder (159).

Cellular mechanism of glucocorticoid action. The molecular mechanisms of glucocorticoids have been extensively studied (155). These hormones can readily cross cellular membranes and bind with glucocorticoid (GR) or mineralocorticoid (MR) receptors in the cytosol. GR is a ligand-activated transcription factor, which is associated with “accessory proteins,” for example heat shock proteins (HSP90, p60/Hop, HSP70), to form a protein complex in the absence of ligand binding (41) (Fig. 2). Following binding with glucocorticoids, GR is activated through a conformational shift and dissociation of HSPs (Fig. 2). The complex of receptor and hormone subsequently migrates into the nucleus through a nuclear pore. Homodimer of GR interacts with a specific sequence on the promoter of its target gene, called glucocorticoid response elements, causing an increase or decrease in gene expression (159) (Fig. 2). In addition, glucocorticoid-activated monomeric GR is known to induce a nontranscriptional effect through interacting with some protein factors, like nuclear factor-κB (NF-κB) and activating protein-1 (AP1). This mechanism may play a key role in their anti-inflammatory effects (5) (Fig. 2). In addition to their genomic effects, glucocorticoids also act via a non-genomic mechanism, which only takes a few seconds to minutes (25, 68). This nongenomic action does not require glucocorticoids to bind with their classical intracellular steroid receptors and is mediated by transmembrane receptors. During this nongenomic mechanism, numerous cellular processes are activated, including mitogen-activated protein kinases (MAPKs), adenyl cyclase (AC), protein kinase C (PKC), and heterotrimeric guanosine triphosphate-binding proteins (G proteins) (25) (Fig. 2).

Insulin Resistance and Glucocorticoids

In the body, blood glucose levels are determined mainly through the balance between insulin-dependent processes like

---

**Fig. 1.** Cortisol secretion, regulation and metabolism. In the human body, the main endogenous glucocorticoid is cortisol and its basal daily secretion is ~6–8 mg/m². Endogenous glucocorticoid synthesis and release is regulated by the hypothalomo-pituitary-adrenal (HPA) axis. Under physiological conditions, the neuroendocrine neurons in the hypothalamus synthesize and secrete corticotropin-releasing hormone (CRH), which subsequently acts on the pituitary gland, causing release of adrenocorticotropic hormone (ACTH). ACTH is transported to the adrenal gland where it stimulates secretion of glucocorticoids (Fig. 1). Increased glucocorticoids can negatively feed back and inhibit the hypothalamus and pituitary (38). Following HPA-mediated release, glucocorticoids undergo a further intracellular conversion in peripheral tissues (161) (Fig. 1). In this process, active cortisol is converted to its inactive form, cortisone; cortisone can also be converted to cortisol (Fig. 1). This conversion mediates the tissue-specific actions of glucocorticoids. 11β-Hydroxysteroid dehydrogenase (11β-HSD), a family of microsomal enzymes, plays a crucial role in this transformation (167). Two separate isoforms of 11β-HSD have been identified in mammalian tissues (161). 11β-HSD2 is highly expressed in classical aldosterone-selective target tissues, such as the kidney. This enzyme shows a high NAD-dependent dehydrogenase activity and rapidly inactivates glucocorticoids, allowing aldosterone access to mineralocorticoid receptors (92, 198). Unlike 11β-HSD2, 11β-HSD1 is widely expressed in insulin target tissues, such as liver, adipose tissue, and central nervous system (142). Although 11β-HSD1 shows both dehydrogenase and reductase activities in vitro (110), in vivo experiments demonstrate only its reductase action (80), which converts intracellular cortisone to cortisol in the human body and 11-dehydrocorticosterone to active corticosterone in rodents (161).

The biological action of cortisol occurs when it is in the free form. However, the majority of cortisol in the circulation is bound with corticosteroid-binding globulin (CBG, 90%) and albumin (6%) (44, 195) (Fig. 1). CBG is a 383-amino acid glycoprotein, present not only in the blood but also in tissues (17). Intracellular CBG can be localized in the cells through transmembrane uptake by a membrane CBG receptor, or it can be synthesized in extrahepatic organs, like lung, ovary, and endometrium (17). The binding of cortisol to CBG restricts the access of this glucocorticoid to target cells (plasma CBG) or serves to mediate intracellular cortisol action (intracellular CBG) (17). Both cortisol and its inactive form cortisone are metabolized by hepatic A-ring reductases and eventually form 5α- and 5β-tetrahydrocortisol (5α- and 5β-THF) and 5β-tetrahydrocortisone (THE) (2) (Fig. 1). The kidney excretes 95% of these metabolites, and the gut eliminates the remainder (159).

Cellular mechanism of glucocorticoid action. The molecular mechanisms of glucocorticoids have been extensively studied (155). These hormones can readily cross cellular membranes and bind with glucocorticoid (GR) or mineralocorticoid (MR) receptors in the cytosol. GR is a ligand-activated transcription factor, which is associated with “accessory proteins,” for example heat shock proteins (HSP90, p60/Hop, HSP70), to form a protein complex in the absence of ligand binding (41) (Fig. 2). Following binding with glucocorticoids, GR is activated through a conformational shift and dissociation of HSPs (Fig. 2). The complex of receptor and hormone subsequently migrates into the nucleus through a nuclear pore. Homodimer of GR interacts with a specific sequence on the promoter of its target gene, called glucocorticoid response elements, causing an increase or decrease in gene expression (159) (Fig. 2). In addition, glucocorticoid-activated monomeric GR is known to induce a nontranscriptional effect through interacting with some protein factors, like nuclear factor-κB (NF-κB) and activating protein-1 (AP1). This mechanism may play a key role in their anti-inflammatory effects (5) (Fig. 2). In addition to their genomic effects, glucocorticoids also act via a non-genomic mechanism, which only takes a few seconds to minutes (25, 68). This nongenomic action does not require glucocorticoids to bind with their classical intracellular steroid receptors and is mediated by transmembrane receptors. During this nongenomic mechanism, numerous cellular processes are activated, including mitogen-activated protein kinases (MAPKs), adenyl cyclase (AC), protein kinase C (PKC), and heterotrimeric guanosine triphosphate-binding proteins (G proteins) (25) (Fig. 2).

Insulin Resistance and Glucocorticoids

In the body, blood glucose levels are determined mainly through the balance between insulin-dependent processes like
Glucocorticoids, insulin resistance, and cardiac metabolism

Glucocorticoids can readily cross cellular membranes and bind with glucocorticoid receptor (GR) in the cytosol. Under inactivated conditions, GR is associated with heat shock proteins (HSP) to form a protein complex. Following binding with glucocorticoids, GR is activated through a conformational shift and dissociation of HSPs. The complex of receptor and hormone subsequently migrates into the nucleus through a nuclear pore. Homodimer of GR interacts with a specific sequence on the promoter of its target gene, which is usually called glucocorticoid response elements (GRE), causing an increase or decrease in gene expression. In contrast, glucocorticoid-activated monomeric GR is known to induce a nongenomic effect through interacting with some protein factors, like NF-κB and activating protein-1 (AP-1). In addition to their genomic effects, glucocorticoids also act via a nongenomic mechanism, which only takes a few seconds to minutes. This nongenomic action does not require glucocorticoids to bind with their classical intracellular steroid receptors and is mediated by transmembrane receptors. During this nongenomic mechanism, numerous cellular processes are activated including mitogen-activated protein kinases (MAPKs), adenylyl cyclase (AC), PKC, and heterotrimeric G proteins.

Insulin resistance is a condition in which normal insulin secretion from the pancreas is insufficient to induce a biological response in these peripheral tissues. Once this disorder occurs, excess insulin is secreted from the pancreas to maintain blood glucose. Insulin resistance is associated with a large number of risk factors that also contribute to the incidence of type 2 diabetes (65). These include a family history of diabetes or gestational diabetes (76), sedentary lifestyle (171), high circulating FA (9), reduced physical activity, aging (54), tobacco smoking (35), or drugs such as steroids (170).

Glucocorticoids, as endogenous hormones and prevalent anti-inflammatory and immunosuppressive drugs, have been reported to induce Cushing’s syndrome, which is characterized by central obesity and insulin resistance (159). Some more recent observations have also indicated that endogenous glucocorticoids play a key role in the incidence and development of the metabolic syndrome (111, 190). In addition, chronic treatment with synthetic glucocorticoids like dexamethasone (DEX) has been associated with hyperinsulinemia in both animal and human research (13, 151). In our laboratory, even though acute DEX injection (4 h) in rats was not associated with hyperinsulinemia, the euglycemic hyperinsulinemic clamp still showed a decrease in glucose infusion rate, suggesting the presence of whole body insulin resistance. Adrenalectomy is effective in improving insulin sensitivity in patients with hypercortisolism (119) and in Zucker fatty rats (57). Blockade of GRs by a specific inhibitor, RU-486, was able to abolish high-fat-induced insulin resistance (89). Taken together, these findings now support the possibility that systems that regulate glucocorticoids, including GR, 11β-HSD1, and CBG, all play an important role in the incidence and progression of insulin resistance.

GRs and insulin resistance. GR plays a crucial role in the regulation of glucocorticoid effects. This cytoplasmic receptor family has been identified and includes three isoforms, α, β and γ, but only the GRα isopform modulates the expression of glucocorticoid response element (GRE) (149). The GR gene lies on the long arm of chromosome 5, and its sequence occupies ~80 kb of genomic DNA (46, 56). A positive correlation has been recognized between the expression of GRα and levels of insulin resistance. For example, human skeletal muscle cells with high expression of GR suggested a reduction of insulin sensitivity (196). In patients, high levels of skeletal muscle GR mRNA were associated with hypertension and insulin resistance measured by a euglycemic hyperinsulinemic clamp (196). Mutations in the GR gene lead to a decreased glucocorticoid-binding affinity (149). Although many of these mutations have been identified as being beneficial to insulin sensitivity, there are still some mutations in the GR gene that are believed to play a role in the incidence and progression of the metabolic syndrome (149). For instance, a well-known GR mutation, Bcl/I polymorphism, has been suggested to be associated with hyperinsulinemia, visceral obesity, and hypertension (21, 192). Currently, the mechanism involved in this relationship is still unclear. Although the mechanism of hormone-independent activation is currently a subject of great interest (193), GR has been suggested to be refractory to ligand-independent activation. Unlike progesterone and estrogen receptors, GR is not activated or phosphorylated via cross talk with other signal transduction pathways (116, 132). Therefore, the mechanism of glucocorticoid-independent activation is currently not considered to be a major mechanism in the development of insulin resistance. GRβ is one of the GR isoforms, but unlike GRα, it does not bind with any glucocorticoid ligands, and therefore it fails to regulate gene transcription (141). Normally, this isoform binds with GRα and interferes with its function. This unique action will limit the sensitivity of glucocorticoids (141). Currently, it is still unclear whether GRβ plays a role in the development of glucocorticoid-induced insulin resistance.

11β-HSD1 and insulin resistance. 11β-HSD1 potentially contributes to obesity and insulin resistance in both rodents (103, 111) and humans (85, 98). This effect is probably related to its role in augmenting intracellular glucocorticoid action (161). 11β-HSD1 activity increases in adipose tissue and decreases in the liver in several genetic obese animal models, such as obese Zucker rats (102) and ob/ob mice (101). A similar elevation of 11β-HSD1 and an increased generation of cortisol from cortisone in skeletal muscle (197) and adipose tissue (138, 139) have also been identified in obese insulin-resistant humans, although plasma concentration of cortisol is not above the normal range. In transgenic mice, hepatic 11β-HSD1 overexpression shows a modest insulin resistance, dyslipidemia, and hypertension but no obesity (126). The effects
of selective adipose overexpression are more pronounced, with these animals demonstrating symptoms typically seen with the metabolic syndrome (111). On the contrary, whole body knockout of this enzyme (114, 115) or overexpression of 11β-HSD2 (86) in mouse adipose tissues exhibited improved insulin sensitivity and resisted the development of visceral obesity on exposure to a high-fat diet. Interestingly, administration of carbenoxolone (CBX), an inhibitor of 11β-HSD1 (and to some extent 11β-HSD2), increased glucose utilization and uptake in the liver and led to an enhanced glucose infusion during euglycemic hyperinsulinemic clamps in healthy (189) and nonobese type 2 diabetic humans (1). However, because CBX had no effect on 11β-HSD1 in adipose tissue, it did not improve insulin resistance and obesity in obese Zucker rats (104). A more recent study tested another 11β-HSD1 inhibitor, “compound 544,” and suggested that it lowered body weight and plasma glucose and insulin in obese mice (70). Although the association between 11β-HSD1 and insulin resistance has been consistently observed in monogenic obese models and in different groups of obese subjects, it is still too early to determine the role of 11β-HSD1 in the incidence and progression of insulin resistance in humans. Thus, a clinical study using obese subjects failed to confirm an increase in 11β-HSD1 in adipose tissue (177). In addition, the presence of insulin resistance induced by high-fat feeding, even though adipose 11β-HSD1 dropped, also challenges this conclusion (43). Finally, weight loss increases 11β-HSD1 expression in human adipose tissues (176). Collectively, these studies imply that 11β-HSD1 is likely not a key mechanism in all conditions of insulin resistance.

**CBG and insulin resistance.** As CBG restricts free glucocorticoids in the circulation and alters the quantity of hormone reaching target tissues, its levels are negatively correlated with glucocorticoid activity (17) and even insulin resistance (51). The effect of CBG is mainly dependent on its cortisol-binding capacity, affinity, and/or specificity. In animal experiments, tissue-specific reduction of CBG has been reported to augment free local corticosterone (64). Several genetic mutations induce a marked reduction of CBG affinity in humans (45, 180). However, this decreased affinity still maintained relatively normal serum-free cortisol concentrations. This result suggested that the decreased CBG affinity not only increases free cortisol release but may also stimulate the negative regulation of the HPA axis (45). Under the conditions of CBG deficiency, increased local glucocorticoids stimulate cellular proliferation and differentiation in adipose tissue (82). Therefore, in the human population, the reduction of CBG is inversely correlated with BMI, waist-to-hip ratio, and insulin resistance (51). Interestingly, overexpression of CBG in target organs cannot produce a beneficial effect in insulin resistance, as an increase in local CBG capacity will produce a compensatory response from the HPA axis, leading to increased total and free glucocorticoid levels (125). It should be noted that, unlike cortisol, most synthetic glucocorticoids (except prednisolone) have a low affinity to CBG, and they are normally bound to albumin (159). Therefore, CBG mediation has a low possibility of occurring in those insulin resistance models induced by synthetic glucocorticoid treatment.

**Glucocorticoids and Peripheral Tissue Metabolism**

Glucocorticoid-induced whole body insulin resistance is tightly correlated to its metabolic effects in individual organs. Currently, most investigations on glucocorticoids and peripheral metabolism have targeted skeletal muscle, liver, and adipose tissue. The metabolic events that occurred in these tissues, for example, decreased glucose utilization and increased glucose output and lipogenesis, play a key role in the incidence of whole body abnormalities in the metabolic syndrome. This section will briefly highlight the mechanisms of glucocorticoid effects on skeletal muscle, liver and adipose tissue metabolism.

**Skeletal muscle.** Skeletal muscle accounts for 80% of insulin-induced glucose disposal in the human body (37); thus, it is a major target for glucocorticoid-induced insulin resistance. In skeletal muscle, insulin stimulates glucose uptake, utilization, and storage. As cortisol administration did not alter the number of insulin receptors in skeletal muscle (15), it is likely that glucocorticoids alter glucose metabolism through its postreceptor effects on downstream insulin signaling or glucose utilization. Following chronic DEX treatment, even though its gene expression is unchanged, the phosphorylation of Akt/protein kinase B (PKB) induced by insulin significantly decreases (151). This reduction in insulin signal is paralleled by a decreased glucose uptake and disposal (39, 151, 183). The decreased PKB phosphorylation may be attributed to decreased insulin receptor tyrosine phosphorylation and insulin receptor substrate (IRS) protein expression (62). Interestingly, the reduction of glucose uptake in skeletal muscle was unrelated to alteration of glucose transporters GLUT1 and GLUT4 (175). Both total mRNA and content of GLUT1 in skeletal muscle remained unchanged following DEX (175). With GLUT4, although total protein and its functional fraction at the plasma membrane have been demonstrated to be normal or even increased (32, 67, 117), insulin-stimulated GLUT4 transport in soleus muscle decreases following DEX treatment (39, 194). These results suggest that glucocorticoids decrease glucose transport in skeletal muscle through a lowering of insulin-stimulated GLUT4 translocation. With regard to glycogen synthesis, both an increase and a decrease in skeletal muscle have been reported following DEX (39, 50, 151). Pyruvate dehydrogenase kinase-4 (PDK-4) is known to inactivate pyruvate dehydrogenase (PDH), a key enzyme that regulates pyruvate uptake into mitochondria, followed by oxidation (73). Glucocorticoid treatment in mice induced gene expression of forkhead-type transcription factor (FOXO) in skeletal muscle, which potentially upregulates PDK-4 and decreases glucose oxidation (60).

Decreased glucose metabolism is often correlated with elevation in FA metabolism. Thus, glucocorticoid treatment significantly increases FA transporter (CD36) gene expression (88) in gastrocnemius and FA oxidation in diaphragm muscle (183). However, the effect of glucocorticoids in controlling skeletal FA metabolism has yet to be completely elucidated. Peroxisome proliferator-activated receptor-α (PPARα) is an early-response gene of glucocorticoids (95) that plays a key role in the regulation of FA metabolism. Recent studies have indicated that PPARα also contributes toward development of insulin resistance. PPARα null mice were protected from diet-and glucocorticoid-induced insulin resistance (52). In contrast, overexpressed PPARα mice exhibited an augmented FA oxi-
Adipose tissue. Similar to skeletal muscle, glucose utilization in adipose tissue also affects whole body glucose disposal. Following DEX treatment, a GR-dependent and transcription-independent mechanism that attenuates insulin signal has been identified recently in cultured adipocytes (107). The decreased insulin sensitivity was attributed to postreceptor signaling defects (156). Thus, incubation with DEX significantly inhibits total mRNA and tyrosine phosphorylation of IRS-1 (156, 181). The decreased IRS-1 reduces activation of phosphatidylinositol 3-kinase (PI 3-kinase), which plays a key role in the regulation of GLUT4 transport (156). Although the total amount of GLUT4 protein in 3T3-L1 adipocytes was unchanged, basal and insulin-induced transport of GLUT4 decreased following DEX (156). Total GLUT1 protein decreased in this experiment (156). Interestingly, even though IRS-1 and PI 3-kinase were normalized via IRS-1 overexpression, insulin-induced impairment of glucose uptake by DEX did not significantly improve (156). The authors concluded that glucocorticoids might decrease glucose uptake through their inhibition of glucose transport rather than insulin signal transduction. A more recent investigation extended this research and found that DEX probably inhibits the activation of GLUT4 in the plasma membrane through a p38 MAPK process (8).

In addition to affecting glucose metabolism, glucocorticoids also play a key role in regulating lipid metabolism in adipose tissue. Glucocorticoids stimulate adipose differentiation and increase body fat mass. Thus, Cushing’s syndrome is normally characterized by central obesity. This increased visceral fat could indirectly increase 11β-HSD and reinforce local metabolic effects of glucocorticoids. Adipocyte differentiation and proliferation is also known to be associated with expression of PPARγ. In vitro experiments indicated that DEX treatment triggered expression of PPARγ in adipocytes (205). This increased PPARγ regulates a number of downstream genes, like fatty acid-binding protein (178), adipocyte fatty acid binding-protein (aP2) (179), lipoprotein lipase (LPL) (83), diacylglycerol (184), and leptin (71,84), thereby contributing toward lipid accumulation. More recently, GR has been identified to cross-talk with PPARγ in its anti-inflammatory effects (118). Whether this combination can also occur in adipogenesis is currently unknown. It should be noted that not only do glucocorticoids mediate PPARγ, but PPARγ also affects glucocorticoid activity. A PPARγ agonist, thiazolidinediones, inhibits 11β-HSD1 and decreases the conversion of cortisone to cortisol in adipocytes (11). Glucocorticoids also influence adipokine release from adipose tissue, including adiponectin, resistin (136), and leptin (19). However, the role that these hormones play in the development of glucocorticoid-induced insulin resistance has yet to be completely elucidated (69) (185) (136).

As an early marker of differentiation and an important determinant of triglyceride (TG) storage in adipose tissue, LPL catalyzes circulating lipoprotein hydrolysis and facilitates uptake of FA into adipose tissue (112), which is eventually reesterified and stored as TG. Following DEX per se or DEX plus insulin treatment, both total mRNA and activity of LPL significantly increased in animal and human adipose tissue (58, 120). Adipose tissue from prednisolone-treated patients also indicated that the increased LPL activity following glucocorticoids might be a result of inhibiting degradation of the active-dimeric form (87). It should be noted that increased LPL in adipose tissue is not always found consistently, and opposite effects are observed in both intact animal and isolated cells (121). TG content in adipose tissue is also dependent on TG assembly from acyl-CoA and glycerol, a process mediated by acyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (172). Following DEX treatment, FAS expression, activity, and gene transcription rate were significantly enhanced in human adipose tissue (191). However, this de novo lipogenesis is minor compared with FA uptake derived from plasma lipoprotein (40). Under physiological conditions, insulin decreases lipolysis in adipose tissue through the inhibition of hormone-sensitive lipase (HSL) (105). HSL can be phosphorylated and activated through stimulating a cAMP-dependent protein kinase (72). However, DEX is known to increase HSL activity in rat adipocytes through an increase in mRNA (165). This increase in HSL augments lipolysis, which could contribute to the development of insulin resistance, hypertension, and hyperlipidemia.

Liver. Liver is a key organ that contributes to insulin resistance through increasing glucose output. The liver is also the primary metabolic target of glucocorticoid action. A positive relationship has been proposed between glucocorticoid effects in the liver and whole body insulin resistance. Specific inactivation of hepatic GRs reduces elevated glucose output and improves hyperglycemia and hyperlipidemia in streptozotocin (STZ)-induced diabetic (122) and type 2 diabetic animal models (79, 97). Unlike other tissues, altered hepatic glucose metabolism following glucocorticoids involves the enhancement of glucose output and reduction of glucose utilization (66). DEX treatment does not change insulin receptor and IRS-1 (34), but it decreases PI 3-kinase activity in the liver. Whether this decreased PI 3-kinase contributes to a reduction of GLUT4 is currently unknown. Following glucose uptake and glycolysis, the PDH complex (PDC) facilitates entry of pyruvate into the mitochondria for subsequent oxidation. PDK inactivates PDC through phosphorylation of this enzyme. In cultured hepatoma cell lines, DEX treatment significantly increases PDK-4 gene and protein expression, which can be reversed by insulin (74). The FOXO may participate in the stimulation of PDK-4 (90). Following DEX treatment, activated GR and p300/CBP complex are recruited to the PDK-4 gene. FOXO factors in by interacting with p300/CBP complex to activate PDK-4 gene expression (90). Some more recent studies also demonstrated that the PPARγ coactivator (PGC-1α) might also be involved in the activation of PDK-4 (108). However, unlike diabetes and fasting, glucocorticoids do not directly stimulate PDK-4 gene expression through recruiting PGC-1α on the promoter (108). Interestingly, FOXO1 plays some role in the recruitment of PGC-1α (108). Whether this indirect recruitment contributes to PDK-4 gene expression is currently unclear. The inactivated PDC through PDK inhibits glucose utilization and switches the liver to synthesize glucose and store glycogen. Indeed, in vivo experiments, DEX treatment increases glycogen in the liver (61). The elevated hepatic gluconeogenesis is associated with
the effects of glucocorticoids on the rate-limiting enzymes, like phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) (3, 59). Glucocorticoids enhance the gene expression of PEPCK and G-6-Pase, resulting in increased glucose output from the liver, which contributes to whole body insulin resistance (3, 59). Activation of PPARα also plays a key role in glucocorticoid-regulated gluconeogenesis. Thus, following DEX treatment, knockout of PPARα shows unchanged hepatic PEPCK and G-6-Pase, whereas reconstitution of PPARα increases these enzymes (12). Apart from PPARα, PGC-1α is another important regulator of gluconeogenesis. However, its expression appears to be regulated by PPARα. Overexpressed PGC-1α in liver caused hyperglycemia in mice with PPARα expression, but not in PPARα null animals (12).

In addition to altering glucose metabolism, glucocorticoids also promote hepatic TG storage. Normally, the TG level reflects the balance between lipogenesis and lipolysis. Some early studies have suggested that hepatic stored TG undergoes lipolysis to release FA, which is reesterified to form TG in the endoplasmic reticulum. This resynthesized TG eventually incorporates into a VLDL particle with apolipoproteins (91, 199). In this process, triglyceride hydrolase (TGH) is a key enzyme that regulates lipolysis (94), whereas diacylglycerol acyltransferase (DGAT) catalyzes the final stage of TG synthesis (24, 166). Following DEX treatment, a decrease in the expression of TGH and an increase in DGAT2 activity occurs in the liver, which is coupled with an amplified TG storage (42). Whether this increased TG storage promotes lipoprotein secretion is still controversial.

**Glucocorticoids and Cardiac Metabolism**

**Normal cardiac metabolism.** To maintain normal physiological function, heart needs to consistently produce energy in the form of ATP. This procedure may utilize various substrates, like FA, glucose, lactate, and ketone bodies, in which glucose and FA are the most important substrates consumed by cardiac tissue (123, 124, 137). Glucose oxidation provides the heart with ~30% of its energy requirements (18). Following insulin-dependent glucose uptake and glycolysis, PDC facilitates pyruvate translocation and subsequent oxidation in the mitochondria. PDP activates, whereas PDK inactivates PDC, with resultant augmentation or inhibition of glucose oxidation, respectively (18) (Fig. 3). Compared with glucose, FAs are the preferred substrate consumed by cardiac tissue. FA is mainly derived through three pathways: 1) release from adipose tissue and transport to the heart after complexing with albumin (152), 2) provision through the breakdown of endogenous cardiac TG stores (127), and 3) hydrolysis of TG-rich lipoproteins by LPL positioned at the endothelial surface of the coronary lumen (14). Of these mechanisms, LPL-facilitated TG hydrolysis is suggested to be the principal source of FA for cardiac utilization (4) (Fig. 3).

**Glucocorticoids and glucose metabolism in the heart.** Glucocorticoids play a key role in the regulation of glucose metabolism in the heart. Although incubation of cardiomyocytes with corticosteroids for 24 h increased both GLUT1 and GLUT4 gene expression (27), most in vivo investigations suggest that glucocorticoids may not affect insulin-regulated glucose transport in the heart (32, 117). Hence, following chronic DEX treatment, GLUT4 does not show any changes at the plasma membrane (32, 117). Recently, our studies indicated that acute DEX injection is associated with unchanged glycolysis, suggesting that glucocorticoids do not affect glucose uptake (135). It should be noted that, although DEX had no effects on glucose transport, glucose oxidation was decreased following DEX (135). This decreased glucose utilization is probably more correlated with downstream regulation in the glucose metabolic pathway. A possible target, which is affected by glucocorticoids, is PDK, an enzyme that inactivates PDC and subsequently attenuates glucose oxidation. Of the four different isoforms of PDK that have been identified, PDK-2 and -4 are the main isoforms present in the heart (16). Our previous studies reported that the decreased rate of glucose oxidation following DEX was associated with augmented expression of PDK-4 mRNA and protein but without effect on the basal level of PDK-2 (135). Apart from increased PDK, a reduction in PDP may also be important in the glucocorticoid-mediated glucose metabolism. This enzyme has an attenuated activity in the heart from STZ-induced diabetic animals (75). Whether it also plays a role in explaining the effect of DEX on cardiac glucose metabolism is currently unknown.

![Cardiac metabolism](http://ajpendo.physiology.org/)
Glucocorticoids and Cardiac FA Metabolism

FA contributes ~70% of the ATP necessary for normal heart function (106, 182). During metabolic stress, such as diabetes and insulin resistance characterized by inadequate glucose utilization, cardiac FA consumption supersedes glucose oxidation. As an insulin resistance model, with decreasing glucose oxidation, elevated glucocorticoids are also associated with a dysfunction of FA metabolism in the heart (137). In the following sections, the effects of glucocorticoids on FA delivery, oxidation, and composition will be discussed.

FA delivery. Cardiac FA can be derived from three sources, as mentioned previously. Of these, FA released from TG-rich lipoproteins catalyzed by coronary luminal LPL is currently believed to be the most important for cardiac utilization (4). Endothelial cells do not synthesize LPL (23); hence, the enzyme is synthesized in cardiomyocytes (14). Secreted LPL binds to myocyte cell surface heparan sulfate proteoglycans (HSPGs) before it is translocated onto comparable HSPG binding sites on the luminal side of the vessel wall (14, 157, 168). According to in vitro experiments, DEX treatment per se does not increase LPL release from cardiomyocytes, whereas the combination of DEX and insulin significantly enhances LPL secretion from the cells (47, 48). Using an acute-DEX model, our laboratory has reported that glucocorticoid treatment leads to enlargement of the coronary LPL pool (135). This effect is associated with the enhanced LPL mRNA expression (135). More recently, our studies also indicated that DEX not only increases LPL on the coronary lumen but also augments basal and post-heparin plasma lipolytic activity (134). This finding suggests that DEX may produce transcriptional effects on LPL gene in multiple organs. Following acute DEX injection, the increased LPL is related to a progressive clearance of plasma TG (134, 135). Interestingly, this decreased TG level is not linked to changes in plasma FA (135). Given increased cardiac FA and TG following DEX (134, 135), our data suggest that augmented LPL facilitates FA delivery into the heart. It should be noted that the effects of glucocorticoids on the gene expression of FA transporters like CD36 might also contribute to this mechanism (27).

FA oxidation. According to the hypothesis of Randle et al. (137), FA competes with glucose for mitochondrial oxidation. Following the inhibition of glucose metabolism by glucocorticoids, FA oxidation in the heart is likely to be elevated. Corticosteroid treatment in cardiomyocytes increases the gene expression of carnitine palmitoyltransferase I (CPT I), which is an enzyme that regulates FA uptake into the mitochondria (27). Our recent investigation using an in vivo model demonstrates that 4- to 8-h DEX injection increases FA oxidation in the heart (134). This increased oxidation is associated with a number of mechanisms. One of them, reported by our laboratory, is the activation of AMP-activated protein kinase (AMPK) (134). AMPK, a heterotrimeric enzyme, is known to promote FA oxidation (202). DEX, through transcriptional regulation, augments total AMPK protein, and thus phosphorylation (134). This increased phosphorylation of AMPK then increases ACC phosphorylation, and cardiac palmitate oxidation (134). In addition to AMPK, PPARα may also play a role in the increased oxidation of FA following glucocorticoids. PPARα can be activated by a high level of FA. However, in our previous study (134), the enhanced FA following DEX is not associated with any changes in PPARα gene. The short time after DEX exposure during this experiment may explain why PPARα expression did not change. However, this finding cannot exclude the possibility that DEX-induced metabolic changes were due to PPARα activation. A recent study in liver cells also indicated that FOXO1 plays a key role in the mediation of FA metabolism (7). This is probably through the effects of FOXO1 on mediating some gene expression like acyl-CoA oxidase (ACO) and PPARδ (7). In the heart removed from DEX-treated animals, total FOXO1 phosphorylation decreases and nuclear FOXO1 protein increases (unpublished data). This altered FOXO1 augments ACO gene expression (unpublished data), which may also contribute to increased FA oxidation.

FA composition. Increased FA delivery following glucocorticoids alters FA composition in the heart. Some saturated (palmitate) and single unsaturated (oleic) FAs are preferred substrates utilized by the heart. Thus, changes in their composition are tightly correlated with increased FA oxidation (134). Unlike saturated FA necessary for ATP generation, polysaturated FA are also required to manufacture and repair cell membranes and regulate functions like heart rate, blood pressure, and clotting (78, 128). In our recent study (134), following DEX, we reported a drop in cardiac linoleic (LA) and ω-linolenic acid (LNA), with an increase in arachidonic acid (AA; Table 1). Given the function of glucocorticoids to inhibit phospholipase A2, the increase in cardiac AA was unexpected (158). It is possible that, as DEX decreased LA and LNA over time, these FAs are either being oxidized or converted to AA. Other studies have reported that in rat testis, DEX can stimulate Δ-6 desaturase, the rate-limiting enzyme for converting linoleic acid to arachidonic acid (153). In our study, as DEX inhibited cardiac Δ-6 desaturase, it is likely that the decrease in LA and LNA is due to increased FA oxidation (134). At present, the mechanism for the increase in cardiac AA is unknown. Irrespective of the mechanism, excess amounts of AA are known to alter insulin signaling and sensitivity (78) and to induce cell death (130, 144) directly through the mitochondrial permeability transition (160) or indirectly through con-
version of AA to toxic byproducts like epoxyeicosatrienoic acids (26, 93). Unlike ω-6 FA, DEX had limited effects on ω-3 FA like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reported to protect heart from cardiovascular disease (134) (Table 1).

It should be noted that most of the studies describing glucocorticoids and cardiac metabolism have used glucocorticoids for short periods of time. Whether these effects can be reproduced following chronic administration has yet to be determined.

Cardiac Metabolism and Heart Disease

Although promotion of atherosclerosis and other vascular diseases is commonly associated with diabetes and insulin resistance, increasing clinical and experimental evidence has established that metabolic abnormalities in the cardiomyocytes during glucose stress also play a crucial role in the development of heart diseases (96, 145). This specific cardiac phenotype is named “cardiomyopathy” (145). Diabetic cardiomyopathy is commonly initiated by a short-term and severe modification in fuel metabolism and is followed by a chronic myocardial damage and measurable contractile dysfunction (133). Although the mechanisms of cardiomyopathy are not completely understood, most investigators considered that this pathophysiological process is related to hyperinsulinemia, hyperglycemia, and increased FA (131). In general, hyperinsulinemia induces cardiac hypertrophy through insulin-mediated Akt-1-dependent and -independent pathways, whereas hyperglycemia mediates cardiac injury through the generation of reactive oxygen species (ROS) (131). As the most important risk factor, FA has been identified as triggering the development of cardiac hypertrophy via induction of insulin resistance or altering myocardial contractility and cell death (131).

Lipotoxicity and Cardiomyopathy

Overloaded FA contributes toward the initiation and development of cardiomyopathy. Thus, in rodent studies, specific elevation of FA uptake or utilization in the heart is associated with cardiac toxicity, in the absence of any systemic metabolic disturbances. Cardiac-specific overexpression of LPL or fatty acid transport protein-1 significantly increased FA delivery, with ensuing lipid storage, lipotoxic cardiomyopathy, and contractile dysfunction (28, 201). Augmented FA utilization by heart-specific overexpression of PPARα or ACS (acyl-CoA synthetase; an enzyme that converts FA to acyl-CoA for subsequent β-oxidation) brought about cardiomyopathy and cardiac dysfunction, similar to that seen during diabetes (29, 53). In another strategy, reducing FA supply or utilization prevented the development of cardiomyopathy in obese or diabetic animals. In ZDF (Zucker diabetic fatty rodent model of genetic obesity and type 2 diabetes) rats or transgenic mice with cardiac overexpression of LPL, a PPARγ agonist decreased plasma and cardiac intracellular lipids, and ameliorated cardiomyopathy (186, 204).

Lipid-induced cardiomyopathy is a complicated disorder, and several factors have been identified as being associated with its development following glucose stress (10, 49, 63, 145, 154, 173). Increased intracellular FA activates PPARα, which promotes the expression of genes involved in FA oxidation. Elevated FA oxidation increases mitochondrial action potential, leading to overproduction of ROS, cardiomyocyte damage, and cell death (apoptosis) (6, 22, 204). Another potential mechanism for lipotoxicity is accumulation of lipids, when FA uptake supercedes its oxidation, and TG are built up. Although a recent study has suggested that TG formation provides protection against the deleterious effects of fatty acyl-CoA (99), it has been established that this lipid storage is associated with lipotoxicity in both animal models and human studies (30, 164, 204). In general, changes in cardiac metabolism are observed before cardiomyopathy. For example, in STZ-induced diabetes, altered cardiac metabolism is observed as early as 4 days following diabetes induction (164), whereas evidence of cardiomyopathy is apparent only after 4–6 wk (146, 147). Recent studies also suggest that the pathophysiological process observed in diabetic heart might also occur following insulin resistance in animal models (20) and humans (200).

Glucocorticoids and Heart Disease

Due to their effects on inflammation and cellular proliferation, glucocorticoids have been considered beneficial in heart diseases (174). However, excessive endogenous (129, 150) and exogenous (163, 169) glucocorticoids are linked to insulin resistance. In addition, glucocorticoids per se have been implicated in the pathogenesis of cardiac diseases. Epidemiological studies suggest that atherosclerosis and myocardial infarction occur in patients with long-term glucocorticoid treatment or Cushing’s syndrome (33, 109, 187). Recent clinical reports also indicated that glucocorticoid treatment in newborn fetuses and aged patients potentially induced cardiomyopathy (113, 203). Although this pathological process is not yet completely elucidated, some early morphological evaluations indicated that glucocorticoid-induced cardiomyopathy is characterized by increased accumulation of lipid droplets, cardiomyocyte hypertrophy, and dissolution of myofibrils (31, 36). The role of glucocorticoid signal in the development of cardiomyopathy is less clear. One investigation suggests that glucocorticoid-induced cardiomyocyte hypertrophy is probably related to cross talk between glucocorticoid signaling and hypertrophic signaling pathways (100). The enhanced glucocorticoid signal up-

![Fig. 4. Glucocorticoids and cardiac FA metabolism. Glucocorticoid treatment leads to enlargement of coronary and plasma LPL activity. This effect is probably associated with the enhanced mRNA expression in multiple organ systems. This increased LPL facilitates FA delivery into the heart, which subsequently augments FA oxidation and alters FA composition. Glucocorticoids, through transcriptional regulation, activate AMPK or FOXO1, which then upregulate cardiac FA oxidation. Augmented FA utilization also contributes to the alteration of FA composition. Finally, glucocorticoid treatment induced a drop in cardiac linoleic and γ-linolenic acid, with an increase in arachidonic acid (AA). This increased AA accumulation may produce a toxic effect in cardiomyocytes.](http://ajpendo.physiology.org/)

AJP-Endocrinol Metab • VOL 292 • MARCH 2007 • www.ajpendo.org
regulates serum- and glucocorticoid-induced kinase-1, which may augment α-adrenergic-induced hypertrophy (100). Additionally, exogenous glucocorticoid treatment has been reported to induce mitochondrial dysfunction in both liver and muscle tissues (143). Whether this alteration in ATP production and mitochondrial genes also occurs in the heart following glucocorticoids is currently unknown.

Summary

Glucocorticoids have been recognized to play a key role in the development of insulin resistance. This whole body abnormality is associated with altered metabolisms in multiple organ systems, such as skeletal muscle, liver, and adipose tissues. These metabolic alterations and whole body insulin resistance are widely believed to be important factors in the morbidity and mortality of cardiovascular disease. More importantly, clinical and experimental studies have established that cardiac metabolic abnormalities per se play a crucial role in the development of heart failure. Following administration of glucocorticoids, glucose oxidation in the heart is compromised due to augmentation of PDK-4, whereas amplification of LPL increases lipoprotein triglyceride clearance, likely providing the heart with excessive FA that are then stored as intracellular triglyceride, and alters cardiac FA composition (Fig. 4). In the heart, elevated FA use has been implicated in a number of metabolic, morphological, and mechanical changes and, more recently, in “lipotoxicity.” During lipotoxicity, when the capacity to oxidize FA is saturated, FAs accumulate and can, either by themselves or via production of second messengers such as ceramides, provoke cell death.

REFERENCES


134. Saito F, Kawaguchi M, Izumida J, Asakura T, Maehara K, Maruyama Y. Alteration in haemodynamic and pathological changes in the...


