The Hypothalamo–Pituitary–Adrenocortical Axis – An Overview of the Role of Glucocorticoids in the Pathophysiology of Endocrine Disorders and Perspectives for the Future

Christopher D John[1] and Julia C Buckingham[2]

1. Senior Lecturer in Pharmacology; 2. Pro-Rector for Education and Academic Affairs and Professor of Pharmacology, Department of Medicine, Imperial College London

Abstract

Glucocorticoids (GCs) are the end products of the hypothalamo–pituitary–adrenocortical axis (HPA) and, via activation of the ubiquitously expressed GC receptor, influence numerous physiological processes. GCs are also involved in the regulation of basal homeostasis as well as mediating adaptive responses to stress that act to restore homeostasis. This article discusses the various factors that are important in regulating plasma and intracellular GC concentrations and describes the genomic and non-genomic mechanisms used by GCs to influence cellular processes. We describe the concept of allostatic overload associated with chronic HPA activation and the subsequent development of tissue dysfunction and disease. While allostasis is associated with acute stress and a restoration of homeostasis, chronic stress is likely to induce allostatic overload owing to the sustained activation of adaptive processes. Increased wear and tear in GC-sensitive tissues can eventually lead to tissue dysfunction and disease. Chronic elevations in GCs can also induce dysfunction or disease associated with decreased tissue function owing to the prolonged inhibitory effects of GCs or the redistribution of metabolic resource away from physiological systems not involved in restoring homeostasis. Numerous endocrine-related disorders are associated with aberrant GC levels and in terms of pathophysiology may be linked with chronic tissue-specific alterations in GC actions.

Keywords

Hypothalamo–pituitary–adrenocortical axis, glucocorticoids, allostasis, allostatic overload, endocrine disorders

Disclosure: The authors have no conflicts of interest to declare.
Received: 5 October 2010 **Accepted:** 2 February 2011 **Citation:** *European Endocrinology*, 2011;7(1):47–52 DOI:10.17925/EE.2011.07.01.47
Correspondence: Christopher D John, Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Commonwealth Building, Imperial College London, Hammersmith Campus, Du Cane Road, London, W12 0NN, UK. E: c.john@imperial.ac.uk

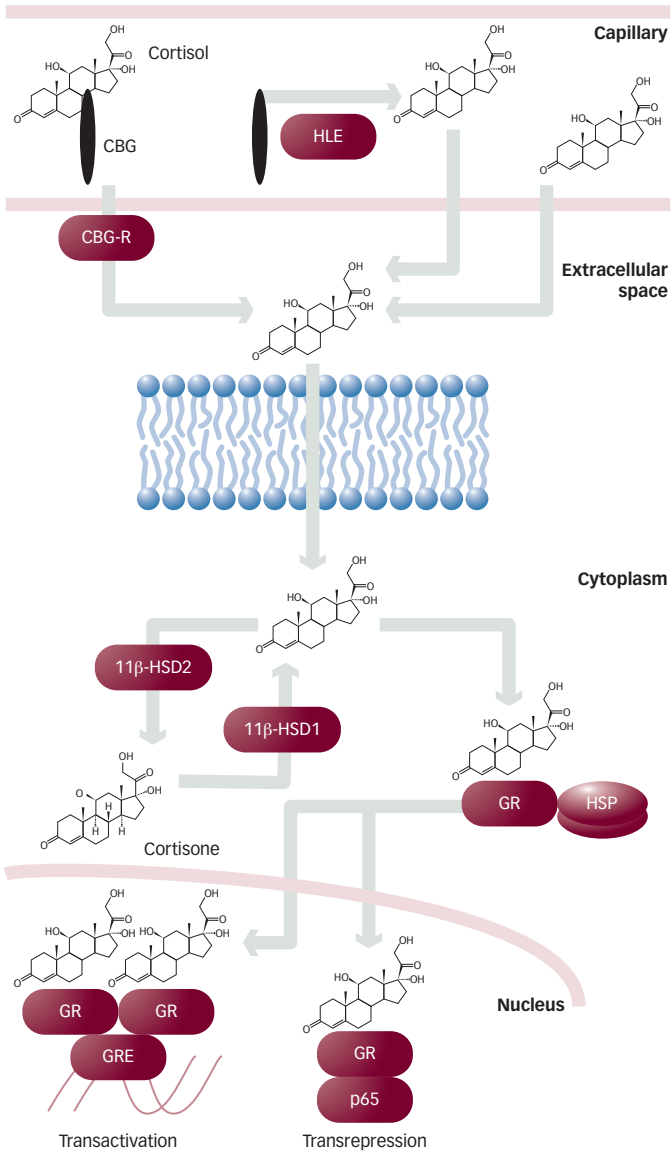
The ability of an organism to respond to stressful stimuli is fundamentally important to that organism's continuing survival. Recognition of a stressor elicits a range of physiological changes that enable the organism to cope and to facilitate the restoration of homeostasis. Many of these physiological changes are mediated via activation of the hypothalamo–pituitary–adrenocortical (HPA) axis and the consequent secretion of glucocorticoids (GCs) by the adrenal gland. Stimulation of the HPA axis is triggered by neural and humoral mechanisms that converge on the parvocellular neurones in the hypothalamic paraventricular nucleus (PVN) and cause release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypothalamo–hypophyseal portal complex for transportation to the anterior pituitary gland. Here, these neurohormones bind to specific CRH and AVP receptors (CRH-R1 and V1b, respectively) on corticotroph cells to induce the release of corticotrophin (adrenocorticotrophic hormone [ACTH]) into the systemic circulation. ACTH acts within the adrenal glands to increase the synthesis and release of GCs, cortisol (in man and other primates) and corticosterone (in rodents). The secretion of these steroid hormones is further regulated by complex negative feedback effects of the GCs themselves on the pituitary gland, hypothalamus and extra-hypothalamic centres in the brain (e.g. hippocampus, brainstem). GCs were originally named on the basis of their influence on metabolic processes, specifically the generation of glucose from protein and lipids. However, GCs also exert a plethora of effects that together serve to maintain homeostasis. GCs thus prepare the organism to respond to stress and also protect the organism from the stress itself, in part by limiting the pathophysiological responses (e.g. inflammation) to the stress that, if left unchecked, may themselves threaten homeostasis.[1]

Glucocorticoids

Regulation of Plasma and Intracellular Glucocorticoid Levels

How do stressful stimuli activate the HPA axis and thus precipitate GC secretion? The PVN is the key site within the brain where many stress-sensitive ascending and descending neural pathways converge and trigger HPA activation. Fibres originating in the brainstem, prefrontal cortex, hippocampus, raphe nucleus and amygdala are particularly important in this regard. These pathways use a range of neurotransmitter/neuromodulator substances to modulate the secretion of CRH and AVP, including acetylcholine, noradrenaline, 5-hydroxytryptamine, gamma-aminobutyric acid, neuropeptide Y, endogenous opioids and various growth factors and cytokines. However, while categorically distinct stressors (i.e. physiological versus emotional) use distinct and specific pathways and transmitters, they recruit largely the same select group of genes within the PVN[2] to induce release of CRH and AVP and, thus, secretion of GCs.

Figure 1: Mechanism of Glucocorticoid Action



Cortisol binding globulin (CBG) limits access of the steroid to intracellular glucocorticoid receptors (GR). Both human leukocyte elastase (HLE) and cortisol binding globulin receptors (CBG-R) can increase cortisol delivery into the cell. Intracellular concentrations of cortisol are further influenced by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which can increase (type 1) or decrease (type 2) cortisol levels. Cortisol binds to GR causing dissociation from heat shock proteins (HSPs) and subsequent translocation to the nucleus. Cortisol bound GR influences gene transcritpion either via an interaction between GR homodimers and glucocorticoid response elements (GREs) within genes (transactivation) or via the binding of GR monomers to relevant transcription factors (transrepression).

Secretion of GCs into the circulation occurs in a pulsatile and circadian fashion. Pulse frequency is approximately one to three pulses per hour[3] in man; variations in pulse amplitude over the 24-hour cycle underpin the circadian profile of maximal GC levels in the morning, prior to awakening (approximately 800nM), and low levels in the evening (approximately 200nM).[4–6] Plasma cortisol levels are further increased by stress and, depending on the nature and intensity of the stress, may rise as much as ten-fold above basal levels.[1]

GCs in the bloodstream are largely bound to plasma proteins (~90%), in particular cortisol-binding globulin (CBG).[7] Only the free steroid can cross cell membranes and gain access to the intracellular receptors that mediate the biological effects of the steroids. Therefore, binding to plasma proteins limits the access of circulating GCs to their receptors by restricting entry to target tissues. However, in certain conditions (e.g. inflammation), cortisol may be released from CBG in the target tissues by the actions of human leukocyte elastase, which cleaves CBG.[8] In addition, some tissues possess membrane-bound CBG receptors, which can internalise both the binding protein and the associated cortisol (see *Figure 1*).[9]

Two further mechanisms determine the bioavailability of free cortisol within the cell. The first, termed pre-receptor ligand metabolism, is mediated by two intracellular enzymes, 11β-hydroxysteroid dehydrogenase 1 and 2 (11β-HSD1 and 11β-HSD2), which regulate the interconversion of cortisol and its biologically inert metabolite, cortisone. 11β-HSD1 acts as a reductase and thus regenerates bioactive cortisol from inactive cortisone and increases the local cortisol concentration. Conversely, 11β-HSD2 catalyses the conversion of cortisol to cortisone and thus reduces the availability of cortisol within the cells. These two enzymes are expressed in a highly tissue-specific manner. 11β–HSD1 is particularly prevalent in GC-responsive metabolic tissues such as the liver and central nervous system,[10,11] while 11β-HSD2 is predominantly located within the kidney and protects high-affinity mineralocorticoid receptors from cortisol.[10,12] The second mechanism is the multidrug-resistant drug (mdr) transporter protein, P-glycoprotein, which is also expressed in a highly tissue-specific manner and exports cortisol from cells, thus reducing the intracellular concentration of the steroid. The tissue-specific patterns of expression of 11β-HSD1, 11β-HSD2 and P-glycoprotein thus provide effective mechanisms for local regulation of the access of GCs to their receptors.

Mechanism of Glucocorticoid Action

GCs act mainly via intracellular receptors, of which there are two main types: the mineralocorticoid receptor (MR) and the GC receptor (GR). These receptors mostly act as transcription factors, regulating the expression of specific target genes. The number of target genes is large – possibly as high as 1% of the genome. MR is a high-affinity receptor that cannot distinguish cortisol from the mineralocorticoid aldosterone. The MRs have a highly tissue-specific pattern of expression, and in tissues classically associated with aldosterone actions (e.g. kidney) are protected from cortisol by 11β–HSD2. By contrast, the GR is a low-affinity receptor with a high specificity for GCs. It is widely distributed in the body. In many tissues, particularly those associated with metabolism (e.g. liver), access of cortisol is facilitated by 11β-HSD1.

GRs are cytoplasmic receptors that, in the absence of ligand, exist in a complex with accessory proteins, such as heat shock proteins, which act as chaperones to retain the GR within the cytoplasm.[13] Binding of cortisol to the ligand-binding domain within the C-terminal of GR[14] induces a conformational change that promotes the dissociation of the heat shock proteins, exposure of the nuclear localisation signal and translocation of the ligand–receptor complex to the nucleus via an importin-mediated mechanism.[15] Ligand-bound GR uses two principal mechanisms to influence transcription of specific target genes: transactivation and transrepression. Transactivation requires homodimerisation of GR subunits and interaction of the GR DNA-binding domain with conserved GC response elements within the promoter region of responsive genes,[16] a process facilitated by the recruitment of transcriptionally active proteins.[14] Interestingly, it appears that small changes in the DNA

recognition sites for GR can subtly alter GR transcriptional activity, suggesting that there may be gene-specific GR effects within tissues.[17] GR-induced transrepression occurs principally via a mechanism independent of DNA binding,[18] with GR monomers specifically binding to and interfering with the actions of transcription factors such as nuclear factor kappa B (NF-κB) or activating protein-1 (AP-1).[19] For example, the ability of the NF-κB p65 subunit to induce expression of pro-inflammatory mediators is suppressed by binding of GR.[20] These differences are highly cell-specific and can determine GC responses, with specific genes demonstrating activation or repression depending on circumstances.

In addition to influencing gene transcription directly, GCs may also act via non-genomic mechanisms.[21,22] For example, GCs promote the cellular exportation of the anti-inflammatory protein annexin 1 from pituitary folliculostellate cells,[23,24] predominantly through a non-genomic mechanism.[24] Croxtall and colleagues demonstrated that this action involves the rapid release of Src kinase from cytoplasmic GR heterocomplexes and subsequent inhibition of arachidonic acid release.[25] It is also possible that some non-genomic actions are associated with activation of a membrane-bound GR. These receptors are present in small numbers per cell, but are actively upregulated after immunostimulation. It has been suggested that overstimulation of the immune system would lead to upregulation of membrane-bound GR, which would act in a feedback manner to reduce the excessive immune reaction.[26] The non-genomic mechanisms of GC action remain poorly understood; therefore, further studies are warranted, particularly since manipulation of these events may prove therapeutically useful.

Glucocorticoids and Human Disease

The clinical features associated with conditions of severe GC excess (Cushing's syndrome) and deficiency (Addison's disease) are well established, but these conditions are relatively rare. However, considerable evidence points to a role for GCs in the pathophysiology of numerous other endocrine-related disorders such as type 2 diabetes, dyslipidaemia and metabolic bone disease. Prolonged increases in physiological GC production are most likely to be the result of exposure to chronic stress. Alternatively, alterations in the local intracellular mechanisms that regulate the access of GCs to their receptors may cause local disturbances in GC homeostasis that influence disease processes.

Acute stress is an allostatic process that aims to restore homeostasis via adaptation, using mediators from numerous systems including the HPA axis. Chronic stress is likely to be associated with allostatic overload, where adaptive processes are used in a sustained manner. It is this prolonged inappropriate use of adaptive physiological processes that can result in dysfunction or disease. For example, increased food intake and fat deposition can be seen as an allostatic response to ensure there is sufficient metabolic resource to maintain homestatic processes, whereas in allostatic overload, this might result in abdominal obesity. Prolonged increases in cortisol due to exposure to chronic stress are likely to exact an allostatic load, with increased wear and tear apparent in certain GC-senstitive tissues, whereas decreased function will be apparent in other tissues owing to prolonged inhibitory effects of GCs or redistribution of metabolic resource to physiological systems involved in restoring homeostasis. However, it should be noted that tissue-specific alterations in GC concentrations without corresponding increases in circulating GC levels can also influence disease processes. It is interesting to note that the high circulating levels of GCs caused by Cushing's syndrome are associated with a number of negative metabolic outcomes,[10,27] whereas near normal serum GC levels are usually found in patients with the more prevalent metabolic syndrome.[28] It has been suggested that an alteration in tissue sensitivity to GCs underlines the metabolic syndrome, specifically an alteration in the expression of 11β-HSD1. Numerous animal studies have demonstrated that 11β-HSD1 expression within metabolic tissues (e.g. adipose tissue, liver) is correlated with an adverse metabolic outcome,[29–31] and metabolic disease within humans is commonly associated with elevated 11β-HSD1 expression/activity.[32,33] Therefore, tissue-specific alterations in 11β-HSD1 expression coupled with increased intracellular GC concentrations and subsequent GR activation may be a common feature of metabolic disease. It has also been demonstrated that there are a number of polymorphisms within the GC receptor gene itself that influence GC sensitivity.[34–36] Several of these GC receptor variants are associated with hypersensitivity to GCs[37–39] and therefore might predispose an individual to negative health outcomes associated with GC overexposure.

The developing organism is particularly sensitive to GCs, and unwanted increases in foetal GR activation due to maternal stress or synthetic GC administration (often used in peri-natal medicine to mature the lung in conditions of pre-term birth) have the potential to induce programming effects on multiple body systems. Studies performed on laboratory animals have shown that exposure of the developing foetus or neonate to supraphysiological GC levels or synthetic GCs results in irreversible morphological and physiological changes in the organism, which predispose it in adulthood to diseases that are endemic in the developed world, such as type 2 diabetes, cardiovascular disease, depression and other mental health disorders. More limited data from clinical studies support these conclusions. The maternal–foetal unit is designed to prevent excessive foetal exposure to GCs, with placental 11β-HSD-2 acting as a barrier to the passage of maternal GCs.[40–42] However, this mechanism may become saturated if endogenous GC levels rise excessively, or can be ineffective, as is the case with synthetic GCs. A key feature of GC programming in early life is prolonged, tissue-specific change in the expression of GR. Reduced GR expression within the HPA axis leads to impairment of GC negative feedback in adulthood, leading to raised GC levels and exaggerated HPA responses to stress. Conversely, GR expression in the liver is increased, thus predisposing the individual to hyperglycaemia.

Glucocorticoids and Endocrine-related Disorders

This section deals with a number of endocrine-related disorders that are associated with aberrant GC levels and in terms of pathophysiology may be linked with chronic tissue-specific alterations in GC actions.

Glucocorticoids and Hyperglycaemia/Type 2 Diabetes

Patients with Cushing's syndrome or on long-term GC therapy classically present with hyperglycaemia[43,44] and symptoms of type 2 diabetes[43] – in this case termed steroid diabetes. It is important to note that the development of type 2 diabetes is usually multifactorial, but this article will discuss steroid diabtetes induced by increased GC levels. GCs act within the liver to upregulate the rate-limiting enzyme for gluconeogensis, phosphoenol-pyruvate carboxykinase (PEPCK), providing a mechanism to explain GC-induced hyperglycaemia. Insulin

resistance is the major biological risk factor for type 2 diabetes,[45] and is associated with both a reduced secretion of insulin by the endocrine pancreas and a reduction in insulin sensitivity within peripheral tissues. GCs decrease insulin secretion[46] and also act on multiple targets to influence insulin sensitivity, downregulating components of insulin signalling such as insulin receptor substrate proteins 1 and 2,[47] phosphoinositide 3 kinase activity[48] and Akt phosphorylation.[49] In addition, there is a strong positive correlation between GR expression levels and the degree of insulin resistance.[50] The link between hyperglycaemia/diabetes and GCs is further strengthened by studies focused on GC pre-receptor metabolism. 11β-HSD-1 overexpression in mice, which increases local GC concentrations in specific target tissues, is associated with modest insulin resistance,[51] whereas 11β-HSD-1 knockout mice show a reduced ability to regenerate intracellular GCs, improved insulin sensitivity,[52,53] impaired induction of PEPCK, a reduced hyperglycaemic response to stress[29] and an improvement in several aspects of GC-induced diabetes.[54] In humans, increased HPA activity is associated with type 2 diabetes,[55–57] with high circulating cortisol levels positively correlated with more severe complications from type 2 diabetes.[58]

Glucocorticoids and Dyslipidaemia/Obesity

GCs are important physiological regulators of energy balance. It is therefore not surprising that the development of metabolic pathologies such as obesity has been strongly associated with dysfunctional GR signalling. Cushing's patients present with centripetal obesity, which is directly linked to excessive GC action.[59] Adipocytes in the abdominal fat pads are more GR-rich than peripheral adipocytes and thus more sensitive to GCs.[60] Within central fat, GCs increase pre-adipocyte differentiation and promote the pro-lipogenic pathways, thereby increasing cellular hypertrophy.[61,62] In non-cushingoid patients the development of obesity is not necessarily associated with increased circulating levels of GCs;[10] however, in some cases at least, there is evidence of altered tissue sensitivity to GCs owing to tissue-specific upregulation of 11β-HSD-1 activity.[63,64] There is evidence that 11β-HSD-1 activity is impaired in the liver but enhanced in adipose tissue in obese human patients.[33,65] Furthermore, the adverse metabolic complications of obesity in mice are prevented by 11β-HSD-1 gene deletion,[66] whereas overexpression of the enzyme in adipose tissue results in metabolic abnormalities.[67] Studies on obese and lean human individuals have demonstrated increased adipose tissue 11β-HSD-1 expression in obese subjects[68] and a direct association between 11β-HSD-1 levels and metabolic abnormalities in obese women.[69] However, tissue-specific overexpression of 11β-HSD-1 in the liver is associated with numerous metabolic alterations but not with changes in fat depot mass.[51] Furthermore, other authors have failed to find an association between obesity and 11β-HSD-1 activity in adipose tissue.[70] Thus, while there is evidence to link tissue-specific alterations in GC bioavailability with the metabolic abnormalities associated with the development of obesity, further studies are necessary to understand fully the role of GCs.

Obesity is associated with an increased risk of coronary heart disease due partly to impaired trapping and breakdown of fatty acids by adipocytes, which facilitates atherogenic dyslipidaemia and is associated with low levels of high-density lipoprotein cholesterol, (HDL-C), elevated triglycerides and increased low-density lipoproteins (LDL). Synthetic GR agonists increase serum triglyceride levels and cause accumulation of hepatic lipid droplets *in vivo*, whereas disruption of GR action specifically decreases serum triglyceride in a mouse model of fatty liver.[71] These GC-mediated effects are most likely due to a decrease in β-oxidation of fatty acids and increased hepatic uptake and storage of fatty acids as triglyceride. Recent studies indicate that GR activation influences the expression of multiple genes directly involved in fatty acid and triglyceride metabolism that may contribute to systemic dyslipidaemia. Enhanced GR activity is also associated with decreased pancreatic lipase activity and fatty acid β-oxidation, profound inhibition of adipose tissue lipoprotein lipase (which would normally act to increase uptake of triglyceride-derived fatty acids) and increased liver cholesterol.[71–73]

Glucocorticoids and Depression

Depression is a complex illness characterised by a spectrum of clinical symptoms including low mood, alterations in appetite and weight, psychomotor agitation or retardation, sleep disruption and suicidal ideation. The development of the disease is influenced by genetic and psychosocial factors as well as biological disturbances.[74,75] There is undoubtedly a very strong correlation between disturbances in HPA function levels and the development of depression. Over 50% of Cushing's patients present with depressive symptoms,[76] and a similar percentage of depressed patients present with hypercortisolaemia.[77] Onset of depression is correlated with stressful life events associated with prolonged elevations in circulating GCs, such as divorce or unemployment.[78] Patients with major depression have been shown to exhibit increased concentrations of cortisol in the plasma, urine and cerebrospinal fluid,[79,80] exaggerated cortisol responses to exogenous ACTH[81] and an enlargement of both the pituitary and the adrenal glands.[82] In addition, a multitude of studies have demonstrated that GC-mediated feedback inhibition of the HPA axis is impaired in depression; thus, unlike normal patients, approximately 50% of depressed patients fail to respond to synthetic GCs with a reduction in serum cortisol.[83] In addition, many effective antidepressant treatments have been shown to modulate cortisol secretion.[84] Indeed, the GR is now an important target for novel antidepressants, and some compounds that specifically reduce the effects of cortisol have produced successful results in clinical trials.[85] Depression is associated with structural abnormalities in a number of cortico-limbic structures that play important roles in cognition and emotional processing, such as the hippocampus, amygdala and prefrontal cortex.[86,87] Each of the aforementioned brain regions is rich in GR,[88–90] and increases in salivary cortisol induced by acute stress are associated with decreased activity within the hippocampus and amygdala.[91] Interestingly, volumetric reductions in the hippocampus are observed in Cushing's patients, and these reductions are partially reversed if the hypercortisolaemia is corrected.[91–94]

While these clinical data are suggestive of a link between hypercortisolaemia and depression, they do not demonstrate true causation. Animal models have attempted to demonstrate a direct link between high circulating GC levels and depressive-like symptoms. However, there is some debate as to whether animals can be classified as depressed and whether the tests used to assess the disease in animals can truly be correlated with clinical symptoms of depression, which are subjective and highly variable. Generally, a good animal model of depression will demonstrate some of the behavioural and neurochemical changes associated with the disease as well as responding to well-established antidepressant treatments. In addition, the ability to examine the aetiology of depressive illness is another favourable asset for any animal model.[95]

Glucocorticoids and Osteoporosis

GC treatment is associated with rapid bone loss, and fractures are a common side effect of long-term GC therapy.[96] Bone remodelling is dependent on the absorption of old bone matrix by osteoclasts, followed by the generation of new bone matrix by osteoblasts that subsequently enter the resorptive lacuna. GCs interfere with bone matrix formation via induction of osteoblast apoptosis via activation of caspase-3, in addition to decreasing the number of osteoblast precursor cells available for differentiation.[97] Chronic GC treatment induces the expression of macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL), both of which are necessary for osteoclast development. GCs also increase osteoclast maturation and survival, which is classically associated with increased bone resorption and rapid loss of trabecular bone.[98,99] However, recent studies suggest that osteoclasts induce a more complex effect on bone remodelling. Osteoclasts act to resorb old bone, an action that requires cytoskeletal organisation. As osteoclasts absorb the old bone matrix, they release factors that promote the movement of osteoblasts into the resorptive lacuna, which then act to synthesise new bone. Long-term GC therapy suppresses specific osteoclast functions related to cytoskeletal organisation and recruitment of osteoblasts, and therefore greatly reduces new bone formation.[100]

Future Perspectives

GCs are predominantly used as anti-inflammatory and immunosuppressive agents. Despite the undoubted clinical benefit obtained from the use of these drugs, the side-effect profile associated with their use remains a huge problem.[43] Anti-inflammatory and immunosuppressive actions of these drugs predominantly feature GC-mediated transrepression, whereas the side effects often involve transactivation. Selective GR agonists with a pharmacological action mostly based on transrepression with little effect on activation could retain the desirable clinical effects of these drugs while considerably reducing side effects.[43] Animal models demonstrating tissue-specific knockout or overexpression of GC targets will also help provide a more accurate picture of GC action *in vivo*.

Antistress gene therapy is also a potential tool to protect against tissue impairment due to prolonged elevations in circulating GCs. Dumas and colleagues[101] have recently demonstrated that tissue-specific expression of 11-β-HSD-2 within the hippocampus offsets neurophysiological disruptions induced by chronically increased GC levels. Thus, a better understanding of tissue-specific GC physiology will allow us to develop more sensitive GC-based therapies and generate treatments aimed at improving outcomes in diseases/disorders associated with chronically increased GC levels. ■

1. Delbende C, Delarue C, Lefebvre H, et al., Glucocorticoids, transmitters and stress, *Br J Psychiatry Suppl*, 1992;15:24–35.
2. Reyes TM, Walker JR, DeCino C, et al., Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus, *J Neurosci*, 2003;23:5607–16.
3. Crown A, Lightman S, Why is the management of glucocorticoid deficiency still controversial: a review of the literature, *Clin Endocrinol (Oxf)*, 2005;63:483–92.
4. Bergendahl M, Iranmanesh A, Mulligan T, Veldhuis JD, Impact of age on cortisol secretory dynamics basally and as driven by nutrient-withdrawal stress, *J Clin Endocrinol Metab*, 2000;85:2203–14.
5. Kerrigan JR, Veldhuis JD, Leyo SA, et al., Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis, *J Clin Endocrinol Metab*, 1993;76:1505–10.
6. Walker BR, Campbell JC, Fraser R, et al., Mineralocorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome, *Clin Endocrinol (Oxf)*, 1992;37:483–92.
7. Stewart P, The adrenal cortex. In: Larsen P, Kronenberg H, Melmed S, Polonsky K (editors), *Williams Textbook of Endocrinology*, 10th ed, Philadelphia, USA: Saunders, 2003;491–551.
8. Torpy DJ, Ho JT, Corticosteroid-binding globulin gene polymorphisms: clinical implications and links to idiopathic chronic fatigue disorders, *Clin Endocrinol (Oxf)*, 2007;67:161–7.
9. Breuner CW, Orchinik M, Plasma binding proteins as mediators of corticosteroid action in vertebrates, *J Endocrinol*, 2002;175:99–112.
10. Seckl JR, Walker BR, Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action, *Endocrinology*, 2001;142:1371–6.
11. Walker BR, Andrew R, Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease, *Ann N Y Acad Sci*, 2006;1083:165–84.
12. Tomlinson JW, Walker EA, Bujalska IJ, et al., 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response, *Endocr Rev*, 2004;25:831–66.
13. Dittmar KD, Demady DR, Stancato LF, et al., Folding of the glucocorticoid receptor by the heat shock protein (hsp) 90-based chaperone machinery. The role of p23 is to stabilize receptor.hsp90 heterocomplexes formed by hsp90.p60.hsp70, *J Biol Chem*, 1997;272:21213–20.
14. van der Laan S, Meijer OC, Pharmacology of glucocorticoids: beyond receptors, *Eur J Pharmacol*, 2008;585:483–91.
15. Freedman ND, Yamamoto KR, Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor, *Mol Biol Cell*, 2004;15:2276–86.
16. Gross KL, Lu NZ, Cidlowski JA, Molecular mechanisms regulating glucocorticoid sensitivity and resistance, *Mol Cell Endocrinol*, 2009;300:7–16.
17. Meijsing SH, Pufall MA, So AY, et al., DNA binding site sequence directs glucocorticoid receptor structure and activity, *Science*, 2009;324:407–10.
18. Clark AR, Anti-inflammatory functions of glucocorticoid-induced genes, *Mol Cell Endocrinol*, 2007;275:79–97.
19. Bamberger CM, Schulte HM, Chrousos GP, Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids, *Endocr Rev*, 1996;17:245–61.
20. Bhavsar PK, Sukkar MB, Khorasani N, et al., Glucocorticoid suppression of CX3CL1 (fractalkine) by reduced gene promoter recruitment of NF-kappaB, *FASEB J*, 2008;22:1807–16.
21. Cato AC, Nestl A, Mink S, Rapid actions of steroid receptors in cellular signaling pathways, *Sci STKE*, 2002;2002:re9.
22. Hafezi-Moghadam A, Simoncini T, Yang Z, et al., Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase, *Nat Med*, 2002;8:473–9.
23. Solito E, Mulla A, Morris JF, et al., Dexamethasone induces rapid serine-phosphorylation and membrane translocation of annexin 1 in a human folliculostellate cell line via a novel nongenomic mechanism involving the glucocorticoid receptor, protein kinase C, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase, *Endocrinology*, 2003;144:1164–74.
24. Taylor AD, Cowell AM, Flower J, Buckingham JC, Lipocortin 1 mediates an early inhibitory action of glucocorticoids on the secretion of ACTH by the rat anterior pituitary gland *in vitro*, *Neuroendocrinology*, 1993;58:430–39.
25. Song IH, Buttgereit F, Non-genomic glucocorticoid effects to provide the basis for new drug developments, *Mol Cell Endocrinol*, 2006;246:142–6.
26. Bartholome B, Spies CM, Gaber T, et al., Membrane glucocorticoid receptors (mGCR) are expressed in normal human peripheral blood mononuclear cells and up-regulated after in vitro stimulation and in patients with rheumatoid arthritis, *FASEB J*, 2004;18:70–80.
27. Montague CT, O'Rahilly S, The perils of portliness: causes and consequences of visceral adiposity, *Diabetes*, 2000;49:883–8.
28. Hautanen A, Raikkonen K, Adlercreutz H, Associations between pituitary-adrenocortical function and abdominal obesity, hyperinsulinaemia and dyslipidaemia in normotensive males, *J Intern Med*, 1997;241:451–61.
29. Kotelevtsev Y, Holmes MC, Burchell A, et al., 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress, *Proc Natl Acad Sci U S A*, 1997;94:14924–9.
30. Masuzaki H, Paterson J, Shinyama H, et al., A transgenic model of visceral obesity and the metabolic syndrome, *Science*, 2001;294:2166–70.
31. Masuzaki H, Yamamoto H, Kenyon CJ, et al., Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice, *J Clin Invest*, 2003;112:83–90.
32. Paulmyer-Lacroix O, Boullu S, Oliver C, et al., Expression of the mRNA coding for 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an *in situ* hybridization study, *J Clin Endocrinol Metab*, 2002;87:2701–5.
33. Rask E, Olsson T, Soderberg S, et al., Tissue-specific dysregulation of cortisol metabolism in human obesity, *J Clin Endocrinol Metab*, 2001;86:1418–21.
34. Stevens A, Ray DW, Zeggini E, et al., Glucocorticoid sensitivity is determined by a specific glucocorticoid receptor haplotype, *J Clin Endocrinol Metab*, 2004;89:892–7.
35. van Rossum EF, Lamberts SW, Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition, *Recent Prog Horm Res*, 2004;59:333–57.
36. van Rossum EF, Roks PH, de Jong FH, et al., Characterization of a promoter polymorphism in the glucocorticoid receptor gene and its relationship to three other polymorphisms, *Clin Endocrinol (Oxf)*, 2004;61:573–81.
37. Huizenga NA, Koper JW, De Lange P, et al., A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids *in vivo*, *J Clin Endocrinol Metab*, 1998;83:144–51.
38. Rosmond R, Chagnon YC, Holm G, et al., A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis, *Obes Res*, 2000;8:211–8.
39. van Rossum EF, Koper JW, van den Beld AW, et al., Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index, *Clin Endocrinol (Oxf)*, 2003;59:585–92.
40. Benediktsson R, Calder AA, Edwards CR, Seckl JR, Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure, *Clin Endocrinol (Oxf)*, 1997;46:161–6.
41. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR, 11 beta-Hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects, *Steroids*, 1996;61:263–9.
42. Seckl JR, Chapman KE, Medical and physiological aspects of the 11beta-hydroxysteroid dehydrogenase system, *Eur J Biochem*, 1997;249:361–4.
43. Schacke H, Docke WD, Asadullah K, Mechanisms involved in the side effects of glucocorticoids, *Pharmacol Ther*, 2002;96:23–43.
44. Shibli-Rahhal A, Van Beek M, Schlechte JA, Cushing's syndrome, *Clin Dermatol*, 2006;24:260–5.
45. Grundy SM, Metabolic syndrome: therapeutic considerations, *Handb Exp Pharmacol*, 2005;170:107–33.
46. Lambillotte C, Gilon P, Henquin JC, Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets, *J Clin Invest*, 1997;99:414–23.
47. Caperuto LC, Anhe GF, Amanso AM, et al., Distinct regulation of IRS proteins in adipose tissue from obese aged and dexamethasone-treated rats, *Endocrine*, 2006;29:391–8.
48. Corporeau C, Foll CL, Taouis M, et al., Adipose tissue compensates for defect of phosphatidylinositol 3'-kinase induced in liver and muscle by dietary fish oil in fed rats, *Am J Physiol Endocrinol Metab*, 2006;290:E78–E86.
49. Buren J, Liu HX, Jensen J, Eriksson JW, Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes, *Eur J Endocrinol*, 2002;146:419–29.
50. Whorwood CB, Donovan SJ, Flanagan D, et al., Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome, *Diabetes*, 2002;51:1066–75.

51. Paterson JM, Morton NM, Fievet C, et al., Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice, *Proc Natl Acad Sci U S A*, 2004;101:7088–93.
52. Morton NM, Holmes MC, Fievet C, et al., Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice, *J Biol Chem*, 2001;276:41293–300.
53. Morton NM, Paterson JM, Masuzaki H, et al., Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice, *Diabetes*, 2004;53:931–8.
54. Bhat BG, Younis H, Herrera J, et al., Antisense inhibition of 11betahydroxysteroid dehydrogenase type 1 improves diabetes in a novel cortisone-induced diabetic KK mouse model, *Biochem Biophys Res Commun*, 2008;365:740–5.
55. Hudson JI, Hudson MS, Rothschild AJ, et al., Abnormal results of dexamethasone suppression tests in nondepressed patients with diabetes mellitus, *Arch Gen Psychiatry*, 1984;41:1086–9.
56. Lee ZS, Chan JC, Yeung VT, et al., Plasma insulin, growth hormone, cortisol, and central obesity among young Chinese type 2 diabetic patients, *Diabetes Care*, 1999;22:1450–7.
57. Reynolds RM, Walker BR, Syddall HE, et al., Elevated plasma cortisol in glucose-intolerant men: differences in responses to glucose and habituation to venepuncture, *J Clin Endocrinol Metab*, 2001;86:1149–53.
58. Chiodini I, Adda G, Scillitani A, et al., Cortisol secretion in patients with type 2 diabetes: relationship with chronic complications, *Diabetes Care*, 2007;30:83–8.
59. Walker BR, Cortisol—cause and cure for metabolic syndrome?, *Diabet Med*, 2006;23:1281–8.
60. Rebuffe-Scrive M, Bronnegard M, Nilsson A, et al., Steroid hormone receptors in human adipose tissues, *J Clin Endocrinol Metab*, 1990;71:1215–9.
61. Gaillard D, Wabitsch M, Pipy B, Negrel R, Control of terminal differentiation of adipose precursor cells by glucocorticoids, *J Lipid Res*, 1991;32:569–79.
62. Samra JS, Summers LK, Frayn KN, Sepsis and fat metabolism, *Br J Surg*, 1996;83:1186–96.
63. Stewart PM, Boulton A, Kumar S, et al., Cortisol metabolism in human obesity: impaired cortisone—>cortisol conversion in subjects with central adiposity, *J Clin Endocrinol Metab*, 1999;84:1022–7.
64. Weaver JU, Taylor NF, Monson JP, et al., Sexual dimorphism in 11 beta hydroxysteroid dehydrogenase activity and its relation to fat distribution and insulin sensitivity; a study in hypopituitary subjects, *Clin Endocrinol (Oxf)*, 1998;49:13–20.
65. Katz JR, Mohamed-Ali V, Wood PJ, et al., An in vivo study of the cortisol-cortisone shuttle in subcutaneous abdominal adipose tissue, *Clin Endocrinol (Oxf)*, 1999;50:63–8.
66. De Sousa Peixoto RA, Turban S, Battle JH, et al., Preadipocyte 11beta-hydroxysteroid dehydrogenase type 1 is a keto-reductase and contributes to diet-induced visceral obesity in vivo, *Endocrinology*, 2008;149:1861–8.
67. Stimson RH, Walker BR, Glucocorticoids and 11beta-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome, *Minerva Endocrinol*, 2007;32:141–59.
68. Desbriere R, Vuaroqueaux V, Achard V, et al., 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients, *Obesity* (Silver Spring), 2006;14:794–8.
69. Engeli S, Bohnke J, Feldpausch M, et al., Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss, *Obes Res*, 2004;12:9–17.
70. Tomlinson JW, Sinha B, Bujalska I, et al., Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity, *J Clin Endocrinol Metab*, 2002;87:5630–5.
71. Lemke U, Krones-Herzig A, Berriel Diaz M, et al., The glucocorticoid receptor controls hepatic dyslipidemia through Hes1, *Cell Metab*, 2008;8:212–23.
72. Jia Y, Viswakarma N, Fu T, et al., Conditional ablation of mediator subunit MED1 (MED1/PPARBP) gene in mouse liver attenuates glucocorticoid receptor agonist dexamethasone-induced hepatic steatosis, *Gene Expr*, 2009;14:291–306.
73. Mandard S, Zandbergen F, van Straten E, et al., The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity, *J Biol Chem*, 2006;281:934–44.
74. Kalia M, Neurobiological basis of depression: an update, *Metab Clin Exp*, 2005;54:24–7.
75. Nemeroff CB, The neurobiology of depression, *Sci Am*, 1998;278:42–9.
76. Sonino N, Fava GA, Residual symptoms in depression – An emerging therapeutic concept, *Prog Neuro-Psychoph*, 2002;26:763–70.
77. Sachar EJ, Baron M, Biology of affective-disorders, *Annu Rev Neurosci*, 1979;2:505–18.
78. Kessing LV, Agerbo E, Mortensen PB, Does the impact of major stressful life events on the risk of developing depression change throughout life?, *Psychol Med*, 2003;33:1177–84.
79. Nemeroff CB, Widerlov E, Bissette G, et al., Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients, *Science*, 1984;226:1342–4.
80. Rubin RT, Poland RE, Lesser IM, et al., Neuroendocrine aspects of primary endogenous depression. I. Cortisol secretory dynamics in patients and matched controls, *Arch Gen Psychiatry*, 1987;44:328–36.
81. Parker KJ, Schatzberg AF, Lyons DM, Neuroendocrine aspects of hypercortisolism in major depression, *Horm Behav*, 2003;43:60–6.
82. Owens MJ, Nemeroff CB, The role of corticotropin-releasing factor in the pathophysiology of affective and anxiety disorders: laboratory and clinical studies, *Ciba Found Symp*, 1993;172:296–308, discussion 16.
83. Holsboer F, The corticosteroid receptor hypothesis of depression, *Neuropsychopharmacol*, 2000;23:477–501.
84. Pariante CM, Hye A, Williamson R, et al., The antidepressant clomipramine regulates cortisol intracellular concentrations and glucocorticoid receptor expression in fibroblasts and rat primary neurones, *Neuropsychopharmacol*, 2003;28:1553–61.
85. Schule C, Baghai TC, Eser D, et al., Effects of mirtazapine on dehydroepiandrosterone-sulfate and cortisol plasma concentrations in depressed patients, *J Psychiatr Res*, 2009;43:538–45.
86. Drevets WC, Neuroimaging studies of mood disorders, *Biol Psychiat*, 2000;48:813–29.
87. Drevets WC, Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders, *Curr Opin Neurobiol*, 2001;11:240–9.
88. Herman JP, Figueiredo H, Mueller NK, et al., Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness, *Front Neuroendocrinol*, 2003;24:151–80.
89. Herman JP, Ostrander MM, Mueller NK, Figueiredo H, Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis, *Prog Neuropsychopharmacol Biol Psychiatry*, 2005;29:1201–13.
90. Mitra R, Sapolsky RM, Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy, *Proc Natl Acad Sci U S A*, 2008;105:5573–8.
91. Pruessner JC, Dedovic K, Khalili-Mahani N, et al., Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies, *Biol Psychiatry*, 2008;63:234–40.
92. Fuchs E, Czeh B, Kole MH, et al., Alterations of neuroplasticity in depression: the hippocampus and beyond, *Eur Neuropsychopharmacol*, 2004;14(Suppl. 5):S481–90.
93. Starkman MN, Gebarski SS, Berent S, Schteingart DE, Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome, *Biol Psychiatry*, 1992;32:756–65.
94. Starkman MN, Giordani B, Gebarski SS, Schteingart DE, Improvement in learning associated with increase in hippocampal formation volume, *Biol Psychiatry*, 2003;53:233–8.
95. Willner P, Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS, *Neuropsychobiology*, 2005;52:90–110.
96. Lukert BP, Raisz LG, Glucocorticoid-induced osteoporosis: pathogenesis and management, *Ann Intern Med*, 1990;112:352–64.
97. Smith E, Coetzee GA, Frenkel B, Glucocorticoids inhibit cell cycle progression in differentiating osteoblasts via glycogen synthase kinase-3beta, *J Biol Chem*, 2002;277:18191–7.
98. Lane NE, Yao W, Developments in the scientific understanding of osteoporosis, *Arthritis Res Ther*, 2009;11:228.
99. Lane NE, Yao W, Balooch M, et al., Glucocorticoid-treated mice have localized changes in trabecular bone material properties and osteocyte lacunar size that are not observed in placebo-treated or estrogen-deficient mice, *J Bone Miner Res*, 2006;21:466–76.
100. Kim HJ, New understanding of glucocorticoid action in bone cells, *BMB Rep*, 2010;43:524–9.
101. Dumas TC, Gillette T, Ferguson D, et al., Anti-glucocorticoid gene therapy reverses the impairing effects of elevated corticosterone on spatial memory, hippocampal neuronal excitability, and synaptic plasticity, *J Neurosci*, 2010;30:1712–20.