

# Adrenal Corticosteroid Biosynthesis, Metabolism, and Action

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Adrenal corticosteroids are essential for life, and an appreciation of the mechanisms underpinning their synthesis, secretion, and mode of action in normal physiology is essential if the physician is to diagnose and treat patients who have Cushing's syndromes effectively. In each case, there have been clinically significant advances in the knowledge base over recent years, notably in the understanding of steroidogenesis, cortisol action, and metabolism.

## Adrenal steroids and steroidogenesis

Three main types of hormone are produced by the adrenal cortex: glucocorticoids (cortisol, corticosterone), mineralocorticoids (aldosterone, deoxycorticosterone), and sex steroids (mainly the androgen precursors dehydroepiandrosterone [DHEA] and androstenedione). Cholesterol is the precursor for all adrenal steroidogenesis. The principal source of this cholesterol is provided from the circulation in the form of low-density lipoprotein (LDL) cholesterol [1]. Uptake is by specific cell surface LDL receptors present on adrenal tissue; LDL then is internalized by means of receptor-mediated endocytosis. The resulting vesicles fuse with lysosomes, and free cholesterol is produced following hydrolysis. Cholesterol also can be generated *de novo* within the adrenal cortex from acetyl coenzyme A [2], and there is evidence that the adrenal can use high-density lipoprotein (HDL) cholesterol following uptake through a putative HDL receptor, SR-B1 [3].

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The biochemical pathways involved in adrenal steroidogenesis are shown in Fig. 1. The initial hormone-dependent rate-limiting step is the transport of intracellular cholesterol from the outer to inner mitochondrial membrane for conversion to pregnenolone by cytochrome P450<sub>sc</sub>. Human experiments of nature have confirmed the importance of a 30-kd protein, steroidogenic acute regulatory protein (StAR), in mediating this effect. StAR is induced by an increase in intracellular cAMP following binding of corticotropin (ACTH) to its cognate receptor providing the first important rate-limiting step in adrenal steroidogenesis [4]. Other transporters including the peripheral benzodiazepine-like receptor may be involved.

Steroidogenesis involves the concerted action of several enzymes, all of which have been cloned and characterized. Cholesterol side chain cleavage enzyme and the CYP11B enzymes are localized to the mitochondria and require an electron shuttle system provided through adrenodoxin/adrenodoxin reductase to oxidize/hydroxylate steroids [5]. P450c17, exerting 17 $\alpha$ -hydroxylase and 17,20 lyase activity, and P450c21, exerting 21-hydroxylase activity, are localized to the endoplasmic reticulum (ER). Like all microsomal P450 enzymes, they require electron transfer from nicotinamide adenosine dinucleotide phosphate (NADPH) by the enzyme P450 oxidoreductase. In addition, 17,20 lyase activity of P450c17 is dependent upon the flavoprotein cytochrome b<sub>5</sub> that functions as an allosteric facilitator of P450c17 and P450 oxidoreductase interaction [6]. Mutations in the genes

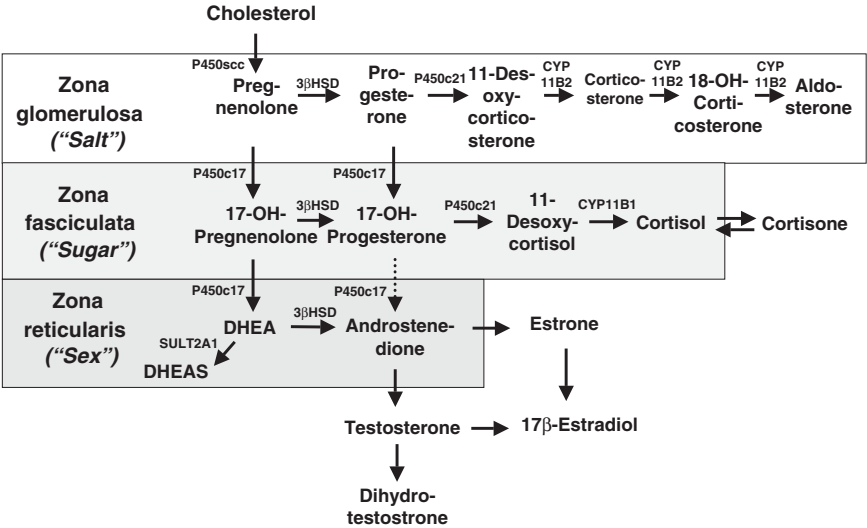


Fig. 1. Steroidogenesis in the human adrenal. Following the StAR-mediated uptake of cholesterol into mitochondria within adrenocortical cells, aldosterone, cortisol, and adrenal androgen precursors are synthesized through the coordinated action of a series of steroidogenic enzymes in a zone-specific fashion.

encoding these enzymes result in human disease [7–13], so some understanding of the underlying pathways and steroid precursors is required.

After uptake of cholesterol to the mitochondrion, cholesterol is cleaved by the P450 cholesterol side chain cleavage enzyme (P450<sub>scc</sub>) to form pregnenolone [14]. In the cytoplasm, pregnenolone is converted to progesterone by the type II isozyme of 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) [15]. Progesterone is hydroxylated to 17OH-progesterone through the activity of P450<sub>c17</sub>, encoded by the *CYP17A1* gene. 17-Hydroxylation is an essential prerequisite for glucocorticoid synthesis, and the zona glomerulosa does not express P450<sub>c17</sub>. P450<sub>c17</sub> also possesses 17,20 lyase activity, which results in the production of the C19 adrenal androgen precursors, dehydroepiandrosterone and androstenedione [16]. In people, however, 17-OH progesterone is not an efficient substrate for P450<sub>c17</sub>, and under physiologic conditions, there is negligible conversion of 17-OH progesterone to androstenedione. Thus, adrenal androstenedione secretion is dependant upon the conversion of dehydroepiandrosterone to androstenedione by 3 $\beta$ -HSD. This enzyme also will convert 17-OH pregnenolone to 17-OH progesterone, but the preferred substrate is pregnenolone.

21-Hydroxylation of either progesterone (zona glomerulosa) or 17-OH-progesterone (zona fasciculata) is performed by the product of the *CYP21A2* gene, P450<sub>c21</sub>, which exerts 21-hydroxylase activity to yield deoxycorticosterone or 11-deoxycortisol, respectively [17]. The final step in cortisol biosynthesis takes place in the mitochondria and involves the conversion of 11-deoxycortisol to cortisol by the enzyme 11 $\beta$ -hydroxylase, encoded by the *CYP11B1* gene [18]. In the zona glomerulosa, 11 $\beta$ -hydroxylase also may convert deoxycorticosterone to corticosterone. The enzyme aldosterone synthase, encoded by *CYP11B2* also may carry out this reaction, however. In addition, it is required for the conversion of corticosterone to aldosterone by means of the intermediate 18-OH corticosterone [19,20].

## Regulation of adrenal steroidogenesis

### *Zonal specific steroidogenesis in the adrenal cortex*

Glucocorticoids are secreted in relatively high amounts (cortisol 10–20 mg/d) from the zona fasciculata under the control of ACTH, while mineralocorticoids are secreted in low amounts (aldosterone 100–150  $\mu$ g/d) from the zona glomerulosa under the principal control of angiotensin II. As a class, adrenal androgens (DHEA, dehydroepiandrosterone sulfate [DHEAS], and androstenedione) are the most abundant steroids secreted from the adult adrenal gland (>20 mg/d). In each case, this is facilitated through expression of steroidogenic enzymes in a specific zonal manner. The zona glomerulosa cannot synthesize cortisol, because it does not express *CYP17*. In contrast, aldosterone secretion is confined to the outer zona

glomerulosa through the restricted expression of *CYP11B2*. Although *CYP11B1* and *CYP11B2* share 95% homology, the 5' promoter sequences differ and permit regulation of the final steps in glucocorticoid and mineralocorticoid biosynthesis by ACTH and angiotensin II, respectively. DHEA is generated by 17,20 lyase activity of P450c17 in the adrenal zona reticularis, which shows ample expression of cytochrome b5, the required cofactor for 17,20 lyase activity [21]. DHEA is converted by the DHEA sulfotransferase (SULT2A1) to form DHEAS, the major secretory product of adrenal steroidogenesis [22].

In the fetal adrenal, steroidogenesis occurs primarily within the inner fetal zone. Because of a relative lack of 3 $\beta$ -HSD and high DHEA sulfotransferase activity, the principal steroidogenic products are DHEA and DHEAS which are then aromatized by placental trophoblast to estrogens. Thus most maternal estrogen across pregnancy is, indirectly, fetally-derived [23].

Classical endocrine feedback loops are in place to control the secretion of glucocorticoids, whereby cortisol inhibits the secretion of both corticotrophin releasing factor and ACTH from the hypothalamus and pituitary, respectively.

#### *Regulation of mineralocorticoid secretion by the renin–angiotensin–aldosterone system*

Aldosterone is secreted from the zona glomerulosa under the control of three principal secretagogues: angiotensin II, potassium, and to a lesser extent ACTH. Other factors, notably somatostatin, heparin, atrial natriuretic factor, and dopamine, can inhibit aldosterone synthesis directly. The secretion of aldosterone and its intermediary 18-hydroxylated metabolites is restricted to the zona glomerulosa because of the zonal-specific expression of aldosterone synthase (*CYP11B2*) [24]. Corticosterone and deoxycorticosterone, while synthesized in both the zona fasciculata and glomerulosa, can act as mineralocorticoids, and this becomes significant in some clinical disease, notably some forms of congenital adrenal hyperplasia and adrenal tumors. Similarly, it is established that cortisol can act as a mineralocorticoid in the setting of impaired metabolism to cortisone performed by the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 2.

Angiotensinogen, an  $\alpha_2$ -globulin synthesized within the liver, is cleaved by renin to form angiotensin I. Angiotensin I then is converted to angiotensin II by angiotensin-converting enzyme (ACE) in lung and many other peripheral tissues. Angiotensin I has no apparent biologic activity, but angiotensin II is a potent stimulator of aldosterone secretion and is a potent vasoconstrictor. The rate-limiting step in the renin–angiotensin–aldosterone system (RAS) is the secretion of renin, which also is controlled through a negative feedback loop [25]. Renin is secreted from juxtaglomerular epithelial cells within the macula densa of the renal tubule in response to underlying renal arteriolar pressure, oncotic pressure, and sympathetic

drive. Thus, low perfusion pressure or low tubular fluid sodium content as seen in hemorrhage, renal artery stenosis, dehydration, or salt loss increases renin secretion. Conversely, secretion is suppressed following a high-salt diet and by factors that increase blood pressure. Autoregulation therefore is maintained, because the increase in renin secretion stimulates angiotensin II and aldosterone production; the concomitant increase in blood pressure and renal sodium retention results in feedback-inhibition of renin secretion. Hypokalemia increases, and hyperkalemia decreases renin secretion; in addition, potassium exerts a direct effect upon the adrenal cortex to increase aldosterone secretion. The sensitivity of the RAS to changes in circulating potassium is high, with changes in potassium concentrations of only 0.1 to 0.5 mmol/L producing marked changes in aldosterone concentrations. Potassium concentrations also determine sensitivity of the aldosterone response to a given infusion of angiotensin II, with high potassium intake increasing responsiveness [26].

Angiotensin II and potassium stimulate aldosterone secretion principally by increasing the transcription of *CYP11B2* in the adrenal zona glomerulosa through common intracellular signaling pathways. Cyclic AMP response elements in the 5' region of the *CYP11B2* gene are activated following an increase in intracellular calcium ion ( $\text{Ca}^{2+}$ ) and activation of calmodulin kinases. The potassium effect is mediated through membrane depolarization and opening of calcium channels, and the AII effect follows binding of angiotensin II to the surface  $\text{AT}_1$  receptor and activation of phospholipase C [24].

The effect of ACTH upon aldosterone secretion is modest and differs in the acute and chronic situation. An acute bolus of ACTH will increase aldosterone secretion, principally by stimulating the early pathways of adrenal steroidogenesis, but circulating levels increase by no more than 10% to 20% above baseline values. ACTH has no effect upon *CYP11B2* gene transcription or enzyme activity. Chronic continual ACTH stimulation has either no effect or an inhibitory effect on aldosterone production, possibly because of receptor down-regulation or suppression of angiotensin II-stimulated secretion caused by a mineralocorticoid effect of cortisol, deoxycorticosterone, or corticosterone.

### *Adrenal androgen secretion*

The adult adrenal secretes approximately 4 mg per day of DHEA, 7 to 15 mg per day of DHEAS, and 1.5 mg per day of androstenedione. DHEA is the crucial sex steroid precursor and only acts androgenic or estrogenic following conversion by the activities of  $3\beta$ -HSD, a superfamily of  $17\beta$ -HSD isozymes and aromatase, expressed in peripheral target tissues. This is of clinical importance in many diseases [27]. In women, more than 50% of active androgens are generated by peripheral conversion from DHEA [28]. In men, this contribution is much smaller because of the testicular

production of androgens, but adrenal androgen excess even in males may be of clinical significance, notably in patients with congenital adrenal hyperplasia. There is a clear gender difference in DHEAS concentrations, with lower concentrations in adult women compared with men [29]. In people and in some nonhuman primates the secretion of DHEAS shows a characteristic pattern throughout the life cycle. DHEAS is secreted in high quantities by the fetal zone of the adrenal cortex, leading to high circulating DHEAS levels at birth. As the fetal zone involutes, a sharp fall in serum DHEAS to almost nondetectable levels is observed postpartum. DHEAS levels remain very low until they gradually increase between the sixth and 10th years of age, owing to increasing DHEA production by the zona reticularis, a phenomenon termed adrenarche [30,31]. DHEAS concentrations peak during the third decade, followed by a steady decline with advancing age so that levels during the eighth and ninth decade are only 10% to 20% of those in young adults [32]. This decline has been termed adrenopause in spite of unchanged or even increased cortisol secretion [33]. The age-related decline in DHEAS levels shows high interindividual variability, and the decline is associated with a reduction in size of the zona reticularis [34].

Corticotropin stimulates adrenal androgen secretion, which may explain why DHEA and androstenedione demonstrate a similar diurnal rhythm to cortisol. Serum DHEAS does not vary throughout the day, most likely because of its longer half-life. The diurnal rhythm and the pulse amplitude of DHEA secretion show an age-associated attenuation [35]. Moreover, the ACTH-induced increase in DHEA secretion is reduced in elderly subjects [36], whereas the cortisol response to an ACTH challenge is constant or even increased. There are many discrepancies between adrenal androgen and glucocorticoid secretion, however, including the age-specific variation of DHEA secretion, which has led to the suggestion of an additional cortical androgen-stimulating hormone (CASH). Many putative CASHs have been proposed, including proopiomelanocortin derivatives such as joining peptide, prolactin, and insulinlike growth factor 1 (IGF-1), but conclusive proof is lacking. Characteristically, adrenal androgen secretion appears stimulated in ACTH-dependent Cushing's disease, while it is suppressed in the case of adrenal glucocorticoid excess.

## **Corticosteroid hormone action**

### *Receptors and gene transcription*

Both cortisol and aldosterone exert their effects following uptake of free hormone from the circulation and binding to intracellular receptors, termed the glucocorticoid and mineralocorticoid receptors (GR and MR) [37–39]. These are both members of the thyroid/steroid hormone receptor

superfamily of transcription factors comprising a C-terminal ligand binding domain, a central DNA binding domain interacting with specific DNA sequences on target genes, and an N-terminal hypervariable region. Splice variants have been described in both cases [40,41], although there is only a single gene encoding the GR and MR.

Glucocorticoid hormone action has been studied in some detail. The binding of steroid to the GR $\alpha$  in the cytosol results in activation of the steroid receptor complex through a process that involves the dissociation of heat shock proteins (HSP 90 and HSP 70) [42]. Following translocation to the nucleus, gene transcription is stimulated or repressed following binding of dimerized GR/ligand complexes to specific DNA sequences (glucocorticoid response elements [GREs]) in the promoter regions of target genes [43]. The GR $\beta$  variant may act as a dominant negative regulator of GR $\alpha$  transactivation [40]. The GR $\gamma$  variant has an additional amino acid within the DNA binding domain of the receptor protein that may reduce GR transactivation [40]. Naturally occurring mutations in the GR (as seen in patients who have glucocorticoid resistance) and in vitro-generated GR mutants have highlighted critical regions of the receptor responsible for binding and transactivation [44,45]. In addition, however, numerous other factors are required (coactivators, corepressors [46]) that may confer tissue specificity of response. In keeping with the diverse array of tissue-specific cortisol action, many hundred glucocorticoid-responsive genes have been identified.

In contrast to the diverse actions of glucocorticoids, mineralocorticoids have been considered to have a more restricted role, principally to stimulate epithelial sodium transport in the distal nephron, distal colon, and salivary glands. Aldosterone binds to the MR, principally in the cytosol (though there is evidence for expression of the unliganded MR in the nucleus), followed by translocation of the hormone-receptor complex to the nucleus. MR and GR share considerable homology, 57% in the steroid-binding domain and 94% in the DNA binding domain. It is perhaps not surprising therefore that there is promiscuity of ligand binding with aldosterone (and the synthetic mineralocorticoid fludrocortisone) binding to the GR and cortisol binding to the MR. For the MR, this is particularly impressive. In vitro, the MR has the same inherent affinity for aldosterone, corticosterone, and cortisol [47]. Specificity upon the MR is conferred through the prereceptor metabolism of cortisol by means of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (*11 $\beta$ -HSD2*), which inactivates cortisol and corticosterone to inactive 11-keto metabolites, enabling aldosterone to bind to the MR [48,49] (Fig. 2).

Mineralocorticoid receptor-induced epithelial sodium conductance is mediated by the apical sodium channel (comprising three subunits:  $\alpha$ ,  $\beta$ , and  $\gamma$ ) and the  $\alpha_1$  and  $\beta_1$  subunits of the basolateral Na<sup>+</sup>K<sup>+</sup>ATPase through transcriptional regulation of an aldosterone-induced gene, serum and glucocorticoid-induced kinase (*sgk*) [50,51]. In addition, recent data have

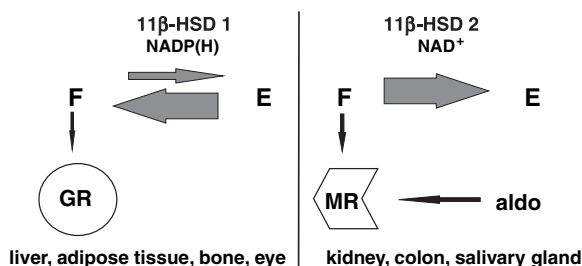


Fig. 2. Schematic role of 11 $\beta$ -HSDs in regulating corticosteroid hormone action at the prereceptor level. 11 $\beta$ -HSD2 in the kidney inactivates cortisol (F) to cortisone (E), enabling aldosterone to bind to the MR *in vivo*. By contrast, in GR-expressing tissues, 11 $\beta$ -HSD1 principally converts inactive E to F, thereby augmenting GR hormone action.

pointed to an extended cardiovascular role for aldosterone and the MR in inducing cardiac fibrosis and vascular inflammation [52,53], although the exact signaling mechanisms have yet to be determined.

For both glucocorticoids and mineralocorticoids, there is accumulating evidence for so-called nongenomic effects involving hormone response obviating the genomic GR or MR. Responses have been reported within seconds/minutes of exposure to corticosteroids and are thought to be mediated by as yet, uncharacterized membrane-coupled receptors [54].

#### *Cortisol-binding globulin and corticosteroid hormone metabolism*

Over 90% of circulating cortisol is bound, predominantly to the  $\alpha_2$ -globulin, cortisol-binding globulin (CBG) [55]. This 383 amino acid protein is synthesized in the liver and binds cortisol with high affinity. Affinity for synthetic corticosteroids (except prednisolone, which has an affinity for CBG, approximately 50% of that of cortisol) is negligible. Circulating CBG concentrations are approximately 700 nmol/L; levels are increased by estrogens and in some patients who have chronic active hepatitis but reduced by glucocorticoids and in patients who have cirrhosis, nephrosis, and hyperthyroidism. The estrogen effect can be marked, with levels increasing two- to threefold across pregnancy. This also should be considered when measuring plasma total cortisol in pregnancy and in women taking estrogens. Inherited abnormalities in CBG synthesis are much rarer than those described for thyroid-binding globulin but include patients who have elevated CBG, partial and complete deficiency of CBG, or CBG variants with reduced affinity for cortisol. In each case, alterations in CBG concentrations change total circulating cortisol concentrations accordingly, but free cortisol concentrations are normal. Only this free circulating fraction is available for transport into tissues for biologic activity. The excretion of free cortisol through the kidneys is termed urinary free cortisol (UFF) and represents only 1% of the total cortisol secretion rate.



The circulating half-life of cortisol varies between 70 and 120 minutes. Cortisol is metabolized through many routes (Fig. 3), but the major routes comprise the interconversion of cortisol (Kendall's compound F) to cortisone (compound E) through the activity of 11 $\beta$ -hydroxysteroid dehydrogenases or reduction of the C4-5 double bond by either 5 $\beta$ -reductase or 5 $\alpha$ -reductase to yield respectively 5 $\beta$ -tetrahydrocortisol (THF) or 5 $\alpha$ -THF (allo-THF). In normal subjects, the 5 $\beta$  metabolites predominate (5 $\beta$ :5 $\alpha$ -THF 2:1). THF, allo-THF and tetrahydrocortisone (THE) are conjugated rapidly with glucuronic acid and excreted in the urine. Downstream, cleavage of THF and THE to the C19 steroids 11-hydroxy- or 11-oxo-androsterone or etiocholanolone can occur. Alternatively, reduction of the 20-oxo group by 20 $\alpha$ - or 20 $\beta$ -hydroxysteroid dehydrogenase yields  $\alpha$  and  $\beta$  cortols and cortolones, respectively, with subsequent oxidation at the C21 position to form the extremely polar metabolites, cortolic, and cortolonic acids. Hydroxylation at C6 to form 6 $\beta$ -hydroxycortisol is described, as is reduction of the C20 position, which may occur without A ring reduction giving rise to 20 $\alpha$ - and 20 $\beta$ -hydroxycortisol.

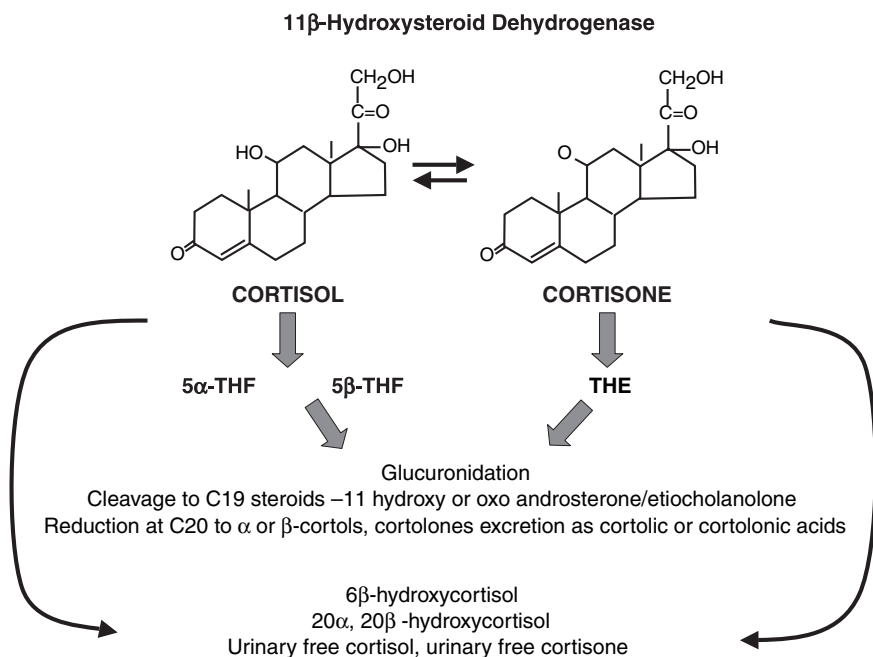


Fig. 3. The principal pathways of cortisol metabolism. Interconversion of hormonally active cortisol to inactive cortisone is catalyzed by two isozymes of 11 $\beta$ -HSD, 11 $\beta$ -HSD1 principally converting cortisone to cortisol and 11 $\beta$ -HSD2 the reverse. A ring reduction is undertaken by 5 $\beta$  or 5 $\alpha$ -reductase and 3 $\beta$ -HSDs to yield tetrahydro metabolites (THF, 5 $\beta$ -THF, and THE) that in turn are conjugated with glucuronic acid or cleared to 11-hydroxy/oxo-androsterone or -etiocholanolone.

Approximately 50% of secreted cortisol appears in the urine as THF, allo-THF, and THE; 25% appears as cortols/cortolones. Ten percent appears as C19 steroids, and 10% appears as cortolic/cortolonic acids. The remaining metabolites are free, unconjugated steroids (cortisol, cortisone, and 6 $\beta$ - and 20 $\alpha$ /20 $\beta$ -metabolites of F and E). Hyperthyroidism results in increased cortisol metabolism and clearance and hypothyroidism in the converse, principally because of an effect of thyroid hormone upon hepatic 11 $\beta$ -HSD and 5 $\alpha$ /5 $\beta$ -reductases [56]. IGF-1 increases cortisol clearance by inhibiting hepatic 11 $\beta$ -HSD (conversion of cortisone to cortisol) [57]. 6 $\beta$ -Hydroxylation is normally a minor pathway, but cortisol itself and some drugs such as rifampicin induce 6 $\beta$ -hydroxylase and increase clearance [58].

Aldosterone also is metabolized in the liver and kidneys. In the liver, it undergoes tetrahydro reduction and is excreted in the urine as a 3-glucuronide tetrahydroaldosterone derivative. Glucuronide conjugation at the 18 position, however, occurs directly in the kidney, as does 3 $\alpha$  and 5 $\alpha$ /5 $\beta$  metabolism of the free steroid [59]. Because of the aldehyde group at the C18 position, aldosterone is not metabolized by 11 $\beta$ -HSD. Hepatic aldosterone clearance is reduced in patients who have cirrhosis, ascites, and severe congestive heart failure.

### *11 $\beta$ -Hydroxysteroid dehydrogenases*

Quantitatively, the interconversion of cortisol to cortisone by 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSD) is the most important cortisol metabolizing pathway [60,61] (Fig. 3). Over the last 10 years, however, 11 $\beta$ -HSD also has emerged as novel factor in the tissue-specific analysis of corticosteroid hormone action, and one that now offers future therapeutic potential. Two distinct 11 $\beta$ -HSD isozymes have been reported. The NAD-dependent dehydrogenase, 11 $\beta$ -HSD2, is coexpressed with the MR in the kidney, colon, and salivary gland and inactivates cortisol to cortisone, thereby enabling aldosterone to bind to the MR in vivo [48,49]. If this enzyme-protective mechanism is impaired, cortisol is able to act as a mineralocorticoid; this explains an inherited form of endocrine hypertension (apparent mineralocorticoid excess) [60] and the hypertension seen in patients who have excessive licorice ingestion and possibly those who have salt-sensitive forms of essential hypertension [62]. Cushing's disease of the kidney reflects a state of local cortisol excess brought about by means of impaired metabolism by 11 $\beta$ -HSD2 despite normal circulating cortisol concentrations.

By contrast, 11 $\beta$ -HSD1 is an NADP(H)-dependent enzyme expressed principally in liver and visceral adipose tissue, but also in brain, bone, gonad, muscle, and other GR-expressing tissues, including the eye [61]. The enzyme is bidirectional, but in intact hepatocytes and adipocytes, oxoreductase activity and thus the generation of F from E predominates [63,64] (see Fig. 2). This has important metabolic consequences, enhancing GR-dependant hepatic gluconeogenesis and glucose output and adipocyte

differentiation, respectively. Therefore, there is great interest in the role of  $11\beta$ -HSD1 in the pathogenesis and future treatment of patients who have the metabolic syndrome [61]. The enzyme that supplies NADPH to  $11\beta$ -HSD1 within the endoplasmic reticulum, thereby conferring  $11$ -oxo-reductase activity upon it, is hexose-6-phosphate dehydrogenase (H6PDH). Combined mutations in the genes encoding  $11\beta$ -HSD1 and H6PDH explain the molecular basis for the putative  $11\beta$ -HSD1 deficient state, cortisone reductase deficiency, an inherited form of polycystic ovary syndrome [65].

Thus at a prereceptor level, the expression and activity of  $11\beta$ -HSDs offer a tissue-specific regulator of mineralocorticoid and glucocorticoid hormone action. This is important not only for the activity of endogenous steroids, cortisol and cortisone, but also the synthetic pharmacologic counterparts, prednisolone and prednisone [66].

## Effects of glucocorticoids

### *Carbohydrate, protein, and lipid metabolism*

Glucocorticoids increase blood glucose concentrations through their action on glycogen, protein, and lipid metabolism (Fig. 4). In the liver,

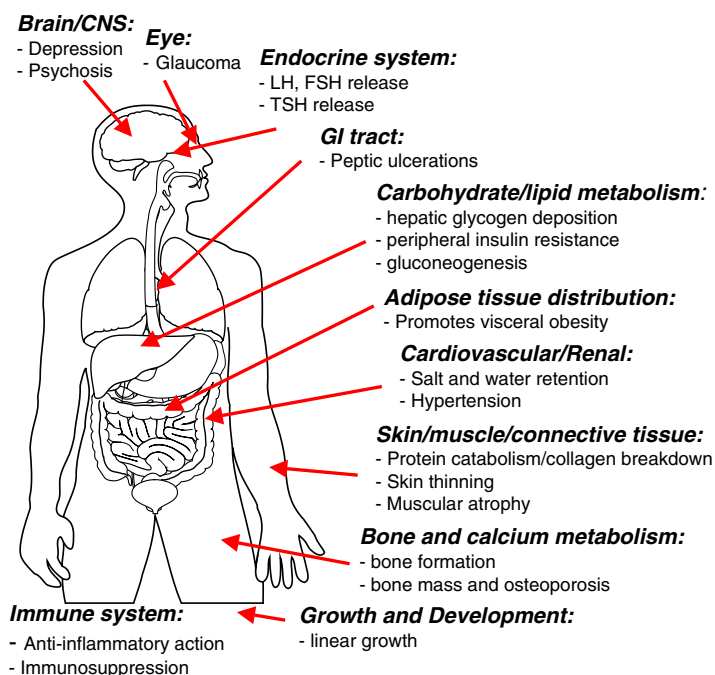


Fig. 4. The principal sites of action of glucocorticoids in people highlighting some of the diverse effects of cortisol upon many tissues.

cortisol stimulates glycogen deposition by increasing glycogen synthase and inhibiting the glycogen mobilizing enzyme, glycogen phosphorylase. Hepatic glucose output increases through the activation of key enzymes involved in gluconeogenesis, principally glucose-6-phosphatase and phosphoenolpyruvate kinase (PEPCK) [67]. In peripheral tissues (muscle and fat), cortisol inhibits glucose uptake and use. In adipose tissue, lipolysis is activated, resulting in the release of free fatty acids into the circulation. An increase in total circulating cholesterol and triglycerides is observed, but HDL cholesterol levels fall. Glucocorticoids also have a permissive effect upon other hormones including catecholamines and glucagon. The resultant effect is to cause insulin resistance and an increase in blood glucose concentrations, at the expense of protein and lipid catabolism. In Cushing's syndrome, glucose intolerance frequently occurs, and overt diabetes mellitus is present in up to one-third of patients in some series. Hepatic lipoprotein synthesis is stimulated, and increases in circulating cholesterol and triglycerides may be found.

Glucocorticoids stimulate adipocyte differentiation, promoting adipogenesis through the transcriptional activation of key differentiation genes including lipoprotein lipase, glycerol-3-phosphate dehydrogenase, and leptin [68]. Chronically, the effects of glucocorticoid excess upon adipose tissue is more complex, at least in people, where the deposition of visceral or central adipose tissue is stimulated, providing a useful discriminatory sign for diagnosing Cushing's syndrome. The explanation for the predilection for visceral obesity may relate to the increased expression of both the GR and type 1 isozyme of 11 $\beta$ -HSD (generating cortisol from cortisone) in omental compared with subcutaneous adipose tissue [63].

Weight gain and obesity are the most common signs in patients who have Cushing's syndrome, and, at least in adults, this is invariably centripetal in nature [69]. Indeed, generalized obesity is more common in the general population than it is in patients who have Cushing's syndrome. One exception to this is childhood, where glucocorticoid excess may result in generalized obesity. In addition to centripetal obesity, patients develop fat depots over the thoraco-cervical spine (buffalo hump), in the supra-clavicular region and over the cheeks and temporal regions giving rise to the rounded moon-like facies. The epidural space is another site of abnormal fat deposition, and this may lead to neurologic deficits.

#### *Skin, muscle, and connective tissue*

In addition to inducing insulin resistance in muscle tissue, glucocorticoids also cause catabolic changes in muscle, skin, and connective tissue. In the skin and connective tissue, glucocorticoids inhibit epidermal cell division and DNA synthesis and reduce collagen synthesis and production. In muscle, glucocorticoids cause atrophy (but not necrosis), and this seems to be specific for type II or phasic muscle fibers. Muscle protein synthesis is

reduced. As a result, patients who have Cushing's syndrome present with muscle weakness and atrophy, characteristically affecting the gluteal and femoral proximal muscles.

### *Bone and calcium metabolism*

The effects of glucocorticoids upon osteoclast function are debated, but osteoblast function is inhibited by glucocorticoids, and this is thought to explain the osteopenia and osteoporosis that characterize glucocorticoid excess [70]. With 0.5% to 1% of Western populations taking chronic glucocorticoid therapy, glucocorticoid-induced osteoporosis is becoming a prevalent health concern. Glucocorticoids also induce negative calcium balance by inhibiting intestinal calcium absorption and increasing renal calcium excretion. As a consequence, parathyroid hormone secretion usually is increased.

In childhood, the most common presentation of Cushing's syndrome is with poor linear growth and weight gain. Many patients who have long-standing Cushing's syndrome have lost height because of osteoporotic vertebral collapse. This can be assessed by measuring the patient's height and comparing it with his or her span; in normal subjects, these measurements should be equal. Pathologic fractures, either spontaneous or after minor trauma, are not uncommon. Rib fractures, in contrast to those of the vertebrae, are often painless. The radiograph appearances are typical, with exuberant callus formation at the site of the healing fracture. In addition, aseptic necrosis of the femoral and humeral heads, a recognized feature of high-dose exogenous corticosteroid therapy, can occur in endogenous Cushing's syndrome. Hypercalciuria may lead to renal calculi, but hypercalcemia is not a feature, because glucocorticoid excess prevents gastrointestinal (GI) calcium absorption.

### *Salt and water homeostasis and blood pressure control*

Glucocorticoids increase blood pressure by several mechanisms involving actions on the kidney and vasculature [71]. In vascular smooth muscle, they increase sensitivity to pressor agents such as catecholamines and angiotensin II while reducing nitric oxide-mediated endothelial dilatation. Angiotensinogen synthesis is increased by glucocorticoids. In the kidney, depending upon the activity of the type 2 isozyme of  $11\beta$ -HSD, cortisol can act on the distal nephron to cause sodium retention and potassium loss (mediated by means of the MR). Elsewhere across the nephron, glucocorticoids increase glomerular filtration rate, proximal tubular epithelial sodium transport, and free water clearance [72]. This latter effect involves antagonism of the action of vasopressin and explains the dilutional hyponatremia seen in patients who have glucocorticoid deficiency [73].

Hypokalemic alkalosis is found in 10% to 15% of patients who have Cushing's disease but over 95% of patients who have ectopic ACTH syndrome. Several factors may contribute to this mineralocorticoid excess state, including corticosterone and deoxycorticosterone excess, but the principal culprit is thought to be cortisol itself. Depending upon the prevailing cortisol production rate, cortisol swamps the protective 11 $\beta$ -HSD2 in the kidney to act as a mineralocorticoid. Hypokalemic alkalosis is more common in ectopic ACTH syndrome, because cortisol production rates are higher than in patients who have Cushing's disease [74,75]. This can be diagnosed by documenting an increase in the ratio of urinary cortisol/cortisone metabolites. In addition, hepatic 5 $\alpha$ -reductase activity is inhibited, resulting in a greater excretion of 5 $\beta$ -cortisol metabolites.

Hypertension is another prominent feature in Cushing's syndrome, occurring in up to 75% of cases. Even though epidemiologic data show a strong association between blood pressure and obesity, hypertension is much more common in patients who have Cushing's syndrome than in those who have simple obesity [76]. This, together with the established metabolic consequences of the disease (diabetes, hyperlipidemia), is thought to explain the increased cardiovascular mortality in untreated cases. In addition, thromboembolic events may be more common in Cushing's disease patients.

#### *Anti-inflammatory actions and the immune system*

Glucocorticoids suppress immunologic responses, and this has been the stimulus to develop a series of highly potent pharmacologic glucocorticoids to treat several autoimmune and inflammatory conditions. The inhibitory effects are mediated at many levels. In peripheral blood, glucocorticoids reduce lymphocyte counts acutely (T lymphocytes > B lymphocytes) by redistributing lymphocytes from the intravascular compartment to the spleen, lymph nodes, and bone marrow [77]. Conversely, neutrophil counts increase following glucocorticoid administration. Eosinophil counts rapidly fall, an effect, which historically was used as a bioassay for glucocorticoids. The immunologic actions of glucocorticoids involve direct actions on both T and B lymphocytes, and these include inhibition of immunoglobulin synthesis and stimulation of lymphocyte apoptosis [78]. Inhibition of cytokine production from lymphocytes is mediated through inhibition of the action of nuclear factor kappa B (NF- $\kappa$ B). NF- $\kappa$ B plays a crucial and generalized role in inducing cytokine gene transcription; glucocorticoids can bind directly to NF- $\kappa$ B to prevent nuclear translocation, and, in addition induce NF- $\kappa$ B inhibitors, which sequester NF- $\kappa$ B in the cytoplasm, thereby inactivating its effect [79].

Additional anti-inflammatory effects involve inhibition of monocyte differentiation into macrophages and macrophage phagocytosis and cytotoxic activity. Glucocorticoids reduce the local inflammatory response by preventing the action of histamine and plasminogen activators.

Prostaglandin synthesis is impaired through the induction of lipocortins that inhibit phospholipase A2 activity.

Infections are more common in patients who have Cushing's disease [79,80]. In many instances, these are asymptomatic and occur because the normal inflammatory response is suppressed. Reactivation of tuberculosis has been reported and has even been the presenting feature in some cases. Fungal infections of the skin (notably tinea versicolor) and nails may occur, as may opportunistic fungal infections. Bowel perforation is more common in patients who have extreme hypercortisolism, and in turn, the hypercortisolism may mask the usual symptoms and signs of the condition. Wound infections are common and contribute to poor wound healing.

### *Central nervous system and mood*

Clinical observations on patients who have glucocorticoid excess and deficiency reveal that the brain is an important target tissue for glucocorticoids, with depression, euphoria, psychosis, apathy, and lethargy being important manifestations. Both glucocorticoid and mineralocorticoid receptors are expressed in discrete regions of the rodent, brain including hippocampus, hypothalamus, cerebellum, and cortex [81]. Glucocorticoids cause neuronal death, notably in the hippocampus, and this may underlie the recent interest in glucocorticoids and cognitive function, memory, and neurodegenerative diseases such as Alzheimer's disease. Of note, DHEA has been shown to have neuroprotective effects in the hippocampus region [82,83]. CYP7B, an enzyme metabolizing DHEA to its 7 $\alpha$ -hydroxylated metabolite, was shown to be highly expressed in brain, with increased expression in the hippocampus. Moreover, it has been shown that in the brain of Alzheimer's disease patients CYP7B expression in dentate neurons is decreased significantly [84]. In Cushing's syndrome, a characteristic shrinkage of the hippocampus region has been observed, caused by glucocorticoid excess, and it may be worthwhile to speculate whether the suppression of DHEA in adrenal Cushing's syndrome may result in a more pronounced effect because of the loss of a putative hippocampus protective factor.

Psychiatric abnormalities occur in approximately 50% of patients who have Cushing's syndrome regardless of cause [85]. Agitated depression and lethargy are among the most common problems [86], but paranoia and overt psychosis also are recognized. Memory and cognitive function also may be affected, and increased irritability may be an early feature. Insomnia is common, and both rapid eye movement and delta wave sleep patterns are reduced. Lowering of plasma cortisol by medical or surgical therapy usually results in a rapid improvement in the psychiatric state. Psychosis occurs most frequently in patients who have ACTH-dependent disease, and once this is cured, psychosis usually cannot be provoked by exogenous glucocorticoid treatment.

In the eye, glucocorticoids act to raise intraocular pressure through an increase in aqueous humor production and deposition of matrix within the trabecular meshwork that inhibits aqueous drainage.  $11\beta$ -HSD1 is expressed in the ciliary epithelium, and this may augment corticosteroid-induced aqueous humor production [61]. Steroid-induced glaucoma appears to have a genetic predisposition, but the underlying mechanisms are unknown [87]. Ocular effects in Cushing's syndrome include raised intraocular pressure and exophthalmos (in up to one third of patients in Cushing's original series), the latter occurring because of increased retro-orbital fat deposition. Cataracts, a well-recognized complication of corticosteroid therapy, seem to be uncommon [88], except as a complication of diabetes. In the authors' experience, chemosis is a sensitive and under-reported feature of Cushing's syndrome.

### *Gut*

Chronic, but not acute administration of glucocorticoids increases the risk of developing peptic ulcer disease [89]. Pancreatitis with fat necrosis is reported in patients who have glucocorticoid excess. The GR is expressed throughout the GI tract and the MR in the distal colon, and these mediate the corticosteroid control of epithelial ion transport.

### *Endocrine effects*

Glucocorticoids suppress the thyroid axis, probably through a direct action on thyrotropin secretion. In addition, they inhibit 5' deiodinase activity, mediating the conversion of thyroxine to active triiodothyronine. Growth hormone secretion is reduced, possibly mediated through an increase in somatostatinergic tone. Glucocorticoids also act centrally to inhibit GnRH pulsatility and luteinizing hormone and follicle-stimulating hormone release [90]. These physiologic effects of glucocorticoids explain the functional suppression of the pituitary–thyroid axis and pituitary–gonadal axis in patients who have Cushing's syndrome. Cortisol causes a reversible form of hypogonadotrophic hypogonadism but also directly inhibits Leydig cell function.

In Cushing's syndrome, gonadal dysfunction is very common, with menstrual irregularity in females and loss of libido in both sexes. Hirsutism frequently is found in female patients, as is acne. The most common form of hirsutism is vellous hypertrichosis on the face, and this should be distinguished from darker terminal differentiated hirsutism that may occur, but usually signifies concomitant androgen excess (as may occur secondary to ACTH-mediated adrenal androgen secretion).

### **Summary**

Insights into the physiology and pathophysiology of adrenal steroids help to explain how the clinical sequelae of Cushing's syndrome evolve.



Endogenous glucocorticoid excess does not impact only on glucocorticoid action, but it also affects adrenal steroidogenesis and steroid metabolism, thereby contributing to the manifestations of the disease. Further understanding of the underlying pathophysiology will enable clinicians to improve diagnostic assessment and therapeutic management of patients suffering from Cushing's syndrome.

## References

- [1] Gwynne JT, Strauss JF III. The role of lipoprotein in steroidogenesis and cholesterol metabolism in steroidogenic glands. *Endocr Rev* 1982;3:299–329.
- [2] Borkowski AJ, Levin S, Delcroix C, et al. Blood cholesterol and hydrocortisone production in man: quantitative aspects of the utilization of circulating cholesterol by the adrenals at rest and under adrenocorticotropin stimulation. *J Clin Invest* 1967;46:797–811.
- [3] Acton S, Rigotti A, Landschulz KT, et al. Identification of scavenger receptor SR-BI as a high-density lipoprotein receptor. *Science* 1996;27:518–20.
- [4] Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 1996;17:221–44.
- [5] Bernhardt R. The role of adrenodoxin in adrenal steroidogenesis. *Current Opinion in Endocrinology & Diabetes* 2000;7:109–15.
- [6] Onoda M, Hall PF. Cytochrome b<sub>5</sub> stimulates purified testicular microsomal cytochrome P450 (C<sub>21</sub> side-chain cleavage). *Biochem Biophys Res Commun* 1982;108:454–60.
- [7] Lee TC, Miller WL, Auchus RJ. Cytochrome b<sub>5</sub> augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem* 1998;273:3158–65.
- [8] Miller WL. Molecular biology of steroid hormone synthesis. *Endocr Rev* 1988;9:295–318.
- [9] White PC, Mune T, Agarwal AK. 11 $\beta$ -Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 1997;18:135–56.
- [10] Rheume E, Simard J, Morel Y, et al. Congenital adrenal hyperplasia due to point mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene. *Nat Genet* 1992;1:239–45.
- [11] Geller DH, Auchus RJ, Mendonca BB, et al. The genetic and functional basis of isolated 17,20-lyase deficiency. *Nat Genet* 1997;17:201–5.
- [12] Tajima T, Fujieda K, Kouda N, et al. Heterozygous mutation in the cholesterol side chain cleavage enzyme (p450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. *J Clin Endocrinol Metab* 2001;86:3820–5.
- [13] Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet* 2004;363:2128–35.
- [14] John ME, John MC, Ashley P, MacDonald RJ, et al. Identification and characterization of cDNA clones specific for cholesterol side-chain cleavage cytochrome P-450. *Proc Natl Acad Sci U S A* 1984;81:5628–32.
- [15] Lorence MC, Murray BA, Trant JM, et al. Human 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta^5$ - $\Delta^4$  isomerase from placenta: expression in nonsteroidogenic cells of a protein that catalyses the dehydrogenation/isomerization of C<sub>21</sub> and C<sub>19</sub> steroids. *Endocrinology* 1990;126:2493–8.
- [16] Bradshaw KD, Waterman MR, Couch RT, et al. Characterization of complementary deoxyribonucleic acid for human adrenocortical 17 $\alpha$ -hydroxylase: a probe for analysis of 17 $\alpha$ -hydroxylase deficiency. *Mol Endocrinol* 1987;1:348–54.
- [17] White PC, New MI, Dupont B. Cloning and expression of cDNA encoding a bovine adrenal cytochrome P450 specific for steroid 21-hydroxylation. *Proc Natl Acad Sci U S A* 1984;81:1986–90.
- [18] Chua SC, Szabo P, Vitek A, et al. Cloning of cDNA encoding steroid 11 $\beta$ -hydroxylase (P450c11). *Proc Natl Acad Sci U S A* 1987;84:7193–7.
- [19] Mornet E, Dupont J, Vitek A, et al. Characterization of two genes encoding human steroid 11 $\beta$ -hydroxylase (P-450(11) $\beta$ ). *J Biol Chem* 1989;264:20961–7.

- [20] Curnow KM, Tusie-Luna MT, Pascoe L, et al. The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. *Mol Endocrinol* 1991;5:1513–22.
- [21] Suzuki T, Sasano H, Takeyama J, et al. Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. *Clin Endocrinol (Oxf)* 2000; 53:739–47.
- [22] Strott CA. Sulfonation and molecular action. *Endocr Rev* 2002;23:703–32.
- [23] Siiteri PK, MacDonald PC. The utilization of dehydroisoandrosterone sulphate for estrogen synthesis during human pregnancy. *Steroids* 1963;2:713–30.
- [24] Rainey WE, White PC. Functional adrenal zonation and regulation of aldosterone biosynthesis. *Current Opinion in Endocrinology & Diabetes* 1998;5:175–82.
- [25] Quinn SJ, Williams GH. Regulation of aldosterone secretion. *Annu Rev Physiol* 1988;50: 409–26.
- [26] Dluhy RG, Axelrod L, Underwood RH, et al. Studies of the control of plasma aldosterone concentration in normal man: effect of dietary potassium and acute potassium infusion. *J Clin Invest* 1972;51:1950–7.
- [27] Labrie F, Belanger A, Simard J, et al. DHEA and peripheral androgen and oestrogen formation: intracrinology. *Ann N Y Acad Sci* 1995;774:16–28.
- [28] Longcope C. Adrenal and gonadal secretion in normal females. *Clin Endocrinol Metab* 1986;15:213–28.
- [29] Orentreich N, Brind JL, Rizer RL, Vogelmann JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab* 1984;59:551–5.
- [30] Reiter EO, Fuldauer VG, Root AW. Secretion of the adrenal androgen, dehydroepiandrosterone sulfate, during normal infancy, childhood, and adolescence, in sick infants, and in children with endocrinologic abnormalities. *J Pediatr* 1977;90:766–70.
- [31] Sklar CA, Kaplan SL, Grumbach MM. Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab* 1980;51:548–56.
- [32] Orentreich N, Brind JL, Vogelmann JH, et al. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. *J Clin Endocrinol Metab* 1992;75: 1002–4.
- [33] Laughlin GA, Barrett-Connor E. Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo Study. *J Clin Endocrinol Metab* 2000;85: 3561–8.
- [34] Parker CR, Mixon RL, Brissie RM, et al. Aging alters zonation in the adrenal cortex of men. *J Clin Endocrinol Metab* 1997;82:3898–901.
- [35] Liu CH, Laughlin GA, Fischer UG, et al. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for a reduced 17,20-desmolase enzymatic activity. *J Clin Endocrinol Metab* 1990;71:900–6.
- [36] Parker CR, Slayden SM, Azziz R, et al. Effects of aging on adrenal function in the human: responsiveness and sensitivity of adrenal androgens and cortisol to adrenocorticotropin in premenopausal and postmenopausal women. *J Clin Endocrinol Metab* 2000;85:48–54.
- [37] Weinberger C, Hollenberg SM, Rosenfeld MG, et al. Domain structure of the human glucocorticoid receptor and its relationship to the v-erb-A oncogene product. *Nature* 1985; 318:670–2.
- [38] Arriza JL, Weinberger C, Cerelli G, et al. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 1987;237:268–75.
- [39] Gustafsson JA, Carlstedt-Duke J, Poellinger L, et al. Biochemistry, molecular biology, and physiology of the glucocorticoid receptor. *Endocr Rev* 1987;8:185–234.
- [40] Lu NZ, Cidlowski JA. The origin and function of multiple human glucocorticoid receptor isoforms. *Ann N Y Acad Sci* 2004;1024:102–23.

- [41] Zennaro MC, Farman N, Bonvalet JP, et al. Tissue-specific expression of  $\alpha$  and  $\beta$  mRNA isoforms of the human mineralocorticoid receptor in normal and pathological states. *J Clin Endocrinol Metab* 1997;82:1345–52.
- [42] Pratt WB. The role of heat shock protein in regulating the function, folding and trafficking of the glucocorticoid receptor. *J Biol Chem* 1993;268:21455–8.
- [43] Beato M, Sanchez-Pacheco A. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr Rev* 1996;17:587–609.
- [44] Hollenberg SM, Giguere V, Segui P, et al. Colocalization of DNA-binding and transcriptional activation functions in the human glucocorticoid receptor. *Cell* 1987;49:39–46.
- [45] Rusconi S, Yamamoto KR. Functional dissection of the hormone and DNA binding activities of the glucocorticoid receptor. *EMBO J* 1987;6:1309–15.
- [46] McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* 1999;20:321–44.
- [47] Krozowski ZS, Funder JW. Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc Natl Acad Sci U S A* 1983;80:6056–60.
- [48] Edwards CR, Stewart PM, Burt D, et al. Localisation of 11 $\beta$ -hydroxysteroid dehydrogenase—tissue-specific protector of the mineralocorticoid receptor. *Lancet* 1988;ii:836–41.
- [49] Funder JW, Pearce PT, Smith R, et al. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988;242:583–5.
- [50] Verrey F, Kraehenbuhl JP, Rossier BC. Aldosterone induces a rapid increase in the rate of Na, K-ATPase gene transcription in cultured kidney cells. *Mol Endocrinol* 1989;3:1369–76.
- [51] Chen SY, Bhargava A, Mastroberardino L, et al. Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proc Natl Acad Sci U S A* 1999;93:6025–30.
- [52] Rocha R, Funder JW. The pathophysiology of aldosterone in the cardiovascular system. *Ann N Y Acad Sci* 2002;970:89–100.
- [53] Pitt B, Remme W, Zannad F, et al. Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study investigators. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 2003;348:1309–21.
- [54] Christ M, Haseroth K, Falkenstein E, et al. Nongenomic steroid actions: fact or fancy? *Vitam Horm* 1999;57:325–73.
- [55] Hammond GL. Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev* 1990;11:65–79.
- [56] Zumoff B, Bradlow HL, Levin J, et al. Influence of thyroid function on the in vivo cortisol–cortisone equilibrium in man. *J Steroid Biochem* 1983;18:437–40.
- [57] Moore JS, Monson JP, Kaltsas G, et al. Modulation of 11 $\beta$ -hydroxysteroid dehydrogenase isozymes by growth hormone and insulin-like growth factor: in vivo and in vitro studies. *J Clin Endocrinol Metab* 1999;84:4172–7.
- [58] Yamada S, Iwai K. Induction of hepatic cortisol-6-hydroxylase by rifampicin. *Lancet* 1976;2:366–7.
- [59] Morris DJ, Brem AS. Metabolic derivatives of aldosterone. *Am J Physiol* 1987;252:F365–73.
- [60] White PC, Mune T, Agarwal AK. 11 $\beta$ -hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 1997;18:135–6.
- [61] Tomlinson JW, Walker EA, Bujalska IJ, et al. 11 $\beta$ -Hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004;25:831–66.
- [62] Quinkler M, Stewart PM. Hypertension and the cortisol–cortisone shuttle. *J Clin Endocrinol Metab* 2003;88:2384–92.
- [63] Bujalska IJ, Kumar S, Stewart PM. Does central obesity reflect Cushing's disease of the omentum? *Lancet* 1997;349:1210–3.

- [64] Kotelevtsev Y, Holmes MC, Burchell A, et al.  $11\beta$ -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.
- [65] Draper N, Walker EA, Bujalska IJ, et al. Mutations in the genes encoding  $11\beta$ -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet* 2003;34:434–9.
- [66] Cooper MS, Blumsohn A, Goddard PE, et al.  $11\beta$ -Hydroxysteroid dehydrogenase type 1 activity predicts the effects of glucocorticoids on bone. *J Clin Endocrinol Metab* 2003;88:3874–7.
- [67] Exton JH. Regulation of gluconeogenesis by glucocorticoids. In: Baxter JD, Rousseau GG, editors. *Glucocorticoid hormone action*. New York: Springer-Verlag; 1979. p. 535–46.
- [68] Hauner H, Entenmann G, Wabitsch M, et al. Promoting effects of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. *J Clin Invest* 1989;84:1663–70.
- [69] Rebuffe-Scrive M, Krotkiewski M, Elfverson J, et al. Muscle and adipose morphology and metabolism in Cushing's syndrome. *J Clin Endocr Metab* 1988;67:1122–8.
- [70] Canalis E. Clinical review 83. Mechanisms of glucocorticoid action in bone: implications to glucocorticoid-induced osteoporosis. *J Clin Endocrinol Metab* 1996;81:3441–7.
- [71] Fraser R, Davies DL, Connell JMC. Hormones and hypertension. *Clin Endocrinol (Oxf)* 1989;31:701–46.
- [72] Marver D. Evidence of corticosteroid action along the nephron. *Am J Physiol* 1984;246:F111–23.
- [73] Raff H. Glucocorticoid inhibition of neurohypophysial vasopressin secretion. *Am J Physiol* 1987;252:R635–44.
- [74] Ulick S, Wang JZ, Blumenfeld JD, et al. Cortisol inactivation overload: a mechanism of mineralocorticoid hypertension in the ectopic adrenocorticotropin syndrome. *J Clin Endocrinol Metab* 1992;74:963–7.
- [75] Stewart PM, Walker BR, Holder G, et al.  $11\beta$ -Hydroxysteroid dehydrogenase activity in Cushing's syndrome: explaining the mineralocorticoid excess state of the ectopic ACTH syndrome. *J Clin Endocrinol Metab* 1995;80:3617–20.
- [76] Saruta T, Suzuki H, Handa M, et al. Multiple factors contribute to the pathogenesis of hypertension in Cushing's syndrome. *J Clin Endocrinol Metab* 1986;62:275–9.
- [77] Yu DT, Clements PJ, Paulus HE, et al. Human lymphocyte subpopulations: effect of corticosteroids. *J Clin Invest* 1974;53:565–71.
- [78] McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor- $\kappa$ B and steroid receptor-signalling pathways. *Endocr Rev* 1999;20:435–59.
- [79] Dale DC, Petersdorf RG. Corticosteroids and infectious diseases. *Med Clin North Am* 1973;57:1277–87.
- [80] Graham BS, Tucker WS Jr. Opportunistic infections in endogenous Cushing's syndrome. *Ann Intern Med* 1984;101:334–8.
- [81] McEwen BS, deKloet ER, Rostene W. Adrenal steroid receptors and action in the central nervous system. *Physiol Rev* 1986;66:1121–88.
- [82] MacLusky NJ, Hajszan T, Leranthy C. Effects of dehydroepiandrosterone and flutamide on hippocampal CA1 spine synapse density in male and female rats: implications for the role of androgens in maintenance of hippocampal structure. *Endocrinology* 2004;145:4154–61.
- [83] Hajszan T, MacLusky NJ, Leranthy C. Dehydroepiandrosterone increases hippocampal spine synapse density in ovariectomized female rats. *Endocrinology* 2004;145:1042–5.
- [84] Yau JL, Rasmuson S, Andrew R, et al. Dehydroepiandrosterone 7-hydroxylase CYP7B: predominant expression in primate hippocampus and reduced expression in Alzheimer's disease. *Neuroscience* 2003;121:307–14.
- [85] Jeffcoate WJ, Silverstone JT, Edwards CRW, et al. Psychiatric manifestations of Cushing's syndrome: response to lowering of plasma cortisol. *Q J Med* 1979;48:465–72.

- [86] Dorn LD, Burgess ES, Dubbert B, et al. Psychopathology in patients with endogenous Cushing's syndrome: atypical or melancholic features. *Clin Endocrinol (Oxf)* 1995;43: 433–42.
- [87] Clark AF. Steroids, ocular hypertension, and glaucoma. *J Glaucoma* 1995;4:354–69.
- [88] Bouzas EA, Mastorakos G, Friedman G, et al. Posterior subcapsular cataract in endogenous Cushing's syndrome: an uncommon manifestation. *Invest Ophthalmol Vis Sci* 1993;34: 3497–500.
- [89] Messer J, Reitman D, Sacks HS, et al. Association of adrenocorticosteroid therapy and peptic ulcer disease. *N Engl J Med* 1983;309:21–4.
- [90] Saketos M, Sharma N, Santoro NF. Suppression of the hypothalmo-pituitary-ovarian axis in normal women by glucocorticoids. *Biol Reprod* 1993;49:1270–6.