Adrenal Function
Cortisol
Analyte Information
Cortisol

Introduction

Cortisol is a steroid hormone and is the main glucocorticoid hormone in humans (representing 75 – 90% of the plasma corticoids). It is released in response to stress or low blood glucocorticoid level. Its primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in the metabolism of fats, proteins and carbohydrates. It also decreases bone formation.

The chemical name of cortisol is 17,21-dihydroxypregn-4-ene-3,11,20-trione. Its molecular weight is 362.46 Da and its summary formula is C_{21}H_{30}O_{5}. The structural formula of cortisol is shown in Fig.1.

Fig.1: The structural formula of cortisol

![Structural formula of cortisol](image)

This chemical compound is also known as 11-beta-hydrocortisone; hydrocortisone; 4-pregnene-11alpha, 21-triol 3,20-dione and 17alpha-hydroxycorticosterone.

Biosynthesis

Cortisol is produced in the zona fasciculata and reticularis of the human adrenal cortex. As with other adrenal steroids, cortisol is synthesized from cholesterol via a series of enzyme-mediated steps (Fig.2). The first (and rate-limiting) step is the conversion of cholesterol to pregnenolone by the enzyme P450scc (side chain cleavage). There are two subsequent mechanisms of cortisol biosynthesis in which three hydroxylase enzymes are involved: 17α-hydroxylase, 21α-hydroxylase and 11β-hydroxylase.
The hypothalamic-pituitary-adrenal axis (HPA) regulates the secretion of cortisol and other adrenal glucocorticoids and androgens. Cortisol is secreted in response to adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH itself is secreted under the control of the hypothalamic peptide corticotropin-releasing hormone (CRH). The central nervous system is thus the commander-in-chief of glucocorticoid responses, providing an excellent example of close cooperation between the nervous and endocrine systems (Fig.3).
Fig. 3: Regulation of cortisol secretion

AVP – arginine vasopressin
CRH – corticotropin-releasing hormone
ACTH – adrenocorticotropic hormone
Metabolism

Typical cortisol production is 25 mg/day. The majority (~80%) of cortisol circulates bound with high affinity to corticosteroid-binding globulin (CBG, transcortin). Most of the remainder (~15%) is weakly bound to albumin and less than 5% of circulating cortisol is free (unbound). The free and albumin-bound portions of cortisol are biologically active. As a result of its tight binding to CBG, cortisol is metabolized slowly (biological half-life ~ 100 minutes). The principal site of catabolism is within the liver. Metabolism of cortisol involves reduction of the double bond between C-4 and C-5 to form dihydrocortisol or dihydrocortisone. Further metabolism of cortisol and cortisone produces tetrahydrocortisol (THF) and tetrahydrocortisone (THE), which are in turn metabolized to the cortic acids, cortol and cortolone. A minor metabolite of cortisol is 6β-hydroxycortisol, which appears unconjugated in urine (Fig.4). More than 95% of cortisol and cortisone metabolites are conjugated by the liver, by glucuronidation or sulfation, and excreted in urine. Glucuronide metabolites are more abundant than sulfates.

Less than 2% of cortisol is secreted unchanged in urine.

Fig.4: Metabolism of cortisol

![Diagram of cortisol metabolism]

Unchanged cortisol

Tetrahydrocortisol (+50)

Tetrahydrocortisone

6β-hydroxycortisol

CORTISOL

11β-hydroxyetiocholanolone

Cortol (+β cortol)

Cortolone (+β cortolone)
Physiological Function (Fig.5)

Cortisol is a stress hormone that protects the organism against any abrupt changes in physiological equilibrium by affecting carbohydrate, protein and lipid metabolism as well as electrolyte balance.

The name glucocorticoid derives from early observations that these hormones were involved in glucose metabolism. In the a state of fasting, cortisol stimulates several processes that collectively serve to increase and maintain normal concentrations of glucose in the blood. These effects include:

- **Stimulation of gluconeogenesis, particularly in the liver**: This pathway results in the synthesis of glucose from non-hexose substrates such as amino acids and lipids. Enhancing the expression of enzymes involved in gluconeogenesis is probably the best known metabolic function of cortisol.
- **Mobilization of amino acids from extrahepatic tissues**: These serve as substrates for gluconeogenesis.
- **Inhibition of glucose uptake in muscle and adipose tissue**: A mechanism to conserve glucose.
- **Stimulation of fat breakdown in adipose tissue**: The fatty acids released by lipolysis are used for energy production in tissues like muscle, and the released glycerol provides another substrate for gluconeogenesis.

Cortisol plays a very important role in the immune system.

Cortisol can weaken the activity of the immune system. Cortisol prevents proliferation of T-cells by rendering the interleukin-2 producer T-cells unresponsive to interleukin-1 (IL-1), and unable to produce the T-cell growth factor. Cortisol also has a negative feedback effect on interleukin-1. Cortisol and other glucocorticoids have potent anti-inflammatory and immunosuppressive properties. This is particularly evident when they are administered at pharmacological doses, but is also important in normal immune responses.

**Other cortisol effects**
- Cortisol has an impact on blood pressure and water/electrolyte balance.
- Cortisol slows bone formation, thus leading to the development of osteoporosis in the long term.
- Cortisol moves potassium out of cells in exchange for an equal number of sodium ions\textsuperscript{6}.
- Cortisol reduces calcium absorption in the intestine\textsuperscript{7}.

Cortisol and other glucocorticoids have multiple effects on fetal development. An important example is their role in promoting the maturation of the lungs and production of the surfactant necessary for extrauterine lung function.

**Fig.5: Summary of cortisol physiological function\textsuperscript{11}**
Levels
Cortisol secretion does not change significantly with age. Newborns produce more cortisone than cortisol. A few days after birth, cortisol production predominates in a pattern similar to that of adults. In old age, both the production and metabolic clearance rates of cortisol are by about 25% lower than in younger adults. Urinary excretion of cortisol and other 17-hydroxy metabolites decreases with age, but ACTH stimulation of cortisol remains normal in the elderly.

During pregnancy, there is a progressive rise due to increased concentrations of cortisol-binding globulin. In late pregnancy, there is a 2.5-fold increase.

Cortisol levels are regulated by adrenocorticotrophic hormone (ACTH) which is synthesized by the pituitary in response to corticotropin-releasing hormone (CRH). CRH is released in a cyclic fashion by the hypothalamus resulting in diurnal peaks (6 a.m - 8 a.m.) and nadirs (11 p.m.) in plasma cortisol and ACTH levels (Fig.6).

![Fig.6: Diurnal cortisol level course](image)

Typical total cortisol levels in serum/plasma and free cortisol in urine of children and adults are given in table 1 and table 2 respectively. For each assay, the relevant values are shown in the appropriate Instruction for Use (IFU).
Table 1: typical cortisol total levels in serum or plasma

<table>
<thead>
<tr>
<th>Specimen (serum or plasma)</th>
<th>Reference interval (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood:</td>
<td>138 - 469</td>
</tr>
<tr>
<td>0-2 years:</td>
<td>27.6 - 966</td>
</tr>
<tr>
<td>2-6 years:</td>
<td>27.6 - 718</td>
</tr>
<tr>
<td>6-11 years:</td>
<td>27.6 - 1049</td>
</tr>
<tr>
<td>11-15 years:</td>
<td>55.2 - 690</td>
</tr>
<tr>
<td>15-19 years:</td>
<td>27.6 - 856</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>8:00 a.m.:</td>
<td>138 - 635</td>
</tr>
<tr>
<td>4:00 p.m.:</td>
<td>83 - 441</td>
</tr>
<tr>
<td>8:00 p.m. &lt; 50% of 8:00 a.m.</td>
<td></td>
</tr>
<tr>
<td>Maternal (at birth)</td>
<td>1500 ± 86</td>
</tr>
</tbody>
</table>

Table 2: typical cortisol free levels in urine

Urine cortisol levels may also be monitored. Samples are usually collected over the course of 24 hours.

<table>
<thead>
<tr>
<th>Specimen (urine)</th>
<th>Reference interval (nmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10 years:</td>
<td>6.0 - 74</td>
</tr>
<tr>
<td>11 - 20 years:</td>
<td>14 - 152</td>
</tr>
<tr>
<td>Adult</td>
<td>55 - 248</td>
</tr>
</tbody>
</table>

Equation for the conversion of the units: 1 nmol/L = 0.362 ng/mL

Diagnostic utility — prospects and possibilities

Elevated cortisol levels and lack of diurnal variation have been identified in patients with Cushing’s disease and also in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (Addison’s disease, congenital adrenal hyperplasia) and in ACTH deficiency. Due to normal circadian variation in cortisol levels, distinguishing normal and abnormally low cortisol levels can be difficult. Therefore, various tests to evaluate the pituitary-adrenal system.
axis (ACTH-cortisol), including insulin-induced hypoglycaemia, ACTH stimulation, CRF stimulation and artificial blockage of cortisol synthesis with metyrapone are performed.

Relative cortisol and ACTH levels under different conditions are shown in Table 3.

**Table 3: Cortisol and ACTH levels in different conditions**

<table>
<thead>
<tr>
<th>Plasma Cortisol</th>
<th>Plasma ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary hypercortisolism (Cushing's syndrome)</td>
<td>Secondary hypercortisolism (pituitary or ectopic tumor, Cushing's disease, pseudo-Cushing's syndrome)</td>
</tr>
<tr>
<td>Secondary hypocortisolism (pituitary tumor, Sheehan's syndrome)</td>
<td>Primary hypocortisolism (Addison's disease, Nelson's syndrome)</td>
</tr>
</tbody>
</table>

Hypercortisolism is often (70 % of cases$^9$) caused by stimulation of adrenal cortex by increased ACTH production as a result of basophil adenoma of the anterior pituitary – known as Cushing’s disease. This disease affects women five times more often than men. The onset is insidious and usually occurs 2 to 5 years before a clinical diagnosis is made.

Hypercortisolism can be also caused by ectopic ACTH syndrome. In such case benign or more often cancerous tumors arising outside of pituitary can produce ACTH. Lung tumors cause more than half of these cases, and men are affected three times more often than women.

In rare cases, an abnormality of the adrenal glands, most often an adrenal tumor, causes Cushing’s syndrome. Adrenal tumors are five times more common in women than in men, and the average age of onset is about 40.

The most common signs of hypercortisolism are obesity, moon face, high blood pressure, increased blood glucose concentrations, fragile skin, acne, osteoporosis and hirsutism.

Acute stress (including hospitalization and surgery), alcoholism, depression, and many drugs (e.g., exogenous cortisones, anti-convulsants) can prevent normal
diurnal variation, affect response to suppression/stimulation tests, and cause elevated baseline levels.

The most common cause of increased cortisol levels in women is a high circulating concentration of estrogen (e.g., in cases of estrogen therapy or pregnancy) resulting in an increased concentration of CBG.

Elevated cortisol levels are associated with the following disorders:

- Cushing’s disease (pituitary adenoma, secondary hypercortisolism)
- Cushing’s syndrome (primary hypercortisolism)
  - adrenal adenoma (noncancerous tumors)
  - adrenal carcinoma (adrenal cancers, the least common cause of Cushing’s syndrome)
- ectopic ACTH syndrome (secondary hypercortisolism)
- depressive disorders (some patients have a hyperactive hypothalamic-pituitary-adrenal axis)
- schizophrenia
- hypoglycemia
- obesity
- hyperthyroidism
- starvation
- mental anorexia
- alcoholism

Adrenal insufficiency is an endocrine disorder that occurs when the adrenal glands do not produce enough of certain hormones. Adrenal insufficiency can be primary or secondary.

Primary adrenal insufficiency, also called Addison’s disease, occurs when the adrenal glands are damaged and cannot produce enough of the hormone cortisol and often the hormone aldosterone. Addison’s disease affects one to four of every 100,000 people in all age groups and both sexes.

Secondary adrenal insufficiency occurs when the pituitary gland fails to produce enough adrenocorticotropin (ACTH), a hormone that stimulates the adrenal glands to produce cortisol. If ACTH output is too low, cortisol production drops. The adrenal glands may eventually shrink due to lack of ACTH stimulation.
Decreased cortisol levels are associated with the following disorders:

- Addison’s disease (primary adrenal insufficiency)
- tuberculosis (this infection can destroy the adrenal glands and be a cause of Addison’s disease)
- secondary adrenal insufficiency (more common than Addison’s disease)
  - pituitary insufficiency
  - hypothalamic insufficiency
- congenital adrenal hyperplasia
- cirrhosis
- hepatitis

**Diagnostic utility – Practical applications**

**Differential diagnosis of Cushing’s syndrome**

The three most common tests used to diagnose Cushing’s syndrome are the 24-hour urinary free cortisol test, the measurement of midnight plasma cortisol and the low-dose dexamethasone suppression test. Another test, the dexamethasone-corticotropin-releasing hormone tests, may be needed to distinguish Cushing’s syndrome from other causes of cortisol excess.

Cushing’s syndrome is characterized by increased serum cortisol levels. However, the 24-hour urinary free cortisol test is the preferred screening method for Cushing’s syndrome. Urinary free cortisol is proportional to the free (non-bound) cortisol in blood and is the most sensitive and specific test for initial screening of Cushing’s syndrome. Midnight plasma cortisol levels may be measured as well. Cortisol production is normally suppressed at night, but in Cushing’s syndrome, the suppression does not occur.

**Low-dose dexamethasone suppression test (LDDST)**

In the LDDST, a person is given a low dose of dexamethasone, a synthetic glucocorticoid, orally every 6 hours for 2 days. Urine is collected before dexamethasone is administered and several times on each day of the test. A modified LDDST uses a onetime overnight dose.
Cortisol and other glucocorticoids signal the pituitary to release less ACTH, so the normal response after taking dexamethasone is a drop in blood and urine cortisol levels. If cortisol levels do not drop, Cushing’s syndrome is suspected.

The LDDST may not show a drop in cortisol levels in people with depression, alcoholism, high estrogen levels, acute illness, or stress, leading to a false indication of Cushing’s syndrome. On the other hand, drugs such as phenytoin and phenobarbital may cause cortisol levels to drop, falsely indicating that Cushing’s is not present in people who actually have the syndrome. For this reason, it is usually advised to stop taking these drugs at least 1 week before the test.

**Dexamethasone-corticotropin-releasing hormone (dexamethasone-CRH) test**

Some people have high cortisol levels but do not develop the progressive effects of Cushing’s syndrome, such as muscle weakness, fractures, and thinning of the skin. These people may have pseudo-Cushing’s syndrome, a condition sometimes found in people who have depression or anxiety disorders, drink excess alcohol, have poorly-controlled diabetes or are severely obese. Pseudo-Cushing’s does not have the same long-term effects on health as Cushing’s syndrome and does not require direct treatment of the endocrine glands.

The dexamethasone-CRH test rapidly distinguishes pseudo-Cushing’s from mild cases of Cushing’s. This test combines the LDDST and a CRH stimulation test. In the CRH stimulation test, an injection of CRH causes the pituitary to secrete ACTH. Pretreatment with dexamethasone prevents CRH from causing an increase in cortisol in people with pseudo-Cushing’s. Elevations of cortisol during this test suggest Cushing’s syndrome.

**Metyrapone test**

This test helps to distinguish between primary and secondary hypercortisolism. Methyrapon inhibits 11-β-hydroxylase secretion and thus reduces the conversion of 11-deoxycortisol to cortisol. ACTH secretion is stimulated by cortisol declines; increased ACTH production increases 11-deoxycortisol, but it cannot be metabolized to cortisol. Elevated 11-deoxycortisol levels indicate primary adrenal insufficiency, whereas
decreased levels (due to decreased ACTH production) indicate secondary adrenal insufficiency. This test is also used for discrimination of primary and secondary adrenal insufficiency.

**Discrimination between primary and secondary adrenal insufficiency**

In primary adrenal insufficiency, cortisol levels are decreased and ACTH levels are increased; in secondary adrenal insufficiency both cortisol and ACTH levels are decreased.

When symptoms of glucocorticoid deficiency are present and the 8 a.m. plasma cortisol value (or the 24-hour urinary free cortisol value) is lower than expected, further tests are needed to establish the diagnosis. First, the basal plasma ACTH concentration should be measured, followed by the short ACTH stimulation test. Other frequently-used tests are the metyrapone test and the insulin-induced hypoglycemia test.

**ACTH stimulation test**

The ACTH stimulation test is the most commonly used test for diagnosing adrenal insufficiency. In this test, blood cortisol, urine cortisol or both are measured before and after a synthetic form of ACTH is administered by injection. The normal response after an ACTH injection is a rise in blood and urine cortisol levels. People with Addison’s disease or long-standing secondary adrenal insufficiency have little or no increase in cortisol levels.

**CRH stimulation test**

When the response to the ACTH test is abnormal, a CRH stimulation test can help determine the cause of adrenal insufficiency. In this test, synthetic CRH is injected intravenously and blood cortisol levels are measured both before and at 30-minute intervals for two hours after the injection. People with Addison’s disease respond by producing high levels of ACTH but no cortisol. People with secondary adrenal insufficiency have absent or delayed ACTH responses. CRH will not stimulate ACTH secretion if the pituitary is damaged, so an absence of ACTH response points to the pituitary as the cause. A delayed ACTH response points to the hypothalamus as the cause.
Diagnosis during an emergency

In patients suspected of having an Addisonian crisis, treatment must begin with injections of salt, glucose-containing fluids, and glucocorticoid hormones immediately. Although a reliable diagnosis is not possible during crisis treatment, measurement of blood ACTH and cortisol during the crisis—before glucocorticoids are given—is enough to make a preliminary diagnosis. Low blood sodium, low blood glucose, and high blood potassium are also usually present at the time of an adrenal crisis. Once the crisis is controlled, an ACTH stimulation test can be performed to obtain the specific diagnosis.

In the diagnosis of congenital adrenal hyperplasia (CAH)

Cortisol is measured together with DHEA, 17α-hydroxyprogesterone and androstenedione.

Patients taking prednisone

These patients may have falsely increased cortisol levels because prednisone is converted to prednisolone after ingestion. Prednisolone can have a quite high level of cross-reactivity with cortisol.

If there is a suspicion of hypercortisolism it is recommended to test:
- free cortisol in urine
- circadian profile of cortisolemia
- dexamethasone suppression test
1. Tao Le et al: First Aid USMLE Step 1 2009, Tao Le et al.