Biochemistry of Cushing's Syndrome

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Before studying this lecture:

Don't memories any number or percentage

Don't memories any procedure

Focus mainly on the Interpretations and results of each test

As we already know:

Anatomically:

- The adrenal gland is situated on the anteriosuperior aspect of the kidney

Histologically:

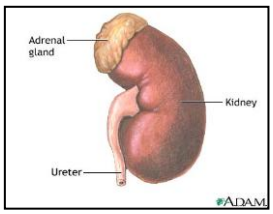
- The adrenal gland consists of two distinct tissues of different embryological origin, the outer cortex and inner medulla.

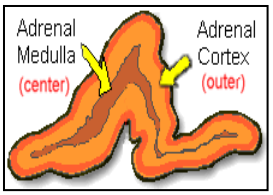
The adrenal cortex comprises three zones based on cell type and function:

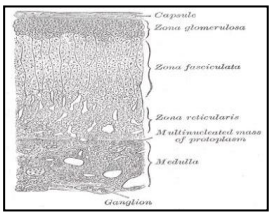
- **Zona glomerulosa** The outermost zone → **aldosterone** (the principal mineralocorticoid).

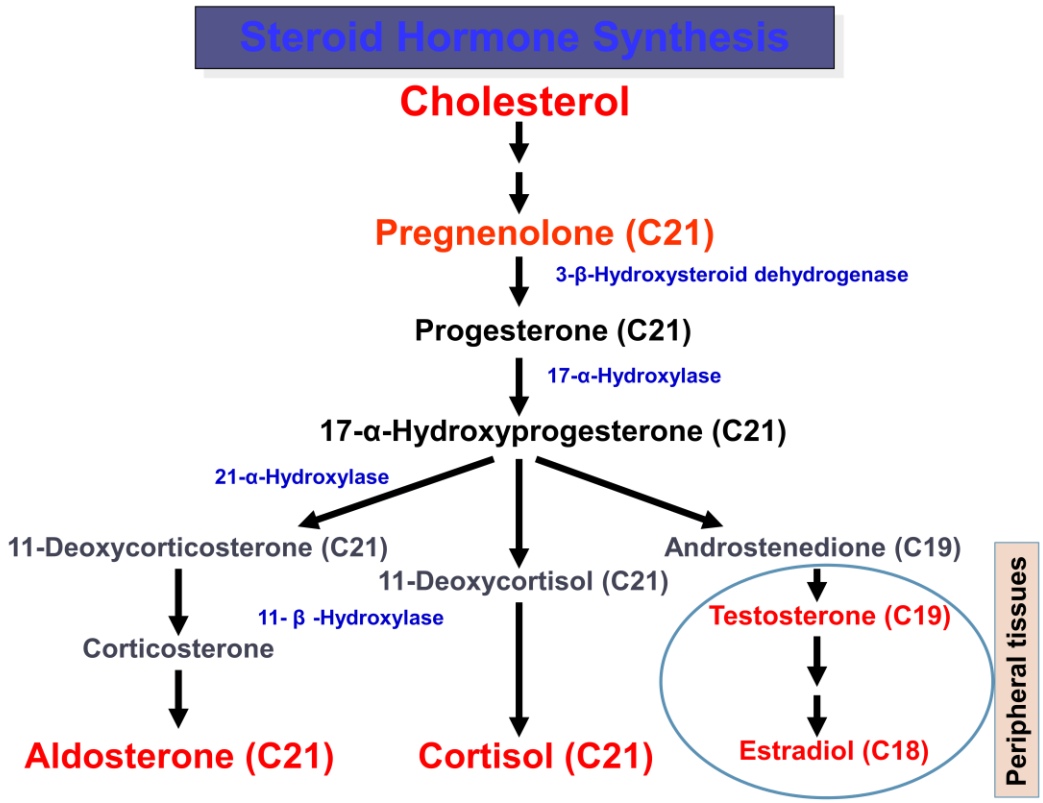
The deeper layers of the cortex:

- **Zona fasciculata** → glucocorticoids – mainly **cortisol** (95%) our main concern in Cushing's syndrome
- **Zona reticularis** → Sex hormones









All you need to know from the previous diagram is that:

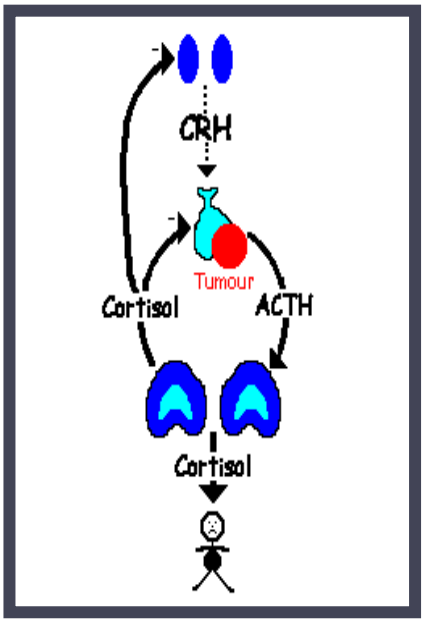
- Cholesterol is the precursor for all steroid hormones.
- One of the sources of adrenal androgens that could be converted to a sex hormone is **Androstenedione.**
- **Androstenedione** is produced by the cortex and is converted to **Testosterone** or **Estradiol** in peripheral tissues.
- That's why we can find **Testosterone** in females, it comes from the **Androstenedione**, and sometimes **Testosterone** could be converted to **Estradiol** *(the female sex hormone).*

Hypothalamic-Pituitary-Adrenal (HPA) Axis:

- The hypothalamus secretes corticotrophin-releasing hormone (**CRH**) which stimulates the anterior pituitary gland to release **ACTH.**
- **ACTH** acts on the zona fasiculata cells → release of **glucocorticoids (*Cortisol*).**

Regulation of ACTH and Cortisol Secretion:

1. Negative feedback control: (when there are high cortisol levels)



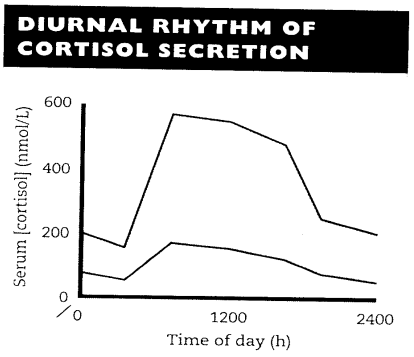
- ACTH release from the anterior pituitary is stimulated by hypothalamic secretion of corticotrophin releasing hormone (CRH).
- CRH → ↑ ACTH → ↑[Cortisol]
- **↑[Cortisol] or synthetic steroid suppress CRH & ACTH secretion**
- That's why the doctor should stop the exogenous cortisol gradually.

2. Stress

(e.g. major surgery, emotional stress) Stress → ↑↑ CRH & ACTH → ↑↑ Cortisol

* The patient should be settled down before withdrawal of blood sample to avoid false a high of cortisol.

3. The diurnal rhythm of plasma cortisol:



- **Highest** Cortisol level in the **morning** (8 - 9 AM).
- **Lowest** Cortisol level in the late **afternoon and evening** (8 - 9 PM).

The diurnal rhythm of cortisol secretion; *The area between the curves represents values that lie within the reference range.* This rhythm will be disturbed in case of cushing.

Case: when measuring a patient's cortisol level, at morning it was 400 and during night 350. This situation is referred to as ***"Loss of diurnal rhythm".***

Plasma [CBG]:

- In the circulation, glucocorticoids are mainly protein-bound (about 90%) because the precursor is cholesterol which is a hydrophobic molecule, chiefly to cortisol-binding globulin (CBG or transcortin).
  - ↑↑ In pregnancy and with estrogen treatment (e.g. oral contraceptives).
  - ↓↓ In hypoproteinemic states (e.g. nephrotic syndrome).
- The **biologically active** fraction of cortisol in plasma is the **free (unbound)** component. 10%

Cortisol and ACTH measurements

For your information: What is the difference between serum and plasma samples? Serum= gets coagulated, Plasma= the tube contains anticoagulant or in other words it lacks fibrin.

Serum [cortisol] and plasma [ACTH]:

- Serum measurement is preferred for cortisol and Plasma for ACTH.

> **Serum:** Consist of clotting factors and thus the blood is coagulated.
>
> **Plasma:** Consist of Anticoagulants that inhibits the clotting factors.

- Samples must be collected (without venous stasis because there will be an increase in protein that will lead to increase the CBG) between 8 a.m. and 9 a.m. and between 10 p.m. and 12 p.m. because of the diurnal rhythm.
- Temporary ↑↑ in these hormones may be observed as a response to emotional stress.

Urinary cortisol excretion:

- Cortisol is removed from plasma by the liver → metabolically inactive compounds → excreted in urine mainly as conjugated metabolites (e.g. glucuronides).
- A small amount of cortisol is excreted unchanged in the urine (UFC).
- In normal individuals:
  - Urinary free cortisol (UFC) is < 250 nmol/24 h.
  - Cortisol / Creatinine ratio in an early morning specimen of urine is < 25 μmol cortisol / mol creatinine.

To sum up: Mainly, you should know that cortisol is 90% bound to CBG, the free form is the biologically active form.

Urinary cortisol is a screening test to include or exclude Cushing disease.

Causes Of Adrenocortical Hyperfunction: Cushing's syndrome (questions may come in the form of cases from the coming parts)

- **ACTH - dependent :** High ACTH Levels

1. ↑ Pituitary (adenoma or carcinoma) ACTH 70% (Cushing's ***disease***).

2. Ectopic ACTH by neoplasms 10%. Especially bronchial cancer, patients may present with weight loss and difficulty in breathing.

3. ACTH therapy.

- **ACTH - independent :** Low ACTH Levels

1. Adrenal tumor 20% (adenoma or carcinoma)

2. Glucocorticoid therapy.

Cushing's Disease is a condition in which the pituitary gland releases too much ACTH, while **Cushing's Syndrome** refers to the general state characterized by excessive levels of the steroid hormone cortisol in the blood.

Causes of elevated serum cortisol concentrations:

1. Increased cortisol secretion:
- Cushing's syndrome
- Exercise
- Stress, Anxiety, Depression
- Obesity
- Alcohol abuse
- Chronic renal failure

2. Increased cortisol binding globulin (CBG):
- Congenital
- Estrogen therapy
- Pregnancy

*Why to mention all the previous states of increased cortisol? To differentiate between Cushing and pseudocushing.

Glucocorticoid functions:

- Glucocorticoids have widespread metabolic effects on carbohydrate, fat and protein metabolism.
- Upon binding to its target, CORTISOL enhances metabolism in several ways:
  - In the liver, Cortisol is an insulin antagonist and has a weak mineralocorticoid action :
    - ↑↑ Gluconeogenesis → production of glucose from newly-released amino acids and lipids
    - ↑↑ Amino acid uptake and degradation
    - ↑↑ Ketogenesis.
  - In the adipose tissue: Cortisol → ↑↑ Lipolysis through breakdown of fat .
  - In the muscles: Cortisol →↑↑ proteolysis (breakdown of proteins) and amino acid release.

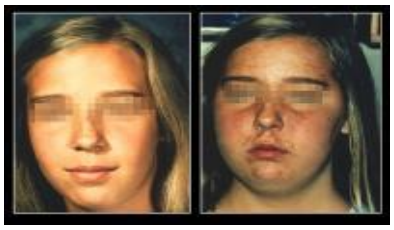
– Conserving glucose: by inhibiting uptake into muscle and fat cells.

Cushing's syndrome

Symptoms:

- Weight gain: trunk and face with sparing of the limbs (central obesity)
- Buffalo's hump.
- Moon face
- Excessive sweating
- Atrophy of the skin and mucous membranes
- Purple striae on the trunk and legs
- Proximal muscle weakness (hips, shoulders)
- Hirsuitism
- The excess cortisol may also affect other endocrine systems → ↓ libido, amenorrhoea and infertility
- Patients frequently suffer various psychological disturbances ranging from euphoria to frank psychosis.

Moon face →



Signs:

- Loss of diurnal rhythm of cortisol and ACTH.
- Hypertension (due to the aldosterone - like effects)
- Hyperglycemia or diabetes due to insulin resistance.
- Hypokalemic alkalosis
- ↑ Protein metabolism.
- Impaired immunity.

Hyperpigmentation is the only common thing in both Cushing and Addison disease is because they both have the same precursor for melanocyte stimulating hormone and ACTH.

Investigations of suspected adrenocortical hyperfunction

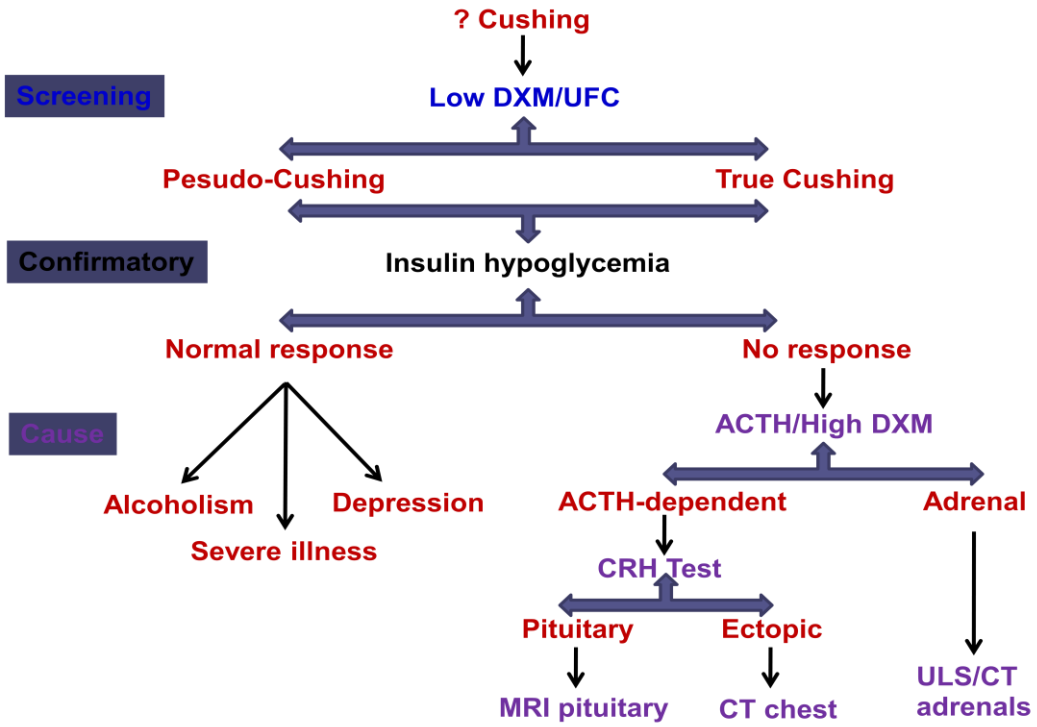
A. Screening tests (out-patient): to assess the clinical diagnosis of adrenocortical hyperfunction.

B. Confirmatory tests (in-patient): to confirm or exclude the provisional diagnosis

C. Tests to determine the cause: to ascertain:

(a) The site of the pathological lesion (adrenal cortex, pituitary or elsewhere?)

(b) The nature of the pathological lesion. (Benign or malignant)



A-Screening test:

Effective screening tests need to be <u>sensitive</u> but <u>do not have to be highly specific</u>.

It includes:

1. **Low-dose dexamethasone suppression test**: (Overnight suppression test)

   Dexamethasone → ↓ CRH → ↓ ACTH → ***<u>↓cortisol</u>*** (normal result)

For your own knowledge: <u>Low-dose dexamethasone (DXM) suppression test</u>: (outpatient procedure)

<u>Procedure:</u> One mg DXM administered at 11-12 PM the night before attending the clinic.

Serum cortisol is measured at 8-9 AM.

<u>Result:</u> <u>Cortisol < 50 nmol/L (suppression)→ exclude Cushing's disease</u>

<u>Precautions:</u> Drugs that induce hepatic microsomal enzymes (Phenobarbitone & phenytoin) → ↑ DXM metabolism and ↓ DXM blood level to achieve CRH suppression (false diagnosis of Cushing)

2. **24-hour urinary free cortisol**

For your own knowledge: 24- hour urinary free cortisol:

<u>Result:</u> <u>Cortisol < 250 nmol/day → exclude Cushing's disease.</u>

<u>Disadvantage:</u> incomplete collection of urine → a false-negative result

- <u>An alternative is to determine</u> the urinary cortisol: creatinine ratio on an early morning specimen

You MUST know the results in order to determine the exact cause.

Interpretation of screening tests:

- **The screening tests serve to:** distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome**.**
- **Confirmatory tests (in-patient basis)** are required to rule out pseudo-Cushing's syndrome
- **Pseudo-Cushing's syndrome:**
  - Depressed or extremely anxious patients
  - Severe intercurrent illness
  - Alcoholism

B- Confirmatory tests: (Inpatient):

- **Insulin-induced hypoglycemia**

Pseudo-Cushing patients show abnormal diurnal rhythm of S. cortisol, but, with Insulin-induced hypoglycemia → ↑ CRH, ACTH and cortisol blood levels

True Cushing patients: No response to hypoglycemia

For your own knowledge Insulin-induced hypoglycemia:

- Hypoglycemia →↑ CRH →↑ ACTH →↑ cortisol
- To test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis.
- To distinguish true Cushing's syndrome from pseudo-Cushing's syndrome
- Contraindicated in: epilepsy or heart disease.
- Procedure:
- Insulin I.V. (0.15 U/kg) to lower blood glucose to 2.2 mmol/L or less .
- Samples for simultaneous measurement of serum glucose and cortisol levels are taken basally (before insulin injection) and at 30, 45, 60 and 90 min after I.V. insulin injection.
- Failure to achieve a glucose level of 2.2 mmol/L invalidates the test and should be repeated with increment in step of 0.05U/kg.

Interpretation of the results of the confirmatory test:

Normally:

- Basal serum cortisol: at least 145 nmol/L
- At 60 - 90 minutes: the level > 425 nmol/L

Do not memories any numbers

Patients with Cushing's syndrome:

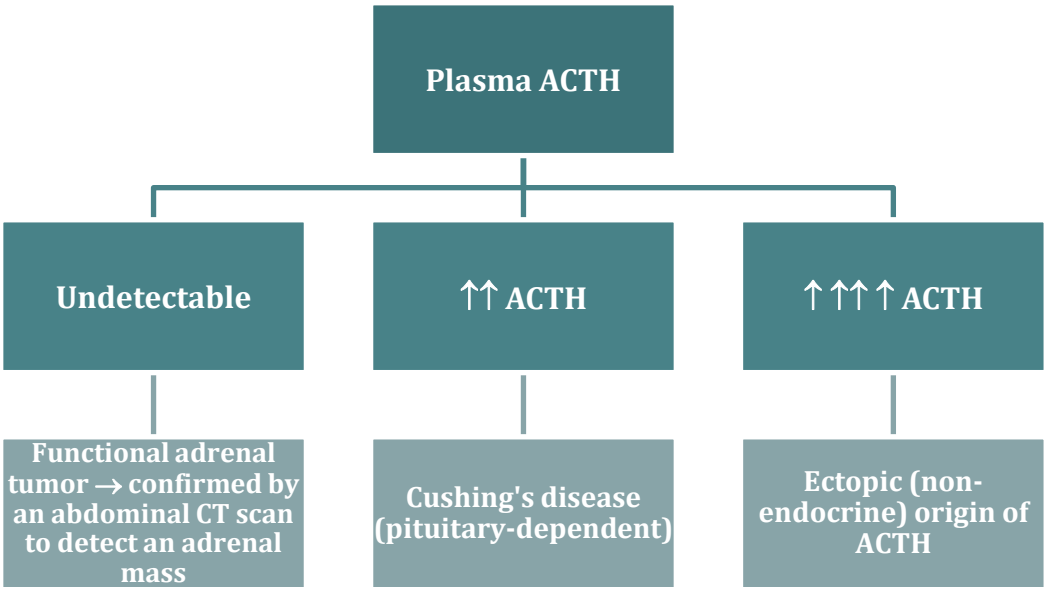
- Whatever the cause, do not respond normally to insulin-induced hypoglycemia.
  - High basal serum cortisol than normal .
  - At 60 - 90 minutes: no increase in S. cortisol, despite the production of an adequate degree of hypoglycemia.

C- Tests used to determine the cause of Cushing's syndrome:

1. Plasma ACTH (Diurnal rhythm)
2. High-dose dexamethasone suppression test
3. CRH stimulation test
4. Radiological tests: MRI of pituitary and ultrasound or CT of adrenals

1. Plasma [ACTH]: *important*

Plasma [ACTH] should be measured on blood specimens collected at 8-9 a.m. and 8-9 p.m.



2. High-dose dexamethasone suppression test: It is used to distinguish Cushing's disease from ectopic ACTH secretion.

- 2 mg dexamethasone six-hourly for 48 hours to suppress cortisol secretion.
- Basal (pre-dexamethasone) serum cortisol or 24-hour urine free cortisol is compared with the results at the end of the 48-hour period.
- Suppression is defined as a fall to less than 50 % of basal value.
- **About 90 % of patients with Cushing's disease show suppression of cortisol output.**

- **In contrast, only 10% of patients with ectopic ACTH production (or with adrenal tumors) show suppression**

3. CRH stimulation test:

Measures the ACTH and cortisol levels basally and 60 minutes after injection of 100 µg CRH.

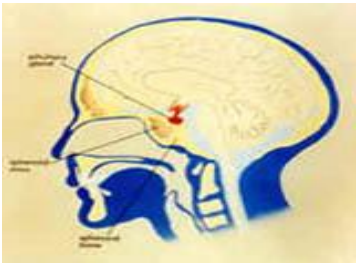
Ectopic ACTH & adrenal tumors	Cushing's disease
• No response • False-positive responses are unusual	• ↑↑ ACTH & cortisol above basal at 60 min • 10% of patients fail to respond

In Cushing's disease: High-dose dexamethasone suppression test + the CRH test → 100 % specificity and sensitivity.

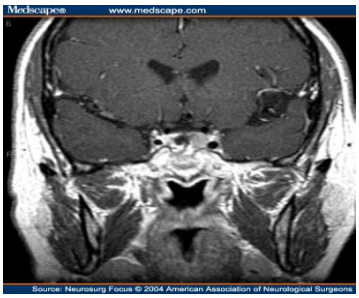
4. Radiological Investigations:

Ultrasound or CT scanning of the adrenal glands

MRI of the pituitary gland



Coronal contrast-enhanced MRI of the sella turcica in a patient with recurrent Cushing's disease



Other blood tests commonly performed for patients suspected to have Cushing's syndrome are:

- Full blood count
- Blood glucose
- Blood electrolytes and pH
- Renal function tests
- Liver function tests

Adrenal Hyperfunction: Summary of Biochemical Tests

Test	Cushing's disease	Adrenal tumor	Ectopic ACTH secreting tumor
S. cortisol	↑	↑	↑
Dexamethasone Low dose test	**Not suppressed**	**Not suppressed**	**Not suppressed**
Urinary cortisol	↑	↑	↑
Diurnal rhythm	**Lost**	**Lost**	**Lost**
Insulin-induced hypoglycemia	**No response**	**No response**	**No response**
Plasma [ACTH]	**Normal or ↑**	**Not detectable**	↑↑↑
Dexamethasone High dose test	**suppressed**	**Not suppressed**	**Not suppressed**
CRH test	↑	**No response**	**No response**

Case study: 58 years old man was admitted with weight loss and respiratory distress. He had increased pigmentation and BP was 140/80.

Lab tests:

Urea	8.6	Normal range (2.5-7 mmol/L)
Sodium	144	Normal range (135-145 mmol/L)
Potassium	2.0	Normal range (3.5-4.5 mmol/L)
Cortisol	1650	Normal range (150-550 nmol/L)
Post overnight DMX	1530	Normal range (<50nmol/L)

Further investigation revealed the following:

DMX suppression test	**Basal**	**after 48 h**	**after 48 h**
		0.5 mg qid	2.0 mg qid
Serum cortisol	1350 8a.m	1420 22.00 pm	1100 No suppression
Plasma ACTH (ng/L)	220	180	Ref. range: 7-51
CRH showed flat response for cortisol and ACTH			

Take Home Message:

- Disorders of the adrenals are uncommon.
- Sensitive screening tests for adrenocortical functions are important.
- Additional confirmatory tests are required to establish the diagnosis and rule out pseudo-Cushing.
- Other biochemical tests and radiological investigation are required to determine the cause of Cushing's syndrome.

Summary:

- Cholesterol is the precursor for: Aldosterone, Cortisol, Testosterone and Estradiol (sex hormones).
- Cortisol is 90% bound to CBG, the free form is the biologically active form.
- Urinary cortisol is a screening test to include or exclude Cushing disease.
- **Investigations of suspected adrenocortical hyperfunction include:**
  - **A-Screening test:**
    - **Low-dose dexamethasone suppression test**

      Result: Cortisol < 50 nmol/L (suppression) → exclude Cushing's disease
    - **24-hour urinary free cortisol**

      Result: Cortisol < 250 nmol/day → exclude Cushing's disease.
  - **B- Confirmatory tests: (Inpatient):**
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