Biochemistry of Cushing's Syndrome

Biochemistry Team

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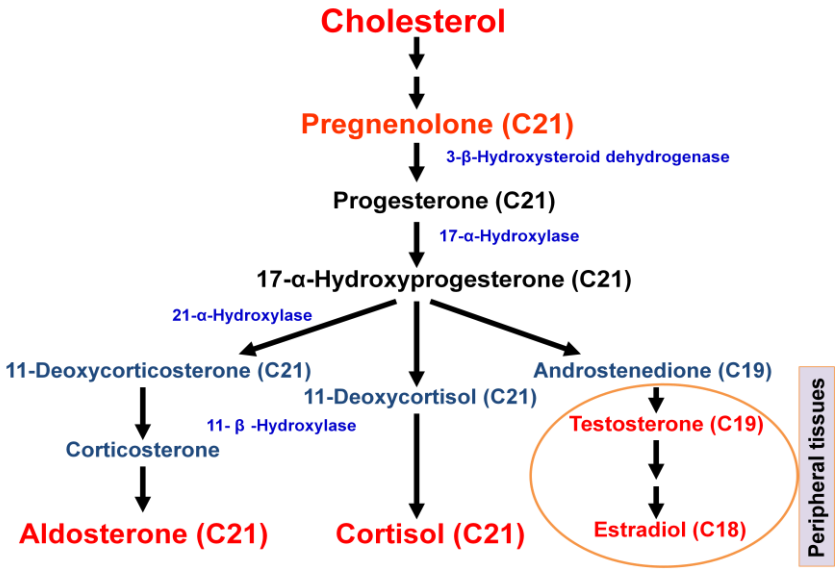
Note : doctor Amr said that the real lecture starts from "Investigations of suspected adrenocortical hyperfunction"
The interpretation of each test is very while the details of test procedure are not important

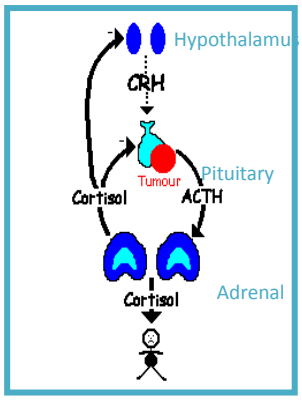
STEROID HORMONE SYNTHESIS

This schematic diagram shows the major steroid biochemistry pathways. Steroid hormones are synthesized in adrenal cortex and gonads from the **initial substrate Cholesterol** via a series of interconnected enzymatic steps



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:

- 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

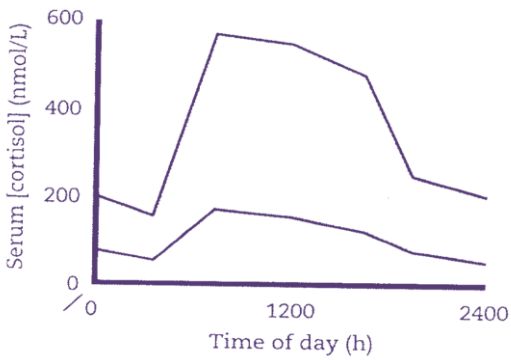
Withdrawal of steroid drugs should be gradual, because HPA axis needs time to get back to normal (allowing the pituitary and adrenal glands to resume their normal function)

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

Normally, the plasma cortisol level at evening should be approximately half of the morning's level. If there is no significant variation, then the patient will present with (loss of Diurnal Rhythm)



The diurnal rhythm of cortisol secretion; *the area between the curves represents values that lie within the reference range*

Plasma [CBG] :

- In the circulation, glucocorticoids are mainly protein-bound (about 90%), 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.

CORTISOL AND ACTH MEASUREMENTS

Serum [cortisol] and plasma [ACTH]:

- Serum measurement is preferred for cortisol and Plasma for ACTH.
- Samples must be collected (without venous stasis) 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).

It will be deactivated then conjugated in the liver.

- 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.

Dr. Rana said: no need to memorize any value, the normal values will be mentioned in the exam's questions.

CAUSES OF ADRENOCORTICAL HYPERFUNCTION; CUSHING'S SYNDROME

- **ACTH - dependent :**

1. ↑ Pituitary ACTH 70% (Cushing's disease).
   (↑**ACTH** - ↑Cortisol)
2. Ectopic ACTH by neoplasms 10%.
   (e.g. small cell carcinoma of the lung)
3. ACTH therapy.

When should we say Cushing's disease and Cushing's Syndrome?

1- Cushing's disease: we say it only when the ACTH is markedly increased from its original secreting gland (Pituitary Adenoma)

2- Cushing's syndrome: could be ACTH-dependent (but not from pituitary) and ACTH –Independent (from the adrenal gland)

- **ACTH - independent :**

1. Adrenal tumor 20% (adenoma or carcinoma)
2. Glucocorticoid therapy. (Treatment for some inflammatory conditions; rheumatoid arthritis)

CAUSES OF ELEVATED SERUM CORTISOL CONCENTRATIONS

1. Increased cortisol secretion:

• Cushing's syndrome (most common one), Exercise, Stress, Anxiety, Depression, Obesity, Alcohol abuse and Chronic renal failure

2. Increased cortisol binding globulin (CBG):

• Congenital, Estrogen therapy and Pregnancy

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 **(non-carbohydrates sources)**
- ↑↑ Amino acid uptake and degradation
- ↑↑ Ketogenesis Due to increased Lipolysis

The most two important words to know about the Cortisol Function are:
1) Insulin Antagonist → Hyperglycemia
2) Catabolic action.

In the adipose tissue: Cortisol → ↑↑ Lipolysis through breakdown of fat.

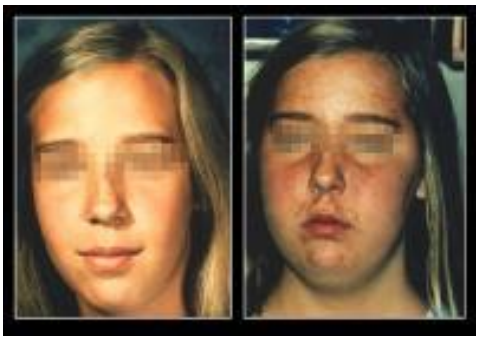
In the muscles: Cortisol →↑↑ proteolysis and amino acid release.

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. (accumulation of fat at the upper back and behind the neck. It is a kind of lipodystrophy)
- Moon face. (due to fat redistribution)
- Excessive sweating
- Atrophy of the skin and mucous membranes
- Purple striae on the trunk and legs (Hyperpigmentation due to ↑ MSH)
- Proximal muscle weakness (hips, shoulders) (due to its catabolic effects on proteins ↑ proteolysis)
- Hirsuitism (Due to ↑ Androgen)
- The excess cortisol may also affect other endocrine systems → ↓ libido, amenorrhea and infertility
- Patients frequently suffer various psychological disturbances ranging from euphoria to frank psychosis.

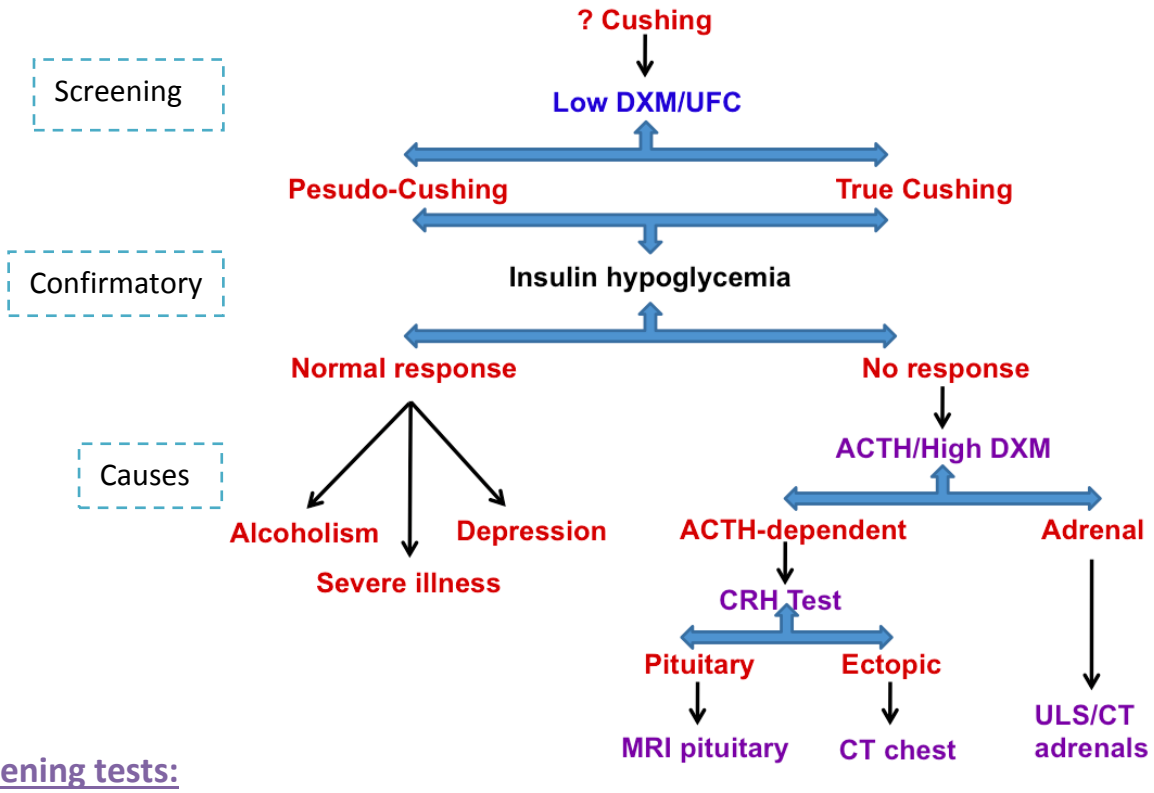


Signs:

- Loss of diurnal rhythm of cortisol and ACTH.
- Hypertension (due to the aldosterone - like effects)
- Hyperglycemia or diabetes due to insulin resistance.
- Hypokalemic alkalosis (Due to the affect of Aldosterone – increased K+ and H+ loss)
- ↑ protein metabolism.
- Impaired immunity.

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.



A- Screening tests:

Effective screening tests need to be sensitive but do not have to be highly specific. It includes:

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

   Dexamethasone → ↓ CRH → ↓ ACTH → ↓ cortisol

After observing the patient and noticing the obesity, This test is considered as first line test which will help the doctor to distinguish between true Cushing and simple non-endocrine obesity … if there is a response to the Low dose of DXM >> Pseudo Cushing's .. if there is not: it is either Cushing's or pseudo Cushing's

2. 24-hour urinary free cortisol

Dexamethasone is a synthetic Cortisol.

1. Low-dose dexamethasone (DXM) suppression test: (outpatient procedure)

- Procedure: One mg DXM administered at 11-12 PM the night before attending the clinic. serum cortisol is measured at 8-9 AM.
- Result: Cortisol < 50 nmol/L (suppression)→ exclude Cushing's disease

Knowing the procedures is not important, only the results.

- Precautions: 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:

- Result: Cortisol < 250 nmol/day → exclude Cushing's disease.
- Disadvantage: incomplete collection of urine → a false-negative result

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

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 (we put the patient in a hypoglycemic state by giving insulin)

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

True Cushing patients: No response to hypoglycemia

The patient must be hospitalized (inpatient) for this procedure, to look after the patient in case of sever hypoglycemic state

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 (confirmatory one)

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:

Normally:

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

Patients with Cushing's syndrome:

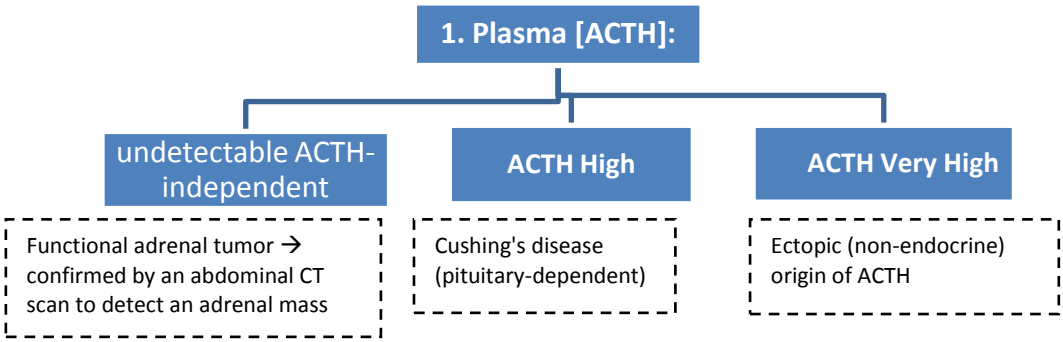
- Whatever the cause, do not respond normally to insulin-induced hypoglycemia.
  - High basal serum cortisol than normal.
  - At 60 - 90 minutes: no increase in serum cortisol, despite the production of an adequate degree of hypoglycemia.

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]:

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.

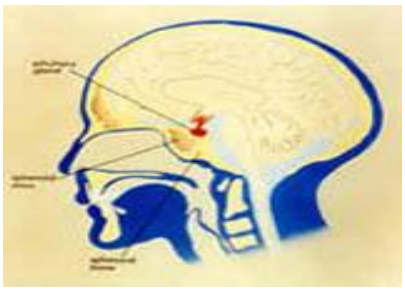
Ectopic ACTH & adrenal tumors	Cushing's disease
• No response • False-positive responses are unusual	• ↑↑ ACTH & cortisol above basal at 60 min • 10% of patients fail to respond

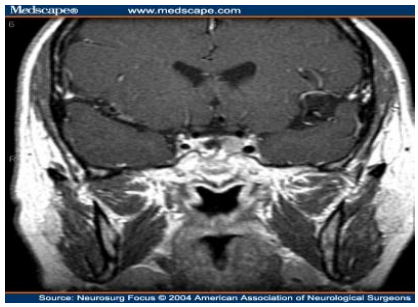
In Cushing's disease:

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
Serum 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 (because ACTH is too low to get suppressed because of negative feedback from cortisol)	Not suppressed (because ACTH is too high to get suppressed)
CRH test	↑	No response	No response

This table is of most importance, as Dr Rana and Dr. have said "you will be able to answer all the questions from here"

Case study & review question

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

Further investigation revealed the following

DMX suppression test	Basal	after 48 h 0.5 mg qid	after 48h 2.0 mg qid	
Serum cortisol	1350 8 am	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

Answer: when we look to the clinical presentation of the patient that stated above :

1- Weight loss
2- Respiratory distress

At this stage we have to think about CANCER .

When we move further :

1- The basal Cortisol level (Before administration of low DXM) is 1650
2- After administration of Low DXM, the result shows no response
3- Further investigation :
   - ❖ After administration of High DXM suppression test : the result shows no response (No suppression)

The diagnosis: Cushing's syndrome (Ectopic ACTH producing tumor) And from the history we find that the patient suffers from RESPIRATORY DISTRESS and that suggest a tumor in the lung (The most common one is Small cell carcinoma – Bronchogenic Carcinoma) to confirm the diagnosis we should do Chest CT scan . (Heavy Smoker can be given as a hint for ectopic tumors of the lungs)

Question

The patient is a 41-year-old Caucasian female who was admitted to the hospital for evaluation of high blood cortisol level. Her complaints were fatigue, weakness, lethargy, decreased concentration and decreased memory over the last 18 months. She also gained 40 lbs over the last two months with central distribution of weight gain and neck obesity. Her physical examination was remarkable for cushingoid appearance with body weight of 211 lbs, palmar erythema and hirsutism. Dexamethasone suppression test results were consistent with Cushing's disease. A magnetic resonance imaging (MRI) examination of the pituitary did not show any abnormal findings . However, CT scan of the chest with contrast revealed a left upper lobe lung nodule. Which one of the following is the cause of patient's problem ?

1- Alcohol induced Pseudo-Cushing's syndrome
2- Adrenal carcinoma
3- Pituitary Adenoma
4- Small cell carcinoma

Answer : 4