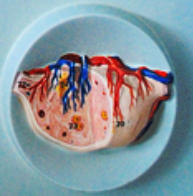


ENDOCRINE SYSTEM



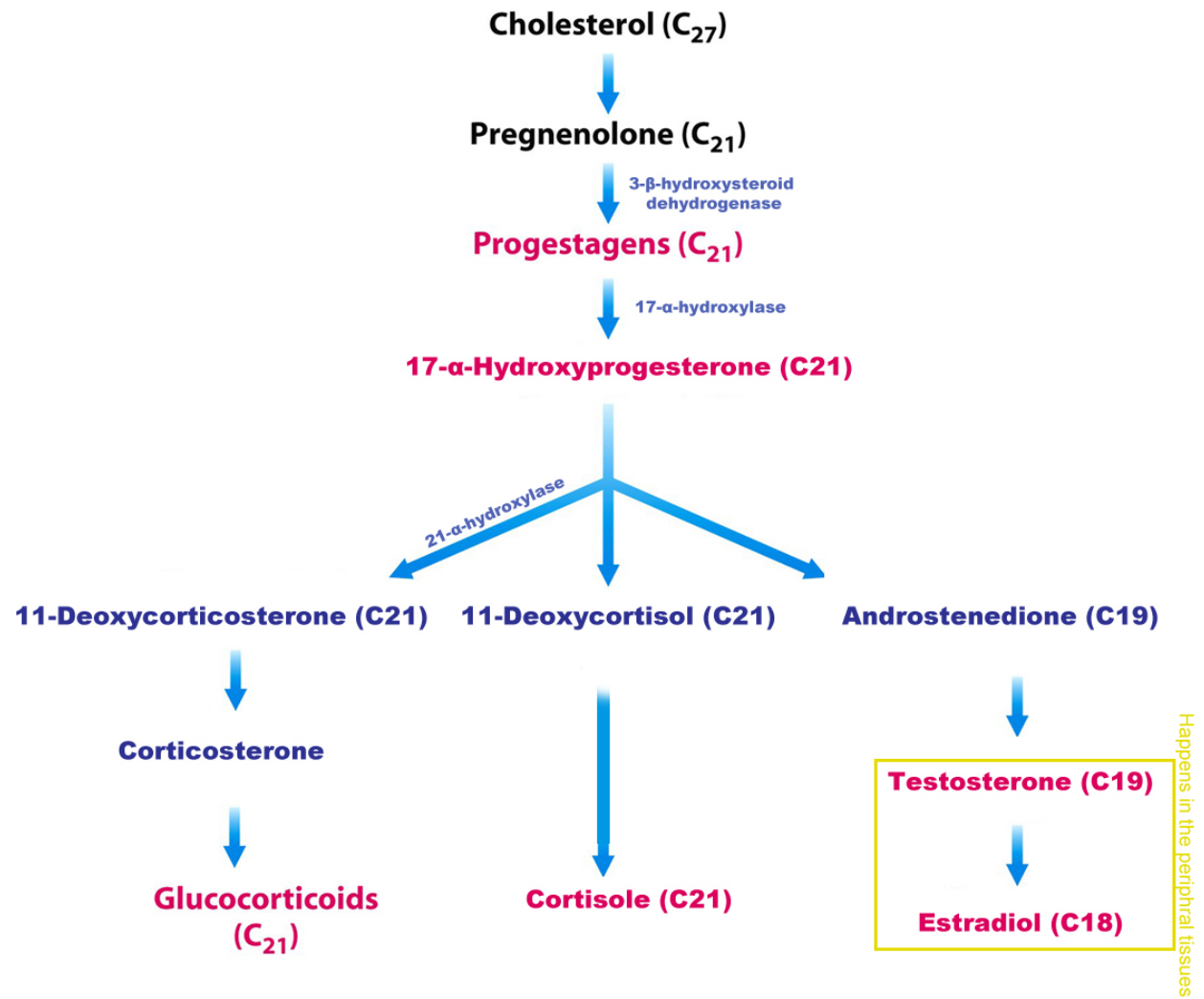
LECTURE 4 :

Cushing Syndrome

Objectives:

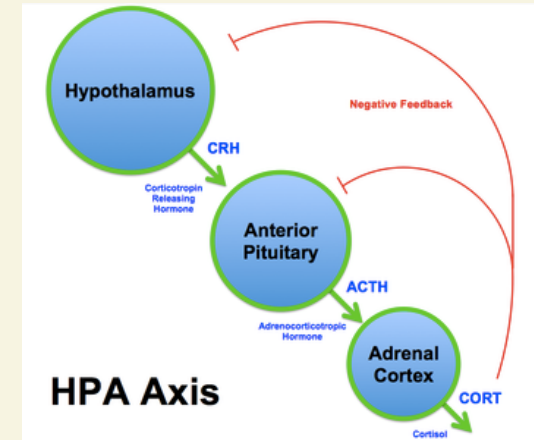
- To identify different causes of Cushing's syndrome
- To understand the diagnostic algorithm for Cushing's syndrome
- To understand the interpretation of laboratory and radiological tests of Cushing's syndrome

Steroid Synthesis



Hypothalamic-pituitary-adrenal (HPA) Axis

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



Regulation of HPA axis:

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

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).

Cortisol in blood

Plasma (CBG):

In the **circulation**, glucocorticoids are **mainly protein-bound** (about 90%), **chiefly to cortisol-binding globulin (CBG or transcortin)**

- CBG ↑ in **pregnancy & estrogen treatment** (e.g. oral contraceptives).
- CBG ↓ in **hypoproteinemic states** (e.g. nephrotic syndrome)

✓ **The biologic active form is the free form (unbound)**

Cortisol and ACTH measurements:

- Serum** measurement is preferred for **cortisol**.
- Plasma** measurement is preferred for **ACTH**
- Samples must be collected between 8 a.m. and 9 a.m. and between 10 p.m. and 12 p.m. because of the diurnal rhythm.
- Temporary increase in these hormones may be observed as response to emotional stress.

Cortisol in Urine

- 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 μ mol cortisol / mol creatinine.

Glucocorticoid functions

Glucocorticoids have widespread metabolic effects on carbohydrate, fat and protein metabolism.

Upon binding to its target, **CORTISOL** enhances metabolism in several ways:

- ❑ **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.
- ❑ **Adipose tissue:** ↑↑ Lipolysis through breakdown of fat .
- ❑ **Muscles:** ↑↑ proteolysis and amino acid release.

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

Causes of Adrenocortical Hyperfunction (Cushing's syndrome)

According to ACTH



ACTH-dependent

↑ Pituitary ACTH 70% (Cushing's disease).

Ectopic ACTH by neoplasms 10%.¹

ACTH therapy.

ACTH-independent

Adrenal tumor 20% (adenoma or carcinoma)

Glucocorticoid therapy

According to serum cortisol & CBG

↑ cortisol secretion

1. Cushing's syndrome
2. Exercise, stress, anxiety, depression
3. Obesity
4. Chronic alcohol abuse
5. Chronic renal failure

↑ cortisol binding globulin (CBG)

1. Congenital
2. Estrogen therapy
3. Pregnancy

1: Mostly by small cell lung carcinoma.

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 mucus membrane

Purple striae (on the trunk and legs)

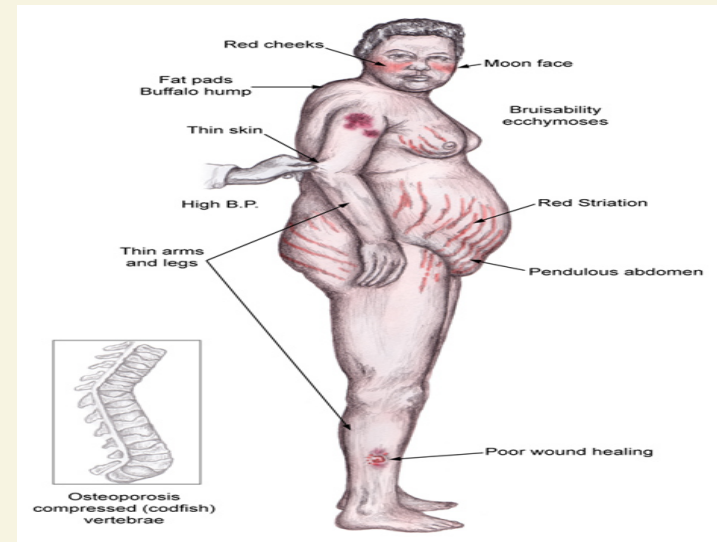
- Proximal muscle weakness (hips & shoulders)

Hirsutism

- ↓ libido, amenorrhea & infertility
- Psychological disturbances (euphoria & psychosis)

Signs

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





Investigations of suspected adrenocortical Hyperfunction

Screening tests: (Out-patients)

- I. To access clinical diagnosis of Hyperfunction
- II. Distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome.

Low-dose dexamethasone suppression test

24-hour urinary free cortisol

Confirmatory tests (In-patients)

- I. Confirm or exclude the provisional diagnosis
- II. To rule out pseudo-Cushing's syndrome

Insulin-induced hypoglycemia:

Tests to determine the cause

- I. The site of the pathological lesion
- II. The nature of the pathological lesion.

- 1- Plasma ACTH (Diurnal rhythm)
- 2- High-dose dexamethasone suppression test
- 3- CRH stimulation test
- 4- Radiological tests:



Screening

Is it Cushing?

Low DXM/UFC

Pseudo-Cushing

True Cushing

Confirmatory

Insulin-induced hypoglycemia

Normal response

No response

Cause

Alcoholism

Depression

Severe illness

ACTH/High DXM

ACTH-dependent

Adrenal

CRH Test

Pituitary

Ectopic

MRI pituitary

CT chest

ULS/CT adrenals

Screening tests

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

- Dexamethasone → ↓ CRH → ↓ ACTH → ↓ cortisol

• 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**

• Precautions:

Hepatic microsomal enzymes inducers (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

Screening tests: Effective screening tests need to be **sensitive** but do **not have to be highly specific.**

Confirmatory test

Contraindicated in
epilepsy or heart
disease.

Insulin-induced hypoglycemia:

- Hypoglycemia \rightarrow \uparrow CRH \rightarrow \uparrow ACTH \rightarrow \uparrow cortisol
- To test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis.

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 S. cortisol, despite the production of an adequate degree of hypoglycemia.

Confirmatory test:

Used to rule out pseudo Cushing's syndrome

True Cushing's patients

No response to hypoglycemia

Pseudo Cushing's patients

Show abnormal diurnal rhythm of Serum cortisol

↑ CRH, ACTH and cortisol blood levels

In response to insulin-induced hypoglycemia

Pseudo-Cushing's syndrome may occur due to:

1. Depressed or extremely anxious patients
2. Severe intercurrent illness
3. Alcoholism

Tests to determine the cause

1. Plasma ACTH:

- Undetectable:

Functional adrenal tumor

→ confirmed by an abdominal CT scan to detect an adrenal mass

- ↑↑ ACTH:

→ Cushing's disease (pituitary – dependent)

- ↑↑↑↑ ACTH(very high):

Ectopic origin of ACTH (non-endocrine) e.g. lung cancer.

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

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.

Tests to determine the cause

3. CRH Stimulation test:

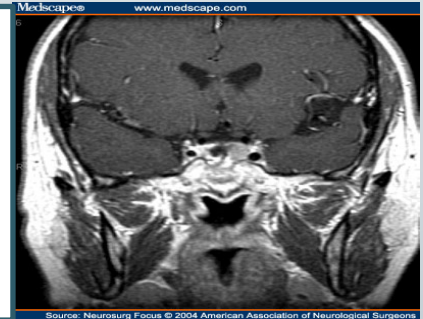
Measures the ACTH and cortisol levels basally and 60 minutes after injection of 100 μ g CRH.

- Ectopic ACTH & adrenal tumors:
→ **No response** (false-positive responses are unusual)
- Cushing disease:
↑↑ ACTH & cortisol above basal at 60 min (10% of patients fail to respond)

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



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

Case study 1

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

DMX suppression test results are shown in the table below:

	Results	Comment	Ref. range
Serum cortisol	Basal : 1350 After 48 h: 1100	No suppression	150-550 nmol/L
Plasma ACTH	8 AM : 220 22 PM: 180	Increased	7-51

CRH showed flat response for cortisol and ACTH

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)

The most-likely cause of her symptom is:

- A- Adrenal gland carcinoma
- B- Cushing's disease
- C- Ectopic ACTH-producing tumor
- D- Pseudo Cushing's

Answer: C

Case study 2

22 years young girl comes because of weight gain although she was trying to lose weight and she easily gets tired especially when climbing stairs . In addition, she has been fired from her job. The general practitioner tried to send her to an endocrinologist but he was on vacation. The GP had to deal with this patient and he did these tests :

Lab tests

Urea	5	(2.5-7 mmol/L)
Sodium	144	(135-145 mmol/L)
Potassium	4	(3.5-4.5 mmol/L)
Cortisol	1650	(150-550 nmol/L)
Post overnight DMX	1530	(<50nmol/L)

Insulin-induced hypoglycemia : After 30 min => ↑ CRH, ACTH and cortisol levels

The most-likely diagnosis is:

- A- Adrenal gland carcinoma
- B- Cushing's disease
- C- Ectopic ACTH-producing tumor
- D- Pseudo Cushing's

Answer: D

Case study 3

- A 38 year-old house wife walks into your office complaining of muscle weakness, weight gain, (particularly in the trunk region), unsightly purple markings across her abdominal region, and hyperpigmentation.

She also tells you that she has been experiencing amenorrhea for the past three months.

On examination, her blood pressure was found to be 160/100 and her pulse was 98/minute.

Hormone profiles revealed serum cortisol levels were 1500 nmol/L (Normal: 150-550 nmol/L) and plasma ACTH levels were 357 pg/ml (normal = < 80 pg/ml at 8:00 am).

She showed no response to insulin-induced hypoglycemia however, her cortisol levels have fallen to 300 nmol/L after 48 hours of administering of high dose of DXM .

Her serum cortisol and ACTH levels have increased 60 minutes after injection of 100 µg CRH.

What other finding do you expect to see in this patient ?

- A. A mass in the left side of the chest on X-ray image
- B. A pituitary mass protruding up into the sella turcica on MRI
- C. Adrenal gland tumor (adenoma or carcinoma)

Answer: B

Summary

(CRH) stimulates the anterior pituitary gland to release ACTH-

- ACTH acts on the zona fasciculata cells to release glucocorticoids (Cortisol)
 - Highest Cortisol level in the morning (8 - 9 AM)-
 - Lowest Cortisol level in the late afternoon and evening (8 - 9 PM)-
 - -In the circulation, glucocorticoids are mainly protein-bound to (CBG or transcortin).
- Serum measurement is preferred for cortisol and Plasma for ACTH.
- -Cortisol excreted in urine mainly as conjugated metabolites (e.g. glucuronides).

The most common cause of Cushing - Pituitary ACTH 70%

- In the liver, Cortisol is an insulin antagonist.

- In the adipose tissue: Cortisol ↑ Lipolysis.

- In the muscles: Cortisol ↑ proteolysis

- Sensitive screening tests.(Low-dose dexamethasone suppression test and 24-hour urinary free cortisol)

-Confirmatory tests(Insulin-induced hypoglycemia) required to rule out pseudo-Cushing's syndrome.

-Radiological Investigations (MRI of the pituitary gland and adrenal US or CT)

-Other biochemical test (Full blood count-Blood glucose- Blood electrolytes and pH- Renal function test-Liver function tests)

TEST YOURSELF!

1- Which of the following is used to distinguish Cushing's disease from ectopic ACTH secretion?

- A. High-dose dexamethasone suppression test
- B. Plasma ACTH
- C. Short ACTH stimulation test
- D. Blood test

2. Which of the following is used to distinguish true Cushing's syndrome from pseudo-Cushing's syndrome (to test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis)?

- A. Insulin-induced hypoglycemia
- B. 24-hour urinary free cortisol (UFC)
- C. Low-dose dexamethasone (DXM) suppression test
- D. CRH stimulation test

3. Insulin-induced hypoglycemia test is contraindicated in

- A. Patients with heart diseases
- B. Patients with kidney stones
- C. Patients with liver disease
- D. All of them

4. Which of the following is used to distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome?

- A. Confirmatory tests
- B. Screening tests
- C. Tests to determine the cause

5. Which of the following is true about High-dose dexamethasone suppression test + the CRH test?

- A. Are Confirmatory tests
- B. Gives 100 % specificity and sensitivity
- C. Are screening tests

6. The action of the cortisol in the live?

- A. Decrease gluconeogenesis
- B. Decrease amino acid uptake
- C. Increase ketogenesis

7. Which of the following causes an increase in cortisol binding globulin (CBG)?

- A. Chronic renal failure
- B. Alcohol abuse
- C. Pregnancy
- D. Obesity

8. Which of the following causes of Cushing syndrome is ACTH-independent?

- A. Increase Pituitary ACTH 70% (Cushing's disease).
- B. Ectopic ACTH by neoplasms 10%.
- C. ACTH therapy.
- D. Glucocorticoid therapy.

9. What time does the Cortisol reach its highest level ?

- A. In the morning between 8-9 AM
- B. Late afternoon and In the evening between 8-9 PM
- C. Early afternoon around 3-4 PM
- D. Late at night between 10-11 PM

- 1.A 2. A 3. A 4. B 5.B
6.C 7. C 8. D 9. A

THANK YOU ...

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