Biochemistry Team 435

جامعة الملك سعود
King Saud University

CUSHING SYNDROME

* Please check out this link to know if there are any changes or additions.

Revised by
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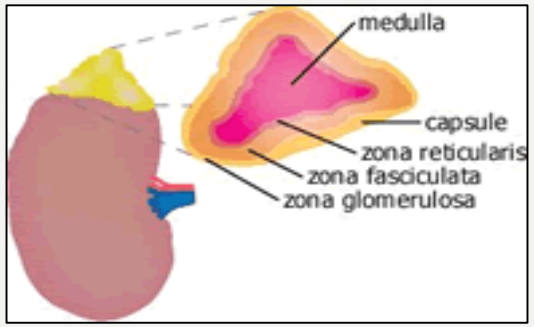
Color index: Important | Doctors notes | Further explanation.

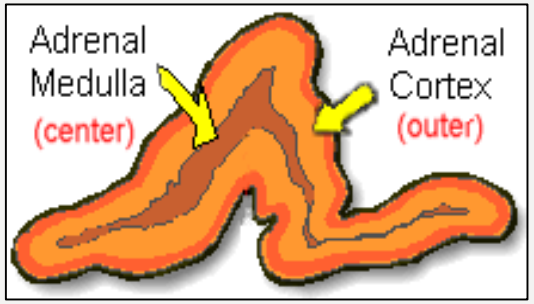
OBJECTIVES:

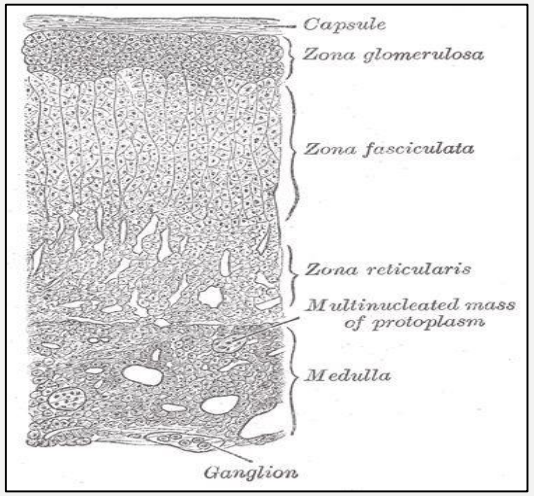
- ✓To identify different causes of Cushing's syndrome
- ✓To understand the diagnostic algorithm for Cushing's syndrome
- ✓To understand the interpretation of laboratory and radiological tests of Cushing's syndrome

Introduction to the adrenal gland:

ANATOMICALLY:		
➢ **The adrenal gland is situated on:** the **anteriosuperior** aspect of the kidney. ➢ **It receives its blood supply from:** the **adrenal arteries**.		
Histologically:		
The adrenal gland consists of <u>two distinct</u> tissues of different embryological origin, the **outer cortex** and **inner medulla**.		
The adrenal cortex comprises three zones based on cell type and function:		
The **outermost** zone	The **deeper** layers of the cortex	
Zona glomerulosa	**Zona fasciculata**	**Zona reticularis**
Aldosterone (the principal mineralocorticoid).	**Glucocorticoids** – mainly cortisol (95%)	**Sex hormones**



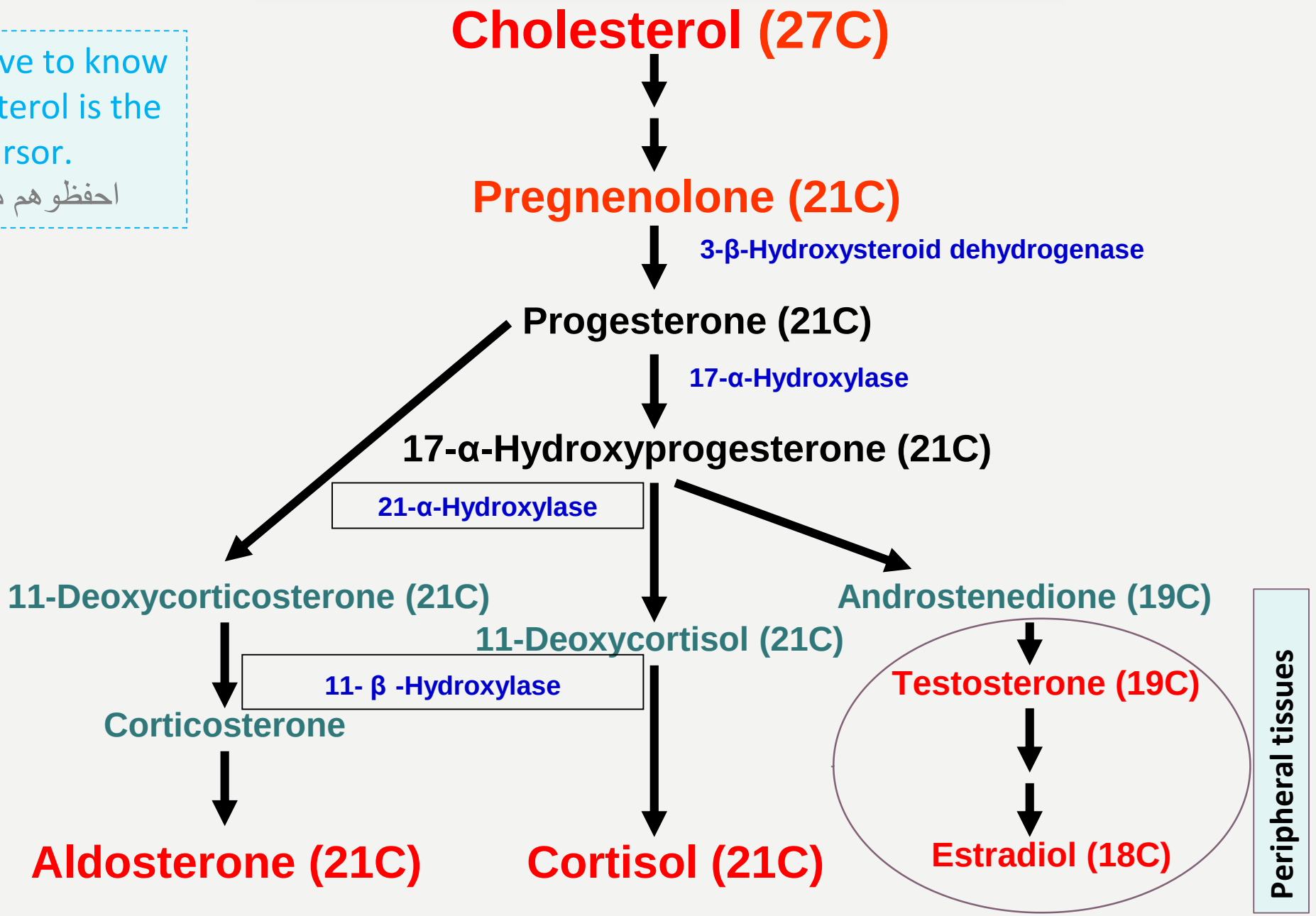




اقروهم على السريع..تخيلوا سؤال بايو ويسأل عن الزونا فسكيولاتا؟ وش بيخلون للهستو؟

Steroid Hormone Synthesis

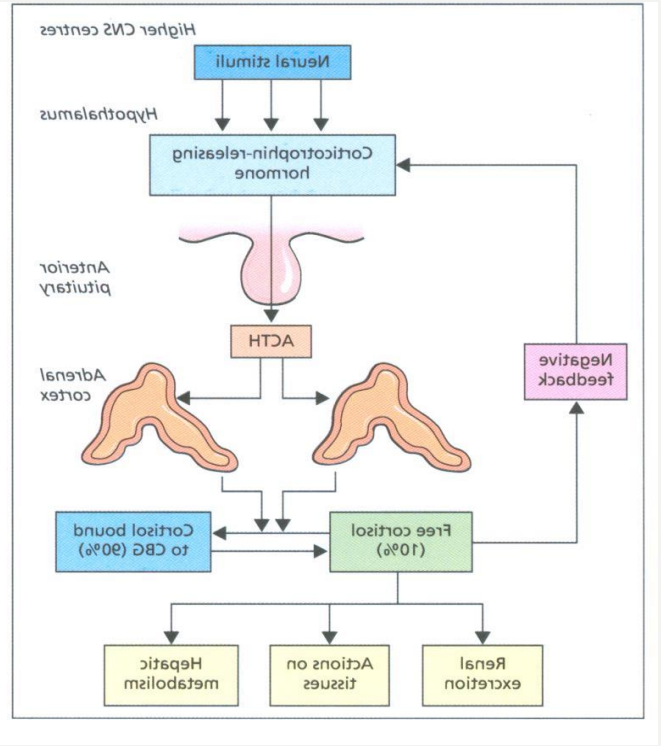
You only have to know that cholesterol is the precursor.
احفظوهم من الفزيو..



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		
Negative feedback control	**Stress**	**The diurnal rhythm of plasma cortisol**
ACTH release from the anterior pituitary is stimulated by hypothalamic secretion of corticotrophin releasing hormone (**CRH**).	e.g. major surgery, emotional stress	➢ **Highest** Cortisol level in the morning (8 - 9 AM). ➢ **Lowest** Cortisol level in the late afternoon and evening (8 - 9 PM).
CRH → ↑ ACTH → ↑[Cortisol] → ↑[Cortisol] or synthetic steroid suppress **CRH** & **ACTH** secretion	Stress → ↑↑ CRH & ACTH → ↑↑ Cortisol	



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 measurement is preferred for cortisol and Plasma 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**.

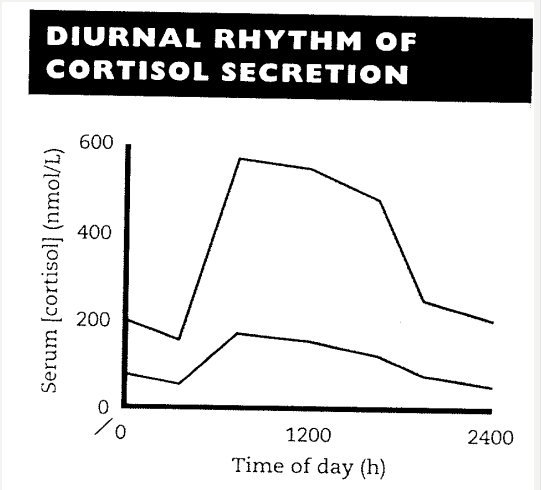
Temporary ↑↑ in these hormones may be observed as a response to **emotional stress**.

Thus we make sure that the patient is emotionally stable before measuring these hormones.

Ignore it

Diurnal rhythm: increase of cortisol in early morning, and decrease in the night.

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



URINARY CORTISOL EXCRETION

Cortisol is removed from plasma by the **liver** → metabolically **inactive** compounds → Excreted in urine mainly as **conjugated metabolites** (e.g. glucuronides).

Cortisol is removed from plasma by the **liver** → A small amount of cortisol is excreted unchanged in the urine (UFC).

Used in cushing test

- **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.**

Notes:

- The early morning specimen in case of babies or old people who cannot obtain the 24 sample.
- We use the ratio to differentiate between the diluted or concentrated urine , and as more diluted as more accurate results.

CAUSES OF elevated serum cortisol concentrations:

Increased cortisol secretion:

- Exercise
- Alcohol abuse
- Obesity
- Chronic renal failure
- Stress, Anxiety, Depression

Increased cortisol binding globulin (CBG):

- Congenital
- Estrogen therapy
- Pregnancy

CUSHING'S SYNDROME

ACTH – dependent

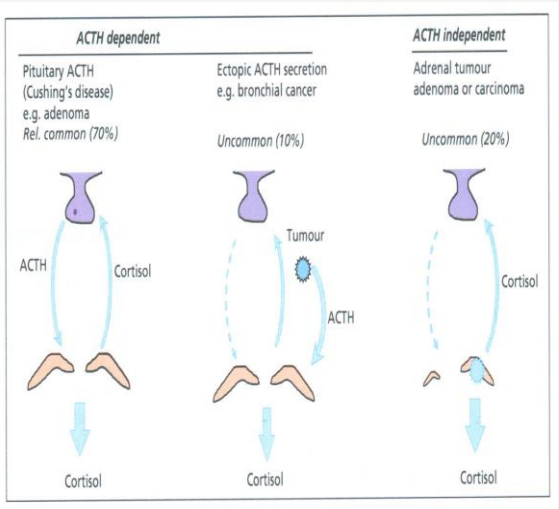
1. ↑ Pituitary ACTH **70% (Cushing's disease)**.
2. Ectopic ACTH by neoplasms 10%. (Example: Bronchial cancer) "Not from the pituitary – Lung cancers"
3. **ACTH therapy**.

هنا افراز الكورتيزول العالي يكون نتيجة لوجود أي سي تي اتش عالي!
اللي قد يكون مصدره من **البتوتاري** "ممكن ادينوما زي ما اخذنا بالمدسن" أو ممكن **ورم خارج هالبتوتاري** "بالرئة على سبيل المثال" وممكن يكون الشخص ياخذه كعلاج وسبب له هالشيء

ACTH – independent

1. **Adrenal tumor** 20% (adenoma or carcinoma)
2. Glucocorticoid therapy.

تفرز الكورتيزول بدون تأثير الاي سي تي اتش!
اما بسبب ورم يفرزه على طول أو لمن الشخص ياخذ جرعات كبيرة من الكورتيزول.



من بداية المحاضرة لازم نتفق على شيء:
الكوشنق سندروم هو لفظ عام لمجموعة من الأمراض اللي كلها تشترك بنفس السبب "افراز الكروتيزول بكميات كبيرة".
الكوشنق ديزيز هو مرض يندرج تحت السندروم "افراز كميات كبيرة من الكوتيزول" وسبب هالزيادة هو الاي سي تي اتش من البتوتاري.

Glucocorticoid Functions

- Glucocorticoids have widespread metabolic effects on **carbohydrate**, **fat** and **protein** metabolism.
- **Conserving glucose**: by **inhibiting** uptake into muscle and fat cells.

CORTISOL enhances metabolism in several ways

In the muscles:.

Cortisol → ↑↑ proteolysis and amino acid release

In the adipose : tissue:

Cortisol → ↑↑ Lipolysis through breakdown of fat

In the liver

Cortisol is an insulin antagonist and has a weak mineralocorticoid action →
1- ↑↑ Gluconeogenesis → production of glucose from newly-released amino acids and lipids
2- ↑↑ Amino acid uptake and degradation
3- ↑↑ Ketogenesis.

Cushing's Syndrome signs & symptoms

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

Signs
1- Loss of diurnal rhythm of cortisol and ACTH.
2- **Hypertension** (due to the aldosterone - like effects)
3- **Hyperglycemia** or **diabetes** due to insulin resistance.
4- **Hypokalemic alkalosis**
5- ↑ protein metabolism.
6- **Impaired immunity**.

Moon face

Cushing syndrome

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 used to determine the cause

to ascertain:
(a) The **site** of the pathological lesion (adrenal cortex, pituitary or lungs).
(b) The **nature** of the lesion.

❖ **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**

USMLE ALGORITHMS: Cushing Syndrome

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests (out-patient)

B. Confirmatory tests

C. Tests used to determine the cause

❖ **We use them to:** assess the clinical diagnosis of adrenocortical hyperfunction.

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

❖ **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.** Thus we can't judge if the patient has Cushing's or not from the screening tests only.

❖ **Pseudo-Cushing's syndrome:** it's a condition in which patients shows the symptoms and the signs of Cushing's syndrome (especially the elevated hormones).

➢ **Examples of pseudo-Cushing's syndrome include:**

- Depressed or extremely anxious patients.
- Severe intercurrent illness.
- Alcoholism.

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests (out-patient)

B. Confirmatory tests

C. Tests used to determine the cause

1- Low-dose dexamethasone (DXM) suppression test (DST):

Also called Overnight suppression test.

DXM will mimic the cortisol, thus it'll act as an exogenous cortisol and send a negative feedback, thus:

Dexamethasone → ↓ CRH → ↓ ACTH → ↓ cortisol

So after the test we measure the cortisol level, if it's suppressed (lower than 50 nmol/L) then we can exclude Cushing syndrome and look for other reasons, but if it's not suppressed, then we think of Cushing and do more investigations.

2- 24- hour urinary free cortisol:

البيشنت في هذا التست راح يجمع اليورين إلي يطلعه لمدة ٢٤ ساعة ، لكن المشكلة هنا أنه احتمال ما يجمع العينة مرة او ثنتين لأي سبب - يمكن مشغول، ناسي، مستعجل، أو متكيسل و يظن إن "مرة ما تضر:) - وعلى كذا ممكن يؤدي هذا إلى False-negative result

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests (out-patient)

B. Confirmatory tests

C. Tests used to determine the cause

1- Low-dose dexamethasone (DXM) suppression test:

Procedure:

1 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:

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

These drugs increase the metabolism of DXM → no or little CRH suppression → which means the test result is wrong, but you will think it's because of Cushing, so the diagnosis will be wrong.

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.

Because I'm a normal individual my cortisol levels after the suppression test will be low , diseased people will have high level due to the absence or impairment of the negative feed-back mechanism .

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests

B. Confirmatory tests (in-patient)

C. Tests used to determine the cause

Insulin-induced hypoglycemia: (Hypoglycemia →↑ CRH →↑ ACTH →↑ cortisol) (DXM عكس)

❖ Goals of the test:

1- To test the integrity of the **hypothalamic-pituitary-adrenal (HPA)** axis.
2- To distinguish true Cushing's syndrome from pseudo-Cushing's syndrome.

❖ Contraindicated in: **epilepsy** or **heart disease**.

❖ Procedure:

1- **Insulin I.V.** (0.15 U/kg) to lower blood glucose to 2.2 mmol/L or less .

2- 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

نقيس الكورتزول عند البشنت وبعدين نعطيه ٠,١٥ انسولين عبر الوريد ونقيس الجلوكوز تقريبا كل نص ساعة من بعد الانجكشن، فإذا وصل الجلوكوز إلى ٢,٢ نانومول لكل لتر نوقف ونشوف الكورتزول، لكن إذا ما وصل ٢,٢ نعيد التست مع زيادة ٠,٠٥ من الانسولين وهكذا ونشوف كم يوصل الجلوكوز، وهكذا إلى أن يوصل للقيمة المطلوبة، ثم نشوف الكورتزول.
لكن إذا ما وصل الجلوكوز لـ٢,٢ ، ما يعني هذا بالضرورة أن المريض عنده كوشنج

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests

B. Confirmatory tests (in-patient)

C. Tests used to determine the cause

Insulin-induced hypoglycemia

When we induce the hypoglycemia by insulin in normal people the cortisol level will increase to compensate and increase the glucose level in the blood , diseased patient will have high cortisol all the time but there will be no further increase in cortisol in response to the the hypoglycemia induced by insulin .

❖ Interpretation of the results:

- **In normal people:**
  - **Basal serum cortisol** → at least 145 nmol/L .
  - **At 60 - 90 minutes** → the serum cortisol level is > 425 nmol/L.
- **In Pseudo-Cushing patients:**
  - they show abnormal diurnal rhythm of Serum cortisol, but with Insulin-induced hypoglycemia → ↑ CRH, ACTH and cortisol blood levels.

ليه يزيد الكورتزول ؟ لأنه مثل ما عرفنا يزيد كمية السكر في الدم بكل الطرق، فهو- المفترض - يستجيب لنقص السكر ويحاول يرفعه.

- **In Patients with Cushing's syndrome:**

Whatever the cause, they **don't** respond normally to insulin-induced hypoglycemia.

High **basal serum cortisol** than normal (much higher than 145nmol/L, usually higher than 400 nmol/L) .

At 60 - 90 minutes: **no increase** in Serum cortisol, despite the production of an adequate degree of hypoglycemia.

In Cushing & pseudo-cushing the diurnal rhythm is abnormal, but when we do this test, the hormones in pseudo-cushing increase, but in Cushing they don't. وكذا نقدر نحدد إذا الزيادة في الكورتيزول هي بسبب كوشنج أو غيره

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests

B. Confirmatory tests

C. Tests used to determine the cause

1- Plasma [ACTH]

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

Plasma ACTH (important)

معناه ان المشكلة من الأدرينال نفسها (الكورتزول زاد بنفسه مب بأمر من فوق)

- **Undetectable amount** → Functional **adrenal tumor** → confirmed by an abdominal CT scan to detect an adrenal mass
- **↑↑ ACTH** → **Cushing's disease** (pituitary-dependent)
- **↑↑↑ ACTH** → **Ectopic origin of ACTH** (non-endocrine origin)

Ectopic could be a small cell carcinoma or bronchial carcinoma (lung is the source).

The ACTH is extremely high, although the cortisol is also high (remember that the cortisol down-regulate the ACTH by negative feedback, but here it didn't, which means that the ACTH is not secreted from pituitary gland, but from somewhere else, usually the lung)

1- Plasma ACTH (Diurnal rhythm)

2- High-dose dexamethasone suppression test

3- CRH stimulation test

4- Radiological tests

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests

B. Confirmatory tests

C. Tests used to determine the cause

2- High-dose dexamethasone suppression test:

- **Goal:** It is used to distinguish Cushing's disease from ectopic ACTH secretion.
- **Procedure:**
  - 2 mg dexamethasone six-hourly for **48 hours** to suppress cortisol secretion.
  - Basal serum cortisol (pre-dexamethasone S. cortisol) or 24-hour urine free cortisol is compared with the results at the end of the 48-hour period.
- **Interpretations:**
  - 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**) also show suppression.

Because of these 10%, we do the CRH stimulation test with this test to exclude the adrenal tumor & ectopic ACTH origin.

- **Suppression**: a fall to less than 50% of **basal value.**

1- Plasma ACTH (Diurnal rhythm)

2- High-dose dexamethasone suppression test

3- CRH stimulation test

4- Radiological tests

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests | **B. Confirmatory tests** | **C. Tests used to determine the cause**

3- CRH stimulation test

❖ **Procedure:**

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

❖ **Interpretation:**

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

In Cushing's disease: High-dose dexamethasone suppression test + the CRH test → 100% specificity and sensitivity. يعني هنا نكون تأكدنا بإذن الله ان المريض عنده كوشنج دزيز ولا سبب آخر

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

Investigations Of Suspected Adrenocortical Hyperfunction

A. Screening Tests

B. Confirmatory tests

C. Tests used to determine the cause

4- Radiological tests

- **1- MRI of pituitary gland :** Coronal contrast-enhanced MRI of the sella turcica in a patient with **recurrent Cushing's disease.**

- **2- CT** scanning of the **adrenal glands or lungs.**

Dr. Rana: I don't want you to go through it

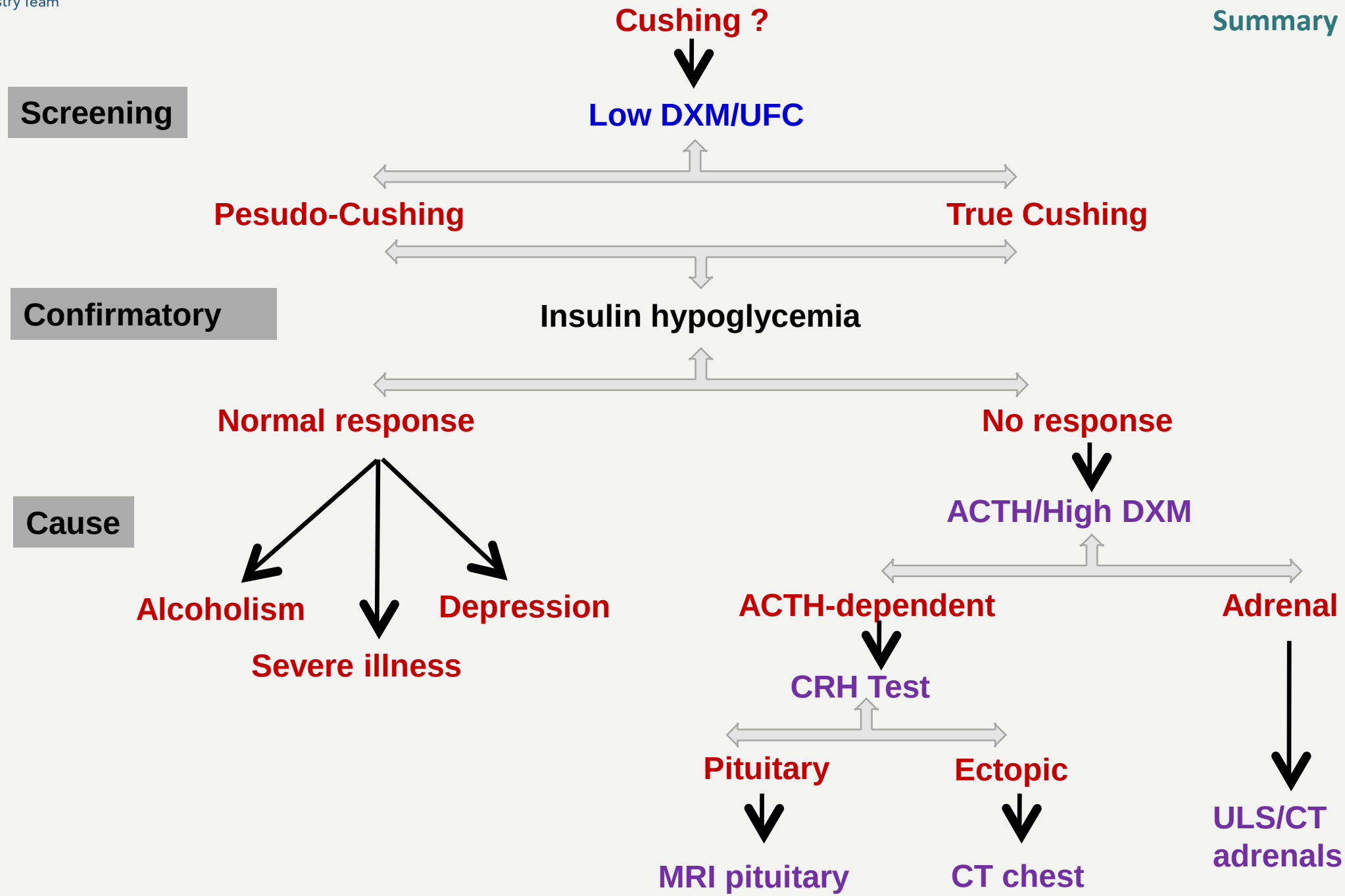
1- Plasma ACTH (Diurnal rhythm)

2- High-dose dexamethasone suppression test

3- CRH stimulation test

4- Radiological tests

Summary :)


Cushing ?
Low DXM/UFC
Screening
Pesudo-Cushing
True Cushing
Confirmatory
Insulin 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

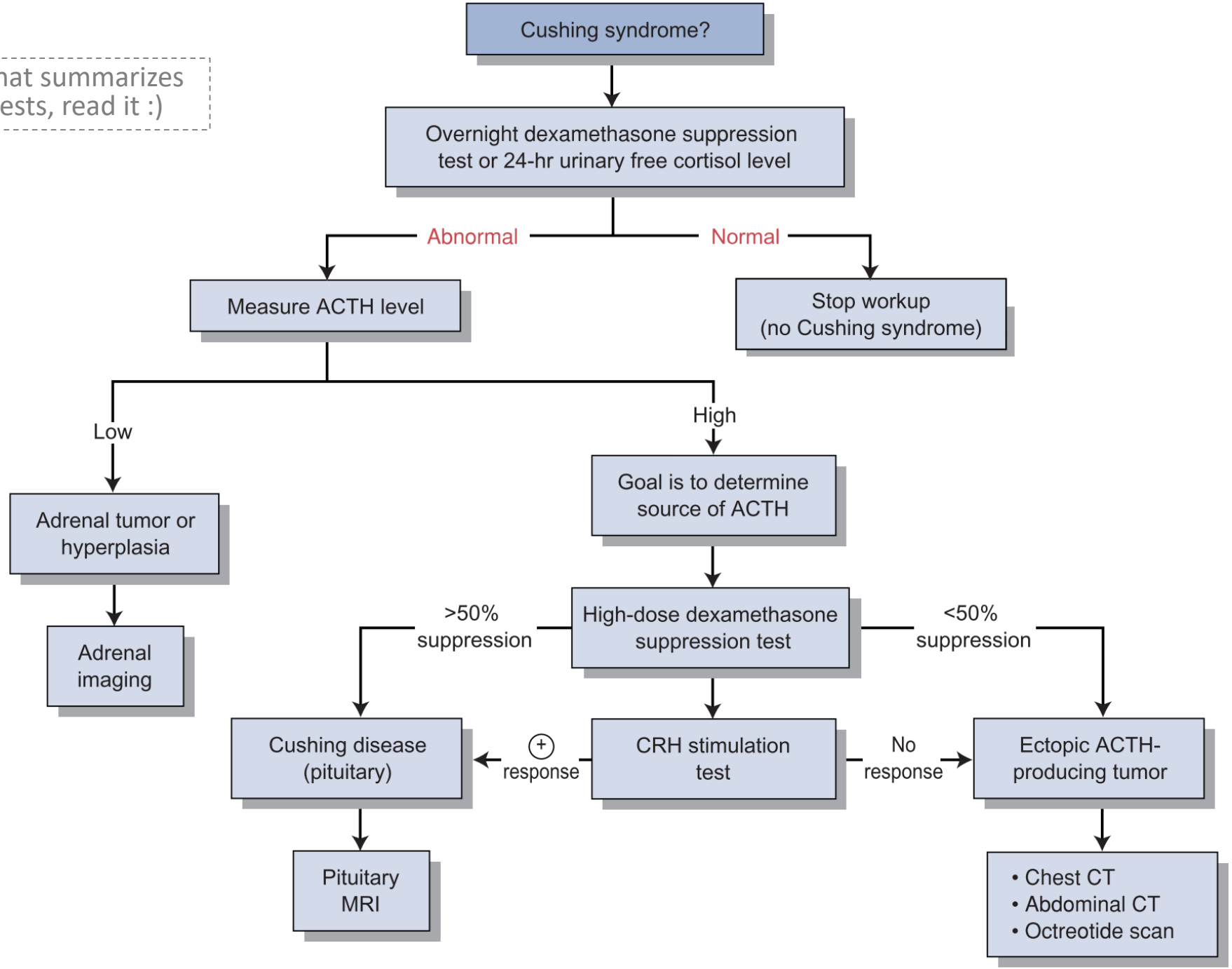
Adrenal Hyperfunction

❖ 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

Biochemistry Team 435

Extra picture that summarizes most of the tests, read it :)



Check your understanding!

Q1: Which one of the following zones release cortisol by the action of ACTH:

A. Zona glomerulosa.
B. Zona fasciculata.
C. Zona reticularis.
D. A & B.

Q2: The cortisol gets removed from the plasma by:

A. Kidney.
B. Pancrease.
C. Liver.
D. None of the above.

Q3: In normal individuals urinary free cortisol is:

A. <250 nmol/24 h.
B. <100 nmol/24 h.
C. <200 nmol/24 h.
D. <50 nmol/24 h.

Q4: Which one of the following is a cause of elevated serum cortisol concentration:

A. Exercise.
B. Stress.
C. Cushing's syndrome.
D. All of them.

Q5: Which one of the following is a function of cortisol:

A. Decrease gluconeogenesis.
B. Increase lipolysis.
C. Decrease proteolysis.
D. Increase lipogenesis.

1.B 2.C 3.A 4.D 5.B

Check your understanding!

Q6: Which one of the following is a sign for cushing's syndrome:

A. Moon face.
B. Hypotension.
C. Buffalo's hump.
D. Weight gain.

Q7: Which one of the following causes of cushing's syndrome is ACTH-independent:

A. Pituitary adenoma.
B. ACTH therapy.
C. Adrenal tumor.
D. Small cell carcinoma of the lung.

Q8: Which one of the following is used to distinguish cushing's disease from ectopic ACTH secretion:

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

Q9: Insulin-induced hypoglycemia test is contraindication in:

A. Patients with kidney stones.
B. Patients with epilepsy.
C. Patients with heart disease.
D. B & C.

Q10: Which one of the following radiological technologists is used to look at the adrenal gland:

A. X-ray.
B. MRI.
C. CT scan.
D. Ultrasound.

6.B 7.C 8.A 9.D 10.C

Done by:

- عبدالله الغزي.
- شهد العنزي.
- ثاني معافا.
- نورة الرميح.
- عبدالله الشنيفي.
- لينا الشهري.
- عبدالله الطويل.
- أحمد الرويلي.

ستواجه صنفًا لايفكر إلا بنفسه، وستواجه صنفًا يخذلك في نهاية الطريق، وصنفًا ينكر معروفًا لك. ستصفعك الحياة بمن وثقت بهم.. فتعلم كيف تنهض بنفسك.

You will meet selfish people, and you will meet who will disappoint you at the end, who will be thankless..
Life will slap you on your face with people who you have trusted to teach you how to rise again and rely only on yourself.

Revised by:

- فراس المؤمن.

Resources:

- 435's slides and notes.
-Clinical Biochemistry – sixth edition.
- Step up for medicine – 6th edition.

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