lecture 1: Introduction to Clinical Chemistry

5th Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department

2020-2021

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- **References:**

✓ Clinical Chemistry & Metabolic Medicine, Crook

✓ - Clinical Chemistry, Kaplan

Clinical Chemistry

- In this class you will gain the range of tests to help clinicians diagnose, treat, and monitor the recovery of patients. You will be taken through a number of organ systems and the diseases and disorders that can affect them. Detecting pathological changes in these systems requires an understanding of the 'biomarker' changes that occur - and the reliability of tests that can detect those changes.

Clinical Chemistry

- **Major organ/disease systems**: pregnancy/infertility, calcium/bone, diabetes, liver, poisoned patient, kidney, lipids, thyroid, heart and cancer. **Aspects of nutrition**: vitamins, dietary advice in obesity, and urine analysis.

- Clinical biochemistry combines the understanding of analytical devices, systems biology in health and disease, and an appreciation of how diagnoses can be practically applied in appropriate treatment decisions.

Clinical Chemistry Laboratory

- The analysis of individual constituents, proteins, enzymes, nutrients, waste products, metabolites, hormones, etc. in blood or body fluids that provides information regarding the function or integrity of a tissue or organ.

- The clinical chemistry laboratory measures change in biochemical compounds as an indicator of health status or disease processes.

- Clinical Laboratory plays an integrated role in the diagnosis, prognosis, treatment, and long-term management of disease.

Objectives of this class

Understanding of:

- The principal methods to characterise patient samples
- The major disorders and pathological states encountered by clinical biochemists
- The major organ systems involved in disease
- Clinical diagnosis and prognosis
- Aspects of therapies used
- To display knowledge of human body chemistry levels under healthy and abnormal conditions

Clinical Biochemistry

- Patient-focused service
- Concerned with changes in the composition of blood
- Analysis blood samples from patients
- Provide rapid results of high quality
- Interpret significance of the data and provide advice
- Offer broad spectrum tests
- Diagnosis and monitoring of disease, and response to therapy

Why are Clinical biochemistry tests ordered?

- Diagnosis
- Monitor progression of disease
- Monitor effectiveness of treatment
- To identify complications of treatment
- To check the accuracy of an unexpected data
- To prevent malpractice
- To conduct research: response to new drugs

What kind of Biological Specimens?

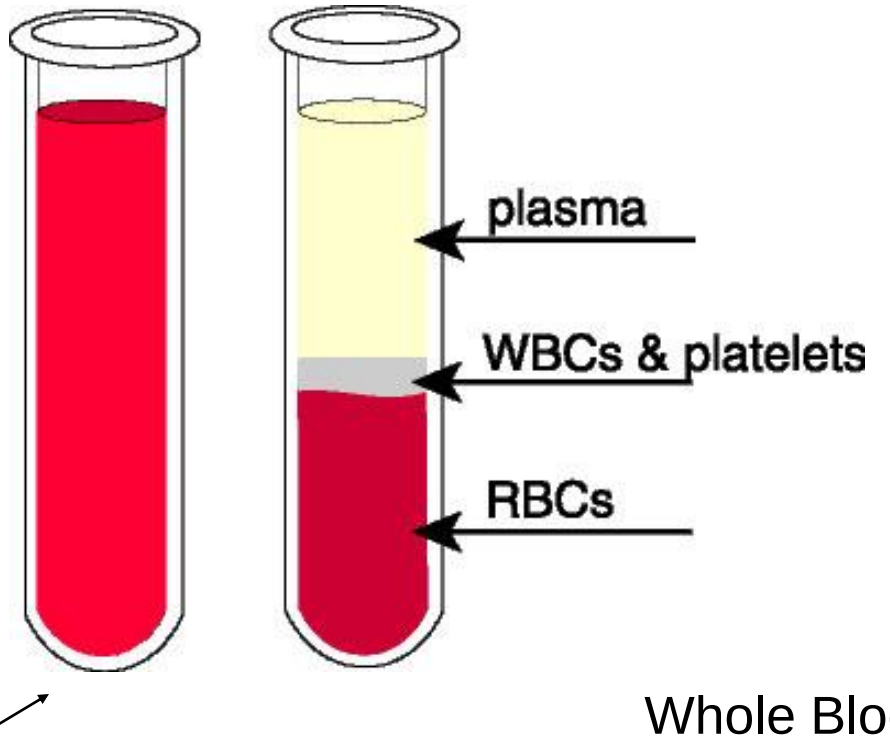
- Blood
- Urine
- Cerebrospinal Fluid
- Amniotic Fluid
- Gastric Juice
- Gall stone
- Kidney Stone
- Stools/Feces
- Sputum & Saliva
- Synovial Fluid (joint fluid)
- Tissue Specimen

Comprise the majority of all specimens analyzed

The Clinical Biochemistry Process

- Clinical history and physical examination
- Blood sample taken
- Tests requested
- Barcode label attached to blood tube
- Transported to lab

Blood Composition



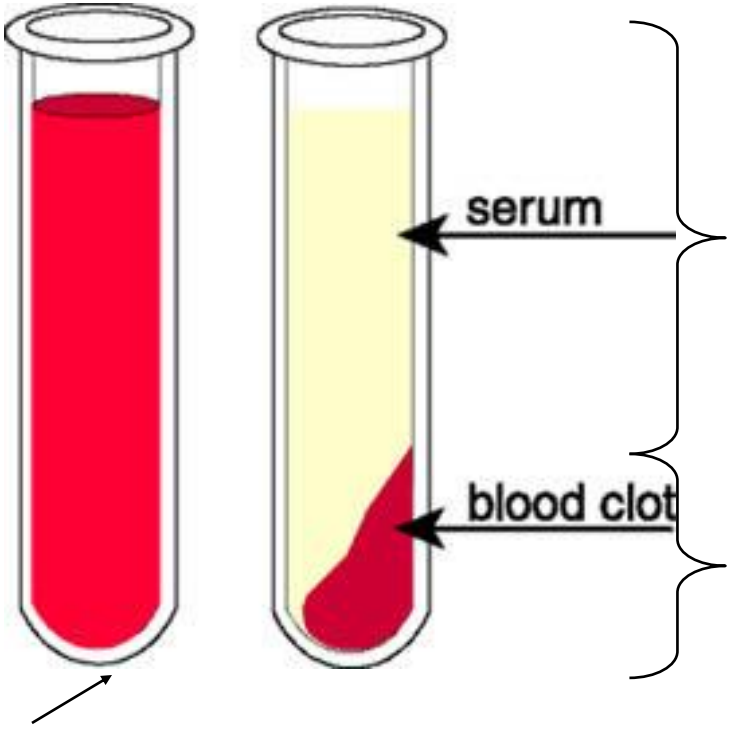
Whole Blood

Plasma is fluid component of blood. Comprises ~55% of total volume of whole blood. Contains proteins, sugars, vitamins, minerals, lipids, lipoproteins and clotting factors.
95% of plasma is water

Whole Blood after centrifugation
Note: clotting has been prevented

Blood Composition

If blood is collected and allowed to stand, it will clot. Formation of an insoluble fibrin clot. If blood is then centrifuged the fluid portion is known as serum



Serum

Blood Clot
-Comprised of clotting factors (Fibrin,platets etc)
-RBCs

Whole Blood after clotting and centrifugation

Changes in blood metabolites on keeping

- Glucose is converted to lactate.
- Glycolysis decreases serum glucose by approximately 5 – 7 % in 1 hr. (5 – 10 mg /dl) in uncentrifuged coagulated blood .
- The rate of glycolysis is higher in the presence of leukocytosis or bacterial contamination.
- substances pass through RBC membrane e.g.K+, Lactate Dehydrogenase (LDH).
- Loss of CO2
- Enzymes activities are lost on long keeping
- Formation of ammonia from nitrogenous substances

Factors affecting of parameters

- Age.
- Sex.
- Ethnicity.
- Altitude.
- Physiological conditions (e.g. at rest, after exercise, standing, lying).
- Sampling methods (e.g. with or without using tourniquet).
- Storage and age of sample.
- Container used, e.g. for blood sample, anticoagulant.
- Method of analysis

Interpreting Clinical Biochemistry Results

Evaluation of all of the below increases the reliability of diagnosis

- No diagnosis should be made on the basis of a single test result
- Information from initial patient consultation
- Physical examination findings
- Personal and family history
- Previous test results

lecture 2: Disorders of Carbohydrates metabolism (Diabetes Mellitus)

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Learning outcomes

- Overview of glucose homeostasis
- Definition of Diabetes
- Classification and causes of Diabetes
- Treatment of Diabetes
- Diagnosis of Diabetes
- Metabolic complications of Diabetes
- Monitoring of Diabetes

Major health problem

In the World (WHO):

- 1980: 108 million people diagnosed
- 2014: 422 million people diagnosed
- 2045: 628.6 million people (estimate)
- 2016: 1.6 million deaths
- 2016: The seventh leading cause of death

Cost to diabetes in the UK and USA:

- £10 billion spent on diabetes annually (UK)
- $327 billion spent on diabetes in 2017 (USA)

Diabetes is a major cause of :

- Blindness
- Kidney failure
- Heart attacks
- Stroke
- Lower limb amputation.

Blindness

- Diabetic patients are **10 – 20 times** more likely to go blind than people without diabetes

Kidney damage

Diabetes is most common cause of end-stage kidney failure

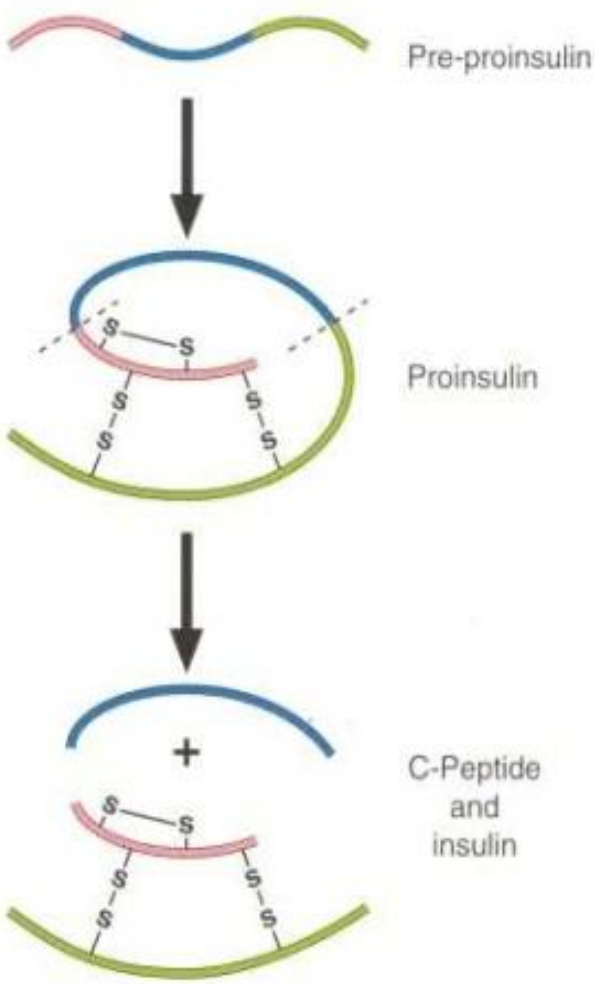
- **Dialysis**
- **Transplant**

Amputation

Diabetes is the most common cause of lower limb amputations

Insulin

- **C-peptide and insulin** are released from the pancreas at the same time and in about equal amounts
- **In the muscle**, insulin stimulates glucose uptake into cells and enhances glycogenesis
- **In adipose tissue**, insulin stimulates glucose uptake into cells and enhances lipogenesis
- **In the liver**, insulin has a negative effect, inhibiting gluconeogenesis and glycogenolysis



Insulin & C peptide

Insulin

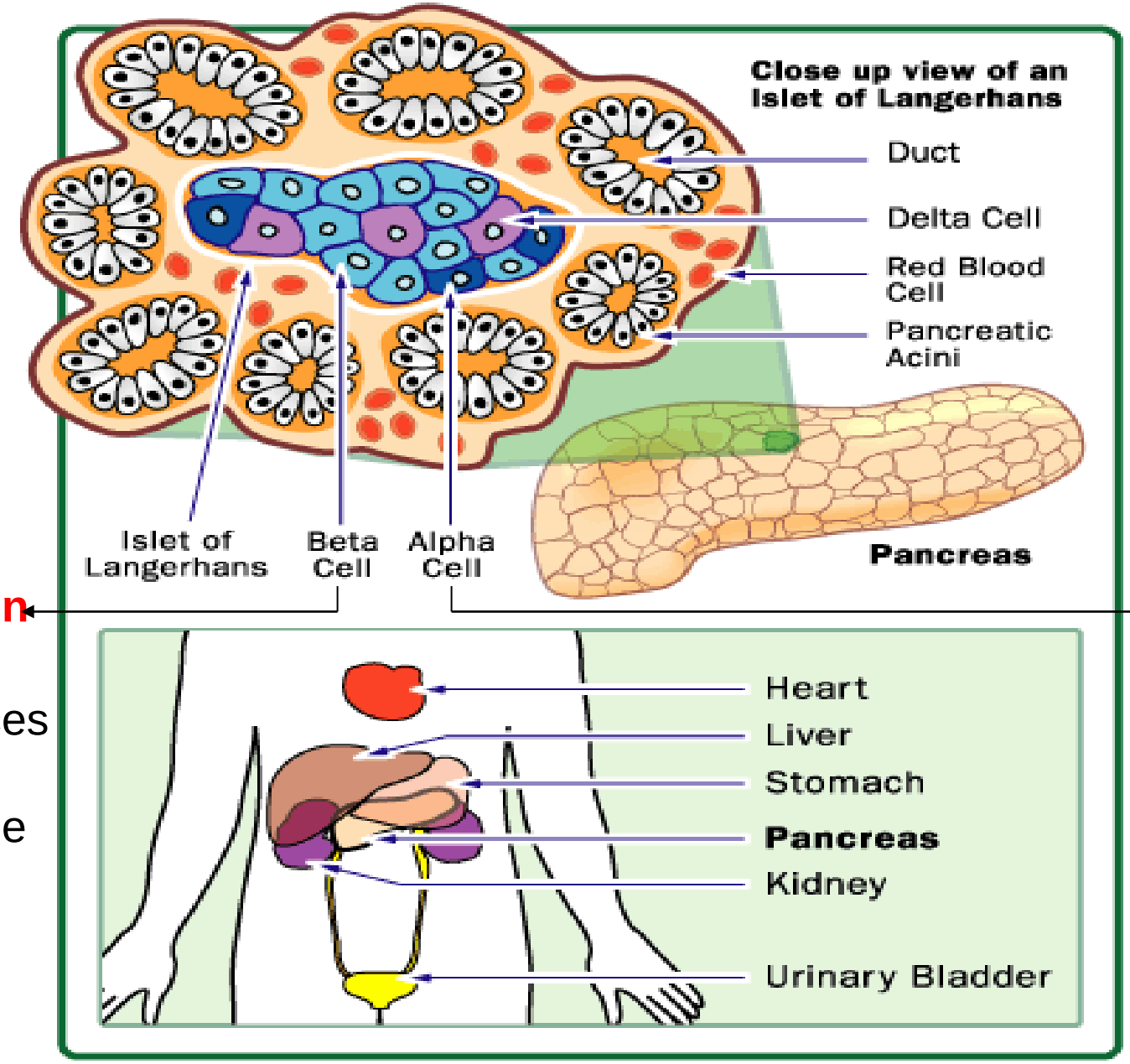
Decreases glucose blood concentration by:

- increasing glycogenesis
- increasing glycolysis
- increasing the entry of glucose into muscle and adipose cells
- inhibiting glycogenolysis

Glucagon

Increases glucose blood concentration by:

- Increasing glycogenolysis in liver
- Increasing gluconeogenesis



Insulin

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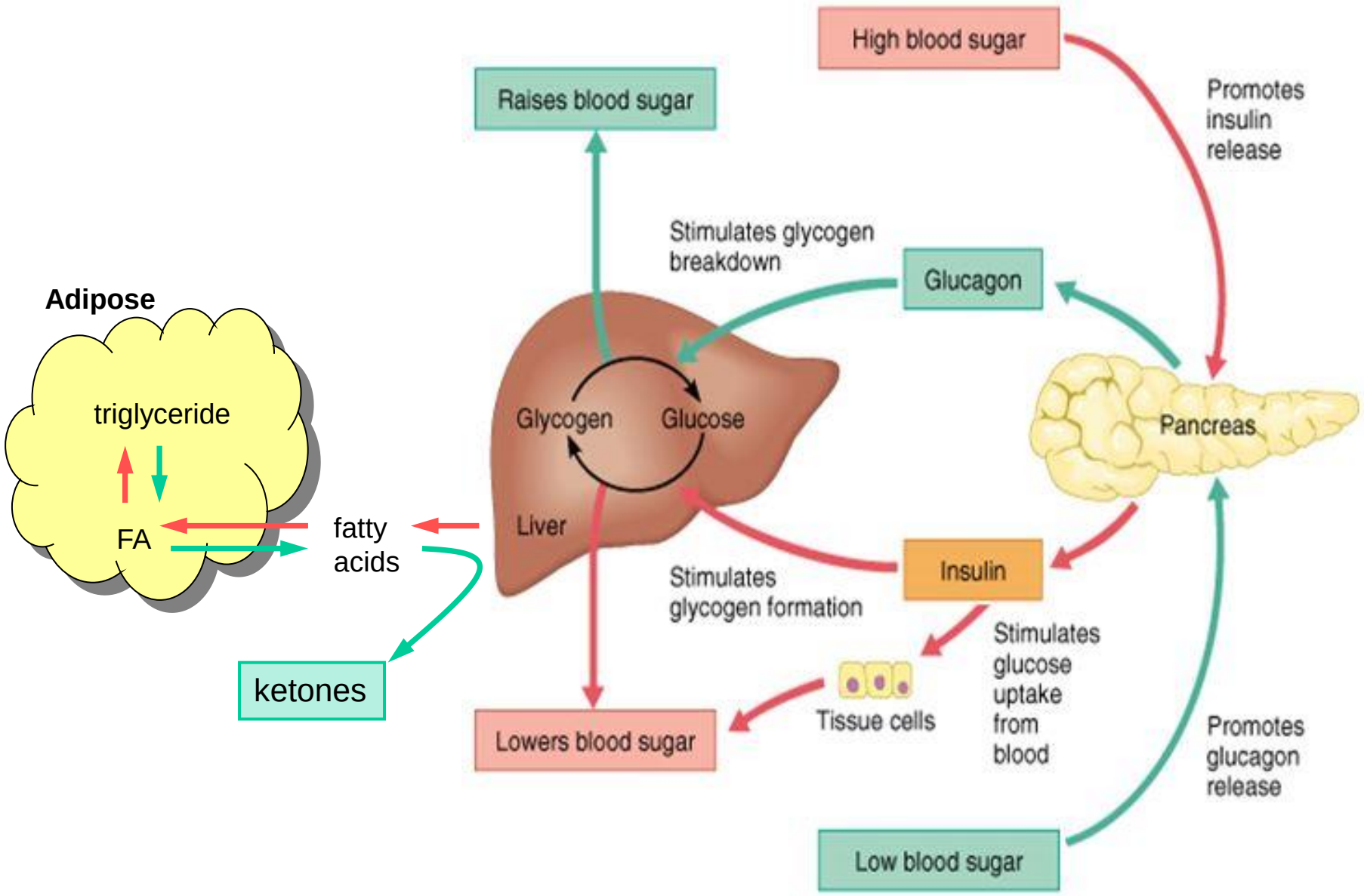
Reduces blood glucose conc.

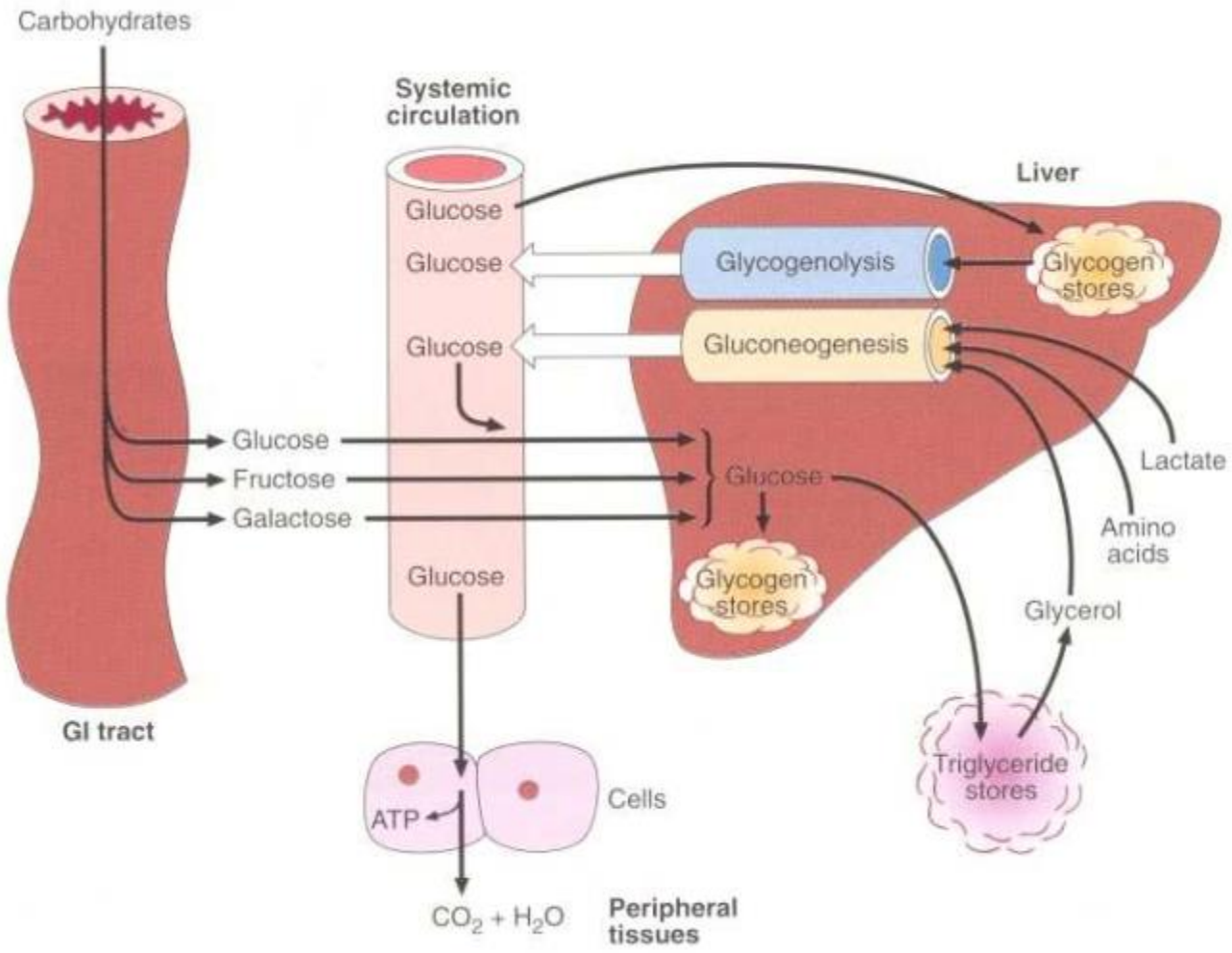
Glucagon

↓

Increases blood glucose conc.


High blood sugar
Raises blood sugar
Promotes insulin release
Stimulates glycogen breakdown
Glucagon
Adipose
triglyceride
FA
fatty acids
Glycogen
Glucose
Liver
Pancreas
Stimulates glycogen formation
Insulin
Stimulates glucose uptake from blood
Tissue cells
ketones
Lowers blood sugar
Promotes glucagon release
Low blood sugar

Glucose homeostasis



- Blood glucose concentration maintained within narrow range (4 - 8 mmol/L)

Carbohydrates as biochemical markers of disease

- The most common carbohydrate disorder in humans is diabetes mellitus.
- This disease is caused by an inability to produce or to respond to the hormone insulin.

What is diabetes mellitus

Syndrome characterized by hyperglycemia due to an absolute or relative lack of insulin, and/or insulin resistance

Classification of diabetes

- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes
- Secondary causes:
  - pancreatic damage
  - endocrine disease
  - hepatic cirrhosis
  - medications (eg. steroids)

Type 1 diabetes

- Previously called insulin dependent diabetes mellitus (IDDM)
- ~10% of diabetic patients
- Most common in young (peak incidence 9-14yrs of age)
- Absolute lack of insulin production and secretion by the beta cells of the pancreas due to autoimmune destruction of beta-cells
- Environmental trigger (e.g. viral infection)

Type 2 diabetes

- Previously called Non-insulin dependent diabetes mellitus (NIDDM)
- Decline in insulin action due to the resistance of tissue cells to the action of insulin
- Majority of diabetic patients (90%)
- Can occur at any age (Usually >40yrs)
- Commonly associated with obesity
- Serum insulin levels may be normal or even high
- Eventual β-cell failure and insulin deficiency

Risk factors for type 2 diabetes

- Weight gain/obesity
- Lack of exercise
- High triglycerides, high cholesterol, low high-density lipoprotein (HDL)
- High blood pressure
- Family history of diabetes

Treatment of diabetes

- **Type 1 diabetes**
  - Insulin
- **Type 2 diabetes**
  - Diet and exercise
  - Oral hypoglycemic drugs
  - Insulin
- **Control of blood pressure and lipids**

Clinical features of diabetes

- Thirst
- Weight loss
- Tiredness
- Infections
- Polyuria (increased urine volume)
  - If plasma [glucose] >10mmol/L → glycosuria

  (glucose in urine) → osmotic diuresis polyuria

Diagnostic criteria of diabetes

- **Indications for investigation:**
  - Symptoms
  - Family history of diabetes
  - Glycosuria

- **Investigations:**
  - Plasma glucose

- Fasting Blood Sugar (FBS)
- Random Blood Sugar (RBS)
- Oral glucose tolerance test (OGTT)

Diagnostic criteria of diabetes

- Normal fasting venous plasma glucose: <6.1mmol/L
- Diabetes symptoms
  - Fasting venous plasma glucose ≥7.0mmol/L
  - Random plasma glucose ≥11.1 mmol/L
  - 2hr plasma glucose ≥11.1 mmol/L (in oral glucose tolerance test)

Oral glucose tolerance test (OGTT)

- Patient: fasting, resting, no smoking
- Fasting venous plasma sample (0 min)
- 75g glucose (in ~300ml water) taken orally (over 5 mins)
- 2nd plasma sample taken 2 hrs after ingestion of glucose

IFG/IGT – prediabetes

- Impaired fasting glycaemia (IFG)
- Impaired glucose tolerance (IGT)

- IFG: blood sugar levels during fasting are above the normal range, but are not high enough to mean that the person has diabetes (≥100 and <126 mg/dl)
- IFG means that the body isn't able to use glucose as efficiently as it should
- a signs of insulin resistance
- Risk of developing type 2 diabetes mellitus in future
- Increased risk of cardiovascular disease (CVD)
- Annual oral glucose tolerance test (OGTT)
- Prediabetes can be a reversible condition with diet and lifestyle changes

Interpretation (venous plasma glucose in mmol/L)

	Fasting sample	2 hr sample
Normal	< 6.1	< 7.8
IFG	≥ 6.1 and < 7.0	< 7.8
IGT	< 7.0	≥ 7.8 and < 11.1
DM	≥ 7.0	≥ 11.1

Metabolic Complications

- Acute
  - hypoglycaemia
  - hyperglycaemia

- Chronic
  - long term effects of chronic hyperglycemia

Acute

Causes of coma in the diabetic patient:

- Hypoglycemia
- Diabetic ketoacidosis (DKA)
- Hyperosmolar non-ketotic hyperglycemia (HONK)

Hypoglycaemia

- Most common cause of coma in diabetics
- Plasma [glucose] <2.5 mmol/L
- Due to too high a dose of insulin or other drugs used in treatment of diabetes
- Missed meal or excessive exercise
- Signs – sweating, anxiety, tremor, confusion, dizziness, hunger
- Raising the blood sugar to normal through

➢ Receiving 10–20 g of carbohydrate can give intravenous dextrose

➢ 1 to 2 mg of glucagon in an intramuscular injection.

Diabetic ketoacidosis (DKA)

- DKA occurs when blood sugar levels are very high and insulin levels are low
- Type 1 diabetes
- The body switches to burning fatty acids for energy, which produces acidic ketone bodies
- DKA is typically diagnosed when testing finds high blood sugar, low blood pH and ketoacids in either the blood or urine
- DKA was first described in 1886 and, until the introduction of insulin therapy in the 1920s, it was almost universally fatal

Health problems that may result from DKA

- Severe dehydration
- Kidney failure
- Fluid buildup in the brain (cerebral edema)
- Heart stops working (cardiac arrest)
- Coma

Treatment usually involves:

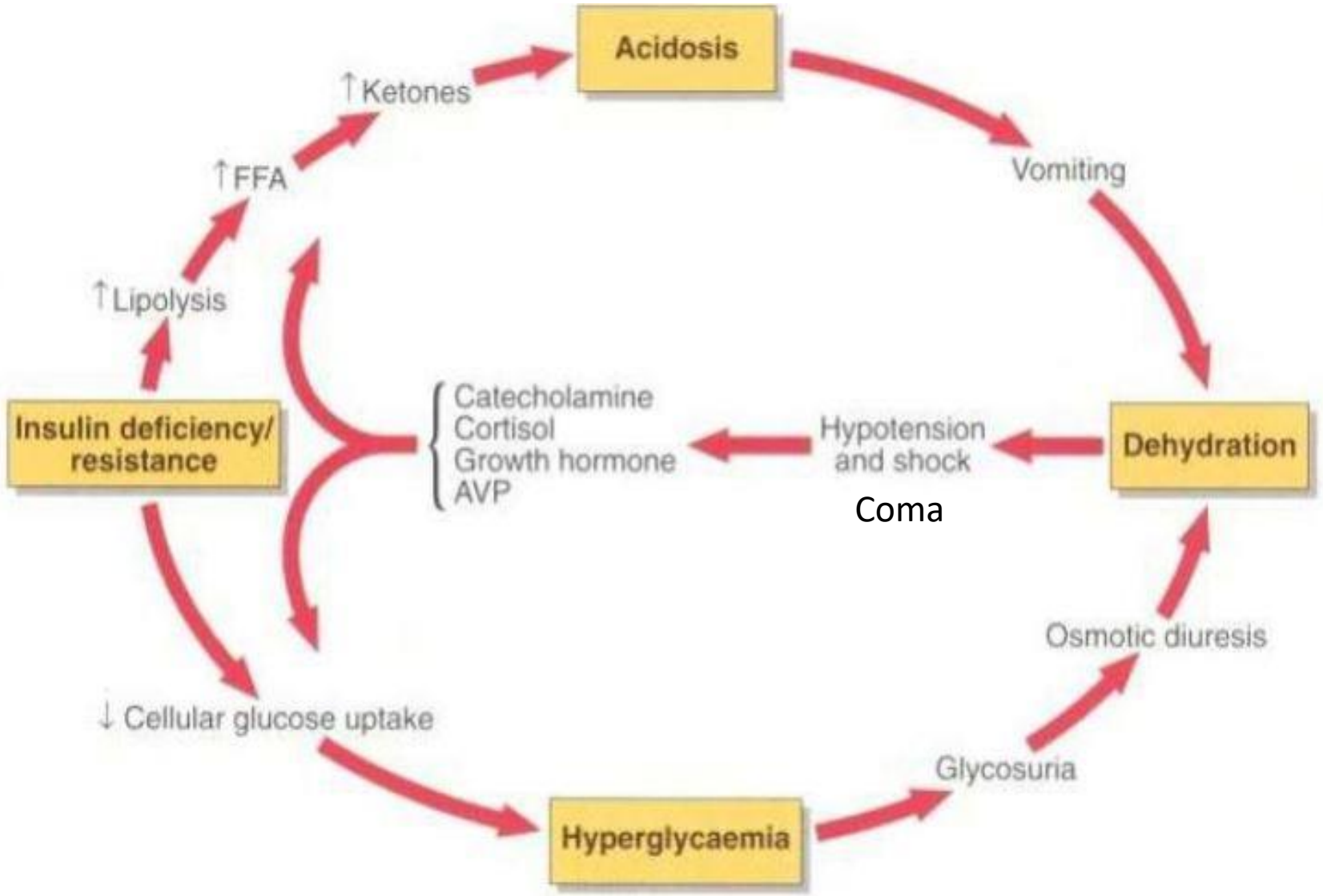
- Rehydration (Fluid replacement)
- Insulin therapy
- Electrolytes replacement (Potassium)

The development of diabetic ketoacidosis (DKA)

The most common precipitating factors in the development of diabetic keto acidosis are:

- Infection
- Omission of insulin
- Heart attack
- Trauma

The development of diabetic ketoacidosis (DKA)



Hyperosmolar non-ketotic hyperglycemia (HONK)

- Coma resulting from very high blood glucose levels in a patient with normal ketone levels and minimal acidosis
- More common in elderly (Type 2 diabetes) due to diabetic medication not being taken [glucose] usually >50 mmol/L
- Severe dehydration
- Risk of thrombosis (blood clots)

Treatment of HONK

- Insulin (low dose)
- Fluid replacement (i.v. saline)
- Potassium replacement
- Heparin (anti-coagulant)

Metabolic complications: Chronic

- Chronic hyperglycaemia can cause long-term damage to blood vessels, nerves and organs
- Macrovascular: large vessel disease
- Microvascular : damage to small blood vessels

Macrovascular complications

- Cardiovascular disease
  - Coronary heart disease (CHD), peripheral vascular disease, stroke

Related to:

  - high blood pressure
  - high cholesterol
  - Smoking

- Coronary heart disease is major cause of death in diabetic patients (~35%)

Microvascular complications

- Retinopathy (eyes)
- Nephropathy (kidneys)
- Neuropathy (nerve tissue)

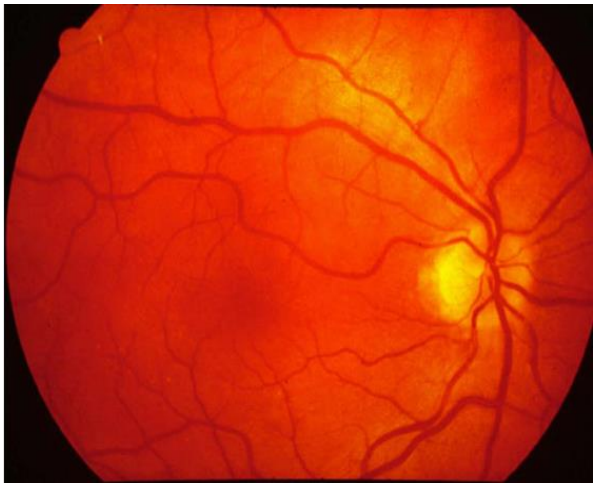
Related to:

- long-term control of blood glucose

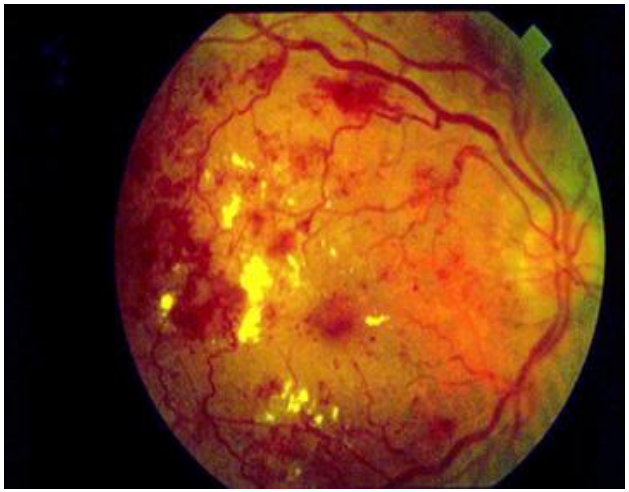
Diabetic retinopathy

- Damage to blood vessels in retina (Retinas have tiny blood vessels that are easy to damage)
- If left untreated, may lead to blindness

Normal retina



Diabetic retinopathy



Symptoms of DR

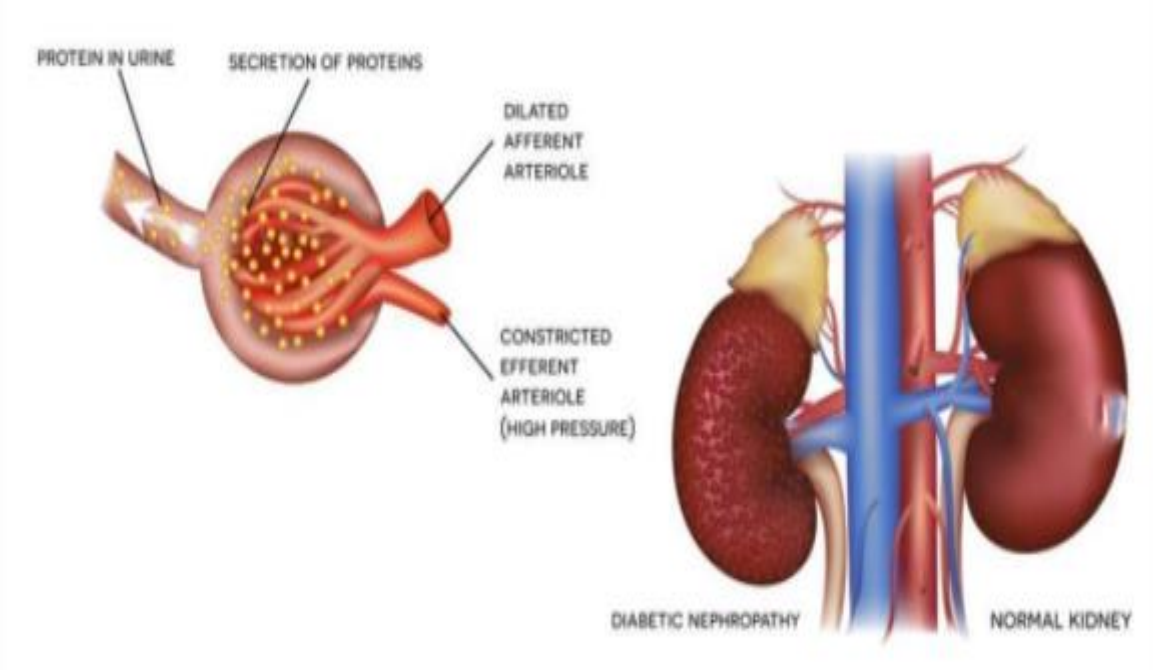
- Seeing spots or floaters
- Blurred vision
- Difficulty seeing well in night
- Seeing blank or dark areas

Treatment of DR

- Anti-VEGF (vascular endothelial growth factor) to reduce abnormal blood vessels
- laser treatment: directly targets the damaged blood vessels

Diabetic nephropathy

- Loss of small amounts of protein in the urine “microalbuminuria” (30-300mg/day) is an important biomarker currently used in the diagnosis of DN
- Progresses to overt proteinuria (>300mg/day) and deterioration in kidney function
- Ultimately leads to renal failure
- Dialysis or transplant

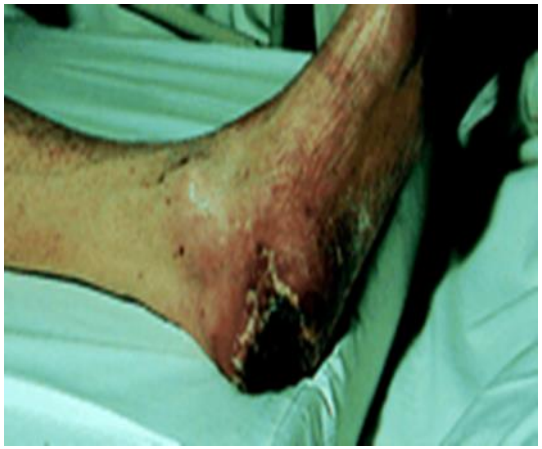


Diabetic nephropathy and Hypertension

- Hypertension is common in patients who are diabetic
- Hypertension alone can result in severe renal failure
- Hypertension associated with hyperglycemia (diabetes) is a major killer for your kidneys.
- Hence it is advised to keep your blood pressure under control.

Diabetic neuropathy

- Occurs due to damage to nerve blood vessels and abnormal glucose metabolism in nerve cells
- e.g. the diabetic foot:
  - Numbness
  - damage
  - ulcer
  - infection
  - gangrene
  - amputation



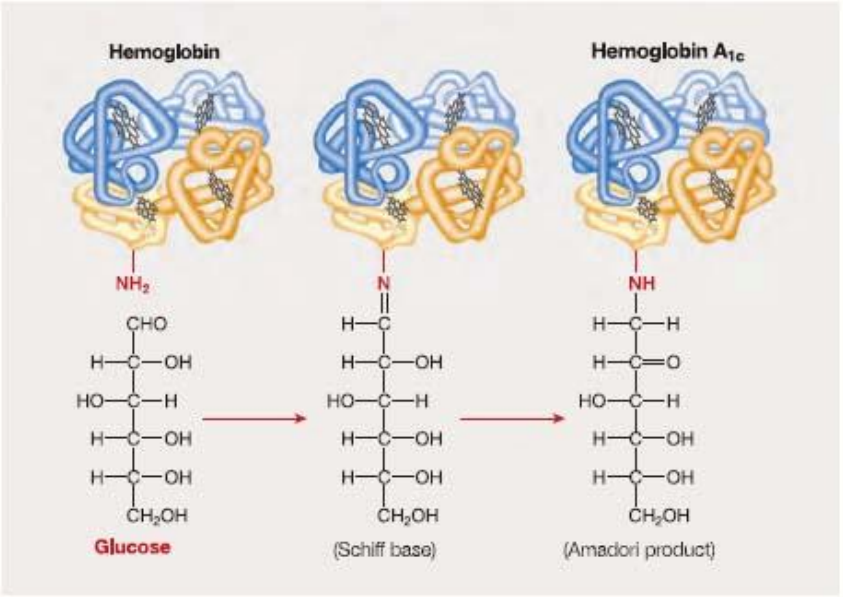
Blood glucose monitoring in diabetes

- An alternative to the clinical biochemistry laboratory testing
- **Point-of-care and self testing (home testing)**

- Monitoring Errors
- Out of date strips
- Contaminated strips
- Incorrect meter coding
- Batteries dead
- Hands not clean

HbA1c

- Formed by non-enzymatic glycation of haemoglobin (Hb)
- Dependent on mean plasma glucose conc. and lifespan of red blood cells
- Retrospective assessment of the mean plasma glucose conc. over previous 6-8 weeks
- Falsely low values may be found if RBC lifespan is shortened (e.g. haemolytic anaemia)
- Expressed as mmol per mol of total Hb
  - Non-diabetic: 20-41 mmol/mol
  - Diabetic patients: 48-59 mmol/mol



lecture 3: Disorders of Lipids Metabolism

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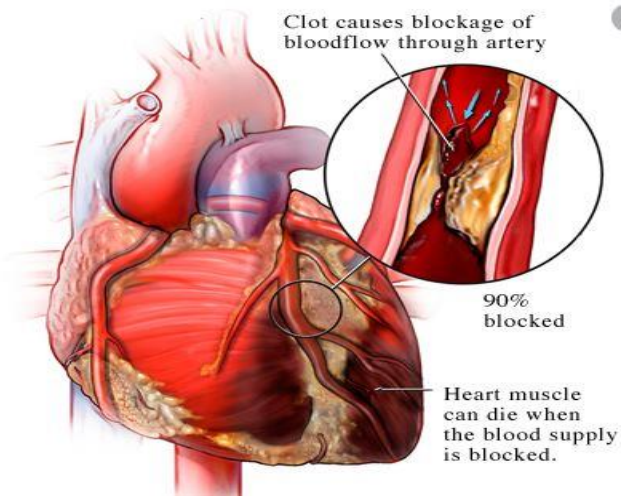
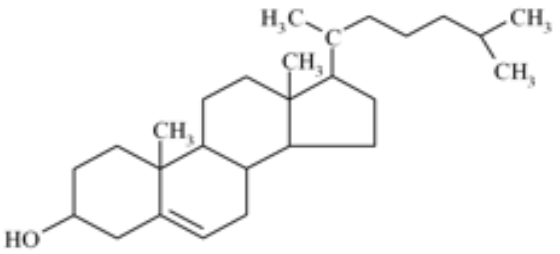


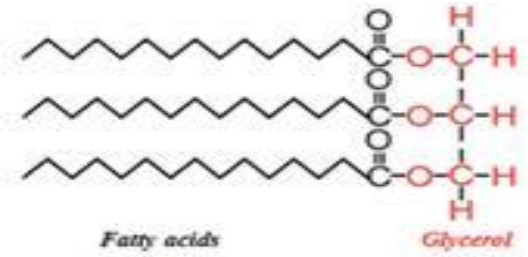
- **Lipids** are a group of substances soluble in organic solvents and virtually insoluble in water
- **Three main groups**

1- Cholesterol



- Essential component of cell membranes
- Essential for synthesis of steroid hormones
- Essential for bile acids synthesis

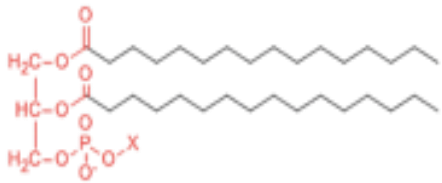
2-Triglycerides

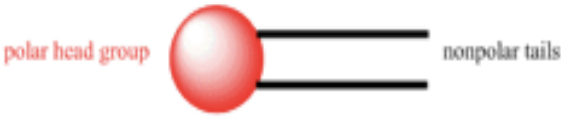


- Used as energy source in tissues
- Used for energy storage in adipose tissue

3- Phospholipids

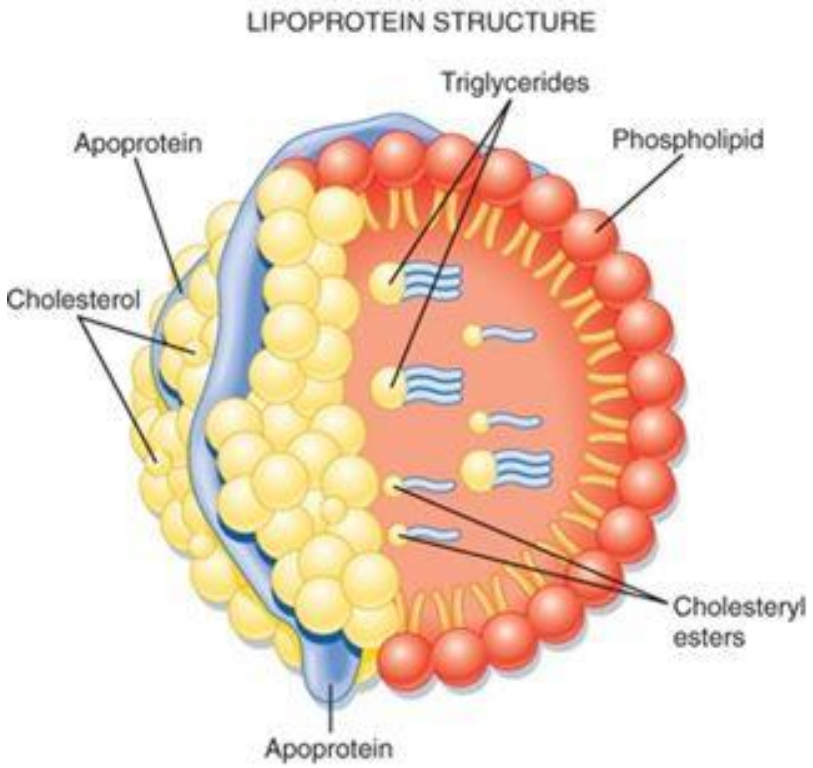
- Form phospholipid bilayers – a major component of the cell membrane
- Participate in the transport of other lipids
- Help transfer biological signals





Lipoproteins

- A lipoprotein is a complex spherical structure
- central core of hydrophobic lipids (triglycerides and cholesterol esters)
- surface layer of polar components (phospholipids, free cholesterol,
- proteins – the apolipoproteins)

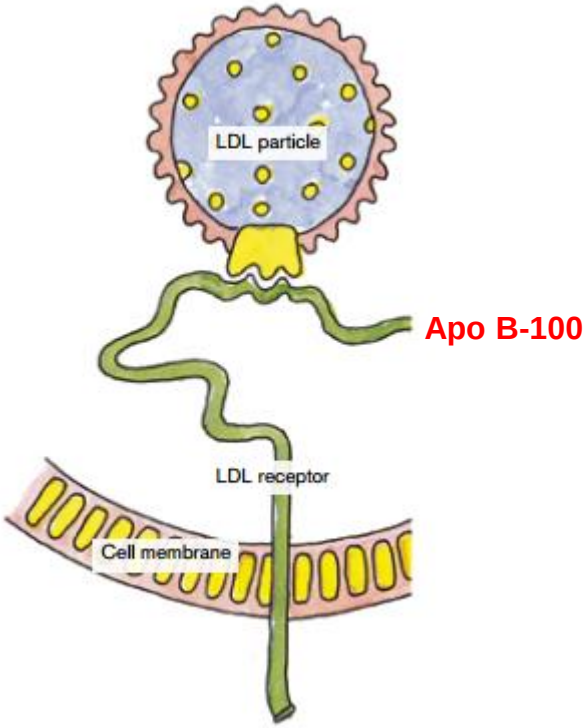


- The lipoprotein system evolved to solve the problem of transporting fats in the aqueous environment of plasma

Apolipoproteins

The proteins associated with lipoproteins are called apolipoproteins (apo)

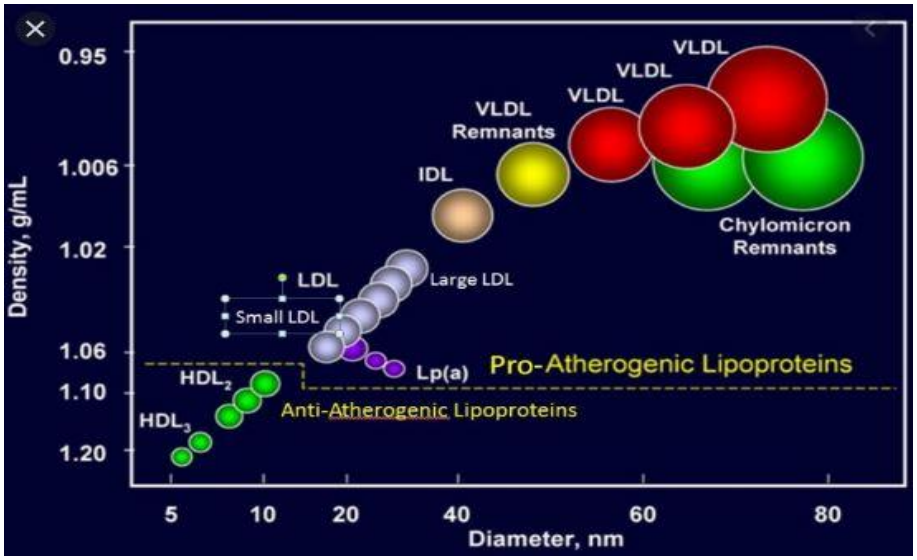
- **4 Major Functions:**
  - Serving a structural role for lipoproteins
  - Acting as ligands for lipoprotein receptors
  - Guiding the formation of lipoproteins
  - Serving as activators or inhibitors of enzymes involved in the metabolism of lipoproteins



- Apolipoproteins thus play a crucial role in lipoprotein metabolism

Main lipoproteins and their functions

Lipoproteins are defined by their density and differ in composition, structure and function



Type	Major apolipoprotein	Function
Chylomicrons	apoB-48	Produced by gut after a meal. Not present in normal fasting plasma. Main carrier of dietary TGs.
Very low density lipoprotein (VLDL)	apoB-100	Produced by the liver. Main carrier of endogenously produced TGs.
Low density lipoprotein (LDL)	apoB-100	Generated from VLDL in the circulation. Main carrier of cholesterol from liver to the tissues
High density lipoprotein (HDL)	apoA-I	Protective function. Transport excess cholesterol from tissues to the liver for excretion.

Main apolipoproteins and their functions

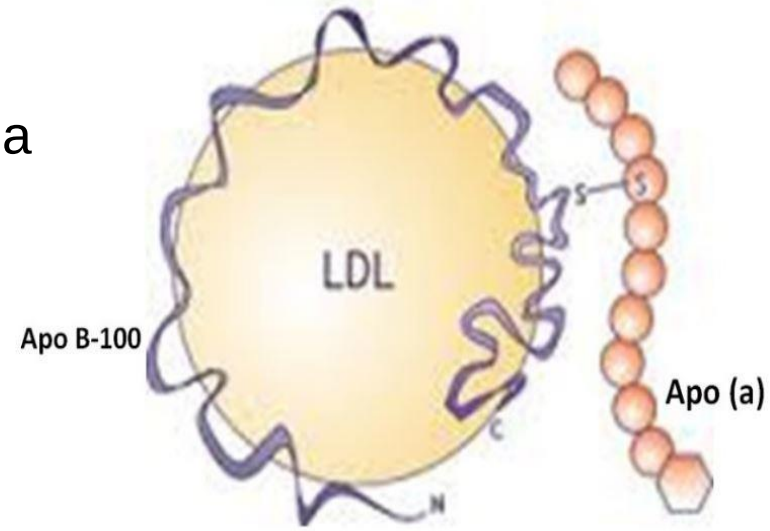
- ✓ **Apolipoprotein B-48: Apo B-48** is synthesized in the intestine and is the major structural protein of chylomicrons and chylomicron remnants.

- ✓ **Apolipoprotein B-100: Apo B-100** is synthesized in the liver and is the major structural component of VLDL, IDL, and LDL. It is a ligand for the LDL receptor and therefore plays an important role in the clearance of lipoprotein particles.

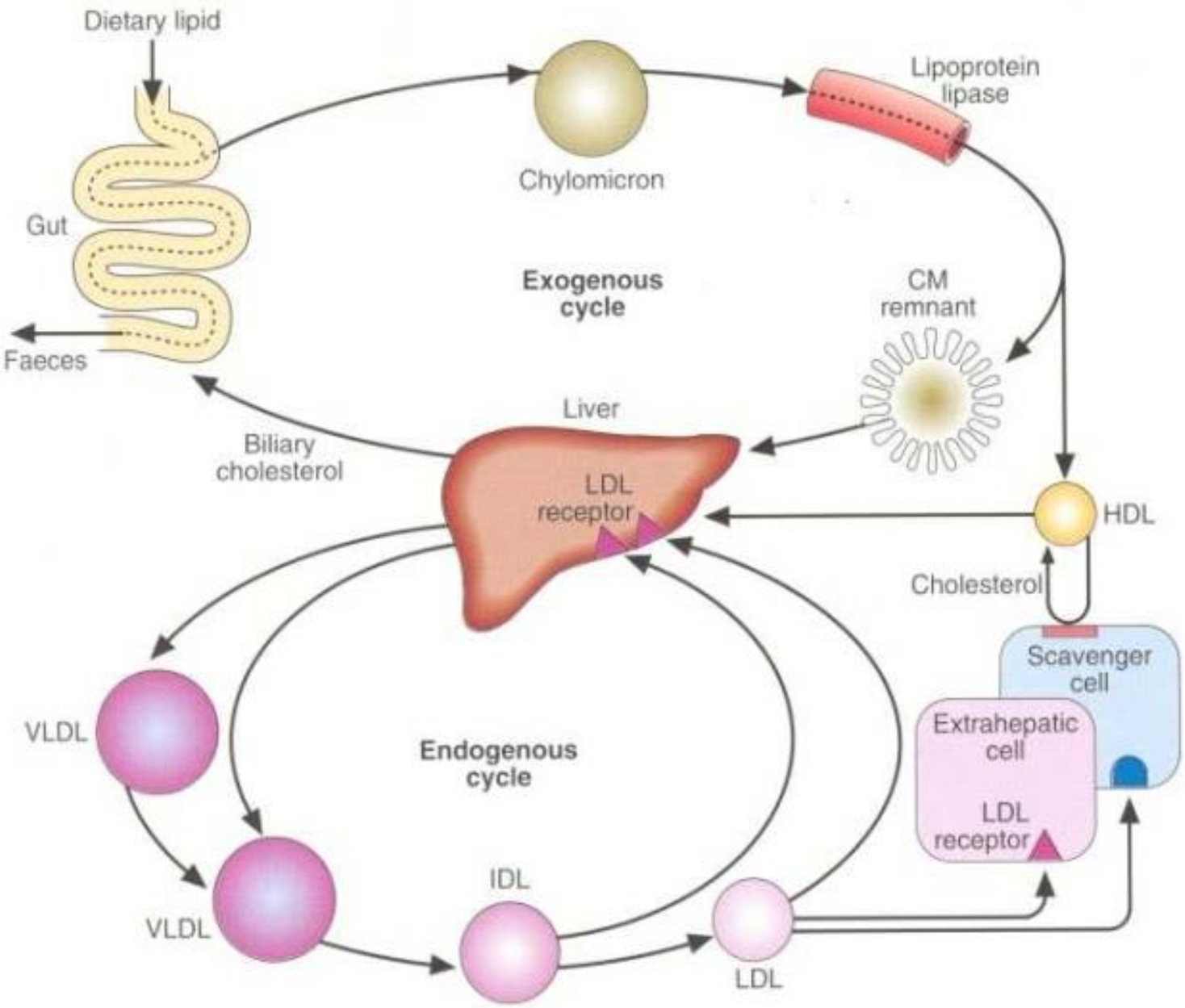
- ✓ **Apolipoprotein A-I: Apo A-I** is synthesized in the liver and intestine and is the major structural protein of HDL accounting for approximately 70% of HDL protein. It is an activator of lecithin: cholesterol acyltransferase (LCAT), an enzyme that converts free cholesterol into cholesteryl ester.

Lipoprotein (a): Lp (a)

- Similar in lipid composition to LDL but has a higher protein content
- Consists of apolipoprotein (a), which is attached to the apo B-100 of the LDL via a single disulfide bond
- The plasma concentration of Lp(a) is normally less than 0.30 g/L
- Elevated plasma Lp(a) levels are associated with an increased risk of CVD (pro-atherogenic lipoprotein)
- The kidney appears to play an important role in Lp (a) clearance as kidney disease is associated with delayed clearance and elevations in Lp (a) levels



Lipoprotein metabolism



Lipoprotein metabolism

- Lipoprotein are complexes of lipids and protein which facilitate lipid transport
- Their metabolism can be thought two interconnected cycles centered on the liver
- Lipoprotein are defined by their density and differ in composition, structure and function
- Apolipoproteins have a function as well as structure importance
- Cholesterol can only be excreted from the body by way of the liver

Lipid Profile test

- Total cholesterol
- HDL-cholesterol (Good cholesterol)
- LDL-cholesterol (Bad cholesterol)
- Triglyceride

Why it is done

- As part of a routine physical exam to screen for a lipid disorder
- To check the response to medicines used to treat lipid disorder
- To help determine risk of coronary heart disease (CHD)

Lipoprotein disorders

- Lipoprotein disorders are caused by abnormal synthesis, processing or catabolism of lipoprotein particles with their various consequences which include:

  - Cardiovascular disease (CVD)

  - Coronary heart disease (CHD) - angina, myocardial infarction (MI), heart failure, sudden death

  -Stroke

  -Peripheral vascular disease

  - Acute pancreatitis

  - Weakness

Lipoprotein disorders

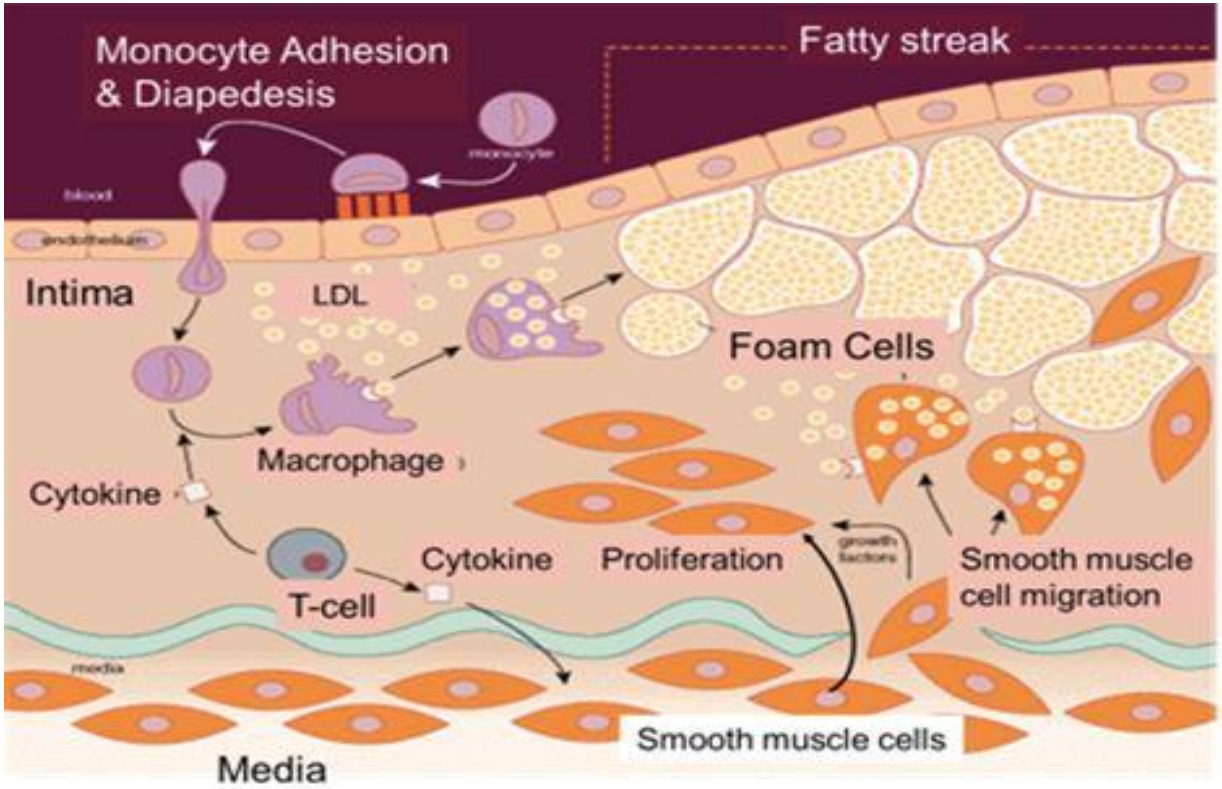
- Lipoprotein disorders are classified into the following categories:
  - Hypercholesterolemia
  - Hypertriglyceridemia
  - Combined (Mixed) hyperlipidemia

- Cause is either
  - Primary (genetically determined) or
  - Secondary (abnormality is result of acquired condition)

Hypercholesterolemia

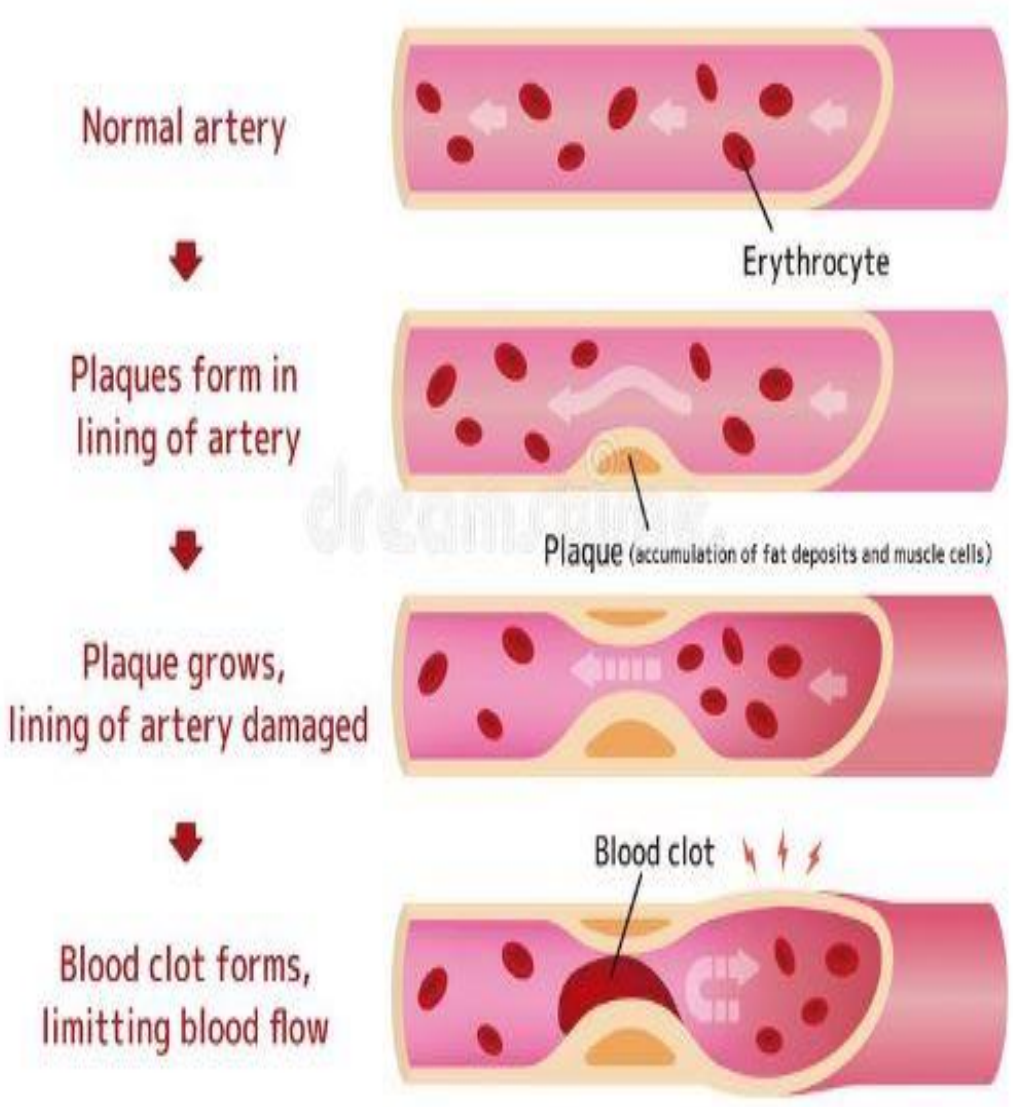
- One of the major causes of atherosclerosis
- For most patients, a combination of genetic factors and environmental (dietary) determines plasma cholesterol levels.

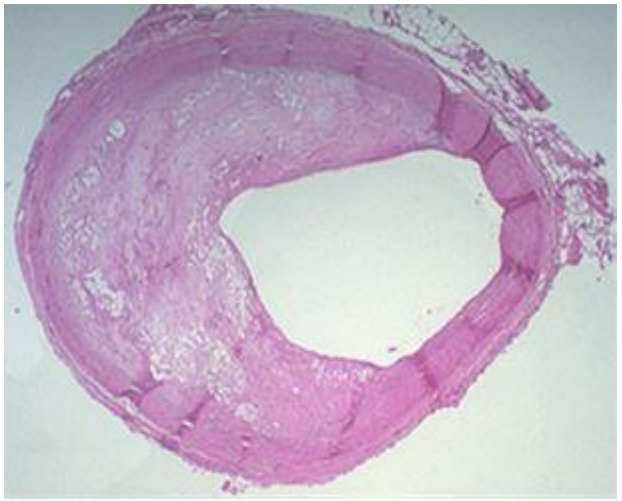
The role of LDL in atherosclerosis



Injury to endothelial cells → monocytes adhere to endothelium and migrate beneath it, LDL particles enter the arterial wall and undergo oxidation → monocytes differentiate into macrophages, take up ox-LDL and become 'foam cells'→ foam cells undergo apoptosis and die but the lipid accumulates → T-cells secrete cytokines that induce smooth muscle cells to migrate to the intima → accumulation of lipid and smooth muscle cells that begins to raise the endothelium

Progression of atherosclerosis





Cross-section of an Artery at the site of an Atherosclerotic

Hypercholesterolemia – Primary causes

Familial hypercholesterolemia - FH

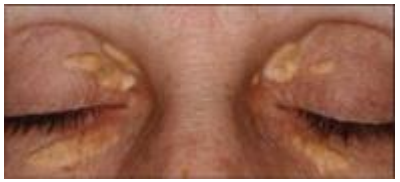
- Inherited condition leading to hypercholesterolaemia with a relatively normal plasma triglyceride
- It affects about 1 in 200 people
- The most common genetic disorder in Afrikaners, Europe and the USA
- FH is a dominantly inherited condition. If a parent has FH, there is 1 in 2 chance that their son or daughter will also have it
- If untreated, most affected people will have symptomatic CHD by the age of 60.
- Treatment: high-dose statins combined with a cholesterol absorption inhibitor

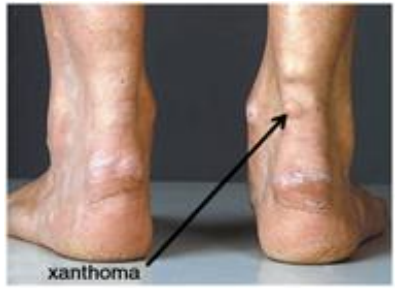
FH diagnosis

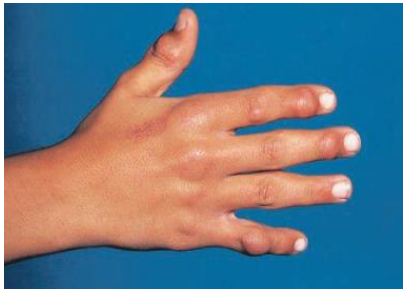
Clinical criteria used to identify patients with FH include:

- Total cholesterol >7.5 mmol/l or LDL-C >4.9 mmol/l in an adult
- Family history of hypercholesterolemia
- Personal and family history of premature CVD
- Deposition of cholesterol in extravascular tissues such as tendon xanthomata, xanthelasma or corneal arcus
- can affect the back of the hands

- Xanthoma: an irregular yellow patch or nodule on the skin, caused by deposition of lipids







Hypercholesterolemia – Secondary causes

- Hypothyroidism
- Nephrotic syndrome
- Pregnancy
- Cushing’s syndrome
- Anorexia nervosa
- Immunosuppressant drugs
- Corticosteroids

Hypertriglyceridemia

- Familial hypertriglyceridemia is often observed with low HDL cholesterol concentration.
- TG <2.3 mmol/L (Optimal level)
- TG >10 mmol/L may cause acute pancreatitis
- Elevated triglycerides cause a cardiovascular risk
- Discovered when samples are found to be lipaemic or through cardiovascular risk screening.
- Dietary measures, and sometimes lipid-lowering drugs such as the fibrates

Hypertriglyceridemia – Secondary causes

- Obesity
- Type 2 diabetes
- Excessive alcohol intake
- Renal disease
- Hypothyroidism
- Pregnancy
- Autoimmune disorders

Combined hyperlipidemia

- Characterized by moderate or marked elevation of both cholesterol and TG
- Often secondary to other diseases or medications
- Most of the conditions indicated as possible causes of hypertriglyceridemia may also present as combined hyperlipidemia
- Primary causes: Familial combined hyperlipidemia, Type III dyslipidemia

Management of Hyperlipidemia

- **Identification and treatment of secondary causes**
- **Lifestyle modification**
  - stopping of smoking – associated with improved lipid
  - Diet – reduced saturated fat and simple carbohydrates. Omega-3 fatty acids can reduce TGs
  - Weight loss – reduction in TGs and LDL, increase in HDL
  - Exercise – improves fitness and decreases in intra-abdominal fat. Small increase in HDL and decreases TGs
- **Cardiovascular risk assessment**
- **Medication**
  - At least two fasting lipid profile should be performed before starting any form of lipid-lowering therapy
  - First-line of therapy always consist of dietary therapy

Monitoring of lipids

- Non-fasting lipid panel

➢ Measures HDL and total cholesterol

- Fasting lipid panel

➢ Measures HDL, total cholesterol and triglycerides

- LDL cholesterol is calculated:

LDL cholesterol = total cholesterol – (HDL + triglycerides/5)

- **LDL**
  - < 100 →Optimal
  - 100-129 → Near optimal
  - 130-159 → Borderline
  - 160-189→ High
  - ≥ 190 → Very High

- **HDL**
  - < 40 → Low
  - ≥ 60 → High

- **Total Cholesterol**
  - < 200 → Desirable
  - 200-239 → Borderline
  - ≥240 → High

- **Serum Triglycerides**
  - < 150 → normal
  - 150-199 → Borderline
  - 200-499 → High
  - ≥ 500 → Very High

Drug therapy

- Dietary management
- Lifestyle changes
- The most commonest drugs for the treatment of primary hypercholesterolemia are HMG CoA reductase inhibitors (Statins)
- Statins (Pravastatin and simvastatin) → reduce mortality of coronary heart patients
- The most commonest drugs for the treatment of primary hypertriglyceridemia or combined hyperlipidemia are Fibrates

Drug therapy

Lipid lowering drugs

Drug group	Principle of Action
Bile acid sequestrant resins	Block the acid reabsorption and lower total and LDL cholesterol
HMG CoA reductase inhibitors	Inhibit cholesterol biosynthesis and lower total and LDL cholesterol
Fibrate	Activate lipoprotein lipase and lower triglyceride, total and LDL cholesterol. May increase HDL cholesterol

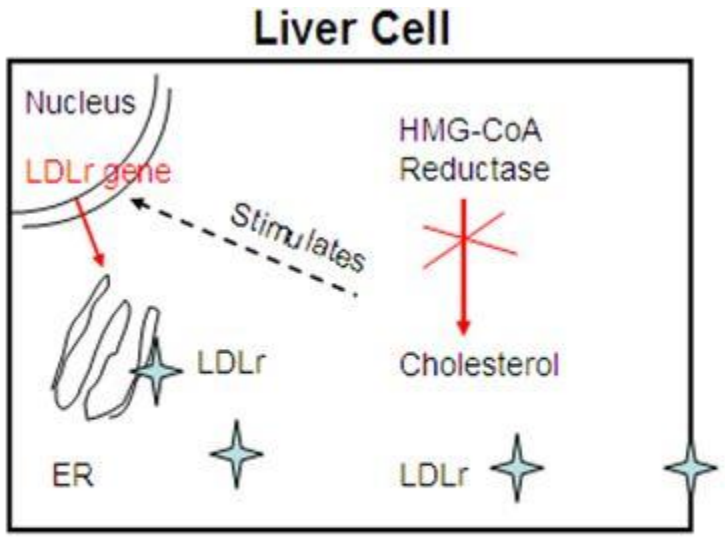
Lipid-lowering medications (Statins)

- Statins inhibit a key enzyme in the cholesterol synthesis pathway (HMG CoA reductase) → reduced cholesterol → LDL-receptor expression is upregulated → increased uptake of apo-B lipoproteins

- Major actions: ↓ LDL-C, ↑ HDL-C modestly, ↓triglycerides

- Side effects: myalgia and myositis

- For every **1 mmol/l** decrease in **LDL-C**, the reduction in risk of major cardiovascular events is **21%**



HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase

LDLr, LDL receptor; ER, endoplasmic reticulum

lecture 4: Kidney Function Tests

5th Class

Anbar University-College of Pharmacy-
Clinical Laboratory Sciences Department

2020-2021

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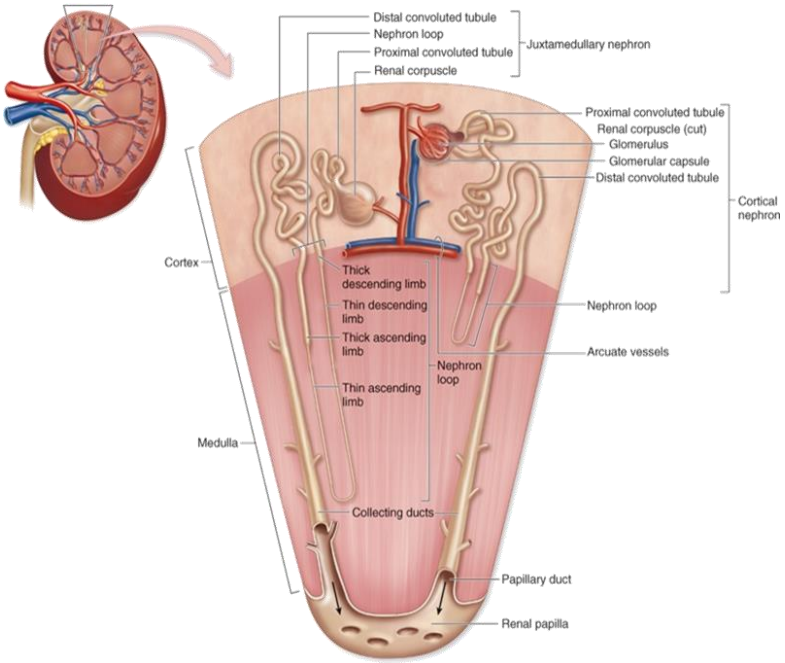
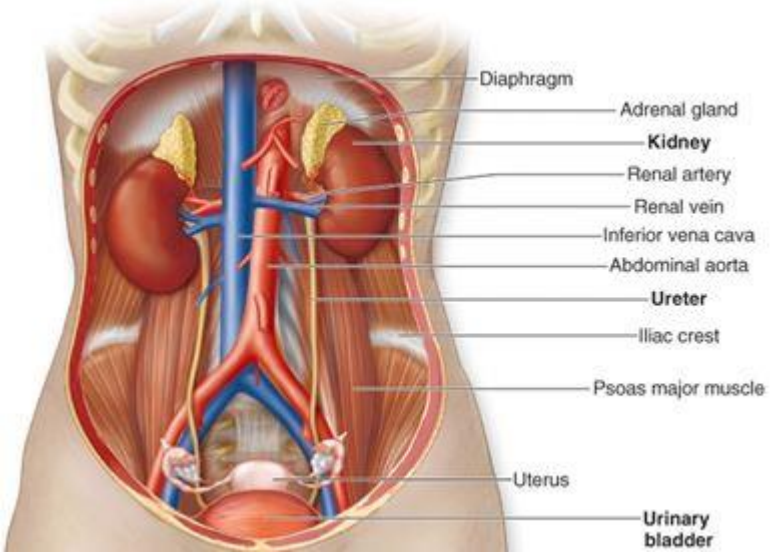
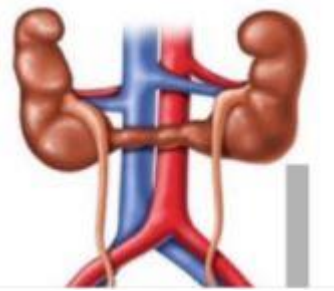


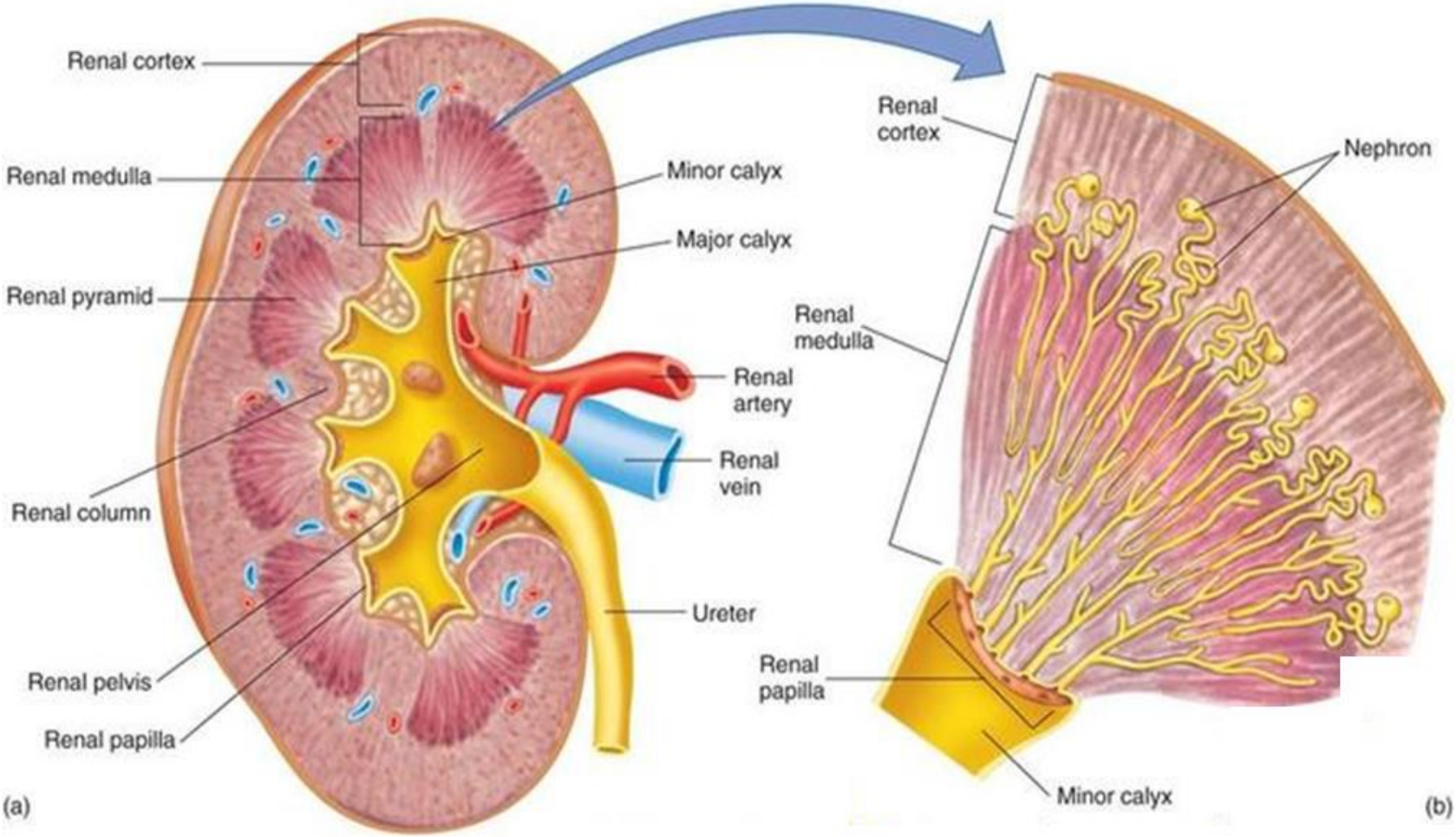
- Kidneys are normally located between 11th thoracic and 3rd lumbar vertebra, either side of vertebral column
- Some people have only one kidney
- 1 in 500 people have a horseshoe kidney
- ~12 cm long
- ~150 g male, ~135 g female
- Kidneys receive blood from the paired renal arteries; blood exits into the paired renal veins
- Each kidney is attached to a ureter





Structure

The structural and functional unit of the kidney is the nephron. It processes the blood supplied to it via filtration, reabsorption, secretion and excretion; the consequence of those processes is the production of urine



The kidney have three main functions

- Regulation of water, electrolyte concentrations, acid base balance, and whole-body homeostasis
- Excretion of waste products of protein and nucleic acid metabolism e.g. urea, creatinine and uric acid
- Hormone synthesis:
  - Erythropoietin (EPO) – haemoglobin synthesis
  - Renin – blood pressure control
  - Activates vitamin D

- The kidney accomplishes these homeostatic functions both independently and in concert with other organs,

Functional unit – the nephron

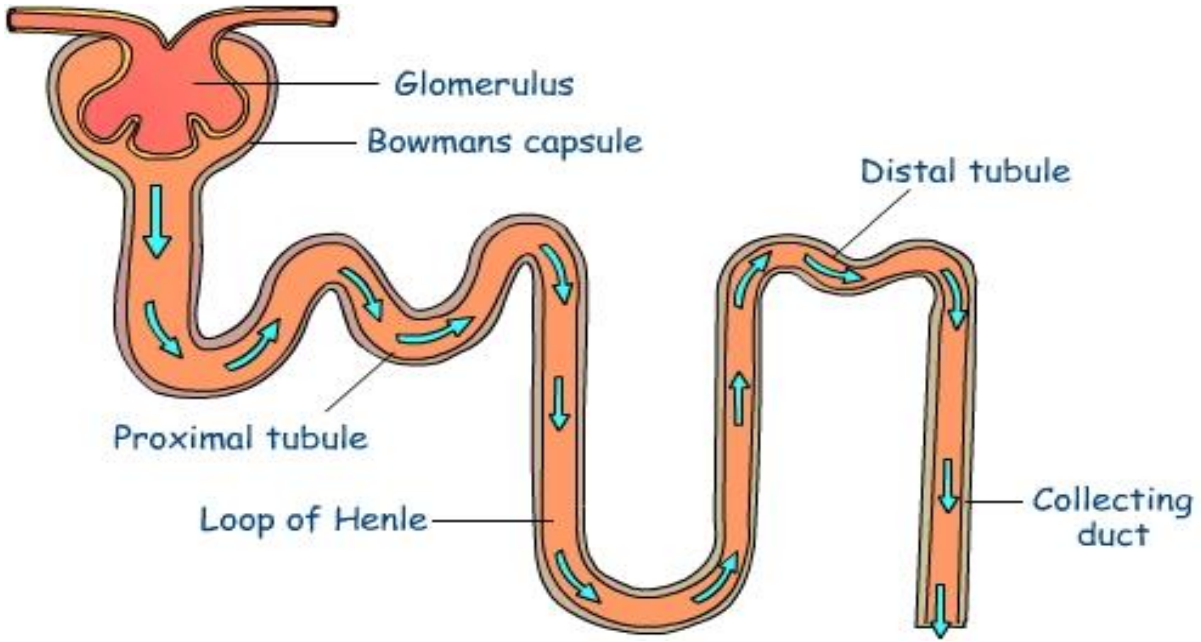
Glomerulus - filters blood

Proximal Tubule - Bulk reabsorption of electrolytes from the glomerular filtrate back into circulation.

Loop of Henle - production of osmotic gradient for control of water reabsorption.

Distal Tubule - fine-tuning of electrolyte

Collecting Duct - water reabsorption/excretion



Evaluation of kidney function

we have three options:

- Evaluation of glomerular filtration

– the ability to remove waste

- Evaluation of glomerular integrity

– the ability to select what is allowed to enter the tubules

- Evaluation of tubular cell function

- the ability to secrete or reabsorb compounds and respond to stimulation

Glomerular filtration rate (GFR)

- GFR is the rate at which substances are filtered from the blood, of the glomeruli into Bowman's capsules of the nephrons (Best indicator of renal function)
- The complications of Chronic Kidney Disease (CKD) increase with decreasing GFR and may progress from gradual reduction in renal function to end-stage renal disease (ESRD)
- Low GFR is risk factor for cardiovascular disease (CVD) mortality

Glomerular filtration rate (GFR)

- Renal clearance of a substance is defined as the volume of plasma from which the substance is completely cleared by the kidneys per unit of time.
- The maximum rate that the plasma can cleared any substance is equal to the GFR

GFR = (US ×V) /PS

GFR = the flow rate in milliliters per minute of plasma through the glomerular membranes.

US = Urinary concentration of the substance

V = Volumetric flow rate of urine in milliliters per minute

PS = Plasma concentration of the substance

Glomerular filtration rate (GFR)

- GFR is best determined under standardized conditions:
  - Discontinuation of medication
  - Prior fasting
  - Sufficient water loading to maintain a urine flow rate >1 mL/min
  - Complete bladder emptying

Evaluation of glomerular function

- Measurement of glomerular filtration rate (GFR):
- GFR is an expression of the quantity of glomerular filtrate formed each minute in the nephrons of both kidneys, calculated by measuring the clearance of specific substances.

➢ Creatinine clearance

➢ Estimated GFR (eGFR)

➢ Exogenous markers

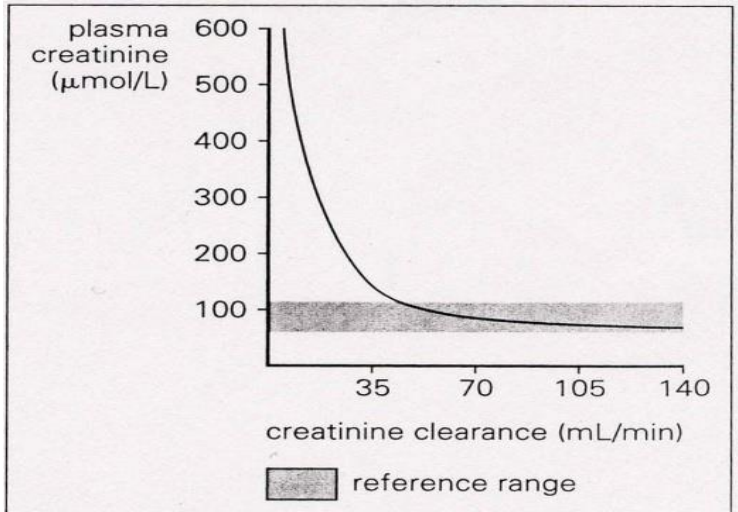
Evaluation of glomerular function

- GFR cannot be measured directly in humans. It has to be determined indirectly by measuring the clearance of an ideal filtration marker
- Ideal marker of glomerular filtration:

- Endogenously produced
- Appears in the plasma at a constant rate (stable plasma concentration)
- Freely filtered at the glomerulus (low molecular weight)
- Not bind to plasma proteins
- Not reabsorbed or secreted by the renal tubules
- Not eliminated extra-renally

Evaluation of glomerular function

- **Creatinine**
  - Produced at a constant rate from muscle creatine breakdown.
  - Not metabolised or excreted from the body other than by the kidney.
  - Can measure its clearance from plasma or excretion in urine.
- Affected by:
- Analytical interference: bilirubin, haemoglobin, ketones, glucose, drugs



Evaluation of glomerular function

Serum/Plasma Creatinine

- The breakdown product of creatine and creatine phosphate released from skeletal muscle at a steady rate.
- One blood sample – cheap and quick
- BUT
  - Not sensitive
    - Serum creatinine only starts to increase above normal when kidney function drops by half.
  - Affected by:
    - Muscle mass
    - Age
    - Gender

Endogenous creatinine produced is proportional to muscle mass

A body builder will have a much higher serum creatinine.

A small person with a low muscle mass will have a much lower serum creatinine.

Evaluation of glomerular function

Urea

- Derived in the liver from amino acids and therefore protein (diet or tissues)
- Removed by kidneys
- However, if rate of production exceeds the rate of clearance plasma concentrations can rise. Rate of production increased:

➢ High protein diet

➢ Gastrointestinal (GI) bleeding

➢ Increased catabolism due to starvation, sepsis, tissue damage

- Conversely, plasma urea concentration can be low:

➢ Low protein diet

➢ Pregnancy

Evaluation of glomerular function

Creatinine Clearance

- More sensitive than plasma creatinine at picking up small changes in renal function.
  - Removes the variables of diet and muscle mass

BUT

- Inconvenient - 24hr urine collection plus serum required.
- Lots of measurements and calculation – lots of room for error!

Calculation:

$$\text{Creatinine clearance (mL/min)} = \frac{\text{urine creatinine (mg/dl) x urine volume (ml)}}{\text{serum creatinine (mg/dl) x 1440 min}}$$

Urine volume is the total urine volume in 24 hour by ml and 1440 is a number of minutes in 24 hour

Evaluation of glomerular function

Estimated GFR (eGFR)

- More sensitive than plasma creatinine
- Uses only one serum sample and a calculation – more convenient

BUT

- Not to be used in certain situations such as:
  - In the acutely ill
  - In amputees
  - Pregnant woman
  - The very elderly
  - The obese
  - Malnourished

Evaluation of glomerular function

Exogenous markers

- In some situations, a very accurate GFR may be need e.g. live kidney donors, pre dangerous chemotherapeutics.

- **Inulin clearance (gold standard):**
- Infusion of inulin, collection of blood and urine samples.
- Calculation of inulin clearance

- **^{51}Cr-EDTA (standard clinical measure of GFR):**
- Injection bolus of ^{51}Cr-EDTA. Collect blood samples and use to calculate eGFR from known amount injected and the decrease in activity over time

Evaluation of glomerular integrity

Proteinuria

- Proteinuria defined as urine protein excretion >3g/24hr.
- Damage to the glomerulus can cause proteins (>66kDa) and blood cells to leak through and present in the urine.
- Normal urinary protein excretion is ~150mg/24hr with the majority consisting of secreted proteins such as Tamm-Horsfall protein (secreted from tubules).

Evaluation of glomerular integrity

Assessment of Proteinuria

- To screen for excess protein in the urine, to help evaluate and monitor kidney function, and to detect kidney damage
- Elevated levels may be seen temporarily with conditions such as infections, stress, pregnancy, diet, cold exposure, or heavy exercise.
- Proteins – which help build muscle and bone, regulate the amount of fluid in blood, combat infection and repair tissue – should remain in the blood.

Evaluation of glomerular integrity

- **Urine Dipsticks (A urine test strip):** A urine dipstick test is a test of urine, using a special strip of paper that is dipped into a sample of urine. The result is available almost immediately.
  - Protein – proteinuria: a sign the kidneys are not working right.
  - Blood – haematuria: a sign of infections
  - Acidity, or pH. If the acid is above normal: a sign the kidneys have stones or a urinary tract infection

Evaluation of glomerular integrity

- **Assessment of Proteinuria**
- **Laboratory measurements**
  - 24hr total protein
  - Protein: creatinine ratio (PCR).
  - Albumin: creatinine ratio (ACR). Albumin is the predominant plasma protein responsible for many physiological functions, normal urinary albumin is less than 30 mg/day. Normally NOT filtered by the glomerulus due to:
- Size (~66kDa)
- Charge (net negative charge)

Evaluation of tubular cell function

- Ability to excrete an acid load
  - Urine pH
  - Acid/base status

- Ability to concentrate urine
  - Osmolality: The kidney conserves water by first diluting urine as it moves through the loop of Henle and then concentrating urine in the distal tubules and collecting duct under the influence of antidiuretic hormone or (ADH).

- Ability to respond to hormones
  - Electrolytes: reabsorption or secretion of electrolytes, specifically Na+, K+, H+. Depends on concentration of electrolytes in plasma under hormonal control (e.g. Aldosterone)

- Ability to reabsorb solutes, electrolytes, proteins
  - Urine glucose
  - Urine amino acids

Common terminology in kidney diseases

- AKI — Acute Kidney Injury (within 3 months)
- CKD — Chronic Kidney Disease (> 3 months)
- ESRF/D — End stage renal failure/disease
- HD — Haemodialysis
- MDRD — Modification of Diet in Renal Disease
- CKD-EPI — Chronic kidney Disease-Epidemiology Collaboration

Acute Kidney Injury (AKI)

- Problems affecting the kidneys and/or their circulation
- It usually presents as a sudden degradation of renal function indicated by rapidly rising serum urea and creatinine concentration.
- Usually, urine output falls to less than 400 ml/24h (The patients may pass No urine at all)

Classification of Acute Kidney Injury

- Acute kidney injury/Failure can be classified as

- **Pre-renal:** the kidney fails to receive a proper blood supply

- Decrease in the glomerular filtration rate (GFR)

- Both kidneys need to be affected as one kidney is enough for normal kidney function

- **Post-renal:** the urinary emptying of the kidneys is impaired because of an obstruction (stones)

- **Renal:** intrinsic damage to the kidney tissue (kidney's structure). This may be due to a set of disease, or prolonged pre-renal or post-renal problems

Acute kidney injury (AKI)

- High plasma urea and creatinine concentrations occur along with fluid retention
- Anuria or oliguria
- Hyperkalaemia
- Hyperphosphataemia
- Metabolic acidosis.

End-stage chronic kidney disease (CKD5)

- Irreversible renal disease
- Raised plasma urea and creatinine concentrations occur initially
- Further hyperkalaemia
- Hyperphosphataemia
- Metabolic acidosis
- Hypocalcaemia
- Anaemia
- This may necessitate renal support such as dialysis

Case 1

A 17-year-old man was involved in a road traffic accident. Both femurs were fractured and his spleen was ruptured. Two days after surgery and transfusion of 16 units of blood, the following results were found:

Plasma

- Sodium 136 mmol/L (135–145)
- Potassium 6.1 mmol/L (3.5–5.0)
- Urea 20.9 mmol/L (2.5–7.0)
- Creatinine 190 µmol/L (70–110)
- Albumin-adjusted calcium 2.40 mmol/L (2.15–2.55)
- Phosphate 2.8 mmol/L (0.80–1.35)
- Bicarbonate 17 mmol/L (24–32)
- The patient was producing only 10 mL of urine per hour and a spot urinary sodium was 8 mmol/L.

What is the main biochemical abnormality?

Case 1

The results are compatible with pre-renal acute kidney injury (AKI).

Note the **oliguria, low urinary sodium concentration, hyperkalaemia, hyperphosphataemia and also low plasma bicarbonate concentration, suggestive of a metabolic acidosis.**

lecture 5: Liver Function Tests

5th Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences
Department 2020-2021

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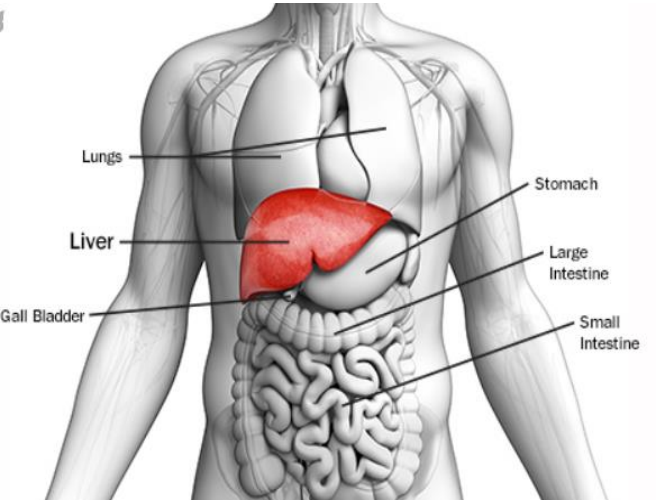


Learning outcomes

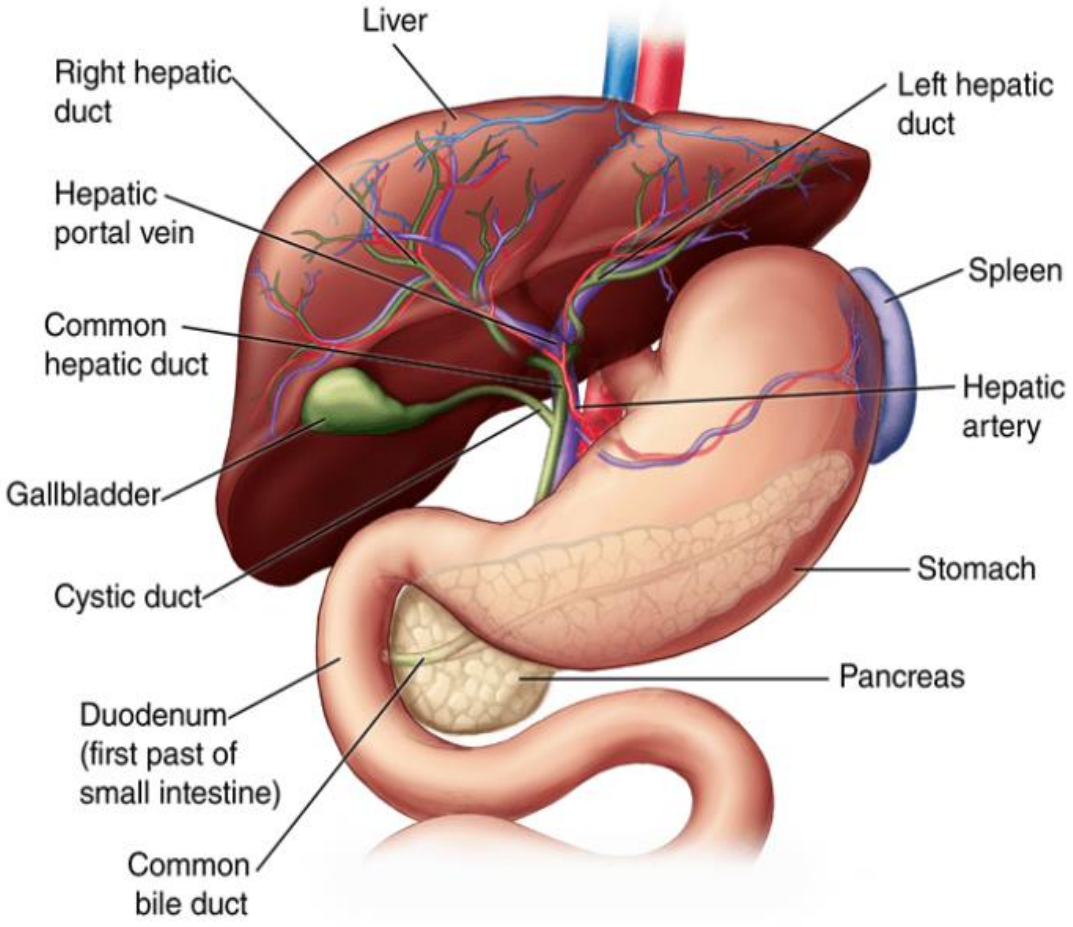
- Overview of Liver
- Functions of the liver
- Tests of liver function
- Acute liver disease
- Chronic liver disease
- Inherited liver disease
- Other liver problems

Liver structure

- Largest internal organ in human body
- Located in the right upper quadrant of the abdominal cavity below the diaphragm
- A reddish brown organ, divided into 4 lobes
- Contains an extensive Reticuloendothelial system for the synthesis and breakdown of blood cells

Liver structure

- Weighs 1.44 - 1.66 kg
- Connected to hepatic artery and portal vein
- Capable of regeneration
- Many functions
- Site of many diseases



Functions of the Liver

- Metabolizes, or breaks down, nutrients from food to produce energy, when needed
- Removes potentially toxic by products of certain medications
- Produces most proteins needed by the body
- Produces bile, a compound needed to digest fat and to absorb vitamin A, D, E and K
- Synthesis of cholesterol, phospholipids and triglycerides
- Produces most of the substances that regulate blood clotting
- Helps the body fight infection by removing bacteria from the blood
- Prevents shortages of nutrients by storing vitamins, minerals and sugar

Liver Function Tests (LFTs)

- Clinical biochemistry laboratory blood tests
- Provide information about the state of a patient's liver
- Can detect presence and follow progress of liver disease
- A request for LFTs will provide results for enzymes, bilirubin and proteins
- Can assist in differentiating biliary tract obstruction, acute, and chronic disease

Liver enzymes

Aminotransferases (AST & ALT)

- Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), Present in cells and leak into the blood when cells are damage due to inflammation, virus infection and cell death
- Some medications (drug overdose) may elevate serum ALT and AST because some drugs cause liver cell damage
- AST is synthesized by liver cells, cardiac muscle and skeletal muscles
- AST is less sensitive biomarker than ALT for liver damage
- Myocardial infarction (heart attack) and muscle damage lead to increase AST level in serum

Alkaline phosphatase (ALP)

- Liver is not sole source of ALP activity
- Present also in bile duct, bone, small intestine, placenta and kidney
- Normal physiological elevation during growing children and during pregnancy (3rd trimester of pregnancy)
- **Increases in**
  - Cholestasis (flow of bile from liver is reduced or blocked)
  - Inflammation
  - Cirrhosis
  - Alcoholic hepatitis

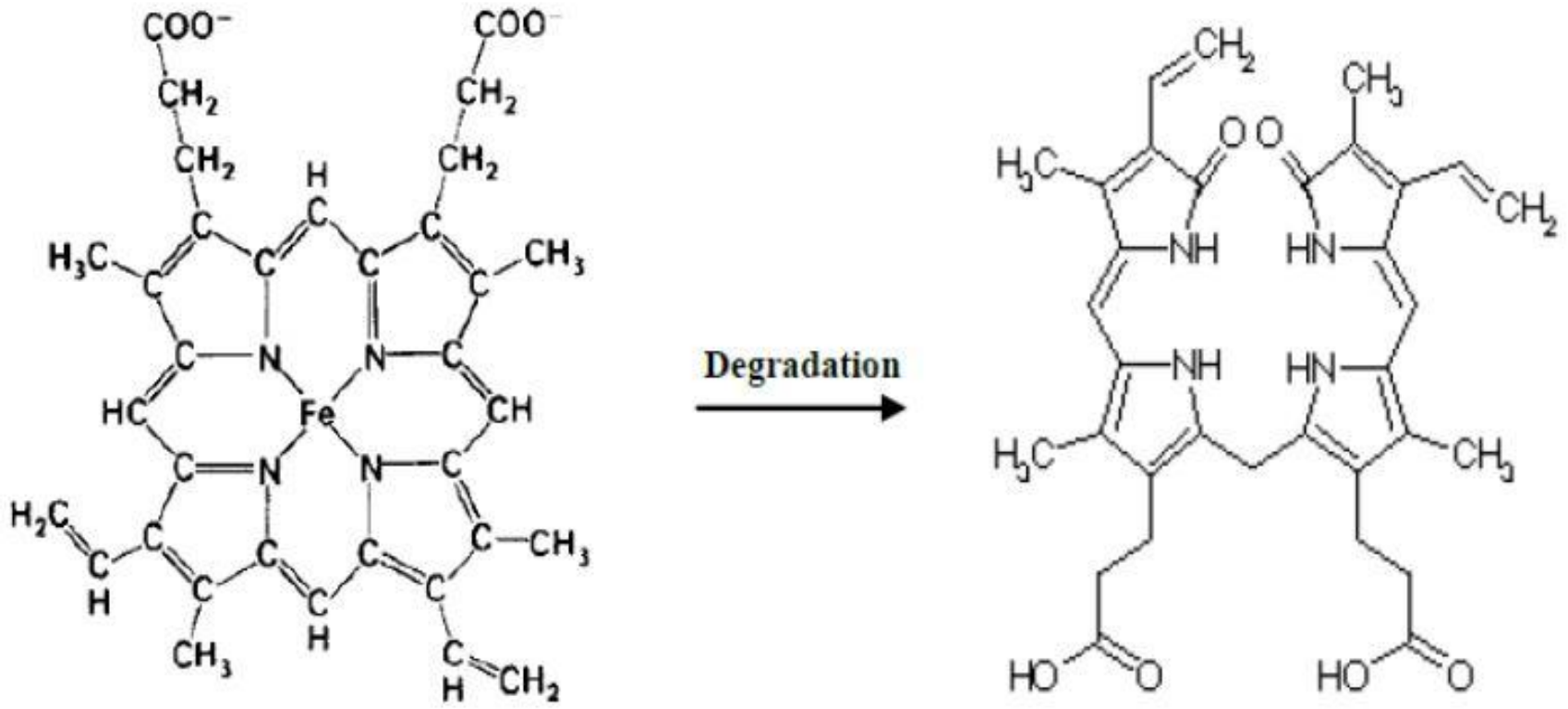
Gamma glutamyl transferase (γGT or GGT)

- Produced by liver, kidney and pancreas
- It is used to confirm hepatic etiology of ALP evaluation
- Increases in cholestasis
- Alcohol and drugs such as phenytoin (anti-epileptic) induce enzyme activity

- Eight hours fasting is recommended because GGT drops after eating

Bilirubin

- Bile pigment
- Bilirubin produced from haem degradation, mainly found in haemoglobin
- Insoluble in water and is transported in the blood bound to albumin
- Taken up by liver cells and conjugated (more water soluble)
- Conjugated bilirubin excreted into bile to help in food digestion



Heam
(Fe-protoporphyrin IX)

Bilirubin
(lipophilic)

Type of bilirubin in serum

- **Direct bilirubin** is **conjugated** or water soluble bilirubin (rapidly reaction)
- **Indirect bilirubin** is **unconjugated** or water insoluble bilirubin (slowly reaction)
- Both conjugated and unconjugated bilirubin are measured given **total bilirubin**
- Unconjugated bilirubin calculated by subtracting Direct from total so called indirect
- Knowing the level of each type of bilirubin has diagnostic important

Jaundice

- Jaundice due to increased levels of bilirubin in bloodstream
- Yellow colour of skin or sclera
- Detectable when bilirubin concentration is > 40 μmol/L

Physiologic jaundice of the newborn (Neonatal Jaundice):

- High bilirubin levels are common in newborns (1-3 days old).
- It is happened because after birth the newborns breaking down the excess RBCs they are born with it and because the newborn's liver is not fully mature, it is unable to process the extra bilirubin, leads to elevate its level in blood and other body tissues.
- This situation usually resolves itself within a few days.
- Usually newborn is treated by **phototherapy** which breakdown bilirubin

Three main reasons for Jaundice

1- Haemolysis

- Increased bilirubin production caused by haemolysis gives a predominantly unconjugated hyperbilirubinemia
- Commonly happen in babies
- Bilirubin is neurotoxic (high levels in babies can result in brain damage)
- A rapidly rising bilirubin in a neonate should be carefully monitored
- Phototherapy used to breakdown bilirubin if > 200 µmol/L

Three main reasons for Jaundice

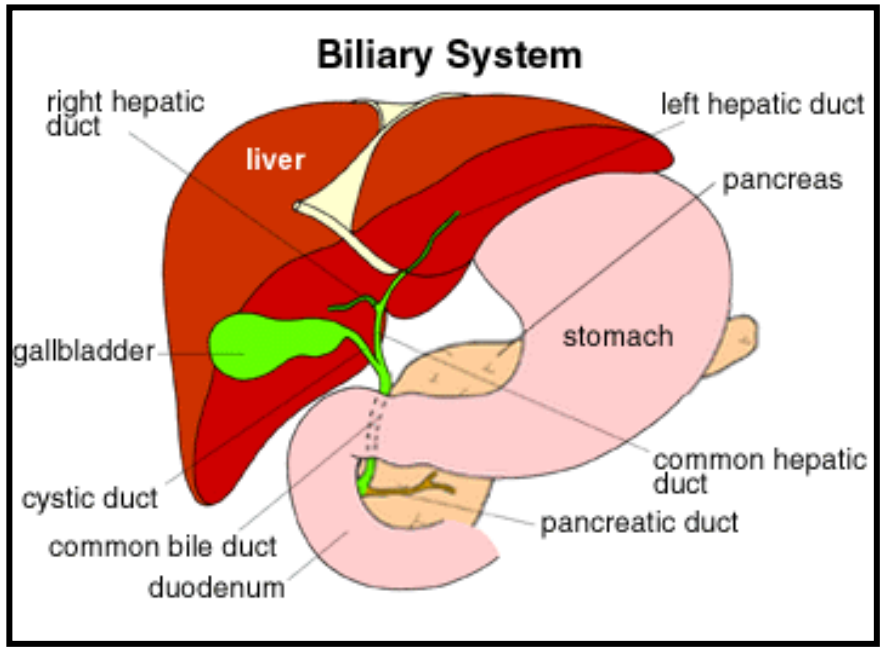
2- Failed conjugation

- Result of hepatocellular damage due to cancer , cirrhosis or hepatitis
- Bilirubin that is conjugated is not efficiently secreted into bile but leaks to blood
- Most common causes of acute jaundice, as a result of hepatocellular damage, seen in adults are:
  - Viral hepatitis
  - Paracetamol poisoning
- Conformational test : AST and ALT are elevated indicating hepatocellular damage

Three main reasons for Jaundice

3. Biliary obstruction (cholestasis)

- Gallstones can partially or fully block the bile duct which prevents passage of bilirubin into intestine
- Direct bilirubin will back to liver and then to circulation elevating its level in blood and urine
- If blockage complete, bilirubin and ALP are raised
- If blockage partial, bilirubin may well be within the reference range



Proteins

- **Albumin** is the major protein product of the liver
- Long biological half-life (20 days)
- Significant falls slow to occur
- Low albumin feature of advanced chronic liver disease
- It is also decreased in nephrotic syndrome
- The consequence of low albumin can be edema

- **Alpha-fetoprotein** (AFP) synthesised by fetal liver
- Low levels in normal adults
- high in liver cancer
- Marker for germ-cell tumour (growth from reproductive cells such as testicular cancer or ovarian cancer)

Acute Liver Disease

- Causes: poisoning (Paracetamol poisoning), infection, inadequate perfusion
- Can progress in three ways:
  - May resolve (majority of cases)
  - May progress to acute hepatic failure
  - May lead to chronic hepatic damage

Paracetamol poisoning

- While paracetamol is safe in normal doses, it is hepatotoxic and potentially fatal in overdose
- Hepatocyte destruction begins after 24h and can progress to acute liver failure
- liver damage is maximal 3-4 days after ingestion
- May also develop encephalopathy, hypoglycemia, renal failure
- Acetylcysteine is a safe and effective antidote to prevent serious hepatic injury after paracetamol overdose if used correctly

Infection

- Both bacteria and viruses can give rise to infective hepatitis
- Hepatitis is inflammation of liver
- Hepatitis A, B and C are most common
- A transmitted by eating and drinking in places with poor hygiene
- B passed on through body fluids
- C transmitted through contact with infected blood
- Elevated aminotransferase activities

Inadequate perfusion

- Poor flow of fluids into liver
- Hypovolemic shock
  - Occurs when the volume of the circulatory system is too depleted to allow adequate circulation to the tissue in the body
- A healthy adult can withstand loss of 0.5 L from a circulation of about 5 L
- Causes include:
  - Loss of blood (e.g. trauma)
  - Sepsis

Acute Liver Failure

- Metabolic functions of liver cannot be compensated for by any other organ
- May give rise to renal failure (kidney exposed to toxins)
- Decreased albumin synthesis leads to hypoalbuminemia and oedema +/or ascites (abnormal buildup of fluid in the abdomen)
  - Without enough albumin, the body can't keep fluid from leaking out of the blood vessels.
- Increased risk of hemorrhage as clotting factors not synthesized
- Recovery may take many weeks

Chronic Liver Disease

Three causes for Chronic Liver Disease

- Chronic alcohol ingestion
- Active hepatitis (B)
- Autoimmune disease

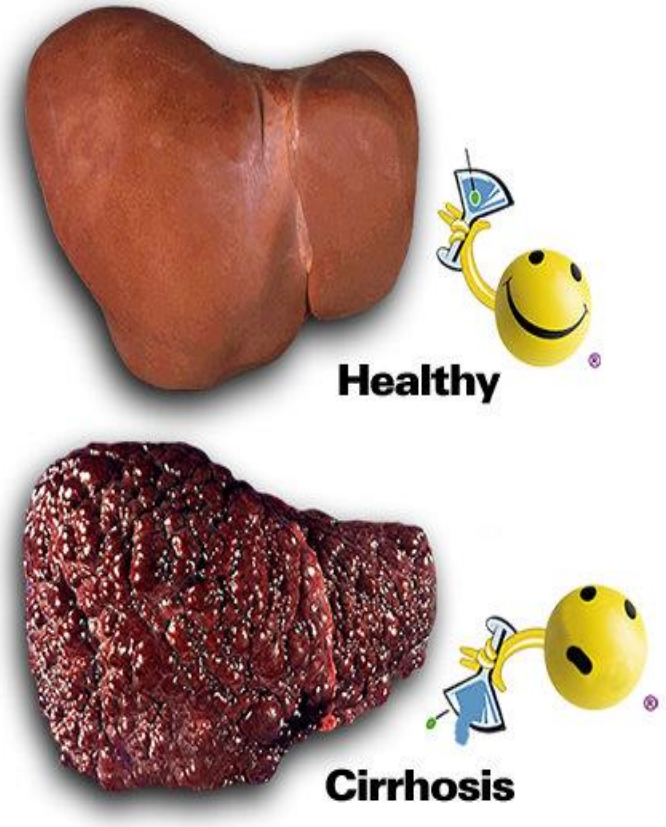
- Immune system makes antibodies against parts of the body
- e.g. primary biliary cirrhosis
- Slow, progressive destruction of bile duct
- All of these conditions progress to cirrhosis
- Low albumin is a feature of advanced chronic liver disease

Alcohol

- Absorbed into bloodstream from stomach and intestines
- Passes through liver before circulating around body
- Highest concentration is in blood flowing through liver
- Cells can process only a certain amount per hour
- Can lead to fatty liver, hepatitis, cirrhosis, all can occur at same time
- May result in liver failure

Cirrhosis

- Characterized by degeneration of cells, inflammation, and liver fibrosis.
- It is not reversible
- Fibrosis is the formation of scar tissue (forms when normal tissue is destroyed by disease, injury, or surgery)
- It is typically a result of alcoholism or hepatitis.
- Progress slow and often no early symptoms
- Jaundice, ascites, bleeding in terminal stages

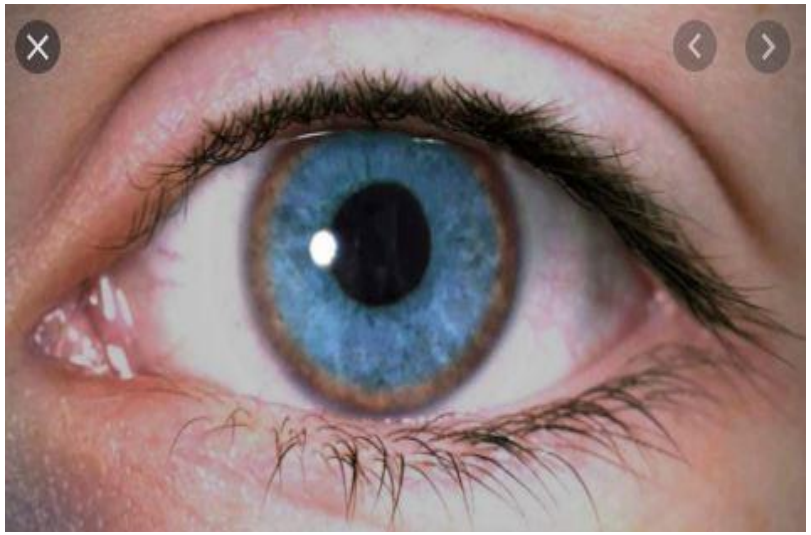


Haemochromatosis

- Most common genetic liver disorder
- Associated with iron accumulation in liver cells (Disorder of iron absorption) . (also heart, joints and pancreas)
- Leads to liver fibrosis and risk of hepatocellular cancer
- It is caused by a mutation in HFE gene (important in regulating iron metabolism in the body)
- Clinical features include diabetes, cardiac failure, joint
- Venesection: half a liter of whole blood removed weekly until excess iron removed.
- Monitoring for hepatocellular cancer

Wilson’s disease

- A rare inherited disorder of copper metabolism
- Copper accumulates in liver and brain and other vital organs
- Often results in hepatic and neurological damage
- Usually presents in children and young adults
- Kayser-Fleischer rings may be observed in eyes (Brownish pigment)
- Due to copper build up in cornea
- Serum copper (low), 24-hour urinary copper (high)
- Neurological disease permanent (tremor)



Other Liver Problems

- Common site of secondary metastases from primary tumours
- Jaundice may be the first indication of the presence of cancer
- Primary hepatocellular carcinoma is a malignant tumour of the liver
- Primary hepatoma is associated with hepatitis and cirrhosis
- Often produce alpha-fetoprotein (AFP) - useful biochemical marker of primary hepatic tumours
  - Elevated at diagnosis in patients with primary liver carcinomas
  - Elevated (little) in hepatitis and cirrhosis

Liver cases (case 1)

A 49-year old woman attended her GP with an 8 day history of anorexia, nausea & flu-like symptoms. Her urine was dark in colour. Physical examination revealed tenderness in upper right quadrant of abdomen. LFTs were as follows

Bilirubin	65 µmol/L	(3-22)
AST	936 IU/L	(12-48)
ALT	2700 IU/L	(3-55)
ALP	410 IU/L	(80-280)
γGT	312 IU/L	(<36)

Comments:

Marked increase in AST and ALT indicate acute hepatocellular damage. Also cholestasis (elevated ALP and γGT).

Most likely diagnosis is **acute viral hepatitis.**

Lecture 6: Tumour Markers

5th Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department 2020-2021

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Learning outcomes

- Know the ideal characteristics of a tumor marker
- Understand the role of tumor markers for diagnosis and management of patients with cancer.
- Understand the role of tumor markers for therapeutic selection

Cancer

- One death in five is caused by cancer
- The effect of tumor growth may be local or systemic
- The cancer cells may secrete toxin locally or in the general circulation
- If tumor growth is not countered by treatment the consequences may be obstruction of blood vessels, lymphatics or ducts, damage to nerves, effusions, bleeding, infection, necrosis of surrounding tissue and eventual death of the patients

Cancer

- Uncontrolled cell growth
- Invasion/destruction
- Metastasis

- Distinguish benign from malignant cancer

Tumour formation

Normal

- Cells reproduce exactly
- Stick together in correct place
- Self destruct if damaged
- Mature/specialise

Cancer

- Continual reproduction
- Fail to obey other cell signals
- Don’t stick together
- Remain immature

Tumour formation

- Uncontrolled reproduction
  - ➢ Loss/damage to genes
  - ➢ Failure of pre-programmed apoptosis
- Ignore cell signals
  - ➢ Genes switched on
- Lack normal adhesion
  - ➢ Loss of cell surface proteins

Cancer genes

- **Oncogenes**

➢ Promote cell replication eg ras

- **Tumour suppressor genes**

➢ Stop cell division – eg p53 and BRCA1

- **DNA repair genes**

➢ Ionising radiation, oxidative damage

Diagnosis of cancer

- Physical Examination
- Blood tests
- Computed tomography (CT scans)
- Biopsy (pathologist)

Diagnosis of cancer

- Diagnosis of carcinoma in the early stages is a difficult task due to lack of symptoms at early stages
- Diagnostic procedures (X-ray, computed tomography (CT), Magnetic resonance imaging (MRI),..) are not suitable for early diagnosis. Methods detect of at least 2cm in size or more

Tumour markers -history

- 30-ies of the 20th century – human chorionic gonadotrophin hCG (physiologically produced by placenta) discovered in young men with testicular tumours
- 70-ies of the 20th century - α1-fetoprotein (AFP) discovered in liver tumours in mice, later on described in human hepatomas

What is a Tumour Marker ?

- Substance related to the presence or progress of a tumour. It found in higher-than-normal level in the blood, urine, or tissues of some people with cancer. This substance can be produce by the tumor or by healthy cells in response to the tumor.
- **Secreted**
  - serum tumour markers (eg PSA, AFP, CEA, etc)
- **Tissue**
  - cell surface (eg leukaemia markers)
  - intracellular (eg steroid receptors)
  - gene expression (eg EGFR, tyrosine kinase)
- **Tumour derived**
  - from the tumour itself (eg philadelphia chromosome)

Ideal serum tumour marker

- Specific for site and a single type of cancer- absent in healthy, other diseases (non-tumours) and benign tumours
- 100% specific: always negative in individuals who do not have the disease
- Highly sensitive for cancerous growth- detectable at early stage of tumor
- 100% sensitive: always positive in patients with the tumour
- Correlation with the tumour mass and prognosis- Change rapidly with change in tumour size
- Short half-life in circulation

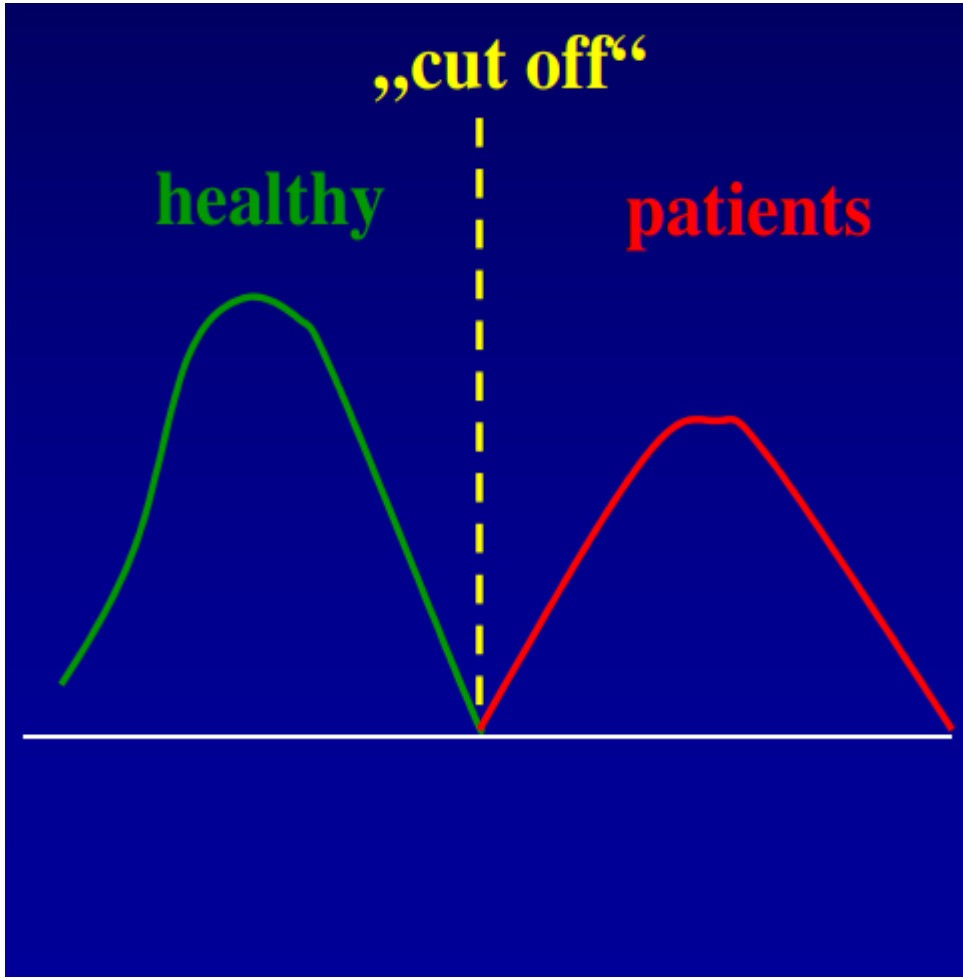
❖ **No marker fulfils these criteria...so far**

Serum Tumour Markers - Pitfalls

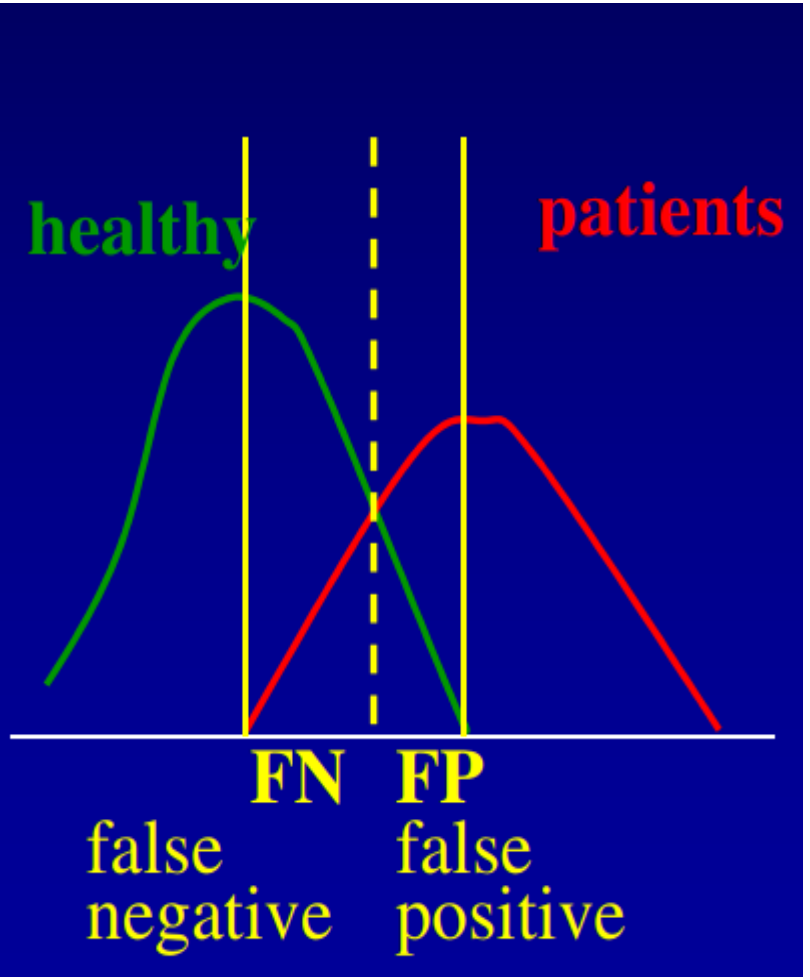
- None specific for malignancy
- Rarely elevated in early malignancy
- No marker absolutely organ specific
- Negative marker does not exclude malignancy

Evaluation of Tumour markers

Ideal situation



Reality



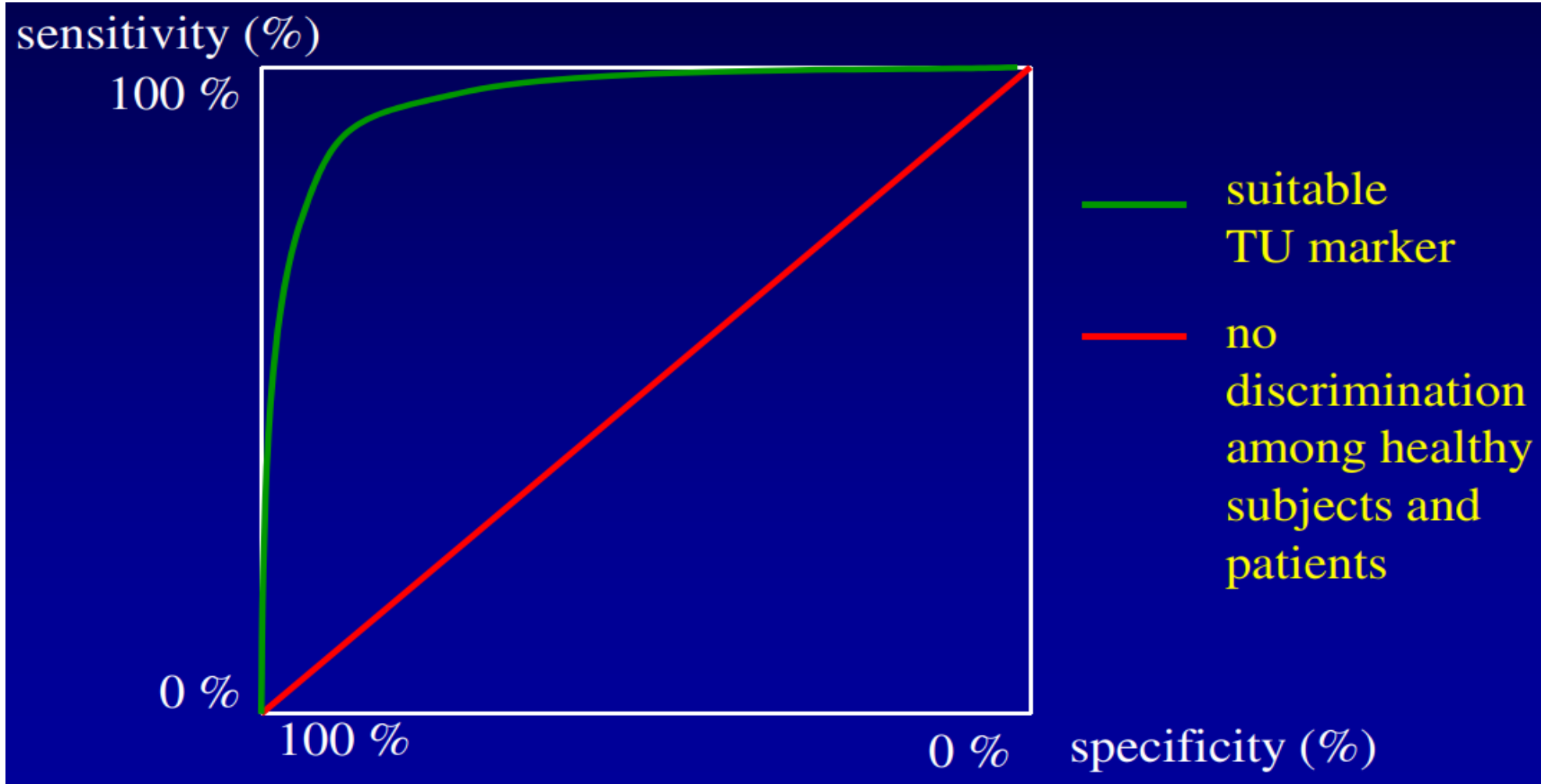
Evaluation of Tumour markers

- Specificity = TN / (TN + FP); (low false-positive tests)
  - probability that a negative test means a negative diagnosis true in healthy subjects
- Sensitivity = TP / (TP + FN); (low false-negative rate)
  - probability that a positive test means a positive diagnosis true in patients with tumours
- Positive predictive value = TP / (TP + FP)
- Negative predictive value = TN / (TN + FN)

- TP – number of true positive examinations
- TN – number of true negative examinations
- FP – number of false positive examinations
- FN – number of false negative examinations

Evaluation of Tumour markers using ROC curves (ROC = receiver operating characteristic)

- If a test gives positive result in 99 patients out of 100 patient (Its sensitivity is 99%)
- If a test gives negative result in 90 individuals out of 100 normal individual (Its specificity is 90%)



Evaluation of Tumour markers

Example

carcinoembryonic antigen (CEA) for colorectal cancer

- 95% specificity – i.e. 5% of healthy subjects are falsely regarded as patients with tumours
- 70% sensitivity – i.e. does not detect 30% of patients with tumours

To increase sensitivity and specificity

- Combination of multiple tumour markers
- Combination tumor marker with other procedure

➢ eg combination carbohydrate antigen CA125 with ultrasonography for early detection of ovarian malignancy

What are the limitations of tumor marker tests?

- Just because a person has tumor markers, it doesn't always mean cancer is present or has come back. Conditions besides cancer can raise tumor marker levels.
- Tumor markers can go up and down over time, making it hard to measure them consistently.
- The level of a tumor maker may not go up until after the cancer is advanced.
- Some cancers don't produce tumor markers
- Some people don't have higher tumor marker levels even if the type of cancer they have usually produces tumor markers.

Applications of Tumour Markers

- Screening
- Diagnosis
- Prognosis
- Residual disease
- Monitoring therapy
  - Individualised treatments
- Follow up

Screening

- **Objective**
  - population screening for malignancy before clinically suspect enabling therapy that will improve outcome
- **Limitation**
  - low predictive value of most tumour markers means that screening is very rarely cost effective
- **Reality**
  - at specific high risk sub-populations

- PSA in elderly men for early detection of prostate cancer
- AFP : patients with liver cirrhosis or chronic hepatitis who are at risk for development of hepatocellular carcinoma

Diagnosis

- **Objective**
  - to make the diagnosis in patients with symptoms, in conjunction with clinical findings / radiology
- **Limitation**
  - poor specificity / sensitivity of most markers
  - negative result does not exclude tumour
- **Reality**
  - few good markers (HCG, calcitonin)

Prognosis

- Should correlate with tumor mass(to assess the aggressiveness)
- Based on aggressiveness of tumor which in turn determines how a patient should be treated, surgery, chemotherapy, radiotherapy.
- High levels of serum tumor markers during diagnosis would indicate the presence of malignant or metastatic tumor associated with poor prognosis

Prognosis

- **Objective**
  - predict outcome of treatment
- **Limitation**
  - poor correlation between size / activity of tumour and marker
- **Reality**
  - few markers have prognostic value AFP, HCG, LDH

Monitoring Response

- Highly sensitive tumor marker test to detect recurrence at early at possible
- The appearance of most of the circulating tumor markers have time of several month (3-6 months) prior to the stage at which many the physical procedures can be used for detection of the cancer
- Therefore, increasing tumor marker levels may detect recurrence of disease before any clinical or radiological evidence of disease is apparent (biochemical recurrence)

Monitoring Response

- **Objective**
  - marker as indicator of size/activity
  - therapy linked to change in marker
- **Application**
  - main application of all markers
  - novel experimental immunotherapies
- **Response**
  - Response = <10% pretreatment
  - Improvement = <50% pretreatment
  - Complete response = return to normal

Follow Up

- **Objective**
  - marker predicts frequency of testing
  - rising marker triggers appropriate intervention
- **Interpretation**
  - reference ranges are of very limited value
  - individuals must be assessed against their own baseline
- **Application**

all markers, frequently for life

Role of Serum Tumour Markers

- Determine risk: screening of specific high risk sub-populations for early cancer (Ovarian Cancer, calcitonin, Hepatocellular Cancer, and prostate cancer)
- Diagnose a type of cancer (hCG, Germ cell tumours)
- Estimate prognosis (Ovarian cancer, Germ cell tumours)
- Monitor for disease progression or recurrence (most widely used function)
- Therapeutic selection (her2/neu, kras)

How tumor marker testing can guide cancer care?

- For instance, early-stage breast cancer has specific tumor markers known as estrogen receptor (ER) and progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). If the tests are positive, the patient is more likely to be treated successfully with hormone therapy.

Tumour markers – clinical-chemical division

- Enzymes – ALP, PSA, LDH
- Oncofetal antigens (AFP)
- Immunoglobulins – IgG, IgM, IgA, β2-microglobulin
- Hormones – growth hormon, calcitonin, ACTH, TG, PRL, PTH, hCG
- Polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS),
- Glycoproteins, glycolipids and saccharides – AFP, hCG, CEA, squamous cell carcinoma antigen (SCC), CA 19-9, CA 125, CA 15-3, CA 549, CA 72-4
- Receptors – estrogen and progesterone receptors,

Clinical Tumour Markers

Tumour	Tumour Markers
Liver cancer	▪ AFP
Prostate cancer	▪ PSA ▪ PSMA
Lung cancer	▪ CEA ▪ CA125
Testicular cancer	▪ AFP ▪ hCG
Ovarian cancer	▪ CA 125
Breast cancer	▪ CA 125 ▪ CA 15-3 ▪ CEA ▪ Estrogen and progesterone receptors ▪ her2/neu
Stomach cancer	▪ CEA
Pancrease cancer	▪ CEA ▪ CA125
Colon cancer	▪ CEA

Prostate specific antigen Measurement

- Two types of PSA:
  - 55-95% PSA complexed with antichymotrypsin (PSA-ACT)
  - 5-45% free PSA (fPSA)
- Total PSA = fPSA + PSA-ACT
- Total PSA ranges: 2.5 - 4 ng/ml (Normal)
- Elevated in Prostate cancer and Benign prostatic hyperplasia (BPH)
- Elevated for at least 6-8 weeks after prostate biopsy
- Highly sensitive marker for prostate cancer but less specific
- In assessment of response to therapy and follow-up of patients with prostate cancer
- Blood level PSA below 4 ng/mL = prostate cancer is unlikely
- An increasing from one year to next mean prostate cancer is more likely
- PSA >10 ng/mL = prostate cancer is likely

Prostate Specific Membrane Antigen (PSMA)

- PSMA blood levels increase with age and prostate cancer
- Very sensitive marker
- So far, it has not proven to be better than PSA