Carbohydrates metabolism & disorders

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Objectives

- After attending a series of lectures on carbohydrate metabolism, the students will:

- Identify/Summarize and explain rule of hormones in glucose metabolism. (TL1)
- Identify/Classify the types of DM. (TL1)
- Evaluate the laboratory test available for DM investigation. (TL1)
- For each test discussed in this lecture, determine the source of error and solve the problem by finding the suitable solution. (TL2)
- Interpret the biochemical data used in the investigation and diagnosis of diabetes, when given problem based case studies. (TL3)

Carbohydrates function

- Major energy source for sustainment of life (storage & generation)
- Cell wall of bacteria & aid in molecular recognition and communication.
  - Such as blood group sugars on cell surface
- Major disease is Diabetes Mellitus DM

Glucose Metabolism

- During a fast, the blood glucose level is kept constant by mobilizing the glycogen stores in the liver.
- During long fasts, gluconeogenesis is required to maintain blood glucose levels because glycogen stores are used up in about 24-48 hours.
- An individual with a fasting blood glucose level >100 mg/dL is referred to as hyperglycemic.
- An individual with a fasting blood glucose level <50 mg/dL is referred to as hypoglycemic.

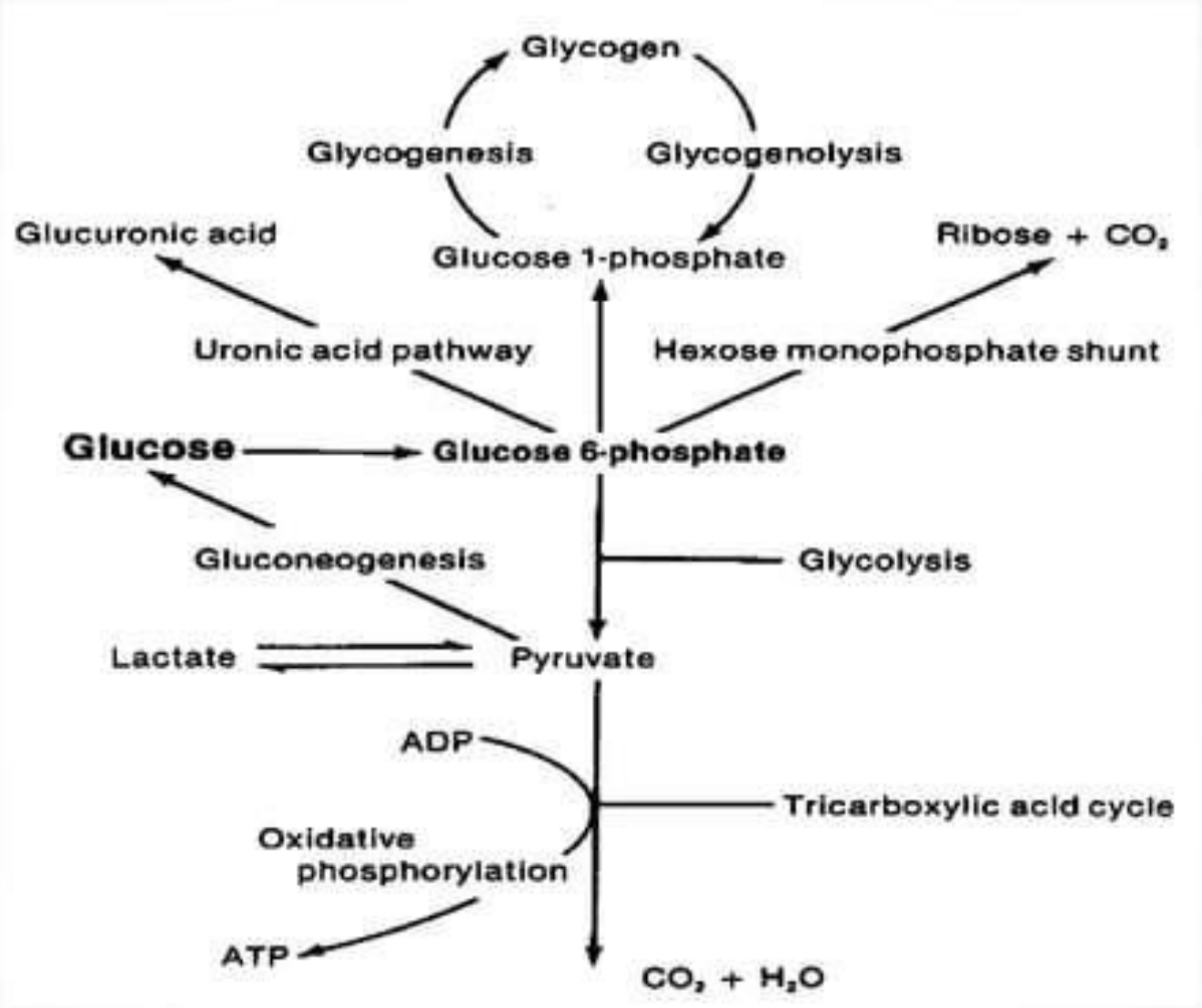
Carbohydrate Classification

Classified into three main groups:

- Monosaccharide's
  - Glucose
  - Fructose
  - galactose
- Disaccharides
  - Maltose
  - Lactose
  - Sucrose
- Polysaccharides
  - glycogen-starch

Major biochemical pathways associated with carbohydrates metabolism

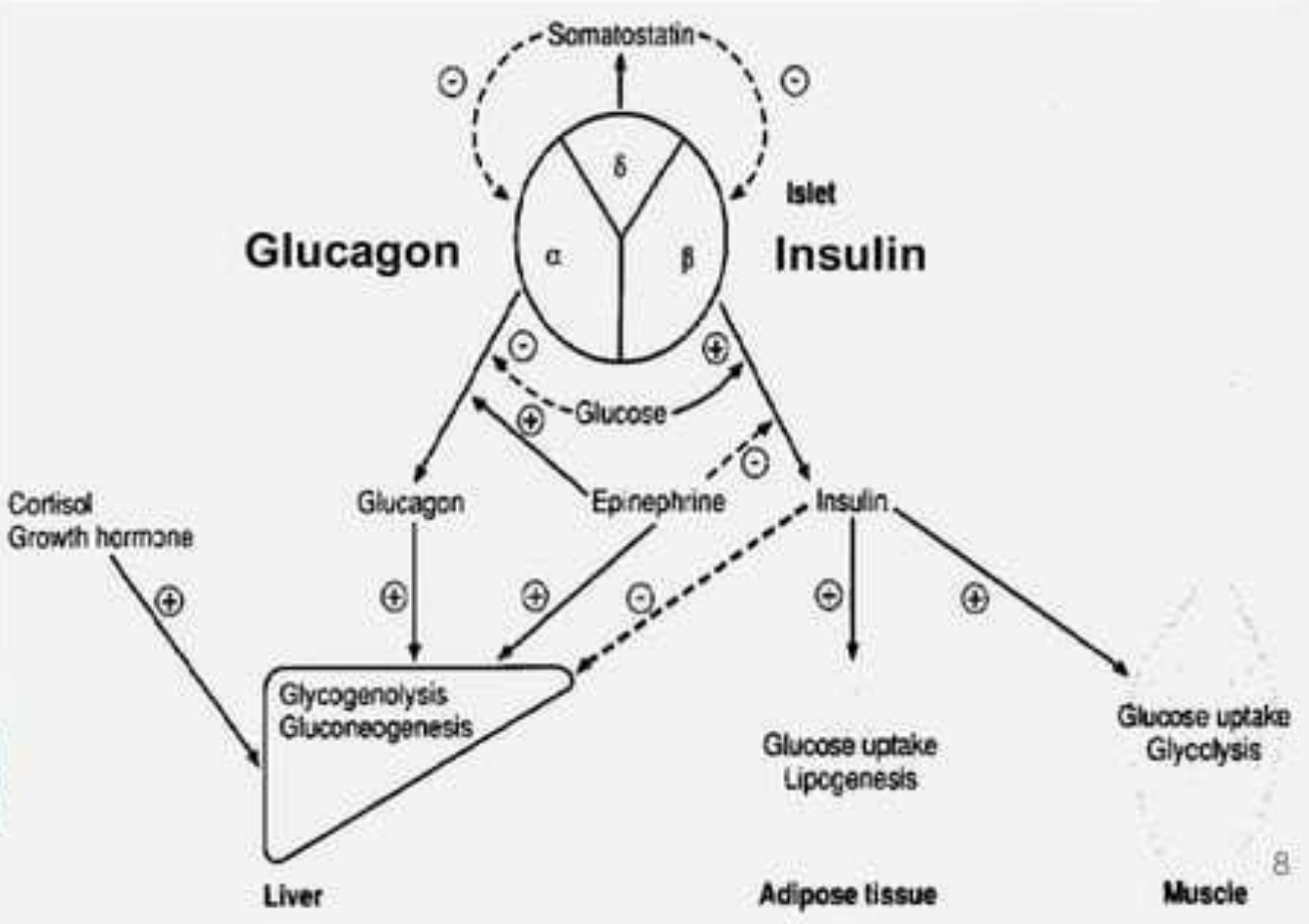
Glycolysis	Glucose ⟶ pyruvate ⟷ lactate
Glycogenesis	Glucose ⟶ glycogen
Tricarboxylic acid cycle	Glucose ⟶ pyruvate ⟶ $CO_2 + H_2O +$ ATP
Pentose Phosphate Pathways/hexose Monophosphate Shunt	Glucose ⟶ ribose + CO_2 + NADPH
Uronic Acid Pathway	Glucose ⟶ glucuronic acid
Glycogenolysis	Glycogen ⟶ glucose
Gluconeogenesis	Non-carbohydrate source, protein, fats ⟶ glucose


Glycogen
Glycogenesis
Glycogenolysis
Glucuronic acid
Glucose 1-phosphate
Ribose + CO_2
Uronic acid pathway
Hexose monophosphate shunt
Glucose
Glucose 6-phosphate
Gluconeogenesis
Glycolysis
Lactate
Pyruvate
ADP
Tricarboxylic acid cycle
Oxidative phosphorylation
ATP
$CO_2 + H_2O$

Hormonal control & major site of glucose regulation

Islets of Langerhans in the pancreas produce:

1. alpha cells secrets glucagon
2. beta cells secrets insulin
3. delta cells - somatostatin



Normal blood glucose

- Fasting blood glucose: 70-105 mg/dL
- May rise to 130-160 mg/dL about 1 hr after a glucose load or after high carbohydrate meal (postprandial).
- After 2 hr, it drops back to normal fasting range

Normal urine glucose/Renal Threshold for Glucose

- Glucose is eliminated from the blood by glomerular ultra-filtration GFR and efficiently reabsorbed by the renal tubules.
- Urine contains only a trace of glucose
- Typical renal threshold for glucose is 180 mg/dL.
- >180 mg/dL glucose will show in the urine (**glucosuria**).
  - **When blood glucose reaches this level or exceeds it, the renal tubular transport mechanism becomes saturated, which causes glucose to be excreted into the urine.**

Hormones Affecting Blood Glucose Levels

- Insulin
- Glucagon
- Epinephrine
- Growth hormone
- Adreno-corticotropic hormone ACTH
- cortisol
- Somatostatin
- T3 &T4

Insulin (hormone)

Dr Elham Sharif

- Secreted by pancreas B cells of islets of Langerhans.
- primary function is to decrease blood glucose & movement of glucose from blood into cells.

Actions:

- Facilitate the entry of glucose into hepatocyte, adipocytes,red cells and monocytes by making the cell membrane permeabile to glucose.

- Increases uptake of glucose by liver promotes glycogenesis and lipogenesis.

- inhibits hepatic output of glucose into circulation

- increases synthesis of protein in the liver, muscle and fat cells.

- decreases gluconeogenesis.

So insulin reduces plasma glucose

So insulin work to return the glucose level back to normal by lowering it and facilitates its entry into the cells.

Glucagon (hormone)

Secreted by α cells of islets, secretion is regulated by plasma glucose concentration.

Insulin inhibits glucagon release.

Primary function:

- increases blood glucose conc and FFA, mobilization of energy stores.

Actions:

- stimulates breakdown of liver glycogen into glucose (Glycogenolysis)
- increase liver gluconeogenesis (from non carb)
- promotes hepatic lipolysis.

Epinephrine (hormone)

- Secreted by adrenal medulla
- Primary function: increases blood glucose and mobilize energy stores.
- **Actions:**

  - Increases glycogenolysis
  - Stimulates glucagon secretion
  - Inhibits insulin secretion
  - Increase TG breakdown in adipose tissue (lipolysis).
  - Physical or emotional stress causes increased secretion of epinephrine and an immediate increase in blood glucose levels.

Growth Hormone

- Polypeptide secreted by the anterior pituitary
- Primary action: increase in blood glucose and mobilize energy stores.
- **Actions:**

  - increases gluconeogenesis
  - antagonizes insulin
  - inhibits lipogenesis from carbohydrates

Adreno-corticotropic hormone ACTH

- Secreted by anterior pituitary
- Primary function: increases blood glucose & and mobilize energy stores.
- **Actions:**

  - antagonizes insulin

Cortisol

- Secreted by adrenal cortex
- Primary function: increases blood glucose & and mobilize energy stores.
- **Actions:**
  - Promotes protein catabolism
  - Promotes deamination of amino acids
  - Promotes gluconeogenesis
  - Inhibits glucose metabolism in the peripheral tissues
  - antagonist of insulin.

Thyroid hormones T4 & T3

- Secreted by the thyroid gland
- Primary function: increases blood glucose & and mobilize energy stores.
- **Actions:**
  - stimulates glycogenolysis
  - increases intestinal absorption of glucose
  - accelerates the degradation of insulin.

Somatostatin

- Peptide hormone synthesized by delta by the δ-cells of the pancreatic islets of Langerhans.
- Inhibits both insulin, glucagon and growth hormone release , resulting in an increase in plasma glucose level

Hormones involved in glucose homoeostasis

Hormone	Principal actions		
insulin	increases	cellular glucose uptake	M, A
		glycogen synthesis	L, M
		protein synthesis	*L, M*
		fatty acid and triglyceride synthesis	*L, A*
	decreases	gluconeogenesis	L
		glycogenolysis	L, M
		ketogenesis	*L*
		lipolysis	*A*
		proteolysis	*M*
glucagon	increases	glycogenolysis	L
		gluconeogenesis	L
		ketogenesis	*L*
		lipolysis	*A*
adrenaline (epinephrine)	increases	glycogenolysis	L, M
		lipolysis	*A*
growth hormone	increases	glycogenolysis	L
		lipolysis	*A*
cortisol	increases	gluconeogenesis	L
		glycogen synthesis	L
		proteolysis	*M*
	decreases	tissue glucose utilization	L, M, A

L, liver; M, skeletal muscle; A, adipose tissue.

So

Glucagon

Epinephrine

Growth hormone

ACTH

Cortisol

T3 & T4

Increases blood glucose & and mobilizes energy stores.

So which hormone increase in Hypoglycaemia???

Abnormalities in Carbohydrates Metabolism

Lactose Intolerance

- Lactose or milk sugar found in the milk of mammals - 4-6% in cow's milk and 5-8% in human milk.
  - It is also a by product in the manufacture of cheese.
- Lactose intolerance is the inability to digest significant amounts of lactose, the predominant sugar of milk.
- This inability results from a shortage of the **enzyme lactase**, which is normally produced by the cells that line the small intestine.
- **Lactase** breaks down the lactose, milk sugar, into glucose and galactose that can then be absorbed into the bloodstream.

Lactose Intolerance cont'd

- **Common symptoms (sever to mild) include:**
  - abdominal pain, abdominal bloating, gas, diarrhea, nausea
  - uncomfortable 30 minutes to 2 hours after consuming milk and milk products
  - Symptoms range from mild to severe, based on the amount of lactose consumed and the amount a person can tolerate.
- **Two tests are commonly used to measure the digestion of lactose:**
- **Hydrogen Breath Test.**
  - The patient drinks a lactose-loaded beverage and then the breath is analyzed at regular intervals to measure the amount of hydrogen.
  - Normally, very little hydrogen is detectable in the breath, but undigested lactose produces high levels of hydrogen.
  - Smoking and some foods and medications may affect the accuracy of the results.
- **Stool Acidity Test.**
  - Used for infants and young children to measure the amount of acid in the stool.
  - Undigested lactose creates lactic acid and other fatty acids that can be detected in a stool sample.
  - Glucose may also be present in the stool as a result of undigested lactose.

Hypoglycemia

Hypoglycemia defined as: fasting blood glucose < 70 mg/dL, the body respond by secreting glucagon and epinephrine

Causes of hypoglycemia

- starvation
- exaggerated insulin release
- hyperinsulinemia, β-cell tumor
- Over administration of insulin or oral hypoglycemic agents
- IBEM --- enzyme deficiency
- drug effects, ethanol inhibits gluconeogenesis
- Sever hepatic dysfunction

Fasting blood levels < 50 mg/dL uncommon.

Rapid drop in blood glucose (< 50 -55 mg/dL) will cause release of epinephrine leading to:

in early stage: anxiety, dizziness, chills, tachycardia and increase perspiration.

Blood levels < 20 mg/dL (later stages) lead to:

impaired nerve function & nerve damage, slurred speech, loss of motor coordination, coma, lethargy, confusion, seizures

Hypoglycaemia

Causes	Clinical features
Reactive hypoglycaemia	***Acute***
post-prandial:	due to neuroglycopenia:
gastric surgery	tiredness
essential (idiopathic)	confusion
drug-induced:	detachment, lack of concentration
insulin	ataxia
sulphonylureas	dizziness
alcohol	paraesthesiae
others	hemiparesis
inherited metabolic disorders:	convulsions
galactosaemia	coma
hereditary fructose intolerance	due to sympathetic stimulation:
	palpitation and tachycardia
Fasting hypoglycaemia	profuse sweating
hepatic and renal disease (uncommon)	facial flushing
endocrine disease:	tremor
adrenal failure	anxiety
pituitary failure	non-specific:
isolated ACTH or GH deficiency	hunger
inherited metabolic disorders:	weakness
glycogen storage disease type I	blurred vision
hyperinsulinism:	
insulinoma	***Chronic neuroglycopenia***
'nesidioblastosis'	personality changes
non-pancreatic neoplasms	memory loss
alcohol-induced fasting hypoglycaemia	psychosis
various forms of neonatal hypoglycaemia	dementia
septicaemia	

Hyperglycemia

- **Remember: normal fasting blood glucose 70 to 105 mg/dL**
- **Hyperglycemia** - fasting blood glucose > 105 mg/dL.
- Diabetes mellitus - a disorder in glucose metabolism producing hyperglycemia.
- **Remember**: hypoglycemia - fasting blood glucose < 70 mg/dL

Causes of Diabetes Miletus

- Decrease or absence of insulin secretion
- Insulin resistance
- Abnormality in the control of insulin secretion
- Insulin action defect on the target cell

General Symptoms of Diabetes Miletus

- ↑3Ps (polyuria, polydipsia, polyphagia)
- Weight loss
- ↑in bacterial and yeast infection
- Fatigue
- Blurred vision
- Impotence in men
- Frequent vaginal infections & cessation of menstruation.

Diabetes Mellitus

- Type 1, IDDM, Juvenile-onset
- Type 2, NIDDM, Adult-onset
- Gestational DM (GDM)

Classification of Diabetes Mellitus

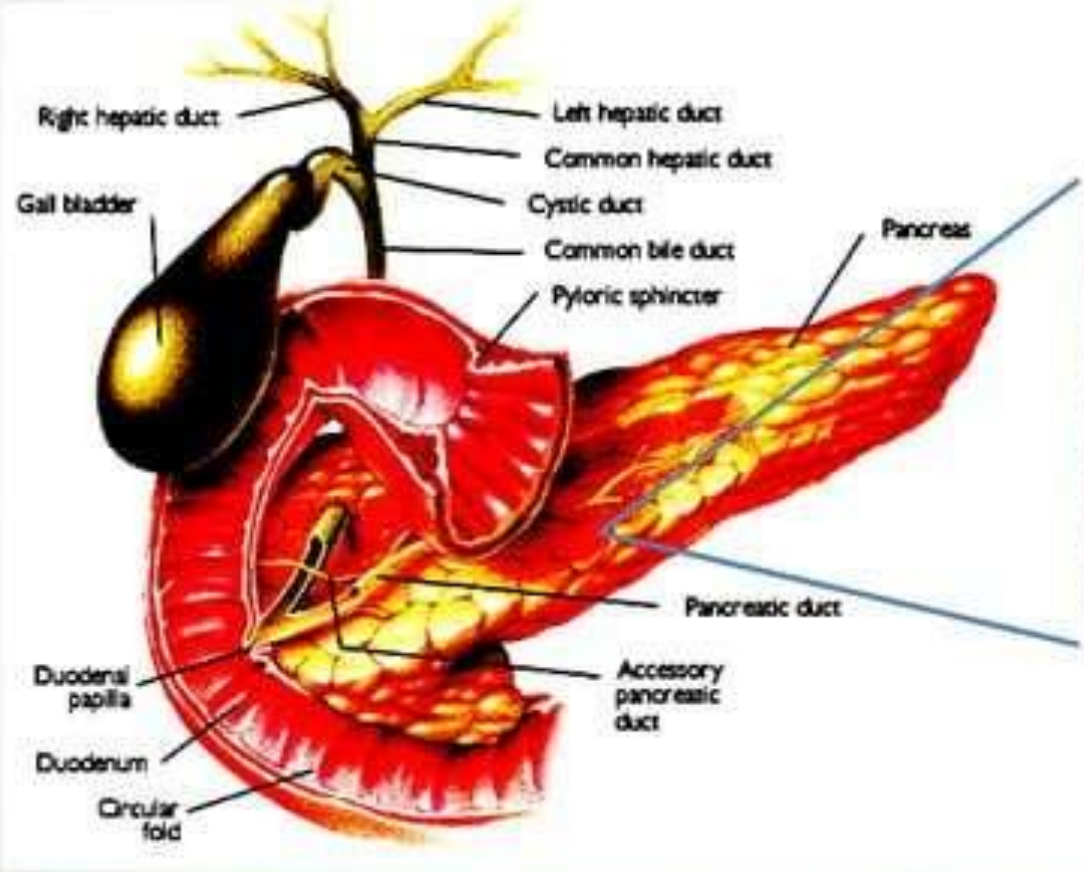
Type 1 (IDDM) Juvenile Diabetes

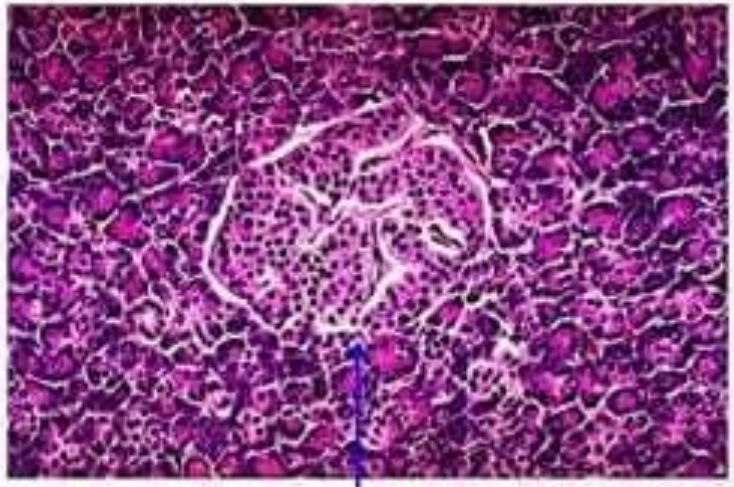
- 10% of all cases with DM
- Frequent in childhood, but often occurs at an early age, hence "juvenile diabetes"
- insulin deficiency due to β-cells destruction.
- Need insulin for survival
- 85-90% of pts are detected with autoantibodies (ICAs, IAAs, GAD, TPA).
- Correlation between viral infection (mumps & measles & type 1 DM.
- Also correlation between HLA e.g. B8, BW15, DR3 ,DR4 and IDDM.
- See table 1-5 in the book for auto-antibodies

Type 2 (NIDDM)

- 90 %
- Occur at old age
- Insulin resistance, with normal to elevated insulin
- Increase with age & 80% obesity
- Women diagnosed with gestational DM is more predisposed to type 2 DM.
- Family history
- ↑ hypertension & dyslipidemia.
- Treated with drug, diet, insulin

Pathophysiology of Type1DM





antibodies attack islets!

Symptoms

Type 1 -IDDM

- hyperglycemia
- ketoacidosis
- polyuria
- polydipsia
- rapid weight loss
- hyperosmotic blood
- urinary glucose positive
- urinary ketones positive
- hyperventilation
- "acetone breath"
- coma
- **Treatment**: insulin injection, pancreas transplant

Type 2-NIDDM

- hyperglycemia
- no keto-acidosis
- obesity
- **Treatment**: dietary control, ora hypoglycemic drugs, insulin injection

Complications

- nephropathy
- neuropathy
- retinopathy
- atherosclerosis
- peripheral vascular disease

Type 1 versus Type 2 Diabetes

Features	Type 1 DM (< 10%)	Type 2 DM (> 90%)
Age of onset	Children, young adults	> 40
Type of onset	Acute	insidious
DKA	Yes	No
Ketone bodies	Yes	No
Weight	Usually lean	80% overweight
Serum insulin concentration	Low or absent	Normal to high
Cause	Autoimmune or unknown	No autoimmune markers
Family history of DM	Uncommon	common

Major characteristics of type 1 and type 2 diabetes mellitus

Feature	Type 1 DM	Type 2 DM
typical age of onset	children, young adults	middle-aged, elderly
onset	acute	gradual
habitus	lean	often obese
weight loss	usual	infrequent
ketosis-prone	usually	usually not
plasma insulin concentration	low or absent	often normal; may be ↑
family history of diabetes	less common	common
HLA association	DR3, DR4; DQ2, DQ8	none

Diabetes Mellitus caused by secondary factors

- **Damage to the pancreas**
- **High hormone levels**
  - cortisol
  - growth hormone
  - thyroxine
  - insulin receptor deficiency

- **Impaired Glucose Tolerance (IGT)**
  - normal fasting glucose levels
  - greater than normal glucose levels after a meal or oral glucose.
  - high risk for development of diabetes mellitus

- **Delayed and Exaggerated Response**
  - impaired glucose tolerance followed by hyperinsulinemia and hypoglycemia

Type 1 diabetes mellitus
Beta cell destruction, usually leading to absolute insulin deficiency
- Immune mediated
- Idiopathic

Type 2 diabetes mellitus
May range from predominant insulin resistance with relative insulin deficiency to predominant secretory defect with insulin resistance

Gestational diabetes mellitus
First onset or recognition of glucose intolerance during pregnancy

Other specific types

Genetic defects of beta cell function
- Chromosome 20, HNF-4alpha (formerly MODY1)
- Chromosome 7, glucokinase (formerly MODY2)
- Chromosome 12, HNF-1alpha (formerly MODY3)
- Mitochondrial DNA
- Others

Genetic defects in insulin action
- Alstrom syndrome
- Leprechaunism
- Lipoatrophic diabetes
- Rabson-Mendenhall syndrome
- Type A insulin resistance
- Others

Diseases of the pancreas
- Cystic fibrosis
- Fibrocalculous pancreatopathy
- Hemochromatosis
- Neoplasia
- Pancreatitis
- Trauma/pancreatectomy
- Others

Endocrinopathies
- Acromegaly
- Aldosteronoma
- Cushing syndrome
- Glucagonoma
- Hyperthyroidism
- Pheochromocytoma
- Somatostatinoma
- Others

Infections
- Congenital rubella
- Cytomegalovirus
- Others

Uncommon forms of immune-mediated diabetes
- Anti-insulin receptor antibodies
- 'Stiff-man' syndrome
- Others

Drug or chemical induced
- Atypical antipsychotics
- Beta-adrenergic agonists
- Diazoxide
- Glucocorticoids
- Interferon alfa
- Nicotinic acid
- Pentamidine
- Phenytoin
- Protease inhibitors
- Thiazide diuretics
- Others

Other genetic syndromes sometimes associated with diabetes
- Down syndrome
- Friedreich's ataxia
- Huntington's chorea
- Klinefelter syndrome
- Laurence-Moon-Bardet-Biedl syndrome
- Myotonic dystrophy
- Porphyria
- Prader-Willi syndrome
- Turner syndrome
- Wolfram syndrome
- Others

Acute and chronic complication of Diabetes Mellitus & hyperglycaemia

Acute

Hypoglycemia : due to over administration of insulin, or oral hypoglycemic agents, impaired glucagon response to hypoglycemia

Diabetic Ketoacidosis: insulin deficiency lead to low intracellular glucose and high blood glucose, the intracellular reacts by mobilisation of fats for energy… the by product is ketoacids acetones, acetoacetate & β-hydroxybutyrate… leading to metabolic acidosis.

Chronic

Neuropathy: ↑IC sorbitol sugar.. Lead to ↑ IC osmotic pressure →water to enter → nerve swelling & damage

Retinopathy

Nephropathy

Tissue necrosis (gangrene): due to poor circulation and reduced healing…. amputations

Angiopathy (coronary artery disease, stroke, MI), due to changes in blood flow due to hyper → high blood pressure → 2-3-fold ↑macrovascular disease & 5-fold ↑cerebral vascular disease

Diabetes: Complications

Macrovascular

Stroke

Heart disease and hypertension
2-4 X increased risk

Peripheral vascular disease

Foot problems

Microvascular

Diabetic eye disease (retinopathy and cataracts)

Renal disease

Erectile Dysfunction

Peripheral Neuropathy

Gestational Diabetes Mellitus (GDM)

- Hyperglycemia or impaired glucose tolerance during pregnancy.
- Glucose tolerance may return to normal after delivery.

- High risk for development of diabetes mellitus later.

- **Complications**:
  - prenatal morbidity and mortality,
  - ↑rate of C-sections,
  - chronic hypertension.
- **Risk factors:**
  - age 25 & over, < 25 and obese
  - history of DM in 1st degree relative
  - ethnic group.

Gestational Diabetes Mellitus (GDM)

Screening

1. Perform between 24 and 28 wk of gestation on all pregnant women not identified as having glucose intolerance.
2. Give 50 g oral glucose load without regard to time of day or time of last meal.
3. Measure plasma glucose at 1 hr.
4. If glucose is ≥ 140 mg/dL, perform glucose tolerance test.

Diagnosis

1. Perform in the morning after a 10–16 hr fast.
2. Measure fasting plasma glucose.
3. Give 100 g of glucose orally.
4. Measure plasma glucose hourly for 3 hr.
5. At least two values must exceed the following:

Fasting	105 mg/dL
1 hr	190 mg/dL
2 hr	165 mg/dL
3 hr	145 mg/dL

6. If results are normal in a clinically suspicious situation, repeat during the third trimester.

update

Gestational diabetes mellitus (GDM) diagnosis

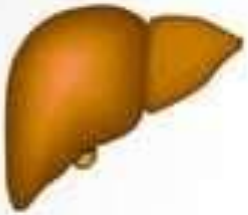
- 1) A woman at high risk for GDM should have an initial screening early in the pregnancy. If she is not found to have GDM during the initial screening, the woman should be retested at 24 to 28 weeks of gestation. For women of average risk, testing should be performed at 24 to 28 weeks of gestation.
- 2) For GDM, fasting plasma glucose >126 mg/dL or a casual plasma glucose >200 mg/dL is diagnostic of diabetes mellitus.
- 3) If unequivocal hyperglycemia is not apparent, retesting must be performed on a subsequent day.
- 4) When using the two-step approach, an initial screening is performed using a 50-g oral glucose load (time of day or time of last meal not relevant). Plasma is tested at 1 hour. This is a glucose challenge test (GCT). If the test value exceeds the glucose threshold value >140 mg/dL, an OGTT is performed. Some experts recommend using a glucose threshold value of 130 mg/dL.
- 5) Gestational diabetes mellitus may be diagnosed using an OGTT with oral ingestion of 100 g of glucose. The glucose results must meet or exceed two or more of the following criteria: a fasting plasma glucose >95 mg/dL, a 1-hour plasma glucose > 180 mg/dL, a 2-hour plasma glucose > 155 mg/dL, or a 3-hour plasma glucose > 140 mg/dL. Alternatively, a 75-g glucose load may be used and glucose measured through the 2-hour period.

Disease Burden of Diabetes Mellitus

- Leading cause of blindness
- 50% of all non-traumatic amputations
- increase risk of stroke
- increase in cardiovascular mortality
- DM responsible for 25% of cardiac surgeries
- Mortality in DM: 70% due to Cardiovascular disease

Sites of Action of Currently Available Therapeutic Options

LIVER



GLUCOSE PRODUCTION

Metformin

Thiazolidinediones

PANCREAS

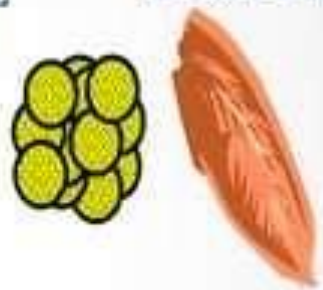
INSULIN SECRETION

Sulfonylureas: Glyburide, Gliclazide, Glimepiride

Non-SU Secretagogues: Repaglinide, Nateglinide

ADIPOSE TISSUE

MUSCLE



PERIPHERAL GLUCOSE UPTAKE

Thiazolidinediones

Metformin

INTESTINE

GLUCOSE ABSORPTION

Alpha-glucosidase inhibitors

Oral Hypoglycemic Agents(OHAs)

Drug	↓ BG	↓ HbA1c	Side-effects
Sulfonylurea	FBG 20%	1.0-2.0%	Hypoglycemia Weight gain
Biguanide	FBG 2.8-3.9 mM	1.0-2.0%	Lactic acidosis GI intolerance
TZD	FBG 2.2-3.6 mM	1.0-1.5%	Edema Weight gain Liver monitoring
Meglitinide	FPG 4 mM PPG 5.6 mM	1.0-2.0%	Hypoglycemia (50% < SU)
α-glucosidase Inhibitor	FPG 14% PPG 25%	0.5-1.0%	GI intolerance

Reducing risk in diabetes

- Glycemic control:
  - New insulins
  - New oral agents
  - Continuous BG monitor + insulin pump
    - "Artificial Pancreas"
- Blood pressure control
- Cholesterol control
- Aspirin
- Smoking cessation

Inherited disorders of carbohydrate metabolism

- **Glycogen storage diseases:**
  - Of which there are 10 types, are inherited diseases involving the deficiency of particular enzymes; these deficiencies cause defects in the normal metabolism of glycogen. Examples:
  - 1) von Gierke, type I: Glucose-6-phosphatase deficiency, leads to glycogen build up in tissues, resulting in low blood sugar.
  - 2) Pompe, type II: a-1,4-glucosidase deficiency
  - 3) Cori, type III: Amylo-1,6-glucosidase deficiency
- **Galactosemia**
  - This is characterized by a deficiency or absence of galactokinase, galactose 1-phosphate uridyl transferase, or uridyl diphosphate glucose-4-epimerase; the enzyme defect prevents metabolism of galactose.
  - Galactose is found in milk as a component of lactose, with galactosemia generally identified in infants.
  - Most commonly, galactose 1-phosphate uridyl transferase is deficient, which leads to excessive galactose in blood and excretion in urine.

Laboratory Diagnosis

Investigation and test procedures

- Screening.. People age 45 & older
- Diagnosis
- Monitoring glycemic status
- Screening for renal & cardiovascular complications
- Detection of acute crisis situations (ketoacidosis)

Screening & diagnostic procedures

- Normal fasting plasma glucose (FPG) < 100 mg/dL
- Impaired fasting glucose (IFG) is defined as a fasting plasma glucose level that ranges between 100 and 125 mg/dL.
- Provisional diagnosis of diabetes mellitus is made when FPG S:126 mg/dL
- **Blood glucose**
- Assessment of glycemic status
- home monitor whole blood glucose is 12-15% ↓ than plasma or serum with normal hematocrit.
- Proper sampling is important to reduce glycolysis.
    - At RT serum or plasma glucose ↓ by 5-7 %/hour due to continued glycolysis, this rate becomes higher in patients with:
        - leukocytosis, or bacterial infection.
    - To prevent glycolysis sodium fluoride or sodium iodoacetate is added to blood collection tube.

Criteria for the Diagnosis of Diabetes Mellitus

Diabetes Mellitus

Any one of the following is diagnostic:

1. Classic symptoms of diabetes and casual† plasma glucose concentration ≥200 mg/dL
2. Fasting‡ plasma glucose ≥126 mg/dL
3. A 2-hour postload plasma glucose concentration ≥200 mg/dL during the OGTT

Impaired Fasting Glucose

Fasting plasma glucose between 110 and 125 mg/dL

Impaired Glucose Tolerance

The following two criteria must be met:

1. Fasting plasma glucose <126 mg/dL
2. A 2-hour OGTT plasma glucose concentration between 140 and 199 mg/dL

Sample Fasting Glucose (mg/dL)

Plasma/serum	
Adults	74 to 106 (4.5 to 5.9 mmol/L)
Children	60 to 100 (3.5 to 5.6 mmol/L)
Premature neonates	20 to 60 (1.1 to 3.3 mmol/L)
Term neonates	30 to 60 (1.7 to 3.3 mmol/L)
Whole blood	65 to 95 (3.5 to 5.3 mmol/L)
CSF	40 to 70 (60% of plasma value) (2.2 to 3.9 mmol/L)
Urine	
24 h	1 to 15 mg/dL (0.1 to 0.8 mmol/L)

Blood glucose methodology

- All are enzymatic methods with precision of ≤ 3%.
- Based on glucose oxidase or hexokinase (reference method).
- Diagnostic criteria for the DM, as defined by the ADA, is based on plasma glucose.

Major enzyme assays

Glucose oxidase:

$$\text{Glucose} + 2\,H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + 2\,H_2O_2$$

$$\underset{\text{(colorless)}}{o\text{-Dianisidine}} + H_2O_2 \xrightarrow{\text{peroxidase}} \underset{\text{(colored)}}{\text{oxidized } o\text{-dianisidine}} + H_2O$$

Hexokinase:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \underset{\text{(or NAD}^+\text{)}}{\text{NADP}^+} \xrightarrow{\text{G-6-PD}} \text{6-phosphogluconate} + \underset{\text{(or NADH)}}{\text{NADPH}} + H^+$$

Glucose dehydrogenase:

$$\text{Glucose} + \text{NAD}^+ \xrightarrow{\text{glucose dehydrogenase}} \text{D-glucono-}\delta\text{-lactone} + \text{NADH} + H^+$$

nical tests used to detect the presence of reducing sugars is called; **Benedict's test:**

$$Cu^{+2} + \text{Glucose} \xrightarrow[OH]{\text{Heat}} \underset{(red)}{Cu_2O\downarrow} + \underset{(yellow)}{CuOH\downarrow}$$

update

Oral glucose tolerance test

- Based on the criteria published by the World Health Organization (WHO). Note: American Diabetes Association does not recommend the OGTT for routine clinical use.
- a. Timed measurements of plasma glucose before and after ingesting a specific amount of glucose
- b. Patient preparation: Unrestricted carbohydrate rich diet for 3 days before the test with physical activity, restrict medication on the test day, 12-hour fast required, no smoking
- c. Adult patient ingests 75 grams of glucose in 300-400 mL of water and children 1.75 g/kg up to 75 g of glucose. For assessment of GDM, 50 g, 75 g, or 100 g of glucose may be used (see previous description for details).
- d. Plasma glucose specimen is collected fasting at 10 minutes before glucose load and at 120 minutes after ingestion of glucose. Urine glucose may be measured.
- e. **Interpretation of OGTT results is based on the criteria published by the WHO.**
  - 1) Impaired fasting glucose (IFG) is diagnosed when fasting plasma glucose ranges between 110 and 125 mg/dL.
  - 2) The following two criteria must be met for diagnosis of impaired glucose tolerance (IGT): Fasting plasma glucose level must be <126 mg/dL and the 2-hour plasma glucose level of the OGTT must fall between 140 and 199 mg/dL.
  - 3) Diabetes mellitus is diagnosed when the fasting plasma is s 126 mg/dL or the 2-hour glucose is >200 mg/dL.

Oral glucose tolerance test OGTT cont'd

- Recommended in case of pregnancy for screening of GDM
- Oral glucose tolerance test (OGTT), 75 g glucose, measure fasting and at 30 min intervals for 2 hr
- **Drawbacks:**
  - Poor reproducibility
  - Expense
  - Time, patients prep requirement
  - Lack of standardisation in glucose load administration
  - Multiple venipunctures

The oral glucose tolerance test

Indications	Procedure
equivocal fasting/random blood glucose concentrations	patient should eat normal diet, containing at least 250 g carbohydrate per day for three days
unexplained glycosuria, particularly in pregnancy	fast patient overnight
clinical features of diabetes mellitus or its complications with normal blood glucose concentrations	take basal blood sample for glucose determination
diagnosis of acromegaly (see p. 145)	give 75 g glucose in water orally; take further blood sample at 120 min for glucose determination
	patient should rest throughout test; smoking not permitted; drinks of water are allowed

Measurements of Reducing sugars

- Screening for reducing sugars in the urine of neonates & infants for IEBM screening.
- E.g.: galactose & fructose appear in the urine detected by the copper reduction methodology.
- A positive test for reducing substance in the urine using reagent tablets (clinitest), is given by a number of substance other than glucose. See the table

Substances giving a positive reducing test in urine

- glucose
- lactose (during lactation and the last trimester of pregnancy)
- galactose (in galactosaemia and galactokinase deficiency)
- fructose (in hereditary fructose intolerance and essential fructosuria)
- pentoses (after eating certain fruits and in essential pentosuria)
- homogentisic acid (in alkaptonuria)
- glucuronides of drugs
- salicylic acid (in aspirin overdose)
- ascorbic acid (with high vitamin C intake)
- creatinine (only in high concentration)

Other Tests for monitoring Diabetic patients

- **Urine dip sticks for urine:** measures glucose level (limited coz the average renal threshold for glucose is 180 mg/dL)…
  - so –ve urine sample would indicate that the blood glucose is < 180 mg/dL given normal GFR.
- Used to monitor and maintain glycemic status
- Better use glucose home monitor.

Self-Monitor of blood glucose SMBG

- Is used for type 1 DM particularly
- Is a key factor in the achievement of reasonable glycemic goals
- & to minimise adverse effect caused by acute complications in type 1 DM.
- In general monitoring is recommended 3 to 4 times/day in Type 1.
- Most SMBG employ glucose oxidase methodology.
- As monitors measure whole blood glucose, concs. is usually 12-15% < plasma glucose determinations.
- During fasting capillary blood glucose is 2-5% mg/dL > venous concentration.
- However after glucose load, capillary concentration rises 20-70 mg/dL > the concurrently drawn venous samples.

Use of SMBG by patients are limited due to:

- Cost
- proper use
- Discomfort from finger-stick
- Complexity of the technique
- Time of testing is inconvenient
- Accuracy is user and instrument dependent
- Calibrator and control should be regularly used.
- **Errors with SMBG:**
- Application, timing, removal of specimen from monitor

UPDATE

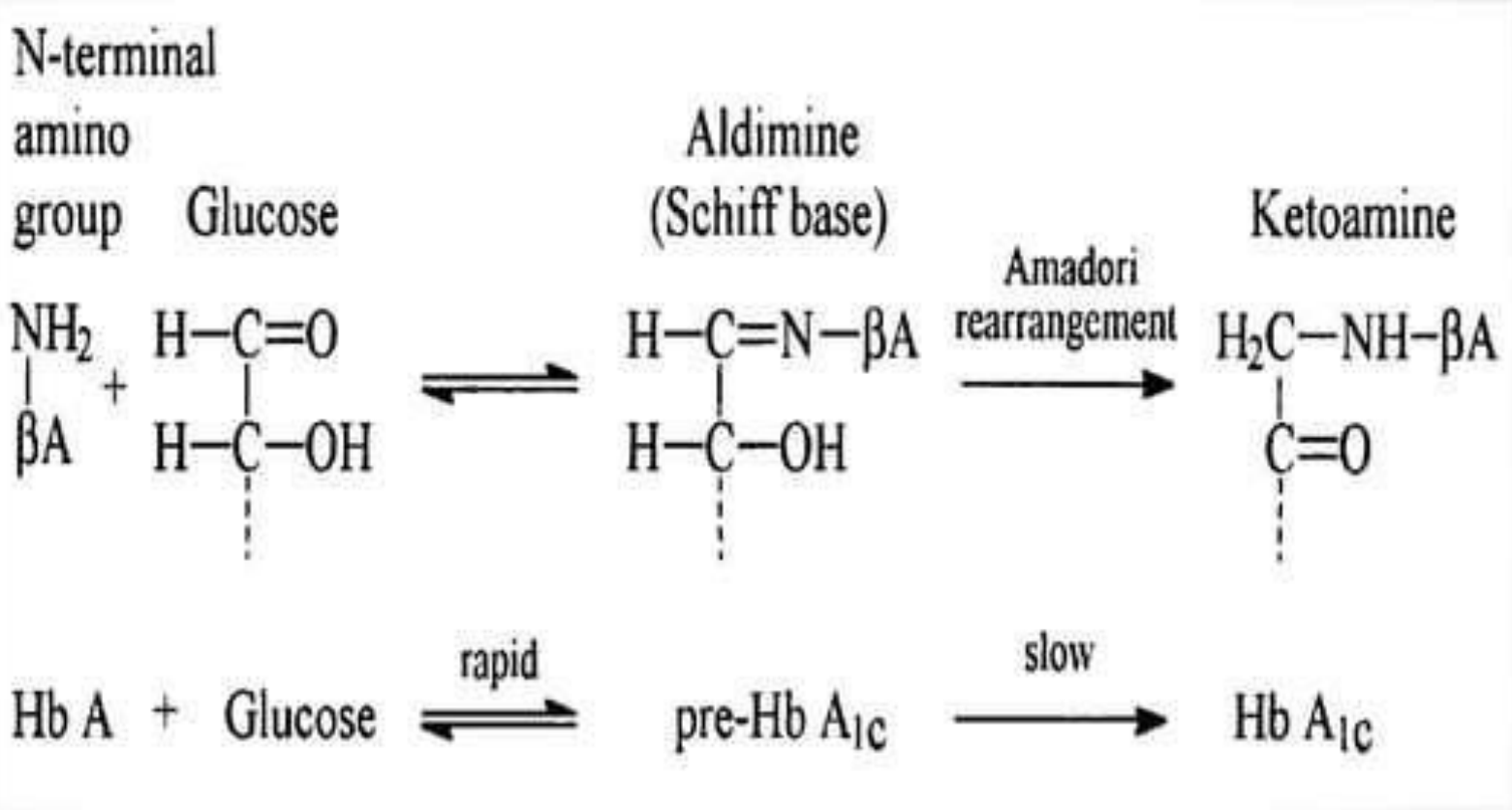
Glycated/glycosylated hemoglobin

- Hemoglobin A is composed of three forms, Hb A1a, Hb A1b, and Hb A1c, which are referred to as glycated or glycosylated hemoglobin.
  - Hb A1c is the main form.
- Glycated hemoglobin is formed from the nonenzymatic, irreversible attachment of glucose to hemoglobin A1.
- Measurement of glycated hemoglobin reflects blood glucose levels for the past 2-3 months.
  - It is useful in monitoring effectiveness of treatment and compliance of diabetic individual to treatment protocol.
- Measured by:
  - affinity chromatography, ion-exchange chromatography IEC, and high-performance liquid chromatography HPLC
- Specimen collection:
  - Non-fasting blood drawn in EDTA tubes
- Reference range:
  - 4-6% Hb Alc; effective treatment range <7% Hb Alc

What is Glycation process

- Slow
- 3 steps
- Non-enzymatic reaction that takes place in the RBCs.
- **Process**:
  - 1st Glucose bind **reversibly** to amino groups on the Hb to form labile **aldimine**.
  - 2nd **aldimine** goes through **irreversible** rearrangement to form a stable ketoamine (Amadori rearrangement).
  - The final step is the glycation which is a conformational change of glucose to a cyclic structure (pH 7.4).
  - SO, **glycated Hb** is the **Ketoamine** (irreversible), but **glycosylated Hb** is the (**reversible**) step.
  - The HbA_{1c} is measured every 3 months based on the RBCs life span (120 days)

Amadori rearrangement



- The primary site for glycation on the HbA_{1c} is the N-terminal valine on the beta chain.

Glycated Haemoglobin HbA_{1c}

- Monitor glycemic status of DM
- **HbA1c Normal range = 5.3 to 7.5% of total Hb**
- Measuring blood glucose conc. fluctuates, So HbA_{1c} is better coz:
- Used 1st for diagnosis and then periodically for monitoring of DM (every 3 months).
- Provides retrospective time average assessment of previous 2-3 months of glycemic control, which is dependent on the life span of RBCs.
- Glycosylated hemoglobin, Hemoglobin A_{1c}, HbA_{1c}
  - HbA - 97% of total hemoglobin
  - "fast hemoglobins", HbA_1, contain carbohydrates bound to Hb
  - HbA_{1c} has 60-80% of HbA_1
- The primary site for glycation on the HbA_{1c} is the N-terminal valine on the beta chain.

Glycated Haemoglobin HbA_{1c}

- **Values are unaffected by:**
  - Day to day changes in glucose conc.
  - Degree of exercise
  - Recent food ingestion
  - Used as predictive for risk of developing chronic complications associated with DM

Glycated Hb/HbA_{1c} methodology

- **Three categories based on structure, charge, & chemical make up.**
- **Techniques based on the charge differences are:**
  - Ion exchange chromatography IEC
  - Isoelectric focusing IEF
  - High performance liquid chromatography HPLC
    - HPLC has an advantage that the chromatogram provides a visual view of the separation of pattern of the Hb fractions, also used as a quality assurance and identification of Hb variants (Hb S, C,E
  - Electrophoresis.
  - **Techniques based on the structure differences are:**
  - affinity chromatography & immunoassays.
  - Spectroscopy is used to perform chemical analysis
  - **All methods uses whole blood collected in EDTA tube.**

Most common methods used are immunoassays and HPLC

Problems associated with Glycated Hb/HbA_{1c} analysis

- Lack of standardization between methods
- Poor precision
- Poor correlation with different labs
- So, the aim of the therapy is to keep the HbA_{1c} concentration < 7 %, as measured by certified method as traceable to reference assay (HPLC).
- Used primarily as an indicator for monitoring the glycemic control of diabetes.

Detection of microalbuminuria

- Defined as low but abnormal levels of albumin in the urine (>30 mg/day).
- Microalbuminuria is present if urinary albumin excretion > 30 mg in a 24-hour collection, or 30mg/g creatinine in a random urine sample.
- Used as early indicator for nephropathy in diabetics.
- +ve microalbuminuria suggest the need to screen for other vascular disease.
- Dipsticks does not detect microalbuminuria.
- Measurement of albumin excretion
  - is useful for patients with renal complications of diabetes mellitus. Performed on random urines, microalbumin analysis always requires the simultaneous analysis of creatinine, and it is reported as an albumin/creatinine ratio.
  - Abnormal values (microalbuminuria) will be >30 mg albumin/g creatinine.

Screening for microalbuminuria is performed by one of the following methods:

- Albumin:creatinine ratio on a random spot urine
- Serum and urine creatinine
- Creatinine clearance on 24-hour urine collection.
- Urine creatinine measured in a timed urine collection.
- Albumin measured in a timed urine collection.

- **Because of the wide day to day variability 2-3 urine collections done in a 3 to 6 months period before a patient is designated as having microalbuminuria**

update

Fructosamine

- Ketoamine linkage forms between glucose and protein, mainly represented by albumin.
- Clinical significance:
  - Measurement of fructosamine reflects blood glucose levels for 2-3 weeks before sampling.
- Measured by:
  - spectrophotometric/colorimetric methods, affinity chromatography, and HPLC
- Reference range:
  - 205-285 |xmol/L

C. Detection of acute crisis (Hypoglycemia & Ketoacidosis)

- Ketones present with type1 DM mostly, BUT also type2
- Ketone present in urine during fasting and in approxi 30% of 1st morning urine sample from pregnant.
- Ketones, ketone bodies:
  - 78% β-hydroxybutyrate, 20% Acetoacetate, 2% Acetone.
  - All commercial test are based on nitroprusside reaction (most sensitive to acetoacetate followed by acetone) (acetest, Ketostix) - acetoacetate - purple complex.
  - Enzyme assay (Ketosite) - β-hydroxybutyrate - purple color.
- Also perform blood gas analysis, electrolyte, anion gap calculation & osmolality.

update

Lactate

- The normal end product of glucose metabolism is pyruvate; however, lactate is produced under conditions of oxygen deficit (**anaerobic metabolism**).
- The production and accumulation of lactate in the blood and its measurement aid in assessing the degree of **oxygen deprivation** that is occurring.
- **Change in the blood lactate level precedes a change in blood pH.**
- Lactate is metabolized by the liver via **gluconeogenesis**.
- **Test methodology:**
  - lactate + 02 ----Lactate oxidase ----→ pyruvate + H2O2
  - H2O2 reduced chromogen ---peroxidase-→oxidized chromogen (colored) + H2O
- **Clinical significance:**
  - **Type A lactic acidosis** is caused by **depressed oxygen** levels that may occur in AMI, CHF, shock, pulmonary edema.
  - **Type B lactic acidosis** is caused by **metabolic** processes that may occur in diabetes mellitus, renal disorders, liver disease, ingestion of toxins (salicylate overdose and excess ethanol), and so on.
- **Source of errors & special handling**: **Avoid** using a **tourniquet** because venous stasis will falsely raise blood lactate levels; place the specimen on **ice** and immediately transport to the laboratory; centrifuge the specimen and **remove** the plasma (additives NaF and K2C2O4) as soon as possible.
- **Reference range (venous):** 0.5-1.3 mmol/L

References

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