# STUDY MATERIAL FOR B.SC MICROBIOLOGY

# BASIC & CLINICAL BIOCHEMISTRY

## I YEAR

## SEMESTER – I



**Academic Year 2023-2024**

**Prepared by**

**MICROBIOLOGY DEPARTMENT**

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## INTRODUCTION

Biochemistry, as the name implies, is the chemistry of living organisms. It has its origin in chemistry and biology. There is a basic unity of biochemistry throughout nature. Although different organisms differ outwardly in their life processes, there are striking similarities in executing different tasks. Genetic code, metabolic pathways, enzymes, coenzymes and even regulatory mechanisms are similar to a large extent in all the living organisms.

# UNIT- I

# BIOMOLECULES – CARBOHYDRATE AND LIPIDS

## CARBOHYDRATES:

- Carbohydrates are the most abundant biomolecules on earth.
- Each year, photosynthesis converts more than 100 billion metric tons of carbon dioxide and water into cellulose and other plant products.
- Carbohydrates are polyhydroxy aldehydes or ketones or substances that yield such compounds on hydrolysis.
- Many but not all carbohydrates have the empirical formula (CH2O) n.
- Some carbohydrates also contain nitrogen, phosphorous or sulfur. So, the carbohydrates are defined as polyhydroxy aldehydes or ketones or their condensation products or derived products.
- The basic condensing bond is glycosidic bond.

### FUNCTIONS OF CARBOHYDRATES:

- Carbohydrates such as sugar and starch are dietary staple in most parts of the world and the oxidation of glucose is the central energy yielding pathway in most cells.
- They are the reserve or storage forms of energy in plants (starch, inulin) and in animals (Glycogen)
- The insoluble carbohydrate polymers serve as structural and protective elements in the cell walls of bacteria and plants and in connective tissue of the animals. i.e., they give structural rigidity. Eg: cellulose in cell wall, chitin in insects and mucopolysaccharides in bacteria.
- They are important components of nucleic acids, coenzymes and flavoproteins (ribose).

**CLASSIFICATION**

Carbohydrates vary greatly in size ranging from smaller glyceraldehyde molecule to larger starch molecule.

Large carbohydrate molecules are polymers of small molecules. The carbohydrates have been classified based on the degree of polymerization into

- **Monosaccharides:** These are the simple sugars which cannot be further broken down. Oligosaccharides: They are composed of short chains of monosaccharides.
- **Polysaccharides:** They are composed of several monosaccharide units.
- **Conjugated Polysaccharides:** In addition to carbohydrates, these polysaccharides contain proteins and lipids also.

**MONOSACCHARIDES**

Monosaccharides also called simple sugars. They consist of a single polyhydroxy aldehyde or ketone units. The most abundant monosaccharides in nature are the 6-carbon sugars like Dglucose and fructose.

**Structure**

Monosaccharide has a backbone, which is un- branched, single bonded carbon chain. One of the carbon atoms is double bonded to an oxygen atom to form carbonyl group. Each of the other carbon atoms has a hydroxyl group. Example.

**Structure of Glucose**

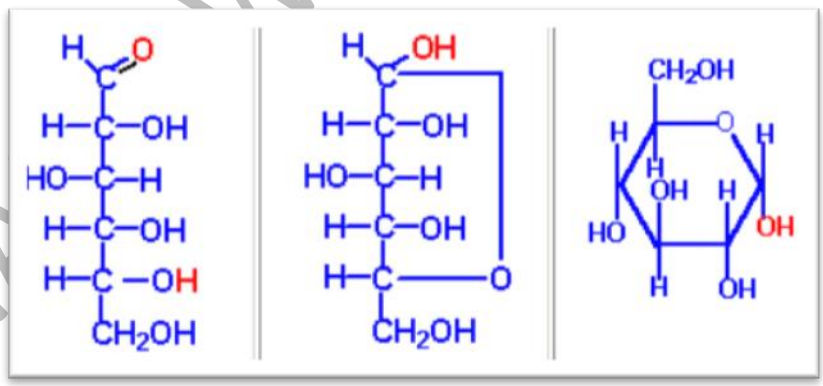



Fig.1 chain D-glucose α-D –glucose (Fisher formula) α-D –glucose (Haworth formula)

There are two families of monosaccharides. Monosaccharides having aldehyde groups are called Aldoses and monosaccharides with Ketone group are Ketoses. Depending on the number of carbon atoms, the monosaccharides are named trioses (C3), tetroses (C4), pentoses (C5), hexoses (C6), heptoses (C7).

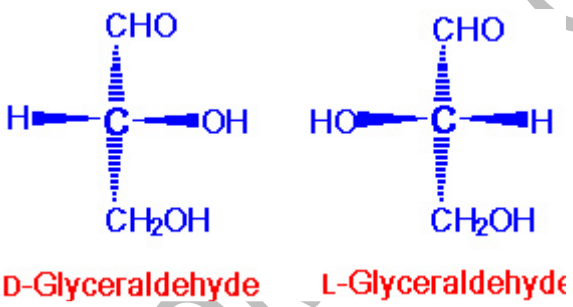
**Physical properties**

**Physical properties of Monosaccharides**

They are colorless, crystalline compounds, readily soluble in water. Their solutions are optically active and exhibit the phenomenon of mutarotation. Carbohydrates spontaneously change between the α and β configuration.

**Asymmetric Center and Stereoisomerism**

- Asymmetric carbon is a carbon that has four different groups or atoms attached to it and having optically activity in solution.
- All the monosaccharides except dihydroxyacetone contain one or more asymmetric or chiral carbon atoms and thus occur in optically active isomeric forms.
- Monosaccharides with n number of asymmetric centers will have (2n) isomeric forms. (n= number of asymmetric carbon atoms).




The two isomeric forms of glyceraldehydes

- The designation of a sugar isomer as the D- form or of its mirror images the L- form is determined by the spatial relationship to the parent compound of the carbohydrate family.
- The D and L forms of Glyceraldehyde are shown in the Figure 2.2.The orientation of- OH and- H groups around the carbon atom adjacent to the terminal primary alcohol carbon determines its D or L form .
- When the - OH group on this carbon is on the right, the sugar is a member of the Desires, when it is on the left, it is a member of the L-series. These D and L configuration are also called Enantiomers.

**OPTICAL ACTIVITY**

- The presence of asymmetric carbon atom causes optical activity. When a beam of plane polarized
- light is passed through a solution of carbohydrate it will rotate the light either to right or to left. Depending on the rotation, molecules are called dextrorotatory (+) (d) or laevorotatory (-) (l).
- Thus, D- glucose is dextrorotatory but D- fructose is laevorotatory.

- When equal amounts of D and L isomers are present, the resulting mixture has no optical activity, since the activities of each isomer cancel one another. Such a mixture is called racemic or DL mixture.

**EPIMERS**

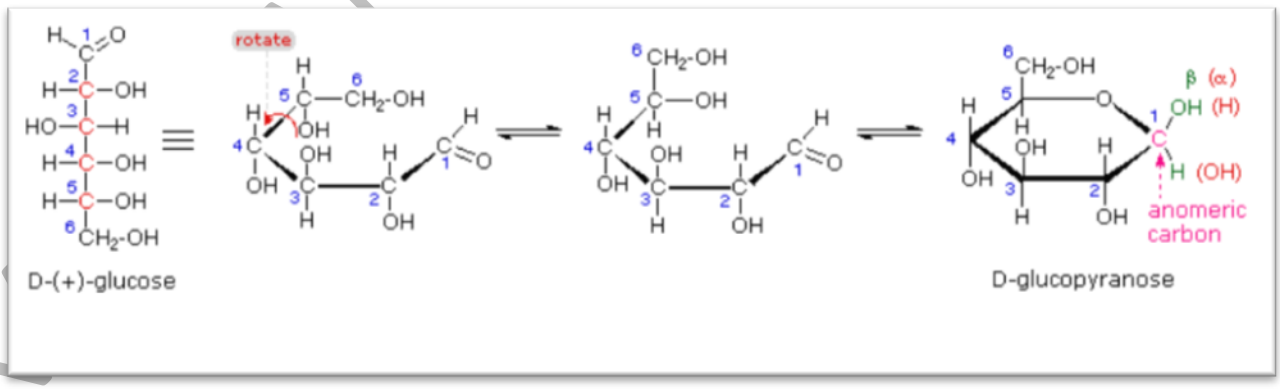
- When sugars are different from one another, only in configuration with regard to a single carbon
- atom (around one carbon atom) they are called epimers of each other.
- For example, glucose and mannose are epimers. They differ only in configuration around C2. Mannose and Galactose are epimers of Glucose

**ANOMERS**

- The two stereoisomers at the hemiacetal (anomeric) carbon are:
- The alpha anomer: Where- OH group is down (Haworth)
- The beta anomer: Where- OH group is up (Haworth)
- Anomers are diastereomers (having different physical properties)

**CYCLIZATION OF MONOSACCHARIDES**

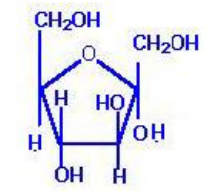
- Monosaccharides with five or more carbon atoms in the backbone usually occur in solution as cyclic or ring structure, in which the carbonyl group is not free as written on the open chain structure.
- But has formed a covalent bond with one of the hydroxyl group along the chain to form a hemiacetal or hemiketal ring. In general, an aldehyde can react with an alcohol to form a hemiacetal or acetal.



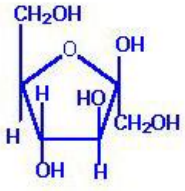

- The C-1 aldehyde in the open-chain form of glucose reacts with the -5th carbon atom containing hydroxyl group to form an intramolecular hemiacetal.
- The resulting six embered ring is called pyranose because of its similarity to organic molecule Pyran.

- Two different forms of glucose are formed when the OH group extends to right it is α-D-Glucose and when it extends to left, it is β-D-Glucose commonly called as Anomers.
- Similarly, a ketone can react with an alcohol to form a hemiketal or ketal. The C-2 keto group in the open chain form of fructose can react with the 5th carbon atom containing hydroxyl group to form an intramolecular hemiketal.
- These five membered rings are called furanose because of its similarity to organic molecule furan.

α and β forms of Fructose



Alpha D. Fructofuranose



Beta D. Fructofuranose

**OLIGOSACCHARIDES**

- Oligosaccharides contain 2 to 10 monosaccharide units. The most abundant oligosaccharides found in nature are the Disaccharides

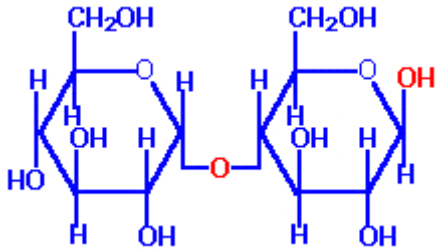
**DISACCHARIDES**

When two monosaccharides are covalently bonded together by glycosidic linkages a disaccharide is formed. Glycosidic bond is formed when the hydroxyl group on one of the sugars reacts with the anomeric carbon on the second sugar. Biologically important disaccharides are sucrose, maltose, and Lactose.

**MALTOSE**

- Maltose, also known as malt, is a disaccharide made up of two alpha D-glucose units.
- The two units of glucose are linked with an alpha 1,4 glycosidic bond. In the small intestinal lining in humans, the enzyme maltase and isomaltase break down the molecules of maltose into two glucose molecules, which is then absorbed by the body.
- Maltose contains two D glucose residues joined by a glycosidic linkage between OH at the first carbon atom of the first glucose residues and OH at the fourth carbon atom of the second glucose forming a α-(1,4) glycosidic linkage as shown in Figure.

- Maltose is the major degradative product of Starch.Maltose is hydrolyzed to two molecules of D- glucose by the intestinal enzyme maltase, which is specific for the α-(1, 4) glycosidic bond.



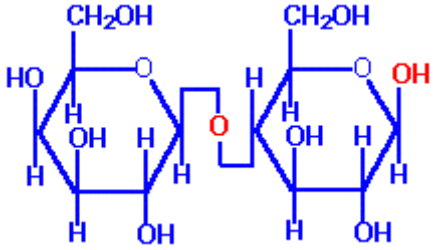
Structure of Maltose

**Properties of Maltose**

- Maltose is a reducing sugar. It tastes sweet but is only 30-60% as sweet as sugar.
- The hydrolysis reaction of maltose in the presence of an acid catalyst gives two molecules of alpha D-glucose.
- There is a free anomeric carbon present in the structure of maltose which can undergo mutarotation in solution. The resultant solution will be a mixture of alpha and beta maltose. Since the aldehydic group is formed in the solution, maltose gives a positive test with Benedicts and Tollens reagents.

**LACTOSE:**

It is a disaccharide formed between the anorexic carbon C-1 of β-D-galactose and C-4 of α- D-glucose. Since the anomeric carbon of galactose molecule is involved in the bond and is in the beta-configuration, this is called β (1Æ4) bond which can be abbreviated as β1Æ4. The etymology is comparable to that of the word lactose in that both contain roots meaning "milk sugar". Lactose is a disaccharide of galactose plus glucose.



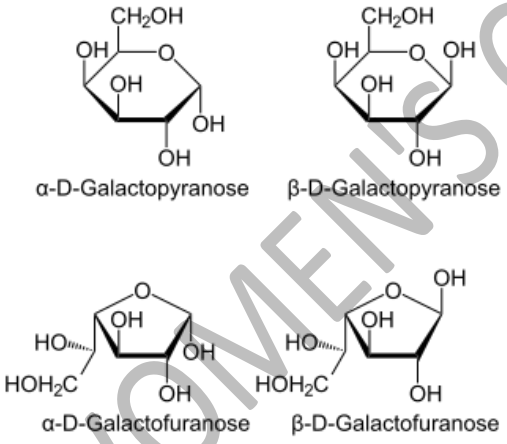
**GALACTOSE**

- abbreviated Gal, is a monosaccharide sugar that is about sweet as glucose, and about 65% as sweet as sucrose.

- It is an aldohexose and a C-4 epimer of glucose. A galactose molecule linked with a glucose molecule forms a lactose molecule.
- Galactan is a polymeric form of galactose found in hemicellulose, and forming the core of the galactans, a class of natural polymeric carbohydrates.
- D-Galactose is also known as brain sugar since it is a component of glycoproteins (oligosaccharide-protein compounds) found in nerve tissue.

**STRUCTURE AND ISOMERISM**

Galactose exists in both open-chain and cyclic form. The open-chain form has a carbonyl at the end of the chain. Four isomers are cyclic, two of them with a pyranose (six-membered) ring, two with a furanose (five-membered) ring.




- A disaccharide composed of two units of galactose, galactose-alpha-1,3-galactose (alpha-gal), has been recognized as a potential allergen present in mammal meat. Alpha-gal allergy may be triggered by lone star tick bites.
- Galactose in sodium saccharin solution has also been found to cause conditioned flavor avoidance in adult female rats within a laboratory setting when combined with intragastric injections.
- The reason for this flavor avoidance is still unknown, however it is possible that a decrease in the levels of the enzymes required to convert galactose to glucose in the liver of the rats could be responsible

**SUCROSE ($C_{12}H_{22}O_{11}$)**

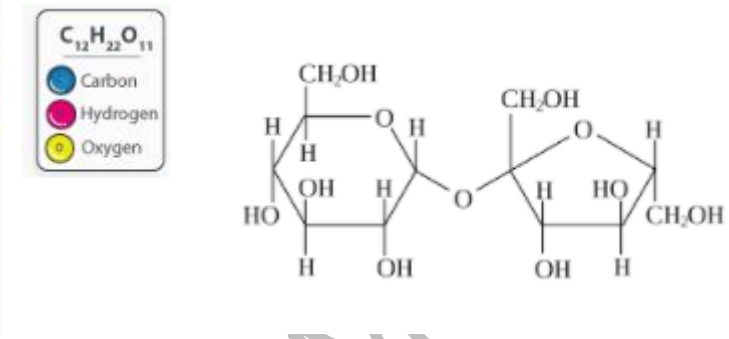
- Sucrose is a molecule composed of two monosaccharides, namely glucose and fructose. This non-reducing disaccharide has a chemical formula of C12H22O11.Sucrose is commonly referred to as table sugar or cane sugar.
- In a C12H22O11 molecule, the fructose and glucose molecules are connected via a glycosidic bond. This type of linking of two monosaccharides called glycosidic

linkage. Sucrose has a monoclinic crystal structure and is quite soluble in water. It is characterized by its sweet taste.

- William Miller, an English chemist, coined the word sucrose in the year 1857. It is widely used as a sweetener in food. C12H22O11 can be obtained from sugar beets or sugar canes, but it must be refined to be fit for human consumption.
- Refined sucrose (or sugar) is a popular ingredient in many food recipes because of its sweet taste.

**STRUCTURE OF SUCROSE**

- Sucrose is a disaccharide which is made up of two monosaccharides. The structure of a sucrose molecule is illustrated below.




Sucrose: It is a disaccharide formed by glycosidic bond formation between the anomeric C-1 of α- D - glucose and the anomeric C-2 of β-D fructose so that sucrose lacks a free reducing group. Thus, sucrose is a non reducing disaccharide. It is formed by condensation of Glucose & Fructose

**PHYSICAL PROPERTIES OF SUCROSE**

- Sucrose has a monoclinic crystal structure.
- When subjected to high temperatures (over 186oC), this compound decomposes, yielding caramel.
- Its solubility in water at a temperature of 20oC is 203.9g/100mL
- The standard enthalpy of combustion corresponding to sucrose is 5647 kJ.mol-1.

**CHEMICAL PROPERTIES OF SUCROSE**

- Sucrose can undergo a combustion reaction to yield carbon dioxide and water.
- When reacted with chloric acid, this compound yields hydrochloric acid, carbon dioxide, and water.
- Upon hydrolysis, the glycosidic bond linking the two carbohydrates in a C12H22O11 molecule is broken, yielding glucose and fructose.
- Sucrose can be dehydrated with the help of H2SO4 (which acts as a catalyst) to give rise to a black solid which is rich in carbon.

**BIOLOGICAL IMPORTANCE**

- Sucrose is one of the most important components of soft drinks and other beverages.
- This compound is used in many pharmaceutical products.
- It serves as a chemical intermediate for many emulsifying agents and detergents.
- It also serves as a food thickening agent and as a food stabilizer.
- The shelf lives of many food products, such as jams and jellies, are extended with the help of this compound.
- The use of sucrose in baking results in the brown colour of the baked products.
- This compound also serves as an antioxidant (a compound that inhibits oxidation).
- Sucrose is widely used as a food preservative.

**POLYSACCHARIDES**

Most of the carbohydrates found in nature occur in the form of high molecular polymers called polysaccharides.

There are two types of polysaccharides. These are:

- Homopolysaccharides that contain only one type of monosaccharide building blocks.
- Heteropolysaccharides, which contain two or more different kinds monosaccharide building blocks.

**HOMOPOLYSACCHARIDES**

Example of Homopolysaccharides: Starch, glycogen, Cellulose and dextrins.

**STARCH**

- It is one of the most important storage polysaccharides in plant cells. It is especially abundant in tubers, such as potatoes and in seeds such as cereals.
- Starch consists of two polymeric units made of glucose called Amylose and Amylopectin but they differ in molecular architecture.
- Amylose is unbranched with 250 to 300 D-Glucose units linked by α-(1, 4) linkages Amylopectin consists of long branched glucose residue (units) with higher molecular weight.
- The inner part of glucose units in amylopectin are joined by α-(1,4) glycosidic linkage as in amylose, but the branch points of amylopectin are α- (1,6) linkages. The branch points repeat about every 20 to 30 (1-4) linkages.
- Starch is a polymer consisting of D-glucose units.
- Starches (and other glucose polymers) are usually insoluble in water because of the high molecular weight. – Because they contain large numbers of OH groups, some starches can form thick colloidal dispersions when heated in water (e.g., flour or starch used as a thickening agent in gravies or sauces).

- There are two forms of starch: amylose and amylopectin.
- Starch — Amylose • Amylose consists of long, unbranched chains of glucose (from 1000 to 2000 molecules) connected (1→4) glycosidic linkages.α by (1→4) glycosidic linkages.
- 10%-20% of the starch in plants is in this form. The amylose chain is flexible enough to allow the molecules to twist into the shape of a helix. Because it packs more tightly, it is slower to digest than other starches.
- Amylose helices can trap molecules of iodine, forming a characteristic deep blue-purple color. (Iodine is often used as a test for the presence of starch.)

**STARCH — AMYLOPECTIN**

- Amylopectin consists of long chains of glucose (up to 105 molecules) (1→4) glycosidicαconnected by (1→6) branches everyαlinkages, with 24 to 30 glucose units along the chain.
- 80%-90% of the starch in plants is in this form.

**GLYCOGEN**

- Glycogen is the main storage polysaccharide of animal cells (Animal starch).
- It is present in liver and in skeletal muscle.
- Like amylopectin glycogen is a branched polysaccharide of D-glucose units in α -(1, 4)
- linkages, but it is highly branched. The branches are formed by α -(1,6) glycosidic linkage that occurs after every 8 -12
- residues. Therefore liver cell can store glycogen within a small space. Multiple terminals of branch points release many glucose units in short time.
- It's, also known as animal starch, is structurally similar to amylopectin, containing both (1→6) branchα(1→4) glycosidic linkages and α points. Glycogen is even more highly branched, with branches occurring every 8 to 12 glucose units.
- Glycogen is abundant in the liver and muscles; on hydrolysis it forms D-glucose, which maintains normal blood sugar level and provides energy.
- Glycogen molecule consists of glucose units which are linked in long chains by α1Æ4 bonds. For every 10 units or so, the chain is branched by the formation of α 1Æ6 glycosidic bond.
- The glycogen chain terminates in a non reducing end with a free 4'-OH group.
- Since the enzyme that degrades glycogen catalyzes the removal of glycosyl units from non reducing end of glycogen chain, the numerous branches, each with a non reducing end, greatly increase the accessibility of the polysachharide to degradation.
- The α1Æ6 branches are removed by debranching enzyme.

## LIPIDS

Lipids comprise very heterogeneous group of compounds which are insoluble in water but soluble in non-polar organic solvents such us benzene, chloroform, and ether. They are present in all living organisms. The group includes fats, oils, waxes and related compounds.

**General Functions of Lipids**

i. They are efficient energy sources.

ii. Serve as thermal insulators.

iii. They are structural components of the cell membrane.

iv. Serve as precursors for hormones (steroid hormones).

v. They also dissolve the vitamins, which are fat-soluble and assist their digestion.

**Classification: -**

There are two ways of classification i.e.,
Classification as storage and structural lipids and some other functional lipids.
Classification based on lipid composition.

**I. Simple lipids**:- esters of fatty acids with different alcohols.

Fats and oils:- These are esters of fatty acids with glycerol.

Waxes:- Esters of fatty acids with high molecular weight monohydric alcohols

**II. Complex lipids:-** Esters of fatty acids and alcohols together with some other head groups.

A. Phospholipids:- Esters of the above type containing phosphoric acid residue.

a) Glycerophospholipids:- The alcohol is glycerol

b) Sphingophospholipids:- The alcohol is shingosine.

B. Glycolipids:- Lipids containing fatty acid, sphingosine and carbohydrate residues.

C. Others:- Include sulfolipids, amino lipids and lipoproteins, which are modified forms of lipids.

**III. Derived lipids:** include the hydrolytic products of the simple and complex lipids. Eg. Fattyacids, cholesterol etc. The simplest naturally occurring lipids are triacylglycerols formed by esterification of fatty acids.with glycerol. Biological membranes are made up of phospholipids, glycolipids and proteoglycans.

**FUNCTIONS OF LIPIDS:** Lipids encompass a very large and diverse group of biological substances and so they perform a wide-ranging functions too. Some major functions of lipids are as follows.

1. Lipids act as reservoir of energy in biological systems. Being more reduced than carbohydrates, lipids can store more energy. The most important storage form of lipids is the triacyl glycerols stored in the oil bodies in plant seeds and adipose tissues in animals.
2. Lipids act as the major components of biological membranes. The most important class of lipids in this regard is the amphipathic phospholipids with a small hydrophilic head and a long hydrophobic tail arranged in a bilayer form.
3. Some lipids act as members of electron transport system in inner mitochondrial membrane viz. ubiquinone and also phosphorylation systems in thylakoid membrane.
4. Lipids act as carriers of sugars viz. dolichol in the biosynthesis of glycoproteins.
5. Lipids materials are used for the biosynthesis of certain hormones in animals & plants.
6. Lipids in the form of bile acids (e.g. cholic acid) help in the digestion and absorption of other lipids.
7. Triacyl glycerols act as heat insulating materials.

**FATTY ACIDS**

- Fatty acids are the carboxylic acids with a hydrocarbon chain. They form the most important constituent of lipids.
- They contain normally 12 to 26 C atoms; however, 16 and 18 C fatty acids are the most common. Most fatty acids contain an even number of carbon atoms. They may be linear or branched or may have cyclic groups.
- They may have additional functional groups like OH, C=O (keto) etc. They may be saturated (SFAs) with no double bond or unsaturated (USFAs) containing up to six double bonds.
- SFAs up to 8 C atoms are liquids at room temperature, while those with more than 8 C atoms are solids. The melting point of fatty acids therefore increases with increase in chain length.
- In USFAs, the double bonds are of cis-type and separated from each other by one or more CH2 groups.
- Those USFAs with more than one double bond are referred to as polyunsaturated fatty acids (PUFA). The position of the double bond is indicated by a superscript Δ followed by the number of C atom counting from the carboxyl end.
- Among the USFAs, oleic and linoleic acids are the most abundant. The USFAs are more condensed in length than the SFAs of equal chain length.

The double bonds give the fatty acid molecules a sharp bend. The double bonds thus prevent tight packing within the membranes. This behavior of USFAs does have profound biological significance. Further, the introduction of double bonds in fatty acids decreases the melting point (M.P) of the fatty acids.

1. Numbering starts from carboxyl carbon. The last carbon is the "n" carbon

2. The second carbon is the "α"and the third the "β" Carbon. The last carbon atom is omega.

Eg:- CH3 (CH2)7 CH2CH2 (CH2)7 COOH stearic acid (saturated fatty acid)

Eg:- CH3 (CH2)7 CH=CH (CH2)7 COOH oleic acid (Unsaturated fatty acid)

Fatty acids can be represented as shown below where the delta indicates the position of the double bond and the next number shows the number of carbon atoms and the last number indicates the number of double bonds. In a different way the position of the double bond(s) canbe indicated as shown in the second expression without the delta. C18:1, Δ9 or 18:1(9)

C18 indicates 18 carbons, 1 indicates the number of double bonds, delta 9(Δ9) indicates the position of double bond between 9th and 10th carbon atoms. - Double bonds in naturally occuring fatty acids are in the cis- configuration and saturated fatty acids of C12 to C24 are solids at body temperature but the unsaturated once are liquids.

PUFA (Polyunsaturated fatty acids): They have two or more double bonds. They are called as essential fatty acids because they are required in the body and cannot be synthesized. So they need to be include in the diet. Linoleic acid 18: 2; 9 (12) 18 Linolenic acid 18: 2; 9 (12, 15) These two are called essential fatty acids. Arachidonic acid : 4; (5, 8, 11, 14)

**ESSENTIAL FATTY ACIDS.**

**Functions:**

1. The fluidity of membrane depends on length and degree of unsaturated fatty acids. Membrane PL contains essential fatty acids. In case of deficiency of EFA, other fatty acids replace them in the membrane; as a result membrane gets modified structurally and functionally.
2. They are required for the synthesis of PL, cholesterol ester and lipoproteins
3. Poly unsaturated fatty acids are released from membranes, diverted for the synthesis of prostaglandins, leukotriens and thromboxanes.
4. They act as fat mobilizing agents in liver and protect liver from accumulating fats (fatty liver).

| Fatty acid | M.P |
|---|---|
| 18 C saturated fatty acid | – Stearic acid 690 C |
| 18 C unsaturated fatty acid | – Oleic acid (one double bond) 130 C |
| 18C unsaturated fatty acid | – Linoleic acid (two double bonds) - 170 C |

**TRIACYLGLYCEROLS**

These are esters of fatty acids with the alcohol glycerol, which are storage forms of lipids (depot lipids). Triacylglycerols or also called as triacyl glycerides, exist as simple or mixed types depending on the type of fatty acids that form esters with the glycerol. Both saturated and/or unsaturated fatty acids can form the ester linkage with the backbone alcohol. Eg. Tripalmitate, Triolein.

- Tristearin is a chief component of beef lipid.
- Butter has short chain fatty acids.
- Unsaturated fatty acids are sensitive to air and oxidized to give rancid smell.
- Triacylglycerols are mainly found in special cells called adipocytes (fat cells), of the mamary gland, abdomen and under skin of animals. They produce twice as much energy as that of carbohydrates per gram.

**STRUCTURE OF LIPIDS**

- Phosphatidate is the parent compound for the formation of the different glycerophospholipids. To the phosphate group different head alcohol may be attached.
- If choline is attached it is called phosphatidyl choline (lecithin), if ethanolamine is attached it is called phosphatidyl ethanolamine.
- The second largest membrane lipids are sphingolipids, which contain two non-polar and one polar head groups.
- Their alcohol is the amino alcohol sphingosine. Sphingolipids have subclasses viz., sphingomyelins, cerebrosides and gangliosides.
- Out of these only sphingomyelins contains phosphorus.

**SPHINGOLIPIDS**

Sphingolipids are also important components of membranes. They however, do not contain glycerol.

- Instead they contain an 18 C amino alcohol called sphingosine or its few derivatives. Sphingolipids are important components in nerve cell membranes where they protect and insulate the nerve fibres.

**WAXES**

- Waxes are water insoluble substances present on the surface of plant and animal bodies. They include a variety of compounds such as hydrocarbons, alcohols, ketones, and carboxylic acids and most importantly the esters of long the chain monohydroxy alcohols (24 to 28 C) with fatty acids (20 to 24 C).
- Waxes are used as carriers in cosmetics and a variety of coloring materials. Waxes contain many unusual fatty acids.
- Waxes are water repelling and impart a protective function against degradation by water. Shining appearance of fruits, leaves, and flower petals is often due to the presence of waxes. Lotus leaves contain high amounts of waxes.
- Examples of natural waxes are bees wax, jojoba wax, ear wax etc.

**FATTY ACID GANGLIOSIDES**:

These are glycolipids most of which are complex containing oligomers of sugars on head groups. One unit shall definitely be N-acetyl neuraminic acid (sialic acid) 6% of grey brain matter is ganglioside.

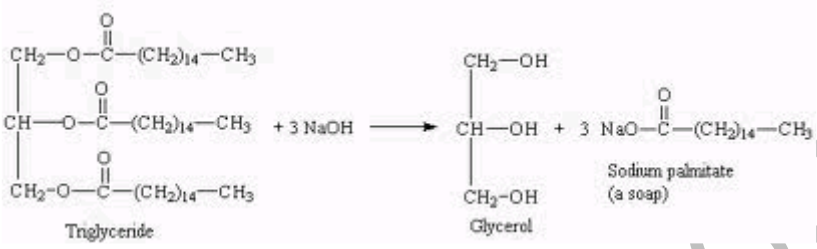
**CEREBROSIDES:-** These are glycolipids which have no phosphate group but neutral head group and contain one or two sugar groups usually glucose or Galactose .

- Dipalmitoyl choline (lecithin) acts as surfactant and lowers the surface tension in alveoli of lungs.
- Lecithin along with sphingomyelin maintains the shape of alveoli and prevents their collapse due to high surface tension of the surrounding medium.
- Some premature infants can't secrete lecithin; therefore, suffer from respiratory distress syndrome.
- Intra cellular signals (for second messengers) like inositol triphosphate and diacylglycerol are generated from membrane PL, during the action of hormones.
- PL anchors certain proteins to cell membranes. PL being amphipathic can interact with nonpolar and polar substances. They link proteins to nonpolar membranes.
- Solubilization of cholesterol is done by amphipathic nature of PL.
- Lipids are transported as lipoproteins, which require PL

## ANALYTICAL PROPERTIES OF LIPIDS:

### SAPONIFICATION

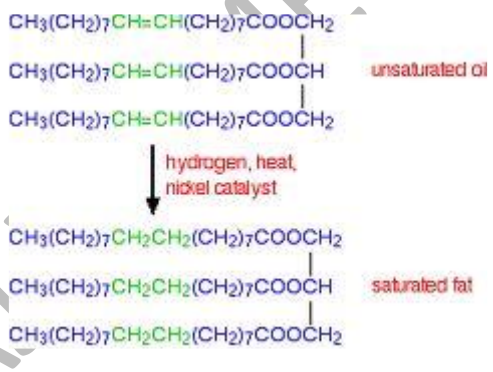
The term 'Saponification' refers to the alkaline hydrolysis of saponifiable lipids - most importantly the fats and oils. In the case of fats and oils, the products are glycerol and the salts of the respective fatty acids.




Thus, R1 COONa, R2 COONa, and R3 COONa, are the sodium salts of the fatty acids. The reaction is utilized in the making of soaps.

### HYDROGENATION

Hydrogenation refers to the addition of hydrogen to the double bonds of the fatty acids in acyl lipids like triacyl glycerols.




The double bonds thus get saturated and the constituent fatty acids are converted to the appropriate saturated fatty acids. Thus, the addition of hydrogen makes the unsaturated fatty acids saturated and the liquid oil gets converted to a solid fat. The reaction process is used industrially in the making of Vanaspathi from edible or even unconventional oils.

### SAPONIFICATION NUMBER

Saponification Number is defined as the number of milligrams of KOH required to saponify 1 g fat or oil. Saponification Number indicates the molecular weight or chain length of the fatty acids present. If the number is high, the molecular weight or chain length of the fatty acids is low and vice versa.

**IODINE VALUE / NUMBER**

Fats and oils do have double bonds in their fatty acids and thus respond to the addition of iodine. The latter adds to the double bonds as follows. Iodine Number is defined as the number of grams of iodine absorbed by 100 g fat or oil. Iodine Number of fats / oils / acyl lipids indicates their degree of unsaturation. If the number is high, the degree of unsaturation is also high i.e. more USFAs are present.

**ACID VALUE / NUMBER**

Acid Number/Value is defined as the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g fat or oil. The free fatty acids may arise due to chemical or microbiological decomposition of the oils or fat during storage i.e. rancidity. Acid Number / Value is used to assess the degree of spoilage (rancidity) of a fat or oil.

**INDUSTRIAL APPLICATIONS OF ACYL LIPIDS:**

**1. SOAPS AND DETERGENTS:** Soaps and detergents are the most important cleansing agents in personal hygiene, hair care, dish washing and laundry. . Soaps are the alkali metal salts of fatty acids. Since all of them contain an acyl group, acyl lipids mostly in the form of unconventional vegetable oils are used in the manufacture of soaps and detergents.

**2. PAINTS:** The paint industry uses acyl lipids in the form of vegetable oils as carriers of colorizing substances. Such oils are drying oils and the special feature is that coloured organic substances are readily dispersed / dissolved in such oils. After application to the surface, the oil gets oxidized by oxygen to form a thin film of solid layer keeping the colorizing matter also in the form of a thin film along with. Among such oils, the linseed oil is the most important. Such oils used in making paints must be highly unsaturated.

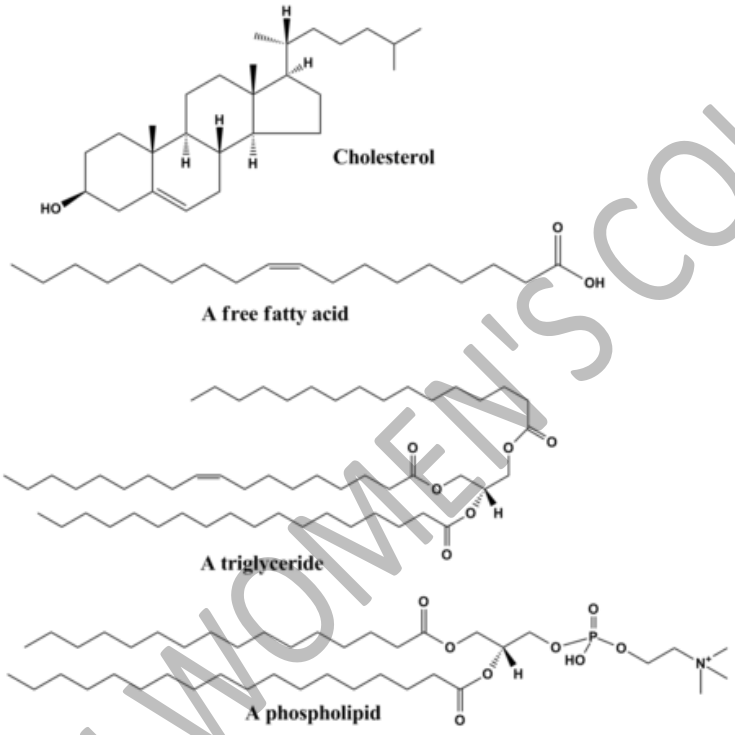
**3. RUBBER:** Natural rubber is obtained from the latex (milky secretion) of a tree Hevea brasiliensis. There are few other species also. Rubber is highly elastic, water repellent, resistant to weak acids and alkalies, tough, impermeable, adhesive and insulator. These characteristics make rubber a very useful in industries. Natural rubber in its latex form however, as such can not be used. To make it usable, it has to be vulcanized. In other words, the naturally occurring polyterpene rubber forms the basic material for the manufacture of better usable synthetic rubber.

**4.BIODIESEL:** Biodiesel is a partial substitute for petroleum diesel and can be blended with the latter. Chemically, biodiesel is monoalkyl esters of fatty acids produced from vegetable oils by transesterification. In India, biodiesel is produced from the oils of Jatropha (wild castor, Jatropha curcus), Karanj (Pongamia pinnata) and few other species. Soybean oil, rape seed oil etc are also used.

**CHOLESTEROL**

Compounds containing 27 carbon cyclopentanoperhydrophenanthrene structures with four rings labeled A to D.

- Steroids are complex fat-soluble molecules, which are present in the plasma lipoproteins and outer cell membrane.
- Cholesterol is one of the important non fatty acid lipid that is grouped with steroids.




- Cholesterol is important in many ways:
- For the synthesis of bile salts that are important in lipid digestion and absorption
- For the synthesis of steroid hormones that are biologically important like the sex hormones estrogen and progesterone.
- For the synthesis of vitamin D3
- As a structural material in biological membranes
- As a component of lipoproteins as transport forms of lipid based energy.

**LOW-DENSITY LIPOPROTEIN**

**Low-density lipoprotein (LDL**) is one of the five major groups of lipoprotein that transport all fat molecules around the body in extracellular water. These groups, from least dense to most dense, are chylomicrons (aka ULDL by the overall density naming convention), very

low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL delivers fat molecules to cells. LDL is involved in atherosclerosis, a process in which it is oxidized within the walls of arteries.

**STRUCTURE**

- Each native LDL particle enables emulsification, i.e. surrounding the fatty acids being carried, enabling these fats to move around the body within the water outside cells.
- Each particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein that has 4536 amino acid residues and a mass of 514 kDa), along with 80 to 100 additional ancillary proteins.
- Each LDL has a highly hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and hundreds to thousands (about 1500 commonly cited as an average) of esterified and unesterified cholesterol molecules.
- This core also carries varying numbers of triglycerides and other fats and is surrounded by a shell of phospholipids and unesterified cholesterol, as well as the single copy of Apo B-100. LDL particles are approximately 22 nm (0.00000087 in.) to 27.5 nm in diameter and have a mass of about 3 million Daltons.
- Since LDL particles contain a variable and changing number of fatty acid molecules, there is a distribution of LDL particle mass and size. Determining the structure of LDL has been a tough task because of its heterogeneous structure.

LDL particles are formed when triglycerides are removed from VLDL by the lipoprotein lipase enzyme (LPL) and they become smaller and denser (i.e. fewer fat molecules with same protein transport shell), containing a higher proportion of cholesterol esters.

**TRANSPORT INTO THE CELL**

- When a cell requires additional it synthesizes the necessary LDL receptors as well as PCSK9, a proprotein convertase that marks the LDL receptor for degradation.
- LDL receptors are inserted into the plasma membrane and diffuse freely until they associate with clathrin-coated pits. When LDL receptors bind LDL particles in the bloodstream, the clathrin-coated pits are endocytosed into the cell.
- Vesicles containing LDL receptors bound to LDL are delivered to the endosome. In the presence of low pH, such as that found in the endosome, LDL receptors undergo a conformation change, releasing LDL. LDL is then shipped to the lysosome, where cholesterol esters in the LDL are hydrolysed.
- LDL receptors are typically returned to the plasma membrane, where they repeat this cycle.
- If LDL receptors bind to PCSK9, however, transport of LDL receptors is redirected to the lysosome, where they are degraded.

**LDL size patterns**

- LDL can be grouped based on its size: large low density LDL particles are described as pattern A, and small high density LDL particles are pattern B. Pattern B has been associated by some with a higher risk for coronary heart disease.
- This is thought to be because the smaller particles are more easily able to penetrate the endothelium of arterial walls. Pattern I, for intermediate, indicates that most LDL particles are very close in size to the normal gaps in the endothelium (26 nm).
- According to one study, sizes 19.0–20.5 nm were designated as pattern B and LDL sizes 20.6–22 nm were designated as pattern A. Other studies have shown no such correlation at all.
- Some evidence suggests the correlation between Pattern B and coronary heart disease is stronger than the correspondence between the LDL numbers measured in the standard lipid profile test. Tests to measure these LDL subtype patterns have been more expensive and not widely available, so the common lipid profile test is used more often.
- There has also been noted a correspondence between higher triglyceride levels and higher levels of smaller, denser LDL particles and alternately lower triglyceride levels and higher levels of the larger, less dense ("buoyant") LDL.

**ESTIMATION OF LDL PARTICLES VIA CHOLESTEROL CONTENT**

- Chemical measures of lipid concentration have long been the most-used clinical measurement. The lipid profile does not measure LDL particles. It only estimates them using the Friedewald equation. by subtracting the amount of cholesterol associated with other particles, such as HDL and VLDL, assuming a prolonged fasting state, etc.

- where H is HDL cholesterol, L is LDL cholesterol, C is total cholesterol, T are triglycerides, and k is 0.20 if the quantities are measured in mg/dL and 0.45 if in mol/L.

**HIGH-DENSITY LIPOPROTEIN**

**High-density lipoprotein (HDL)** is one of the five major groups of lipoproteins.

- Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells.
- They are typically composed of 80–100 proteins per particle (organized by one, two or three ApoA).
- HDL particles enlarge while circulating in the blood, aggregating more fat molecules and transporting up to hundreds of fat molecules per particle.

- Lipoproteins are divided into five subgroups, by density/size (an inverse relationship), which also correlates with function and incidence of cardiovascular events.
- Unlike the larger lipoprotein particles, which deliver fat molecules to cells, HDL particles remove fat molecules from cells. The lipids carried include cholesterol, phospholipids, and triglycerides, amounts of each are variable.
- Increasing concentrations of HDL particles are associated with decreasing accumulation of atherosclerosis within the walls of arteries, reducing the risk of sudden plaque ruptures, cardiovascular disease, stroke and other vascular diseases.
- HDL particles are commonly referred to as "good cholesterol", because they transport fat molecules.
- out of artery walls, reduce macrophage accumulation, and thus help prevent or even regress atherosclerosis.
- However, recent investigations have shown that very high concentrations of HDL particles can be associated with an increased mortality risk and an increased cardiovascular risk, especially in hypertensive patients.

**STRUCTURE AND FUNCTION:**

- With a size ranging from 5 to 17 nm, HDL is the smallest of the lipoprotein particles. It is the densest because it contains the highest proportion of protein to lipids. Its most abundant apolipoproteins are apo A-I and apo A-II. A rare genetic variant, ApoA-1 Milano, has been documented to be far more effective in both protecting against and regressing arterial disease, atherosclerosis.
- A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of the lipoprotein particle, eventually causing the newly synthesized HDL to assume a spherical shape.
- HDL particles increase in size as they circulate through the blood and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins, such as by interaction with the ABCG1 transporter and the phospholipid transport protein (PLTP).
- HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways.
- HDL is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. In humans, probably the most relevant pathway is the indirect one, which is mediated by cholesteryl ester transfer protein (CETP).
- This protein exchanges triglycerides of VLDL against cholesteryl esters of HDL. As the result, VLDLs are processed to LDL, which are removed from the circulation by the LDL receptor pathway.

- The triglycerides are not stable in HDL, but are degraded by hepatic lipase so that, finally, small HDL particles are left, which restart the uptake of cholesterol from cells.
- The cholesterol delivered to the liver is excreted into the bile and, hence, intestine either directly or indirectly after conversion into bile acids.
- Delivery of HDL cholesterol to adrenals, ovaries, and testes is important for the synthesis of steroid hormones.

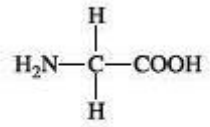
# UNIT –II

# PROTEINS AND AMINO ACIDS

## PROTEINS

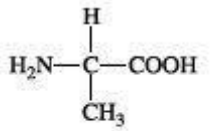
Proteins are made up of different amino acids. Amino acids: In amino acids, there are two functional groups: an amino group and a carboxylic group. Both these groups are attached to the α carbon atom only. Amino acids are alpha (α) amino carboxylic acids. The carbon atom is tetrahedral in shape. The various groups attached to it are placed in different positions. Since the valence of the carbon atom is four, four groups can be attached to the carbon atom. Based on the groups attached to the carbon atom it. There are of two types.

**1. Symmetric carbon atom:** When the valence of the carbon is satisfied by more than one similar atoms/ group then the particular carbon atom is called as symmetric carbon atom. Eg : Glycine



glycine
(Gly, G)

Compounds containing symmetric carbon atoms are optically inactive since they cannot rotate the plane of polarized light. 2. Asymmetric carbon atom: When the valence of the carbon is satisfied by four different groups, then that particular carbon atom is called as asymmetric carbon atom. Eg: Alanine



alanine
(Ala, A)

In amino acids, to α carbon atom, an amino group, a carboxylic group and a hydrogen atom are attached and the fourth group is the R group. This R group varies for each amino acid. All amino acids except glycine have at least one asymmetric carbon atom, hence they are optically active.

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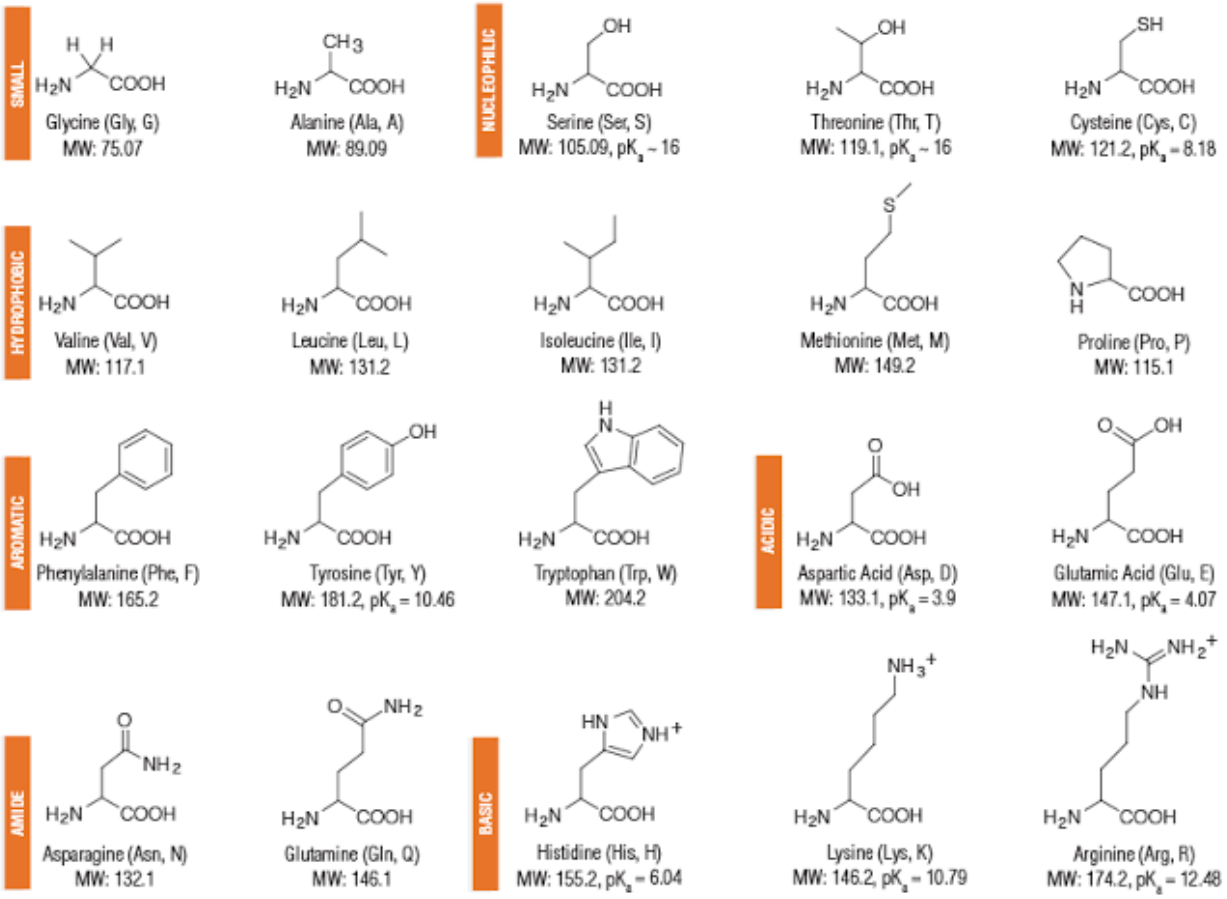
each amino acid. All amino acids except glycine have at least one asymmetric carbon atom, hence they are optically active.

**Classification of amino acids:** Amino acids can be classified in various ways.

1. Based on side chains: Based on the structure of the R groups, all the amino acids are classified as aliphatic, aromatic and heterocyclic amino acids.




**2. BASED ON THEIR PRESENCE OR ABSENCE IN PROTEINS:**

Amino acids are classified as protein amino acids and non-protein amino acids.

**a) Protein amino acids:** - Amino acids that are used for synthesis of proteins are called protein amino acids. All the above mentioned 20 amino acids are present in proteins.

**b) Non protein amino acids:** Apart from the 20 amino acids that are present in proteins, several non protein amino acids are also present in nature. These are obtained by slight modification of 20 protein amino acids.

Eg:- beta alanine, hydroxy proline, N- acetyl glutamic acid etc

**3. BASED ON REQUIREMENT TO THE BODY AS ESSENTIAL AND NON-ESSENTIAL:** Animals cannot synthesis all the 20 amino acids that are present in proteins. Some have to be

provided to the body through external diet. The amino acids which cannot be synthesized by the body, which have to be supplied through diet are called essential amino acids. On the other hand, some amino acids can be synthesized by the body, and they are called as non essential amino acids.

| Essential amino acids | Non essential amino acids |
|---|---|
| Methionine | Alanine |
| Arginine | Asparatic acid |
| Threonine | Glutamatic acid |
| Tryptophan | Cysteine |
| Valine | Glycine |
| Isoleucine | Proline |
| Leucine | Serine |
| Phenylalanine | Tyrosine |
| Lysine | |
| Histidine is essential for children only | |

**4. Based on polarity of the side chains:** This is the most accepted form of classification of amino acids which is based on polarity and hydrophobic nature of R groups.

a) Nonpolar or hydrophobic: The R groups of these amino acids are less soluble in water, or hydrophobic, than those of polar, because they contain bulky side chains. These amino acids play a major role in promoting hydrophobic interactions within protein structures. Eg: Glycine, Alanine, Valine, Leucine, Isoleucine, Proline. Phenylalanine, Tyrosine and Tryptophan.

b) Polar uncharged amino acids: The R groups of these amino acids are more soluble in water, or hydrophilic, than those of non polar, because they contain functional groups that form hydrogen bonds with water. These amino acids possess oxygen, sulfur and/or nitrogen in the side chain and are therefore polar.

The R group of these amino acids cannot be ionised and thus do not carry an overall charge. These amino acids readily interact with water. Eg: Cysteine, Methionine, Serine, Threonine, Asparagine and Glutamine

c) Polar amino acids with positively charged side chains: The R groups of these amino acids are not only polar but they also carry a positive charge and are therefore highly hydrophilic. Eg: Lysine, Histidine and Arginine. They are also called as basic amino acids as they can easily accept a proton.

d) Polar amino acids with negatively charged side groups: The R group of these amino acids is not only polar but they also carry a negative charge. Eg : Aspartic acid and Glutamic acid. They are also called as acidic amino acids as they can easily donate a proton.

**Reactions of amino acids**

**1.Ninhydrin test:** Ninhydrin is an oxidizing agent which oxidatively deaminates the alpha-amino groups of amino acids. It is very important for the detection and the quantitative analysis of amino acids. Ninhydrin also reacts with primary amines. However the formation of carbon dioxide is quite diagnostic for amino acids. Alpha amino acids yield a purple substance (Ruhemann's purple) that absorbs maximally at 570 nm. The reduced ninhydrin then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple colored compound known as Ruhemann's purple.

**2. Peptide bond formation:-**Amino acids are linked together by formation of covalent bonds. The covalent bond is formed between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a peptide bond and the compound formed is a peptide.

A peptide is a chain of amino acids linked together by peptide bonds. Proteins on partial hydrolysis yield the polypeptides and oligopeptides. Polypeptides are usually long peptides whereas oligopeptides are short (< 10 amino acids). Proteins are made up of one or more polypeptides with more than 50 amino acids.

**Nomenclature of the peptides:-**

In a peptide, always the first amino acid has its N terminal free. It will not be involved in the formation of a peptide bond, but the carboxyl group of first amino acid and amino group of second amino acid are involved in the formation of the peptide bond. While naming a peptide, the first amino acid is usually named by adding yl and the second amino acid as it is.

**Example:**


Peptides can be classified based on the structure as linear or cyclic peptides and based on number of amino acids involved in the formation of the peptide.

**Based on structure:**

1. Linear peptide: - When a peptide has its structure in the form of linear structure it is called as linear peptide. Eg: Glutathione and insulin. Number of peptide bonds present in a linear peptide = (n – 1) where n represents the number of amino acids.

a) Glutathione: It is a small molecule made up of three amino acids, which exists in almost every cell of the body. This is called as natural redox tripeptide. There is an unusual peptide bond present between glutamic acid and cysteine and glycine. It is chemically called as gamma glutamyl cysteinyl glycine.

Cyclic peptides: If the carboxyl group at the C-terminus of a peptide forms a peptide bond with the N-terminal amino group, a cyclic peptide is formed. Cyclic peptide has its structure in the form of a ring. The number of peptide bonds in a cyclic peptide can be calculated by the formula No of peptide bonds = n, where n= no of amino acids presents in a peptide Cyclic peptide are most commonly found in microorganisms, and often incorporate some D-amino acids as well as unusual amino acids such as ornithine (Orn).

**PROTEINS**

The word protein was first coined in 1838 to emphasize the importance of this class of molecules. The word is derived from the Greek word proteios which means "of the first-rank. Proteins are polymers of several amino acids. They are folded into specific defined structures, which are maintained by large number of relatively weak bonds. Very small changes in the structure can modify the function. Hence, it is important to study the structure of protein in detail. The biological activity of proteins depends on maintenance of folded conformation.

Proteins fold into well-defined three-dimensional shapes and they are able to recognize their corresponding substrates or antigen molecules and bind them tightly. The protein structure has been classified into four different levels based on the folded confirmation of the protein.

- Primary structure
- Secondary structure

- Tertiary structure
- Quaternary structure

**Primary structure:** Primary structure is the simplest level of structural organization. The sequence of the different amino acids in a protein is called the primary structure of the peptide or protein. Though it is the simplest level of structural organization, in some aspects it is very important. The conformation and function of a protein are determined by the primary structure. Even a change in one amino acid residue may adversly affect the biological activity of protein.

Eg: Hemoglobin is made up of four polypeptide chains, two chains of α type and two chains of β type. When glutamic acid present at the 6th position from Ncterminal end in the β chain is replaced by valine, Hemoglobin-S is formed and causes sickle cell anemia by which there is reduction in capacity to carry oxygen by hemoglobin. In a polypeptide, numbering of residues always starts at the N terminal N- terminalend (NH2-group), where the amino group is not involved in a peptide bond formation.

**Secondary structure:** The secondary structure of protein refers to the conformational patterns of the polypeptide chain. Different types of secondary structures occur widely in proteins. The most prominent are the alpha helix and beta conformations. Linus Pauling and Robert Corey predicted the existence of these secondary structures in 1951. In general, proteins fold into two broad classes of structures, namely globular proteins and fibrous proteins. Globular proteins are compactly folded and coiled, whereas, fibrous proteins are more filamentous or elongated.

**The α-Helix:** The α-helix is a common secondary structure encountered in proteins of the globular class. The formation of the α-helix is spontaneous and is stabilized by H-bonding between amide nitrogen and carbonyl carbons of peptide bonds spaced four residues apart.

**FEATURES OF A-HELIX:**

a) In this, the polypeptide backbone is tightly wound around an imaginary axis drawn longitudinally through the middle of the helix and R groups protrude outward from the helical back bone.

b) Single turn of the helix has 5.4 Amino acids and it is called as the pitch of the helix.

c) There are 3.6 amino acids residues per turn of the helix.

d) Distance between the peptide bonds is 1.5 A every first and the fourth amino acids of the helix.

e) The helix structure is maintained by the hydrogen bond formed between There are three types of α- helices based on the direction and the nature i.e.: left handed α- helix, right handed α- helix and triple helix Eg: collagen

f) The hydrogen bonds are intra molecular and are parallel to the central axis.

**β - Pleated Sheets:** A α-helix is composed of a single linear array of helically disposed amino acids and β-sheets are composed of 2 or more different regions of stretches of at least 5-10 amino acids. The folding and alignment of stretches of the polypeptide backbone aside one another to form β-sheets is stabilized by hydrogen bonding between amide nitrogen and carbonyl carbons. However, the hydrogen bonding residues are present in adjacently opposed stretches of the polypeptide backbone as opposed to a linearly continuous region of the backbone in the α-helix.

B-sheets are said to be pleated. This is due to positioning of the α-carbons of the peptide bond which alternates above and below the plane of the sheet. B-sheets are either parallel or antiparallel. In parallel sheets, adjacent peptide chains proceed in the same direction (i.e. the direction of N-terminal to C-terminal ends is the same), whereas, in antiparallel sheets adjacent chains are aligned in opposite directions. B- Sheets can be depicted in ball and stick format or as ribbons in certain protein formats.

**TERTIARY STRUCTURE:** Tertiary structure refers to the complete three-dimensional structure of the polypeptide units of a given protein. It is the spatial relationship of different secondary structures to one another within a polypeptide chain and how these secondary structures themselves fold into the three-dimensional form of the protein. The tertiary structure is maintained by different forces. These include hydrogen bonding, hydrophobic interactions, electrostatic interactions and Van der Waals forces.

**HYDROGEN BONDING:** Polypeptides contain numerous proton donors and acceptors both in their backbone and in the R-groups of the amino acids. The environment in which proteins are found also contains the ample H-bond donors and acceptors of the water molecule. H-bonding, therefore, occurs not only within and between polypeptide chains but with the surrounding aqueous medium.

**HYDROPHOBIC FORCES:** Proteins are composed of amino acids that contain either hydrophilic or hydrophobic R-groups. It is the nature of the interaction of the different R-groups with the aqueous environment that plays the major role in shaping protein structure. The hydrophobicity of certain amino acid R-groups tends to drive them away from the exterior of proteins into the interior. This driving force restricts the available conformations into which a protein may fold.

**ELECTROSTATIC FORCES:** Electrostatic forces refer to the interaction of ionized R groups of amino acids with the dipole of the water molecule. The slight dipole moment that exists in the polar R-groups of amino acid also influences their interaction with water. It is, therefore, understandable that the majority of the amino acids found on the exterior surfaces of globular proteins contain charged or polar R-groups.

**VAN DER WAALS FORCES:** There are both attractive and repulsive Van der Waals forces that control protein folding. Attractive Van der Waals forces involve the interactions among

induced dipoles that arise from fluctuations in the charge densities that occur between adjacent uncharged non-bonded atoms. Repulsive van der Waals forces involve the interactions that occur when uncharged nonbonded atoms come very close together but do not induce dipoles.

**Quaternary Structure:** Many proteins contain 2 or more different polypeptide chains that are held in association by the same non-covalent forces that stabilize the tertiary structures of proteins. Proteins with multiple polypetide chains are 26 oligomeric proteins. The structure formed by monomer-monomer interaction in an oligomeric protein is known as quaternary structure.

Oligomeric proteins can be composed of multiple identical polypeptide chains or multiple distinct polypeptide chains. Proteins with identical subunits are termed homo-oligomers. Eg: Acetylcholine receptor. Proteins containing several distinct polypeptide chains are termed hetero-oligomers. Hemoglobin, the oxygen carrying protein of the blood, contains two α and two β subunits arranged with a quaternary structure in the form, α2β2. Hemoglobin is, therefore, a heterooligomeric protein.

**LEVELS OF ORGANIZATION OF PROTEIN STRUCTURE**

**Properties of proteins:**

**1. U.V absorption:** Proteins absorb U.V radiation at 280 nm because of the presence of aromatic amino acids like tryptophan and tyrosine. This property is used in estimation of proteins.

**2. Isoelectric point:** Isoelectric point is also called as isoelectric pH. This is the pH at which the number of positive and negative charges is equal in the protein and they are electrically neutral. Solubility of proteins is least at isoelectric pH.

**3. Zwitterions:** Proteins contain both positive and negative charges and hence they are called as zwitterions. Amino acids will act as zwitterions as they can donate a proton and forms cation. They can as well accept a proton and forms an anion. Each amino acid can act as an anion, cation, neutral species and as zwitterion.

**4. Immunological properties:** Proteins exhibit a special property called immunological property, which is useful in defense mechanism. When Denaturation: It is a physical change in which there is a collapse of protein structure. Due to denaturation, there is a decrease in solubility and loss of biological activity of proteins.

Denaturation occurs at extreme temperatures and pH and also by many chemicals like organic solvents, urea, ionic detergents etc. On denaturation, non covalent bonds in the protein are broken and its primary structure remains intact. When the favorable conditions are provided, some peoteins ie: a reversibly denatured protein, will spontaneously return to its native biologically active form and this process is called renaturation.

**Protein folding:** Many proteins fold to their native conformation on their own by self-assembly. However, several other accessory proteins help in this process. They include a) Enzymes: Eg: Peptidyl prolyl cis-trans isomerase introduces reverse bends.

**Molecular chaperons:** They are a special class of proteins which will help in the folding of other proteins. Molecular chaperons will identify the improperly folded proteins and provide a microenvironment in which a polypeptide can progressively fold itself. Molecular chaperons will not impose a structure to proteins but only provide the required environment to the protein. They belong to heat shock protein family which protects polypeptide from denaturation and aggregation at high temperature ever any antigen enters into the body, immediately body releases a special class of proteins called as defense antibodies. The interaction of antigen and antibody to form the antigen-anti body complex is called immune reaction. Antigen may be a protein (protein coat of virus), or a carbohydrate (sugars on the bacterial outer coat) or nucleic acid.

Antibodies are special glycoproteins which will recognize and bind antigens. 7. Solubility: Protein solubility is influenced by pH, heavy metals, salts and organic solvents.

**a) pH:** Solubility of proteins is minimum at isoelectric point (PI). At acidic PH, proteins behave like cations. Anion forms of some compounds like Trichloroacetic acid are effective in precipitating the proteins. Solubility is influenced by the presence of polar hydrophilic groups on the surface.

**b) Heavy metals:** At alkaline pH, proteins behave like anions. Cationic forms of some heavy metals such as Mercury and Lead are attracted by the negative charges present on the free side chains of proteins and precipitate them.

**c) Salts:** By the addition of small quantity of salt like ammonium sulphate or sodium chloride, solubility of protein is usually increased due to increased ionic strength of the solution. On further increase of salt, the solubility decreases.

**d) Organic solvents:** Organic solvents like ethanol, acetone and butanol lower the dielectric constant of the medium and decreases the solubility.

**Sequencing of amino acids:** There are several effective methods by which a polypeptide end groups may be identified. The most effective method in identification of N- terminal residue is Edman degradation method named after its inventor Pehr Edman.

**Purification techniques:** The following techniques are used for purification of proteins.

**a) Salting in and salting out:**

Increase in the solubility of a protein by addition of small quantities of sodium chloride is called as salting in and this is due to increasing the ionic strength of the solution. On the other hand, when excess of salt is added to the solution, there is decrease in the solubility and this is called as salting out.

Salting out occurs due to hydrophobic effect. There are hydrophobic amino acids and hydrophilic amino acids in protein molecules. The hydrophobic amino acids generally are present in the interior of the protein but some are present on the surface also in small patches. Water molecules become ordered, when they are forced to interact with these patches. When the salt concentration is increased, a competition develops for the water between the protein and the salt. Some of the water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with the charged part of the protein.

As the salt concentration increases, the water on protein is removed thus exposing the hydrophobic area of protein molecule. These hydrophobic areas on the protein molecule get attracted to each other by hydrophobic effect. This results in increase in weight of the molecule and its aggregation. Larger the surface hydrophobic area on a protein molecule, quicker will be the precipitation of the protein at a lower concentration of the salt.

**b) Dialysis:** Proteins can be separated by dialysis through a semi permeable membrane such as cellulose membrane which has pores in it. Bigger molecules are retained in the bag and smaller molecules pass through the membranes.

**c) Gel filtration or Size Exclusion Chromatography:** This chromatographic technique is based upon the use of a porous gel in the form of insoluble beads placed into a column. When a solution of proteins is passed through the column, a protein of small size is likely to enter the pores. Small proteins can penetrate into the pores of the beads and, therefore, are retarded in their rate of travel through the column. The larger proteins will move through the gaps present in the column and are likely to move fast and are collected first when compared to the smaller ones. Different beads with different pore sizes can be used depending upon the desired protein size separation profile. Polymer beads made up of dextrose, agarose or polyacrylamide are generally used and the commercial names for few polymer beads are Sephadex, Sepharose and Biogel respectively.

**Classification of proteins:** Proteins are conveniently classified on the basis of their functions

Classification of proteins based on function:

**a) Catalytic proteins:** These are enzyme proteins that catalyze chemical and biochemical reactions within living cell and outside. This group of proteins probably is the biggest and most important group of the proteins. Enzymes are responsible for all metabolic reactions in the living cells. Eg: DNA and RNA polymerases, dehydrogenases etc.

**b) Regulatory proteins:** Few hormones are examples of this class of proteins that are responsible for the regulation of many processes in organisms. Eg: Insulin

**c) Transport proteins:** These proteins are involved in transporting some chemical compounds and ions. Eg: Haemoglobin, myoglobin

**d) Defence proteins:** These proteins are involved in the defence mechanism of the cell. Eg: Gamma globulins.

**e) Structural proteins:** These proteins are involved in maintaining the structure of other biological components like cells and tissues. Eg: Collagen, elastin.

**f) Contractile proteins:** These proteins are involved in contraction of the tissues. Ex: Actin and myosin are responsible for muscular motion.

**g) Storage proteins:** These proteins contain energy, which can be released during various metabolic processes in the organism. Ex: Egg ovalbumin, milk casein.

**h) Receptor proteins:** These proteins act as receptor molecules. They are responsible for signal detection and translation into other type of signal. Ex: GTPases.

Proteins are also divided into two classes based on the solubility in water.

a. Globular proteins which are soluble proteins are made up both hydrophobic and hydrophilic amino acids. In these proteins, hydrophobic amino acids are present internally.

b. Fibrous proteins which are insoluble proteins are made up of mostly hydrophobic amino acids.

**Protein quality evaluation methods:**

Proteins are vital to the living processes and carry out a wide range of functions essential for the sustenance of life. In judging the adequacy of dietary proteins to meet the human needs, not only the quantity, but also the nutritional quality of the dietary protein is important. Proteins present in different foods vary in their nutritional quality because of differences in their amino acid composition. The quality of a protein is evaluated by the following methods.

**Biological methods:** Proteins of raw grains (particularly legumes) are less digestible than that of animal foods. Generally, the low digestibility of plant proteins is due to the presence of trypsin inhibitors which are destroyed on cooking. The overall quality of a protein can be determined by biological methods with laboratory animals like rats as follows;

# UNIT – III

# METABOLISM OF CARBOHYDRATES

**Digestion of Carbohydrates**

Dietary carbohydrates principally consist of the polysaccharides: starch and glycogen. It also contains disaccharides: sucrose, lactose, maltose and in small amounts monosaccharides like fructose and pentoses.

Liquid food materials like milk, soup, fruit juice escape digestion in mouth as they are swallowed, but solid foodstuffs are masticated thoroughly before they are swallowed.

1. Digestion in Mouth Digestion of carbohydrates starts at the mouth, where they come in contact with saliva during mastication. Saliva contains a carbohydrate splitting enzyme called salivary amylase (ptyalin). Action of ptyalin (salivary amylase) It is α - amylase, requires Cl- ion for activation and optimum pH 6-7. The enzyme hydrolyzes α- (1,4) glycosidic linkage at random, from molecules like starch, glycogen and dextrins, producing smaller molecules maltose, glucose and disaccharides maltotriose. Ptyalin action stops in stomach when pH falls to 3 Starch or glycogen

2. Digestion in Stomach No carbohydrate splitting enzymes are available in gastric juice. HCl may hydrolyze some dietary sucrose to equal amounts of glucose and fructose.

3. Digestion in Duodenum Food reaches the duodenum from stomach where it meets the pancreatic juice. Pancreatic juice contains a carbohydrate-splitting enzyme pancreatic amylase.

Action of pancreatic Amylase It is also an α - amylase, optimum pH 7.1. Like ptyalin it also requires Cl- for activity. The enzyme hydrolyzes α-(1,4) glycosidic linkage situated well inside polysaccharide molecule. Other criteria and end products of action are similar of ptyalin.

1. Digestion in Small Intestine Action of Intestinal Juice a. pancreatic amylase: It hydrolyzes terminal α-(1,4), glycosidic linkage in polysaccharides and Oligosaccharide molecules liberating free glucose molecules. b. Lactase It is a β- glycosidase, its pH range is 5.4 to 6.0. Lactose is hydrolyzed to glucose and galactose. Lactose.

**DIABETES MELLITUS / HYPERGLYCEMIA**

- Diabetes mellitus is taken from the Greek word diabetes, meaning siphon - to pass through and the Latin word mellitus meaning sweet.
- A review of the history shows that the term "diabetes" was first used by Apollonius of Memphis around 250 to 300 BC. Ancient Greek, Indian, and Egyptian civilizations

discovered the sweet nature of urine in this condition, and hence the propagation of the word Diabetes Mellitus came into being.

- Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use, etc.
- Symptoms of diabetes may include increased urination and thirst, and people may lose weight even if they are not trying to.
- Diabetes can damage nerves and cause problems with the sense of touch.
- Diabetes can damage blood vessels and increase the risk of heart attack, stroke, chronic kidney disease, and vision loss.
- Doctors diagnose diabetes by measuring blood sugar levels.
- People with diabetes need to follow a healthy diet that is low in refined carbohydrates (including sugar), saturated fat, and processed foods. They also need to exercise, maintain a healthy weight, and usually take medications to lower blood sugar levels and promote weight loss if their weight is above a healthy level.

**Blood sugar**

- The three major nutrients that make up most food are carbohydrates, proteins, and fats. Sugars are one of three types of carbohydrates, along with starch and fiber.
- There are many types of sugar. Some sugars are simple, and others are complex. Table sugar (sucrose) is made of two simpler sugars called glucose and fructose. Milk sugar (lactose) is made of glucose and a simple sugar called galactose.
- Once the body absorbs simple sugars, it usually converts them all into glucose, which is an important source of fuel for the body. Glucose is the sugar that is transported through the bloodstream and taken up by cells. The body can also make glucose from fats and proteins. Blood "sugar" really means blood glucose.

**Insulin**

- Insulin, a hormone released from the pancreas (an organ behind the stomach that also produces digestive enzymes), controls the amount of glucose in the blood.
- Glucose in the bloodstream stimulates the pancreas to produce insulin. Insulin helps glucose to move from the blood into the cells.
- Once inside the cells, glucose is converted to energy, which is used immediately, or the glucose is stored as fat or the starch glycogen until it is needed.

- The levels of glucose in the blood vary normally throughout the day. They rise after a meal and return to pre-meal levels within about 2 hours after eating.
- Once the levels of glucose in the blood return to pre-meal levels, insulin production decreases.
- The variation in blood glucose levels is usually within a narrow range, about 70 to 110 milligrams per deciliter (mg/dL).

**Types**

**Prediabetes** is a condition in which blood glucose levels are too high to be considered normal but not high enough to be labeled diabetes. People have prediabetes if their fasting blood glucose level is between 100 mg/dL (5.6 mmol/L) and 125 mg/dL (6.9 mmol/L) or if their blood glucose level 2 hours after a glucose tolerance test is between 140 mg/dL (7.8 mmol/L) and 199 mg/dL (11.0 mmol/L). Prediabetes carries a higher risk of future diabetes as well as heart disease. Decreasing body weight by 5 to 10% through diet and exercise can significantly reduce the risk of developing diabetes.

**Type 1 diabetes**

In type 1 diabetes (formerly called insulin-dependent diabetes or juvenile-onset diabetes), the body's immune system attacks the insulin-producing cells of the pancreas, and more than 90% of them are permanently destroyed. The pancreas, therefore, produces little or no insulin. Fewer than 10% of all people with diabetes have type 1 disease. Most people who have type 1 diabetes develop the disease before age 30, although it can develop later in life.

**Type 2 diabetes**

In type 2 diabetes (formerly called non–insulin-dependent diabetes or adult-onset diabetes), the pancreas often continues to produce insulin, sometimes even at higher-than-normal levels, especially early in the disease. As type 2 diabetes progresses, the insulin-producing ability of the pancreas decreases.

Type 2 diabetes was once rare in children and adolescents but has become more common. However, it usually begins in people older than 30 years and becomes progressively more common with age. About 30% of people older than 65 have type 2 diabetes.

Obesity is the chief risk factor for developing type 2 diabetes, and 80 to 90% of people with type 2 diabetes have overweight or obesity. Because obesity causes insulin resistance, people with obesity may need large amounts of insulin to maintain normal blood glucose levels.

Certain disorders and medications can affect the way the body uses insulin and can lead to type 2 diabetes.

Examples of common states (conditions) that result in impaired insulin use are

- High levels of corticosteroids (most commonly due to use of corticosteroid medications, such as prednisone, or Cushing syndrome)
- Pregnancy (gestational diabetes)

Diabetes also may occur in people with excess production of growth hormone (acromegaly) and in people with certain hormone-secreting tumors. Severe or recurring pancreatitis and other disorders that directly damage the pancreas can lead to diabetes.

**Symptoms**

Many patients with diabetes may have no symptoms, especially in the early phase of the disease. However, the two types of diabetes can have very similar symptoms if the blood glucose is significantly elevated.

The **symptoms of high blood glucose levels** include

- Increased thirst
- Increased urination
- Increased hunger
- When the blood glucose level rises above 160 to 180 mg/dL (8.9 to 10.0 mmol/L), glucose spills into the urine. When the level of glucose in the urine rises even higher, the kidneys excrete additional water to dilute the large amount of glucose.
- Because the kidneys produce excessive urine, people with diabetes urinate large volumes frequently (polyuria).
- The excessive urination creates abnormal thirst (polydipsia). Because excessive calories are lost in the urine, people may lose weight

**Other symptoms of diabetes include**

- Blurred vision
- Drowsiness
- Nausea
- Decreased endurance during exercise

**Type 1 diabetes**

- In people with type 1 diabetes, the symptoms often begin abruptly and dramatically. A serious condition called diabetic ketoacidosis, a complication in which the body produces excess acid, may quickly develop the initial symptoms of diabetic ketoacidosis also include nausea, vomiting, fatigue, and—particularly in children—abdominal pain.
- Breathing tends to become deep and rapid as the body attempts to correct the blood's acidity (see Acidosis), and the breath smells fruity or like nail polish remover. Without treatment, diabetic ketoacidosis can progress to coma and death, sometimes very quickly.

**Type 2 diabetes**

- People with type 2 diabetes may not have any symptoms for years or decades before they are diagnosed.
- Symptoms may be subtle. Increased urination and thirst are mild at first and gradually worsen over weeks or months.
- Eventually, people feel extremely fatigued, are likely to develop blurred vision, and may become dehydrated.
- Because people with type 2 diabetes produce some insulin, ketoacidosis does not usually develop even when type 2 diabetes is untreated for a long time.
- Rarely, the blood glucose levels become extremely high (even exceeding 1,000 mg/dL [55.5 mmol/L]). Such high levels often happen as the result of some superimposed stress, such as an infection or medication use.
- When the blood glucose levels get very high, people may develop severe dehydration, which may lead to mental confusion, drowsiness, and seizures, a condition called hyperosmolar hyperglycemic state.
- Many people with type 2 diabetes are diagnosed by routine blood glucose testing before they develop such severely high blood glucose levels.

**Complications of diabetes**

- Diabetes damages blood vessels, causing them to narrow and therefore restricting blood flow. Because blood vessels throughout the body are affected, people may have many complications of diabetes. Many organs can be affected, particularly the following:
- Brain, causing stroke
- Eyes (diabetic retinopathy), causing blindness

- Heart, causing heart attack or heart failure
- Kidneys (diabetic nephropathy), causing chronic kidney disease
- Nerves (diabetic neuropathy), causing decreased sensation, mainly in the feet and legs

High blood glucose levels also cause disturbances in the body's immune system, so people with diabetes mellitus are particularly susceptible to bacterial and fungal infections.

**Diagonsis**

**Blood glucose measurement:** Diabetes can be diagnosed if fasting blood glucose levels are 126 mg/dL (7.0 mmol/L) or higher.

**Hemoglobin A1C:** Doctors can also measure the level of a protein, hemoglobin A1C (also called glycosylated or glycolated hemoglobin), in the blood, which reflects the person's long-term trends in blood glucose levels rather than rapid changes. People with a hemoglobin A1C level of 6.5% or more have diabetes. If the level is between 5.7 and 6.4, they have prediabetes and are at risk of developing diabetes.

**Oral glucose tolerance test:** Another kind of blood test, an oral glucose tolerance test, may be done in certain situations, such as screening pregnant women for gestational diabetes or testing older adults who have symptoms of diabetes but normal glucose levels when fasting. In this test, people fast, have a blood sample taken to determine the fasting blood glucose level, and then drink a special solution containing a large, standard amount of glucose. More blood samples are then taken over the next 2 to 3 hours and are tested to determine whether the glucose in the blood rises to abnormally high levels.

**Screening for type 1 diabetes**

Screening for type 1 diabetes is not recommended for all children or adults. Doctors sometimes do tests to screen for type 1 diabetes in people at high risk of type 1 diabetes (such as siblings or children of people who have type 1 diabetes). Testing for insulin antibodies allow doctors to identify people with early stage type 1 diabetes and start preventive measures.

**Screening for type 2 diabetes**

- It is important to do screening tests in people at risk of type 2 diabetes, including those who
- Are 35 years or older,Have overweight or obesity,Have a sedentary lifestyle
- Have a family history of diabetes,Have prediabetes.
- Have had diabetes during pregnancy or had a baby who weighed more than 9 pounds (4,000 grams) at birth

- Have high blood pressure,Have a lipid disorder such as high cholesterol,Have cardiovascular disease.
- Have steatotic liver disease (previously called fatty liver disease)
- Have polycystic ovary disease
- Have racial or ethnic ancestry that is associated with high risk,Have HIV infection
- People with these risk factors should be screened for diabetes at least once every three years.
- Exercise,Weight loss,Education ,In type 1 diabetes, insulin injections.
- In type 2 diabetes, often medications by mouth and sometimes insulin or other medications by injection.
- Diet, exercise, and education are the cornerstones of treatment of diabetes. Weight loss is important for people who have overweight. Some people with type 2 diabetes and mildly elevated glucose levels can start with diet, exercise, and weight loss only. However, in people with more severe glucose abnormalities, or in whom lifestyle modification is not sufficient to normalize glucose, diabetes medications are required. People with type 1 diabetes (no matter their blood glucose levels) require medication when first diagnosed.
- Diabetic ketoacidosis and hyperosmolar hyperglycemic state are medical mergencies because they can cause coma and death. Treatment is similar for both and centers around giving intravenous fluids and insulin.

**General treatment of diabetes**

- People with diabetes should stop smoking and consume only moderate amounts of alcohol (up to one drink per day for women and two for men).
- Diet for people with diabetes
- Diet management is very important for people with either type of diabetes mellitus. Doctors recommend a healthy, balanced diet and efforts to maintain a healthy weight. People with diabetes can benefit from meeting with a dietitian or a diabetes educator to develop an optimal eating plan. Such a plan includes
- Avoiding simple sugars and processed foods
- Increasing dietary fiber
- Limiting portions of carbohydrate-rich and fatty foods (especially saturated fats) People who are taking insulin should avoid long periods between meals to prevent hypoglycemia.

**Treatment to prevent complications of diabetes**

Because diabetes eventually affects blood vessels throughout the body, people with diabetes are likely to develop complications related to problems with blood vessels. Glucose that remains high for long periods causes build-up in the walls of blood vessels, causing them to thicken and leak and risking development of atherosclerosis, stroke, eye problems, and other problems.

Because the risk of complications is so high in people with diabetes, it is important that people carefully control blood glucose levels. Doctors also recommend that people undergo regular monitoring to prevent complications.

**DIABETIC KETOACIDOSIS:**

- Diabetic ketoacidosis is an acute complication of diabetes that occurs mostly in type 1 diabetes mellitus. Glucose is one of the body's main fuels.
- Insulin, a hormone produced by the pancreas, helps glucose move from the blood into the cells.
- Once glucose is inside the cells, it is either converted to energy or stored as fat or glycogen until it is needed.
- When there is not enough insulin, most cells cannot use the glucose that is in the blood. Because cells still need energy to survive, they switch to a back-up mechanism to obtain energy.
- Fat cells begin breaking down, producing compounds called ketones. Ketones provide some energy to cells but also make the blood too acidic (ketoacidosis).
- Ketoacidosis that occurs in people with diabetes is called diabetic ketoacidosis.
- Diabetic ketoacidosis occurs mainly in people who have type 1 diabetes because their body produces little or no insulin. However, rarely, some people with type 2 diabetes develop ketoacidosis.
- People who abuse alcohol also can develop ketoacidosis (alcoholic ketoacidosis). Unlike in diabetic ketoacidosis, blood glucose levels are usually only mildly elevated.

**Causes of Ketoacidosis:**

Diabetic ketoacidosis is sometimes the first sign that people (usually children—see also Diabetes Mellitus (DM) in Children and Adolescents) have developed diabetes. In people who know they have diabetes, diabetic ketoacidosis can occur for two main reasons:

- People stop taking their insulin
- An illness stresses the body

An illness usually increases the body's need for energy. Thus, when people become ill, they often need more insulin to move extra glucose into their cells. If people do not take extra insulin when they are ill, they can develop diabetic ketoacidosis. Common illnesses that can trigger diabetic ketoacidosis include

- Infections (such as pneumonia and urinary tract infections)
- Heart attack
- Stroke
- Pancreatitis
- Rarely, some medications, especially the sodium-glucose co-transporter-2 (SGLT-2) inhibitors, can cause diabetic ketoacidosis, even in people with type 2 diabetes.
- Some people with type 2 diabetes are prone to develop ketoacidosis. This type of diabetes is called ketosis-prone diabetes, but is sometimes referred to as Flatbush diabetes. This type of diabetes is an unusual variant that is more likely to occur in people with obesity and in people of African ancestry.

**Symptoms**

- The initial symptoms of diabetic ketoacidosis include excessive thirst and urination, weight loss, nausea, vomiting, fatigue, and—particularly in children—abdominal pain. Breathing tends to become deep and rapid as the body attempts to correct the blood's acidity. The breath has a fruity odor similar to nail polish remover because of the smell of the ketones escaping into the breath. Without treatment, diabetic ketoacidosis can progress to coma and death (especially in children).

**Diagnosis:**

- Blood and urine tests to determine levels of glucose, ketones, and acid
- Doctors diagnose diabetic ketoacidosis by measuring the level of ketones and acid in the blood and urine. People with diabetic ketoacidosis also have high blood glucose levels, but people may have high glucose levels without having diabetic ketoacidosis (see Hyperosmolar, Hyperglycemic State).
- Doctors typically also do tests, such as a chest x-ray and urine analysis, to look for an underlying infection and electrocardiography (ECG) to look for a heart attack.

**Treatment:**

- Intravenous fluids and electrolytes
- Intravenous insulin
- Diabetic ketoacidosis is a medical emergency. Hospitalization, usually in an intensive care unit, may be necessary. Large amounts of fluids are given intravenously along

with electrolytes, such as sodium, potassium, chloride, and sometimes phosphate, to replace those fluids and electrolytes lost through excessive urination.

- Insulin is generally given intravenously so that it works quickly and the dose can be adjusted frequently.
- Blood levels of glucose, ketones, and electrolytes are measured every few hours. Doctors also measure the blood's acid level.
- Sometimes, additional treatments are needed to correct a high acid level. However, controlling the levels of glucose in the blood with insulin and, giving fluids, and replacing electrolytes usually allow the body to restore the normal acid-base balance.
- Once blood sugars are closer to normal, dextrose (a type of sugar) is given intravenously in combination with insulin to stop production of ketones.

## HYPOGLYCEMIA

**(Low Blood Sugar)**

Hypoglycemia is abnormally low levels of sugar (glucose) in the blood.

- Hypoglycemia is most often caused by medications taken to control diabetes. Much less common causes of hypoglycemia include other medications, critical illness or organ failure, a reaction to carbohydrates (in susceptible people), an insulin-producing tumor in the pancreas, and some types of bariatric (weight loss) surgery.
- A fall in blood glucose causes symptoms such as hunger, sweating, shakiness, fatigue, weakness, and inability to think clearly, whereas severe hypoglycemia causes symptoms such as confusion, seizures, and coma.
- The diagnosis is based on finding low glucose levels in the blood while the person is experiencing symptoms.
- Symptoms of hypoglycemia are treated by consuming sugar in any form.
- Doses of medications that cause hypoglycemia may need to be decreased.

Normally, the body maintains the level of glucose in the blood within a range of about 70 to 110 milligrams per deciliter (mg/dL), or 3.9 to 6.1 millimoles per liter (mmol/L) of blood. In hypoglycemia, the glucose level becomes too low. Although diabetes mellitus, a disorder involving blood glucose levels, is characterized by high levels of glucose in the blood (hyperglycemia), many people with diabetes periodically experience hypoglycemia due to side effects of diabetes treatment. Hypoglycemia is uncommon among people without diabetes.

Very low levels of glucose in the blood may interfere with the function of certain organ systems. The brain is particularly sensitive to low glucose levels because sugar is the brain's major energy source. To prevent glucose levels in the blood from falling too far below their usual range, the brain responds by stimulating the

- Adrenal glands to release epinephrine (adrenaline)
- Adrenal glands to release cortisol
- Pancreas to release glucagon
- Pituitary gland to release growth hormone

All of these hormones cause the liver to release glucose into the blood, but sometimes these hormones do not raise the blood glucose level enough to overcome the hypoglycemia. If the blood glucose level remains too low, the brain will get insufficient fuel, resulting in confusion, seizures, or loss of consciousness.

**Causes:**

- Most cases of hypoglycemia occur in people with diabetes and are caused by insulin or other medications (especially, sulfonylureas such as glyburide, glipizide, and glimepiride.
- They take to lower the levels of glucose in their blood. Hypoglycemia is more common when intense efforts are made to keep the glucose levels in the blood as close to normal as possible, or when people who take insulin do not check blood glucose levels frequently enough.
- People with diabetes who reduce food intake or who develop chronic kidney disease are more likely to have hypoglycemia.
- If, after taking a dose of a medication for diabetes, a person eats less than usual or is more physically active than normal, the medication may lower the level of glucose in the blood too much.
- Certain medications other than those for diabetes, most notably pentamidine, used to treat a form of pneumonia that occurs most often as part of AIDS, and quinine, used to treat muscle cramps, occasionally cause hypoglycemia.

**Symptoms;**

- Mild hypoglycemia: Sweating, nervousness, shaking, faintness, palpitations, and hunger
- Severe hypoglycemia: Dizziness, fatigue, weakness, headaches, inability to concentrate, confusion, slurred speech, blurred vision, seizures, and coma
- Hypoglycemia symptoms rarely develop until the level of glucose in the blood falls below 60 mg/dL (3.3 mmol/L).

- The body first responds to a fall in the level of glucose in the blood by releasing epinephrine from the adrenal glands.
- Epinephrine is a hormone that stimulates the release of glucose from body stores but also causes symptoms similar to those of an anxiety attack: sweating, nervousness, shaking, faintness, palpitations, and hunger.
- More severe hypoglycemia reduces the glucose supply to the brain, causing dizziness, fatigue, weakness, headaches, inability to concentrate, confusion, inappropriate behavior that can be mistaken for drunkenness, slurred speech, blurred vision, seizures, and coma. Severe and prolonged hypoglycemia may permanently damage the brain.
- Symptoms can begin slowly or suddenly, progressing from mild discomfort to severe confusion or panic within minutes.
- In a person with an insulinoma, symptoms are likely to occur early in the morning after an overnight fast, especially if the glucose stores in the blood are further depleted by exercise before breakfast.

**Diagnosis:** Blood tests to measure glucose level.

**Treatment:**

- Consuming sugar to raise the level of glucose in the blood
- Changing medication doses
- Eating several small meals during the day
- Sometimes surgery to remove a tumor
- People prone to hypoglycemia should carry or wear medical identification to inform health care professionals of their condition.
- The symptoms of hypoglycemia are relieved within minutes of consuming sugar in any form, such as candy, glucose tablets, or a sweet drink, such as a glass of fruit juice. People with recurring episodes of hypoglycemia, especially those with diabetes, often prefer to carry glucose tablets because the tablets take effect quickly and provide a consistent amount of sugar.

**GALACTOSEMIA:**

- Some people cannot metabolize galactose. Galactosemia is a condition in which the body is unable to use (metabolize) the simple sugar galactose.

- It is an inherited disorder that the defect may be in the galactokinase, uridlyl transferase or 4-epimerase.Most common is uridyl transferase. Such patients have high concentration of Galactose in blood (Galactosemia).
- In lens, Galactose is reduced to galactitol by aldose reductase.The product accumulates in lense and leads to accumulation of water by osmotic pull. This leads to turbidity of lense proteins (Cataract). If uridyl transferase was absent galactose 1-phosphate accumulates.Liver is depleted of inorganic phosphate. This ultimately causes failure of liver function and mental retardation. If 4-epimerase is absent, since the patient can form UDP-galactose from glucose the patient remains symptom free.

**CAUSES**

Galactosemia is an inherited disorder. If both parents carry a nonworking copy of the gene that can cause galactosemia, each of their children has a 25% (1 in 4) chance of being affected with it.

There are 3 forms of the disease:

- Galactose-1 phosphate uridyl transferase (GALT) deficiency: Classic galactosemia, the most common and most severe form
- Deficiency of galactose kinase (GALK)
- Deficiency of galactose-6-phosphate epimerase (GALE)
- People with galactosemia are unable to fully break down the simple sugar galactose. Galactose makes up one half of lactose, the sugar found in milk.
- If an infant with galactosemia is given milk, substances made from galactose build up in the infant's system. These substances damage the liver, brain, kidneys, and eyes.
- People with galactosemia cannot tolerate any form of milk (human or animal). They must be careful about eating other foods containing galactose.

**Reduction to galactitol**

- In galactosemic patients, the accumulation of galactose becomes the substrate for enzymes that catalyze the polyol pathway of carbohydrate metabolism. The first reaction of this pathway is the reduction of aldoses, types of sugars including galactose, to sugar alcohols. Recent data suggests that aldose reductase is the enzyme responsible for the primary stage of this pathway. Therefore, aldose reductase reduces galactose to its sugar alcohol form, galactitol. Galactitol, Thus, galactitol accumulates in body tissues and is excreted in the urine of galactosemic patients. Accumulation of galactitol has been attributed to many of the negative effects of galactosemia, and high concentrations of galactitol have been found in

people with classic galactosemia (GALT deficiency or Galactose-1-phosphate uridylyltransferase deficiency), galactokinase deficiency, and epimerase deficiency with glucose.

**Oxidation to galactonate**

- Accumulated galactose can also undergo an alternative reaction: Oxidation to galactonate. The mechanism of galactonate formation is still unclear. However, recent studies suggest that galactose dehydrogenase is responsible for converting galactose to galactonolactone, which then spontaneously or enzymatically converts to galactonate. Once formed, galactonate may enter the pentose phosphate pathway. Thus, oxidation to galactonate serves as an alternate pathway for metabolizing galactose. This oxidative pathway renders accumulated galactonate less harmful than accumulated galactitol.

**Symptoms**

Infants with galactosemia may show symptoms in the first few days of life if they eat formula or breast milk that contains lactose. They may develop a serious blood infection with the bacteria E coli.

Symptoms of galactosemia are:

- Convulsions
- Irritability
- Lethargy
- Poor feeding -- baby refuses to eat formula containing milk
- Poor weight gain
- Yellow skin and whites of the eyes (jaundice)
- Vomiting

**Diagonsis**

In many states throughout the world, infants routinely undergo newborn screening (NBS) for galactosemia. This allows a diagnosis to be made while the person is still an infant. Infants affected by galactosemia typically present with symptoms of lethargy, vomiting, diarrhea, failure to thrive, and jaundice. None of these symptoms are specific to galactosemia, often leading to diagnostic delays. If the family of the baby has a history of galactosemia, doctors can test prior to birth by taking a sample of fluid from around the fetus (amniocentesis) or from the placenta (chorionic villus sampling or CVS).

Galactosemia is normally first detected through newborn screening which if available, is able to diagnose the majority of affected infants.

A galactosemia test is a blood test (from the heel of the infant) or urine test that checks for three enzymes that are needed to change galactose sugar that is found in milk

and milk products into glucose, a sugar that the human body uses for energy. A person with galactosemia doesn't have one of these enzymes. This causes high levels of galactose in the blood or urine.

Tests to check for galactosemia include:

- Blood culture for bacterial infection (E coli sepsis)
- Enzyme activity in the red blood cells
- Ketones in the urine
- Prenatal diagnosis by directly measuring the enzyme galactose-1-phosphate uridyl transferase
- "Reducing substances" in the infant's urine, and normal or low blood sugar while the infant is being fed breast milk or a formula containing lactose.In many states, newborn screening tests check for galactosemia.

Test results may show:

- Amino acids in the urine or blood plasma
- Enlarged liver
- Fluid in the abdomen
- Low blood sugar

**Treatment**

People with this condition must avoid all milk, products that contain milk (including dry milk), and other foods that contain galactose, for life. Read product labels to make sure you or your child with the condition are not eating foods that contain galactose.

Infants can be fed:

- Soy formula
- Another lactose-free formula
- Meat-based formula or Nutramigen (a protein hydrolysate formula)

Calcium supplements are recommended.

Galactosemia Foundation -- www.galactosemia.org

**Possible Complications**

These complications can develop:

- Cataracts
- Cirrhosis of the liver
- Delayed speech development
- Irregular menstrual periods, reduced function of ovaries leading to ovarian failure and infertility
- Mental disability
- Severe infection with bacteria (E coli sepsis)
- Tremors (shaking) and uncontrollable motor functions
- Death (if there is galactose in the diet)
- The only treatment for classic galactosemia is eliminating lactose and galactose from the diet (e.g. exclusion of dairy products containing lactose).
- Lactose restricted diet is efficient in resolving acute complications, however, it is not sufficient to prevent long-term complications affecting the brain and female gonads. Some individuals may experience long-term complications such as speech difficulties, learning disabilities, neurological impairment (e.g. tremors, etc.), and ovarian failure.

**LACTOSE INTOLERANCE**

- Lactose is hydrolyzed to galactose and glucose by lactase in humans (by β-Galactosidase in Bacteria).
- Some adults do not have lactase. Such adults cannot digest the sugar.It remains in the intestines and gets fermented by the bacteria. The condition is called as Lactose intolerance.
- Such patients suffer from watery diarrhea, abnormal intestinal flow and chloeic pain. They are advised to avoid the consumption of Lactose containing foods like Milk. C. Maltase.
- The enzyme hydrolyzes the α -(1,4) glycosidic linkage between glucose units in maltose molecule liberating two glucose molecules.
- Its pH range is 5.8 to 6.2. Maltose D. Sucrase PH ranges 5.0 to 7.0. It hydrolyzes sucrose molecule to form glucose and fructose. Sucrose lactase Glucose + Galactose.
- Galactose: Milk sugar contains galactose. Galactokinase converts galatose to galactose-1-P.

- It reacts with UDP-glucose to form UDP-galactose and glucose-1-P.The enzyme is Galactose-1-P uridyltransferase. UDP-galactose can be epimerized to UDP-glucose by 4- epimerase.
- Glycogenesis also requires UDP-glucose. UDP-galactose can be condensed with glucose to form lactose.

**SYMPTOMS OF LACTOSE INTOLERANCE**

Each person's symptoms may vary. Symptoms often start about 30 minutes to 2 hours after you have food or drinks that have lactose.

- Symptoms may include:
- Belly (abdominal) cramps and pain
- Nausea
- Bloating
- Gas
- Diarrhea

**Diagnosis:**

- **Lactose tolerance test:** This test checks how your digestive system absorbs lactose. You will be asked not to eat or drink anything for about 8 hours before the test. This often means not eating after midnight. For the test, you will drink a liquid that contains lactose. Some blood samples will be taken over a 2-hour period. These will check your blood sugar (blood glucose) level. If your blood sugar levels don't rise, you may be lactose intolerant.
- **Hydrogen breath test:** You will drink a liquid containing a lot of lactose. Your breath will be checked several times. High levels of hydrogen in your breath may mean you are lactose intolerant.
- **Stool acidity test:** This test is used for infants and young children. It checks how much acid is in the stool. If someone is not digesting lactose, their stool will have lactic acid, glucose, and other fatty acids.

## HYPERLIPIDEMIA

**INTRODUCTION**

- Hyperlipidemia disease has afflicted humankind since antiquity. In 2002, coronay heart Epidemiological evidence strongly supported the positive correlation between blood lipids, hyperlipidemia and its complications, mainly CHD.

- Cholesterol It is a vital component of the mammalian cell membrane of all tissues and is a precursor of steroid hormones and bile acids. It occurs, either free or as many fatty esters in all animal cells, but is absent in plant fats.
- Triglycerides are esters of glycerol with 3 fatty acid molecules. Data obtained from National Institute of Health, limits triglycerides value to 200 mg/dl as the normal range and 500 mg/dl as an abnormal range. Range higher than 500 mg/dl is considered dangerous for the development of cardiovascular diseases.

**CLASSIFICATION OF HYPERLIPIDEMIA**

On the basis of lipid type Hypercholesterolemia-In this the level of cholesterol is elevated. Hypertriglyceridemia-It is defined as an elevated level of triglycerides.

On the basis of causing factor

**Familial (Primary) hyperlipidemia**–On the basis of causing factors hyperlipidemia can be designated as either primary or secondary.

According to Fredrickson familial hyperlipidemia is classified into five types on the basis of electrophoresis or ultracentrifugation pattern of lipoproteins.

• Type I–Raised cholesterol with high triglyceride levels.

• Type II–High cholesterol with normal triglyceride levels.

• Type III–Raised cholesterol and triglycerides.

• Type IV–Raised triglycerides, atheroma and uric acid.

• Type V–Raised triglycerides.

This classification was later adopted by WHO. This method does not directly account for HDL and also does not distinguish among the different genes that may be partially responsible for some of these conditions. It remains a popular system of classification but is considered dated by many.

**Acquired (Secondary) hyperlipidemia**

Acquired hyperlipidemia (secondary dyslipoproteinemias) results from underlying disorders and lead to alterations in plasma lipid and lipoprotein metabolism. This type of hyperlipidemia may mimic primary forms of hyperlipidemia and can have similar consequences. They may result in increased risk of premature atherosclerosis, pancreatitis and other complications of the chylomicronemia syndrome.

The most common causes of acquired hyperlipidemia are given below.

• Diabetes Mellitus
• Use of drugs such as diuretics, β-blockers and estrogens.

- Alcohol consumption.
- Some rare endocrine disorders and metabolic disorders.
- Hypothyroidism
- Renal failure
- Nephrotic syndrome

Major primary and secondary forms of hyperlipidemia, their lipoprotein abnormalities and drugs used for their treatment.

| Disorder | Lipoprotein abnormality | Drug therapy |
|---|---|---|
| Familial hypercholesterolemia | ↑↑LDL | Lovastatin |
| Polygenic hypercholesterolemia | ↑LDL | Lovastatin |
| Familial lipoprotein lipase deficiency | ↑Chylomicrons | Nicotinic acid |
| Familial hypertriglyceridemia | ↑VLDL | Gemfibrozil |
| Familial combined hyperlipidemia | ↑LDL, ↓HDL | Nicotinic acid, clofibrate |

**COMPLICATIONS OF HYPERLIPIDAEMIA**

**I. Atherosclerosis:** It is a common disorder and occurs when fat, cholesterol and calcium deposits in the arterial linings. This deposition results in the formation of fibrous plaques. A plaque normally consists of three components:

1) atheroma which is a fatty, soft, yellowish nodular mass located in the centre of a larger plaque that consists of macrophages, which are cells that play a role in immunity;

2) a layer of cholesterol crystals; and,

3) calcified outer layer. Atherosclerosis is the leading cause of cardiovascular disease.

**II. Coronary Artery Disease (CAD):** Atherosclerosis is the major cause of CAD. It is characterised by the narrowing of the arteries that supply blood to the myocardium and results in limiting blood flow and insufficient amounts of oxygen to meet the needs of the heart. The narrowing may progress to the extent that the heart muscle would sustain damage due to lack of blood supply. Elevated lipid profile is correlated to the development of coronary atherosclerosis.

**III. Myocardial Infarction (MI):** MI is a condition which occurs when blood and oxygen supplies to the cardiac arteries are partially or completely blocked, resulting in damage or death of heart cells. The blockage is usually due to the formation of a clot in an artery. This condition is commonly known as heart attack. The studies show that one-fourth of survivors of myocardial infarction were hyperlipidemic.

**IV. Angina Pectoris:** Angina is not a disease but a symptom of an underlying heart condition. It is characterised by chest pain, discomfort or a squeezing pressure. Angina occurs as a result of a reduction or a lack of blood supply to a part or the entire heart muscle. Poor blood circulation is usually due to CHD when partial or complete obstruction of the coronary arteries is present.

**V. Ischemic stroke or Cerebrovascular Accident (CVA):** It occurs when blood circulation in part of the brain is blocked or diminished. When blood supply, which carries oxygen, glucose, and other nutrients, is disrupted, brain cells die and become dysfunctional. Usually, strokes occur due to blockage of an artery by a blood clot or a piece of atherosclerotic plaque that breaks loose in a small vessel within the brain. Clinical trials revealed that lowering of LDL and total cholesterol by 15% significantly reduced the risk of first stroke.

**CAUSES OF HYPERLIPIDEMIA**

• A diet rich in saturated fat and cholesterol increases blood cholesterol and triglyceride levels.
• Other disorders as obesity, diabetes mellitus and hypothyroidism increase the risk of hyperlipidemia.
• Smoking and not exercising may lead to hyperlipidemia.
• Excessive use of alcohol also increases the risk of hyperlipidemia.
• Certain drugs as steroids and β–blockers may cause hyperlipidemia.
• Hereditary factor is also one of the common causes for hyperlipidemia.
• In some cases, hyperlipidemia occurs during pregnancy.
• Lipoprotein lipase mutations.

**SYMPTOMS OF HYPERLIPIDEMIA**

Hyperlipidemia usually has no noticeable symptoms and tends to be discovered during routine examination for atherosclerotic cardiovascular disease.

Symptoms may include chest pain (angina), heart attack or stroke.

• When levels are exceedingly high, cholesterol may be deposited in tendons or just beneath the skin under the eyes.

• Swelling of organs such as liver, spleen or pancreas.

• Blockage of blood vessels in brain and heart.

• Higher rate of obesity and glucose intolerance.

• Pimple like lesions across the body.

**PATHOGENESIS OF HYPERLIPIDEMIA**

- Cholesterol, triglycerides, and phospholipids are transported in the bloodstream as complexes of lipid and proteins known as lipoproteins.
- Elevated total and low-density lipoprotein (LDL) cholesterol and reduced high-density lipoprotein (HDL) cholesterol are associated with the development of coronary heart disease (CHD). During the early stages of the hyperlipidemia, blood monocytes and platelets attach to a vessel wall at the sites of endothelial damage.
- The release of the mediators such as platelet derived groth factors leads to a proliferation of smooth cells in the intimal and medial lining of the vessel, collagen synthesis, cholesterol uptake and the beginning of the hyperlipidemic plaque results.
- Plaque ruptures are resulting in the acute syndromes of unstable angina, myocardial infarction and sudden cardiac death.

**DIAGNOSIS OF HYPERLIPIDEMIA**

- Hyperlipidemia typically shows no symptoms and can only be detected by a blood test.
- Screening for hyperlipidemia is done with a blood test called a lipid profile. According to National Cholesterol Education Program (NECP) screening should start at age 20, and if the report is normal, it should be repeated at least every five years. Normal levels for a lipid profile are.

| | | | |
|---|---|---|---|
| Cholesterol | Less than 200 mg/dl | 239 mg/dl | 240 mg/dl |
| Triglycerides | Less than 140 mg/dl | 150-199 mg/dl | 200-499 mg/dl |
| HDL cholesterol | 60 mg/dl | 40-50 mg/dl | Less than 40 mg/dl |
| LDL cholesterol | 60-130 mg/dl | 130-159 mg/dl | 160-189 mg/dl |

**PREVENTION OF HYPERLIPIDEMIA**

• Low fats and cholesterol diet should be taken.

• Eat foods high in soluble fiber such as oats, beans and certain fruits.

• Exercise regularly to maintain a healthy weight. Controllable lifestyle changes are the best way to fight hyperlipidaemia. But when lifestyle changes fail to control the disease then treatment with cholesterol-lowering drugs is required.

**TREATMENT OF HYPERLIPIDEMIA**

In 1987 the National Institute of Health (NIH) established the National Cholesterol Education Program (NCEP) to be directed by the Adult Treatment Panel (ATP) for the purpose of issuing information for health professionals and the general public concerning testing, evaluating, monitoring and treating hyperlipidemia. An important criterion of ATP guidelines is the development of treatment goals for hyperlipidemia based on patient's risk of CHD. ATP recommends two methods of treatment: 1) Therapeutic lifestyle changes; 2) Drug therapy.

**Therapeutic lifestyle** changes Diet modification, regular physical activity, smoking cessation, and weight reduction should be tried as initial treatment, especially in mild cases of hyperlipidemia and in persons without CHD or CHD risk equivalent.

**Drug therapy** High LDL, the presence of risk factors, and documentation of CHD should qualify initiating drug therapy along with TLC. Monotherapy has been shown to be effective in treating hyperlipidemia, but combination therapy may be required for a comprehensive approach.

**HYPERLIPOPROTEINEMIA**

Hyperlipoproteinemia is a common disorder. It results from an inability to break down lipids or fats in your body, specifically cholesterol and triglycerides. There are several types of hyperlipoproteinemia. The type depends on the concentration of lipids and which are affected. High levels of cholesterol or triglycerides are serious because they're associated with heart problems.

**CAUSES OF HYPERLIPOPROTEINEMIA**

Hyperlipoproteinemia can be a primary or secondary condition. Primary hyperlipoproteinemia is often genetic. It's a result of a defect or mutation in lipoproteins. These changes result in problems with accumulation of lipids in your body.

Secondary hyperlipoproteinemia is the result of other health conditions that lead to high levels of lipids in your body. These include:

- diabetes
- hypothyroidism
- pancreatitis
- use of certain drugs, such as contraceptives and steroids
- certain lifestyle choices.

**TYPES OF PRIMARY HYPERLIPOPROTEINEMIA**

There are five types of primary hyperlipoproteinemia:

- **Type 1** is an inherited condition. It causes the normal breakdown of fats in your body to be disrupted. A large amount of fat builds up in your blood as a result.
- **Type 2** runs in families. It's characterized by an increase of circulating cholesterol, either low-density lipoproteins (LDL) alone or with very-low-density lipoproteins (VLDL). These are considered the "bad cholesterols."
- **Type 3** is a recessively inherited disorder in which intermediate-density lipoproteins (IDL) accumulate in your blood. IDL has a cholesterol-to-triglycerides ratio that's higher than that for VLDL. This disorder results in high plasma levels of both cholesterol and triglycerides.
- **Type 4** is a dominantly inherited disorder. It's characterized by high triglycerides contained in VLDL. The levels of cholesterol and phospholipids in your blood usually remain within normal limits.
- **Type 5** runs in families. It involves high levels of LDL alone or together with VLDL.

**SYMPTOMS OF HYPERLIPOPROTEINEMIA**

Lipid deposits are the main symptom of hyperlipoproteinemia. The location of lipid deposits can help to determine the type. Some lipid deposits, called xanthomas, are yellow and crusty. They occur on your skin. Many people with this condition experience no symptoms. They may become aware of it when they develop a heart condition.

Other signs and symptoms of hyperlipoproteinemia include:

- pancreatitis (type 1)
- abdominal pain (types 1 and 5)
- enlarged liver or spleen (type 1)
- lipid deposits or xanthomas (type 1)
- family history of heart disease (types 2 and 4)
- family history of diabetes (types 4 and 5)
- heart attack
- Stroke

**DIAGONSIS**

A doctor can diagnose hyperlipoproteinemia with a blood test. Sometimes, family history is useful. If you have lipid deposits on your body, your doctor will also examine

those. Other diagnostic tests might measure thyroid function, glucose, protein in the urine, liver function, and uric acid.

**TREATMENT:**

Treatment for hyperlipoproteinemia will depend on which type you have. When the condition is the result of hypothyroidism, diabetes, or pancreatitis, treatment will take the underlying disorder into account. Your doctor may prescribe medications like the following to help lower lipid levels:

- atorvastatin (Lipitor)
- fluvastatin (Lescol XL)
- pravastatin (Pravachol)
- ezetimibe (Zetia)

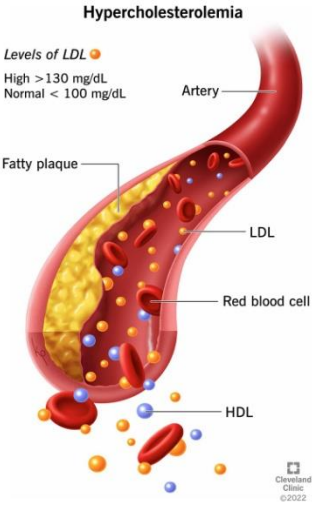
**PREVENTION:**

Certain lifestyle changes can also help with hyperlipoproteinemia. These include:

- a low-fat diet
- increased exercise
- weight loss
- stress relief
- a decrease in alcohol consumption

**HYPERCHOLESTEROLAEMIA**

- Hypercholesterolaemia is one of the major causes of atherosclerosis. Cholesterol plays an important role as the precursor for steroid hormones and bile acids and it is essential for the structural integrity of cell membranes. It is transported in the body in lipoproteins.
- Hypercholesterolaemia usually results from nutritional factors such as obesity and a diet high in saturated fats combined with an underlying polygenic predisposition. There is overproduction of LDL its genetic component is unlikely to be monogenic, unless it is extreme.
- Hypercholesterolaemia can also have an entirely genetic cause. A common example of this is monogenic familial hypercholesterolaemia, an autosomal dominant disorder in which the LDL cholesterol is raised from birth. It is characterised by a dominant pattern of inheritance of premature coronary disease and/or tendon xanthomata.




- Hypercholesterolemia is a disorder known for an excess of low-density lipoprotein (LDL) in your blood. Many people can treat it by making changes to their diet and adding exercise to their lifestyles. Others need to take medicine to bring their LDL level down to a normal level. These treatments lower your risk of heart attacks and strokes.
- Increase in plasma cholesterol (> 200 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders.

1. **Diabetes mellitus:** Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.
2. **Hypothyroidism (myxoedema):** This is believed to be due to decrease in the HDL receptors on hepatocytes.
3. **Obstructive jaundice:** Due to an obstruction in the excretion of cholesterol through bile.
4. **Nephrotic syndrome:** Increase in plasma globulin concentration is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due to increase in plasma lipoprotein fractions in this disorder.

- Hypercholesterolemia is associated with atherosclerosis and coronary heart disease. Atherosclerosis is characterized by deposition of cholesterol esters and other lipids in the intima of the arterial walls often leading to hardening of coronary arteries and cerebral blood vessels.
- More specifically, LDL-cholesterol is positively correlated, whereas HDL-cholesterol is negatively correlated with cardiovascular diseases.

- Bad cholesterol and good cholesterol: Cholesterol is a natural metabolite performing a wide range of functions (membrane structure, precursor for steroid hormones, bile acids).
- The usages good and bad to cholesterol, although inappropriate, are still in use. The cholesterol in high concentration, present in LDL, is considered had due to its involvement in atherosclerosis and related complications.
- Thus, LDL may be regarded as lethally dangerous lipoprotein. On the other hand, HDL cholesterol is good since its high concentration counteracts atherosclerosis. HDL may be considered as highly desirable fipoprotein.

**CONTROL OF HYPERCHOLESTEROLEMIA**

Several measures are advocated to lower the plasma cholesterol level

- Consumption of PUFA: Dietary intake of polyunsaturated fatty acids (PUFA) reduces the plasma cholesterol level. PUFA will help in transoort of cholesterol bv LCAT mechanism. (described earlier) and its excretion from the body. The oils with rich PUFA content include cottonseed oil, soya bean oil, sunflower oil, corn oil, fish oils etc. Ghee and coconut oil are poor sources of PUFA.
- Dietary cholesterol: Cholesterol is found onlv in animal foods and not in plant foods. Dietary cholesterol influence on plasma cholesterol is minimal. However, avoidance of cholesterol-rich foods is advocated to be on the safe side.
- Plant sterols: Certain plant sterols and their esters (e.g. sitostanol esters) reduce plasma cholesterol levels. They inhibit the intestinal absorption of dietary cholesterol.
- Dietary fiber: Fiber present in vegetables decreases the cholesterol absorption from the intesti ne.
- Avoiding high carbohydrate diet: Diets rich in carbohydrates (particularly sucrose) should be avoided to control hypercholesterolemia.
- Impact of lifestyles: Elevation in plasma cholesterol is obseved in people with smoking, abdominal obesity, lack of exercise, stress, high blood pressure, consumption of soft water etc. Therefore, adequate changes in the lifestyles will bring down plasma cholesterol.
- Moderate alcohol cosumption : The beneficial effects of moderate alcohol intake are masked by the ill effects of chronic alcoholism. Red wine is particularly beneficial due to its antioxidants, besides low alcohol content.
- Use of drugs: Drugs such as lovastatin which inhibit HMG CoA reductase and decrease cholesterol synthesis are used. Statins currently in use include atorvastatin,

simvastatin and pravastatin. Certain drugs-cholestyramine and colestipol-bind with bile acids and decrease their intestinal reabsorption. This helps in the conversion of more cholesterol to bile acids and its excretion through feces. Clofibrate increases the activity of lipoprotein lipase and reduces the plasma cholesterol and triacylglycerols.

**HYPERTRIGLYCERIDEMIA**

- Hypertriglyceridemia is the presence of high amounts of triglycerides in the blood. Triglycerides are the most abundant fatty molecule in most organisms.
- Hypertriglyceridemia occurs in various physiologic conditions and in various diseases, and high triglyceride levels are associated with atherosclerosis, even in the absence of hypercholesterolemia (high cholesterol levels) and predispose to cardiovascular disease.
- Chronically elevated serum triglyceride levels are a component of metabolic syndrome and non-alcoholic fatty liver disease (NAFLD), both of which typically involve obesity and contribute significantly to cardiovascular mortality in industrialised countries as of 2021. Extreme triglyceride levels also increase the risk of acute pancreatitis.
- Hypertriglyceridemia itself is usually symptomless, although high levels may be associated with skin lesions known as xanthomas.

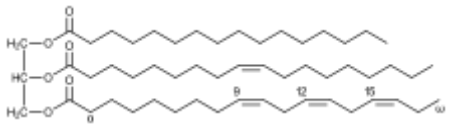
**ETYMOLOGY**

- The word hypertriglyceridemia uses combining forms of hyper- + triglyceride + -emia, thus corresponding to "high triglyceride levels in the blood" or "too many triglycerides in the blood".

**SIGNS AND SYMPTOMS**

- Most people with elevated triglycerides experience no symptoms.
- Some forms of primary hypertriglyceridemia can lead to specific symptoms: both familial chylomicronemia and primary mixed hyperlipidemia include skin symptoms (eruptive xanthoma), eye abnormalities (lipemiaretinalis), hepatosplenomegaly (enlargement of the liver and spleen), and neurological symptoms.
- Some experience attacks of abdominal pain that may be mild episodes of pancreatitis. Eruptive xanthomas are 2–5 mm papules, often with a red ring around them, that occur in clusters on the skin of the trunk, buttocks and extremities.[2] Familial dysbetalipoproteinemia causes larger, tuberous xanthomas; these are red or orange and occur on the elbows and knees. Palmar crease xanthomas may also occur.

- The diagnosis is made on blood tests, often performed as part of screening. Once diagnosed, other blood tests are usually required to determine whether the raised triglyceride level is caused by other underlying disorders ("secondary hypertriglyceridemia") or whether no such underlying cause exists ("primary hypertriglyceridaemia"). There is a hereditary predisposition to both primary and secondary hypertriglyceridemia.



Triglyceride, which cause hypertriglyceridemia at high level

Acute pancreatitis may occur in people whose triglyceride levels are above 1000 mg/dL (11.3 mmol/L) Hypertriglyceridemia is associated with 1–4% of all cases of pancreatitis. The symptoms are similar to pancreatitis secondary to other causes, although the presence of xanthomas or risk factors for hypertriglyceridemia may offer clues.

**Causes [edit**

- Overeating ,Obesity
- Diabetes mellitus and insulin resistance - it is one of the defined components of metabolic syndrome (along with central obesity, hypertension, and hyperglycemia)
- Excess alcohol consumption ,Kidney failure, nephrotic syndrome
- Genetic predisposition; some forms of familial hyperlipidemia such as familial combined hyperlipidemia i.e. Type II hyperlipidemia
- Lipoprotein lipase deficiency - Deficiency of this water-soluble enzyme, that hydrolyzes triglycerides in lipoproteins, leads to elevated levels of triglycerides in the blood.
- Lysosomal acid lipase deficiency or disease, certain medications, e.g. isotretinoin, hydrochlorothiazide diuretics, beta blockers, protease inhibitors
- Hypothyroidism (underactive thyroid),Lupus and associated autoimmune responses .
- Glycogen storage disease type 1.,Propofol,HIV medications

**DIAGNOSIS**

The diagnosis is made on blood tests, often performed as part of screening. The normal triglyceride level is less than 150 mg/dL (1.7 mmol/L). Once diagnosed, other blood tests are usually required to determine whether the raised triglyceride level is caused by other underlying disorders ("secondary hypertriglyceridemia") or whether no such

underlying cause exists ("primary hypertriglyceridaemia"). There is a hereditary predisposition to both primary and secondary hypertriglyceridemia.

**SCREENING:**

- In 2016 the United States Preventive Services Task Force concluded that testing the general population under the age of 40 without symptoms is of unclear benefit.

**TREATMENT**

- Lifestyle changes including weight loss, exercise and dietary modification may improve hypertriglyceridemia. This may include dietary changes such as restriction of fat and carbohydrates (specifically fructose), and increased consumption of omega-3 fatty acids from algae, nuts, and seeds.
- The decision to treat hypertriglyceridemia with medication depends on the levels and on the presence of other risk factors for cardiovascular disease.
- Very high levels that would increase the risk of pancreatitis is treated with a drug from the fibrate class.
- Niacin and omega-3 fatty acids as well as drugs from the statin class may be used in conjunction, with statins being the main drug treatment for moderate hypertriglyceridemia where reduction of cardiovascular risk is required.
- Medications are recommended in those with high levels of triglycerides that are not corrected with lifestyle modifications, with fibrates being recommended first.
- Epanova (omega-3-carboxylic acids) is another prescription drug used to treat very high levels of blood triglycerides.

**SPHINGOLIPIDOSES**

- **Lipid storage diseases**: representing lysosomal storage defects, are inherited disorders. They are characterized by the accumulation of complex lipids. The term sphingolipidoses is often used to collectively refer to the genetic disorders that lead to the accumulation of any one of the sphingolipids (glycosphingolipids and sphingomyelins).
- **Synthesis of sphingomyelins:** These are phospholipids containing a amino alcohol, sphingosine.
- **Degradation of sphingornyelins:** The enzyme sphingomyelinase of lysosomes hydrolyses sphingomyelins to ceramide and phosphoryI choline Ceram ide formed can be further degraded to sphingosine and free fattv acid.
- **Niemann-Pick disease:** lt is an inherited disorder due to a defect in the enzyme sphingomyelinase. This causes accumulation of sphingomyelins in liver and spleen,

resulting in the enlargement of these organs. Victims of Niemann-Pick disease suffer from severe mental retardation, and death may occur in early childhood.

- **Farber's disease:** A defect in the enzyme ceramidase results in Farber's disease. This disorder is characterized by skeletal deformation, subcutaneous nodules, dermatitis and mental retardation. lt is fatal in earlv life".

# UNIT –IV

# DISORDERS OF AMINOACID METABOLISM

## ALKAPTONURIA (BLACK URINE DISEASE)

Alkaptonuria has great historical importance. It was first described by Lusitanus in 1649 and characterized in 1859. Garrod conceived the idea of inborn errors of metabolism from his observation on alkaptonuria. The prevalance of this autosomal recessive disorder is 1 in 25,000.

**Enzyme defect:**

The defective enzyme in alkaptonuria is homogentisate oxidase in tyrosine metabolism. Homogentisate accumulates in tissues and blood, and is excreted into urine. Homogentisate, on standing, gets oxidized to the corresponding quinones, which polymerize to give black or brown colour. For this reason, the urine of alkaptonuric patients resembles coke in colour.

**Biochemical manifestations:**

Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called alkapton.. Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as ochronosis. Many alkaptonuric patients suffer from arthritis and this is believed to be due to the deposition of pigment alkapton (in the joints), produced from homogentisate.

**Diagnosis:**

Change in colour of the urine on standing to brown or dark has been the simple traditional method to identify alkaptonuria. The urine gives a positive test with ferric chloride and silver nitrate. This is due to the strong reducing activity of homogentisate. Benedict's test-employed for the detection of glucose and other reducing sugars-is also positive with homogentisate.

**Treatment:**

Alkaptonuria is not a dangerous disorder and, therefore, does not require any specific treatment. However, consumption of protein diet with relatively low phenylalanine content is recommended.

## PHENYLKETONURIA/PHENYLALANINEMIA

Phenylketonuria (PKU) is the most common metabolic disorder in amino acid metabolism. The incidence of PKU is 1 in 10,000 births. lt is due to the deficiency of the hepatic enzyme, phenylalanine hydroxylase, caused by an autosomal recessive gene.

- In recent years, a variant of PKU-due to a defect in dihydrobiopterin reductase (relatively less)-has been reported.
- This enzyme deficiency impairs the synthesis of tetrahydrobiopterin required for the action of phenylalanine hydroxylase. The net outcome in PKU is that phenylalanine is not converted to tyrosine.

**Phenylalanine metabolism in PKU:**

- Phenylketonuria primarily causes the accumulation of phenylalanine in tissues and blood, and results in its increased excretion in urine.
- Due to disturbances in the routine metabolism, phenylalanine is diverted to alternate pathway, resulting in the excessive production of phenylpyruvate, phenylacetate, phenyllactate and phenylglutamine.
- AII these metabolites are excreted in urine in high concentration in PKU. Phenylacetate gives the urine a mousey odour.
- The name phenylketonuria is coined due to the fact that the metabolite phenylpyruvate is a keto acid (C6H5CH2-CO-COO-) excreted in urine in high amounts.

**CLINICAL/BIOCHEMICAL MANIFESTATIONS OF PKU:**

The disturbed metabolism of phenylalanine resulting in increased concentration of phenylalanine and its metabolites in the bodycauses, many clinical and biochemical manifestation.

**1. Effects on central nervous system:**

- Mental retardation, failure to walk or talk, failure of growth, peizures and tremor are the characteristic findings in PKU. lf untreated, the patients show very low lQ (below 50).
- The biochemical basis of mental retardation in PKU is not well understood. There are, however, many explanations offered.
- Accumulation of phenylalanine in brain impairs the transport and metabolism of other aromatic amino acids (tryptophan and tyrosine).
- The synthesis of serotonin (an excitatory neurotransmitter) from tryptophan is insufficient. This is due to the competition of phenylalanine and its metabolites with tryptophan that impairs the synthesis of serotonin.
- Defect in myelin formation is observed in PKU patients.

**2. Effect on pigmentation:**

- Melanin is the pigment synthesized from tyrosine by tyrosinase. Accumulation of phenylalanine competitively inhibits tyrosinase and impairs melanin formation. The result is hypo pigmentation that causes light skin colour, fair hair, blue eyes etc.

**DIAGNOSIS OF PKU:**

- PKU is mostly detected by screening the newborn babies for the increased plasma levels of phenylalanine (PKU, 20-65 mg/ dl; normal 1-2mg/dl). This is usually carried out by Guthrie fest, which is a bacterial (Bacillus subtilis) bioassay for phenylalanine.
- The test is usually performed after the baby is fed with breast milk for a couple of days. All the babies born in USA are screened for PKU by testing elevated levels of phenylalanine.
- Phenylpyruvate in urine can be detected by ferric chloride test (a green colour is obtained). This test is not specific, since many other compounds give a false positive test.

**TREATMENT OF PKU:**

- The maintenance of plasma phenylalanine concentration within the normal range is a challenging task in the treatment of PKU. This is done by selecting foods with low phenylalanine content and/or feeding synthetic amino acid preparations, low in phenylalanine.
- Dietary intake of phenylalanine should be adjusted by measuring plasma levels. Early diagnosis (in the first couple of months of baby's life) and treatment for 4-5 years can prevent the damage to brain.
- However, the restriction to protein diet should be continued for many more years in life. Since the amino acid tyrosine cannot be synthesized in PKU patients, it becomes essential and should be provided in the diet in sufficient quantity.
- In some seriously affected PKU patients, treatment includes administration of 5-hydroxy tryptophan and dopa to restore the synthesis of serotonin and catecholamines.

**HOMOCYSTINURIAS**

- Homocystinurias are a group of metabolic disorders characterized by the accumulation and increased urinary excretion of homocysteine and S-adenosylmethionine.

- Homocysteine is an intermediate in the synthesis of cysteine from methionine, (Elevation in plasma homocysteine (normal 〈15μm mol/l) has been implicated in coronary artery diseases, although the mechanism is not known.
- lt is believed that homocysteine reacts with collagen to produce reactive free radicals, besides interfering with collagen cross links. Homocysteine is also involved in the aggregation of LDL particles. All this leads to an increased tendency for atherogenesis, and consequently heart complications. Plasma concentration of methionine is increased.
- Supplementation of diet with folic acid, vitamin 812 and vitamin 86 have some beneficial affects in lowering plasma homocysteine levels.
- **Homocystinuria type** I has been more thoroughly investigated. lt is due to a defect in the enzyme cystathionine synthase. Accumulation of homocystetne results in various complications-thrombosis, osteoporosis and, very often, mental retardation.
- Further, the deficiency of cystathionine is associated with damage to endothelial cells which might lead to atherosclerosis.
- Two forms of type I homocystinurias are known, one of them can be corrected with vitamin B6 supplementation (86 responsive) while the other does not respond to 86. The treatment includes consumption of diet low in methionine and high in cystine.
- The patients of homocystinuria have high levels of homocysteine, and usually die of myocardial infarction, stroke, or pulmonary embolism.
- The other homocvstinurias are associated with enzyme defects (as stated below) in the conversion of homocysteine to methionine by remethylation.

**Homocystinuria ll :** N5 –N10 Methylene THF reductase.

**Homocystinuria lll :** N5 –N10 Methyl THFhomocysteine methyltransferase. This is mostly due to impairment in the synthesis of methylcobalamin.

**Homocystinurla lV :** N5-Methyl THF homocysteine methyl transferase. This is primarily due to a defect in the intestinal absorption of vitamin 812.

**TYROSINOSIS OR TYROSINEMIA TYPE I**

- This is due to the deficiency of the enzymes fumarylacetoacetate hydroxylase and/or maleylacetoacetate isomerase.
- Tyrosinosis is a rare but serious disorder. lt causes liver failure, rickets, renal tubular dysfunction and polyneuropathy.
- Tyrosine, its metabolites and many other amino acids are excreted in urine.

- I n acute tyrosinosis, the infant exhibits diarrhea, vomiting, and 'cabbage-like' odor. Death may even occur due to liver failure within one year.
- For the treatment, diets low in tyrosine, phenylalanine and methionine are recommended.

**TYROSINEMIA TYPE II**

- This disorder-also known as RichnerHanhart syndrome, is due to a defect in the enzyme tyrosine transaminase.
- The result is a blockade in the routine degradative pathway of tyrosine.
- Accumulation and excretion of tyrosine and its metabolites-namely p-hydroxyphenylpyruvate, p-hydroxyphenyllactate, phydroxy phenylacetate, N-acetyltyrosine-and tyramine are observed.
- Tyrosinemia type ll is characterized by skin (dermatitis) and eye lesions and, rarely, mental retardation. A disturbed self-coordination is seen in these patients.

**Neonatal tyrosinemia**

- The absence of the enzyme p-hydroxyphenylpyruvate dioxygenase causes neonatal tyrosinemia.
- This is mostly a temporary condition and usually responds to ascorbic acid. lt is explained that the substrate inhibition of the enzyme is overcome by the presence of ascorbic acid.

**AMINOACIDURIA**

Aminoaciduria is an abnormally high amount of amino acids in the urine. Amino acids are the building blocks for proteins in the body. There are many different types of amino acids. It is common for some of each kind to be found in the urine. Increased levels of individual amino acids can be a sign of a problem with metabolism.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Alanine: 9 to 98 | Arginine: 0 to 8 | Asparagine: 10 to 65 | Aspartic acid: 5 to 50 | Citrulline: 1 to 22 | Cystine: 2 to 12 | Lysine: 2 to 16 | Methionine: 2 to 53 |
| Glutamic acid: 0 to 21 | Glutamine: 11 to 42 | Glycine: 17 to 146 | Histidine: 49 to 413 | Isoleucine: 30 to 186 | Leucine: 1 to 9 | Ornithine: 1 to 5 | Valine: 3 to 36 |
| Phenylalanine: 1 to 5 | Proline: 3 to 13 | Serine: 0 to 9 | Taurine: 18 to 89 | Threonine: 13 to 587 | Tyrosine: 3 to 14 | | |

- Aminoaciduria occurs when the urine contains abnormally high amounts of amino acids.
- In the healthy kidney, the glomeruli filter all amino acids out of the blood, and the renal tubules then reabsorb over 95% of the filtered amino acids back into the blood.
- Aminoaciduria Other names Urine amino acids Share of amino acid in various human diets and the resulting mix of amino acids in human blood serum. Glutamate and glutamine are the most frequent in food at over 10%, while alanine, glutamine, and glycine are the most common in blood.

**Complications**

- Severe protein loss in the blood Risk factors Liver disease, malnutrition, kidney disease. In overflow aminoaciduria, abnormally high concentrations of amino acids in the blood plasma overwhelm the resorptive capacity of the renal tubules, resulting in high concentrations of amino acids in the urine.
- This may be caused by congenital disorders of amino acid metabolism, for example, phenylketonuria or may be secondary to liver disease.
- In renal aminoaciduria, the renal tubules are unable to reabsorb the filtered amino acids back into the blood, causing high concentrations of amino acids in the urine.
- This may be caused by a defect in the transport proteins in the renal tubule, for example, as occurs in Hartnup disease, or may be due to damage to the kidney tubule, for example, as occurs in Fanconi syndrome.

# UNIT –V
# DIAGNOSTIC ENZYMES

**LIVER FUNCTION TESTS (L.F.T.)**

- The following set of tests is commonly used to diagnose liver disease.
- Almost all types of liver disease can be isolated by the use of these following tests.
- Liver disease is fairly common today, so these tests are of particular significance in the diagnosing of these related diseases.

**BROMSULPHALEIN TEST**

This is a liver function test used to diagnose general liver disfunction-, including ob-structive liver disease.

**Clinical Implications**: This test uses an injected dye, BSP, for diagnosis of liver disease. After the injection, several blood samples are taken to determine the blood level of the dye. These levels will indicate the liver's ability to excrete the dye and thus the general functioning of the liver. This test is very diagnostic of inactive cirrhosis of the liver.

**SERUM BILIRUBIN**

This test is a measure of the bilirubin in the blood. Normal Value: total bilirubin = less than 1.5 mg/100ml

**Clinical Implications**: Bilirubin is present in blood at all times due to the break-down of hemoglob-in which occurs all the time. Normally, bilirubin is removed from the blood by the liver. Increased serum bilirubin levels indicate obstructive disease of the liver, hemolysis or actual liver cell damage. direct bilirubin--quick, one-minute test for bilirubin (usually not accurate) indirect bilirubin--30 minute test (more accurate)

**ALKALINE PHOSPHATASE**

This is a liver enzyme test. Alkaline phosphatase (ALP) is produc-ed in the liver and bone, it is also derived from the kidney, intes-tine, and placenta. In obstructive biliary disease, there is elevated serum ALP. Normal Values: 20-90 U/L at 30 degrees C. adult 40-300 U/L child Clinical Implications: This test is very useful for diagnosing biliary obstruction. Even in mild cases of obstructive disease, this enzyme is elevated. It is not very useful for diagnos-ing cirrhosis. If a patient has bone disease, this test may be highly inaccurate, as ALP is also found in bone tissue.

**SGOT, SGPT, LDH**

**Definition**: These enzymes are used to help diagnose liver disease (also MI, refer to previous chapter).

**Clinical Implications:** These enzymes can be indicative of liver disease. However, as stated earlier in this text, these enzymes are also found in other body tissues such as bone, heart, kidney, etc. Isoenzyme tests usually must be performed in order to isolate the isoenzyme that is elevated and if the source is the liver.

**SGPT--Serum Glutamic Pyruvic Transaminase normal**: 5-35 U/ml (highest levels seen in liver disease)

**SGOT--Serum Glutamic Oxaloacetic Transaminase normal:** 5-40 U/ml

**BLOOD AMMONIA**

**Test:** Blood Ammonia level of ammonia in the plasma Normal Values: 3.2 - 4.5 g/dl (depends upon the method used) Clinical Implications: Ammonia is formed due to bacterial action in the intestines and by normal metabolism in all body tis-sues. Most of this ammonia is then absorbed by the intestines and goes into the portal circulation, where normally the liver converts it to urea and it is excreted by the kidneys.

This test then, is most useful in diagnosing hepatic failure, although plasma ammonia levels are not elevated in all cases. Reduced portal circulation (through the liver) can also result in very high ammonia levels. CHF and/or acid-osis may also cause a temporary rise in plasma ammonia. Arterial or venous blood may be used for the specimen in most hospitals; some also recommend putting the specimen on ice and transpo-rt to the lab. A greentop tube (heparinized) is usually used. NPO, except for water, 8 hours prior to the test is usually recommended. High or low protein diets may also affect the lab test results. Exercise and certain antibiotics (neomycin and tetracycline) will usually affect the test results.

**PANCREATIC ENZYMES:**

**Test:** Pancreatic Enzymes: Amylase Amylase is an enzyme that is synthesized primarily in the pancreas and salivary glands. Amylase (alphaamylase or AML) helps to digest starch and glycogen in the mouth, stomach, and intestine. In cases of suspected acute pancreatic disease, measurement of serum or urine AML is the most important laboratory test.

- **Normal serum amylase results:** 25 to 160 U/L
- **Please note: There are more than 20 different lab methods for determining the results of this test. Be sure to use the normal values at your facility. Be sure to withhold drugs that elevate AML levels such as aspirin, asparaginase, azathioprine,

corticosteroids, cyprohepadine, narcotic analgesics, oral contraceptives, rifampin, sulfasalazine, and thiazide or loop diuretics.

- If they cannot be withheld, note them on the lab slip. After the onset of acute pancreatitis, AML levels begin to rise within 2 hours, peak within 12 to 48 hours, and return to normal within 3 to 4 days.
- Determination of urine levels should follow normal serum AML results to rule out pancreatitis.
- Moderate serum elevations may accompany obstruction of the common bile duct, pancreatic duct, ampulla of Vater, pancreatic injury from a perforated peptic ulcer, pancreatic cancer, or acute salivary gland disease. Impaired kidney function may increase serum levels.

**TEST: PANCREATIC ENZYME: LIPASE**

- Lipase is produced by the pancreas and secreted into the duodenum, where it converts triglycerides and other fats into fatty acids and glycerol.
- The destruction of pancreatic cells, which occurs in acute pancreatitis, causes large amounts of lipase to be released into the blood.
- This test is used to measure serum lipase levels. It is most useful when performed with a serum or urine amylase test.
- Normal value: 56 to 239 U/L (depending on method)
- Prior to the test, withhold cholinergics, codeine, meperidine, and morphine.
- If these drugs cannot be withheld, note their use on the lab slip when the specimen is sent to the lab.
- High lipase levels suggest acute pancreatitis or pancreatic duct obstruction.
- After an acute attack, levels remain elevated for up to 14 days.
- Lipase levels may also increase in other pancreatic injuries, such as perforated peptic ulcer with chemical pancreatitis due to gastric juices, and in patients with high intestinal obstruction, pancreatic cancer, or renal disease with impaired excretion.

## GASTRIC FUNCTION TEST

- The healthy functioning of our digestive system is very important for the body. Our stomach is a reservoir of ingested food, and it is through digestion that food breaks down and becomes a source of nourishment. One of the tests which are done to get evaluate how well the digestive system is functioning is the gastric function test.
- The tests help to diagnose chronic problems such as diarrhea, constipation, bloating, and other gastrointestinal disorders.
- Gastric juices are nothing but a variable mixture of water, hydrochloric acid, electrolytes, and mucus.
- They are clear, pale yellow, odorless fluids with acidic pH of around 1 and a gravity of approximately 1.007.
- The test is done through qualitative measures such as checking color, odor, and appearance and quantitative measures which implies estimating free and total acidity levels and chlorides.
- Gastric fluids have various functions to perform. While these secretions contribute to protein digestion, gastric acidity improves the absorption of iron besides facilitating the dissociation of iron salts from the consumed food.
- Gastric acid also allows the formation of complexes with ascorbate and sugars which helps to enhance iron absorption by converting the gastric contents to a semi-liquid pulpy fluid (also called chyme).
- These juices get the food ready for further digestion which happens in the duodenum which is a part of the small intestine.

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## TYPES OF GASTRIC FUNCTION TESTS

### *Fractional Test Meal*

- The test involves the collection of stomach contents with Ryle's tube. It is a long thin rubber tube 4 mm in diameter. It has a lead piece at the tip of the tube and perforations at a short distance away from the tip. The tube is passed into the stomach, and gastric juice is collected periodically. After the samples are thus collected, they are examined for free and total acidity.

***Pentagastrin Stimulation Test***

- Pentagastrin is an artificial peptide that can stimulate gastric secretion. In this test, the contents of the stomach are aspirated with the Ryle's tube, collected at 15-minute intervals, and further analyzed for the next hour.

***Augment Histamine Test Meal***

- One of the most powerful stimulants for gastric secretion, this is also aspirated at 15-minute intervals and collected for a detailed analysis. Through the test samples, the levels of acid content are measured.

***Insulin Test Meal***

- It is mainly done to assess the results of vagotomy. Vagotomy is the process of cutting a branch of the vagus nerve to reduce stomach acid secretion. This test involves delivering electrical impulses to the vagus nerve in the brain. This is also known as Hollander's test and in this test, insulin is intravenously administered to the patient.

***Tubeless Gastric Analysis***

- The test is helpful to ascertain the levels of gastric acidity without the use of complicated or cumbersome chemical procedures and intubation. This gastric acidity analysis is helpful to diagnose problems such as duodenal ulcers, achlorhydria, and pernicious anemia.

**Test Preparation**

- There is no special preparation for the test besides fasting. The patient will have to fast for 12 hours overnight through the next morning till the test is completed. In most of the above types of testing, the patient is asked to swallow the bulbous end of the Ryle tube into the throat. When the first mark is at the level of the incisor teeth, the tip is at the esophagus. When the second mark is at the level of the incisor teeth, the tip has reached the stomach's pyloric region. When the third mark is at the level of the incisor teeth, the tip is at the duodenum.

- Treatment for gastroparesis may involve taking certain medicines and/or treating the condition that is causing the problem, such as diabetes. Regardless of the cause, your provider will likely recommend that you make changes to your diet and eating habits. These may include:
- Eating foods low in fiber and fat.
- Avoiding carbonated drinks, smoking, and alcohol.
- Eating smaller meals throughout the day, rather than two or three large meals.
- Chewing food thoroughly.
- Waiting at least two hours after eating before lying down.

**ASSESSMENT OF KIDNEY /RENAL FUNCTION TEST :**

- **Assessment of kidney function** occurs in different ways, using the presence of symptoms and signs, as well as measurements using urine tests, blood tests, and medical imaging.
- Functions of a healthy kidney include maintaining a person's fluid balance, maintaining an acid-base balance; regulating electrolytes including sodium, potassium, and other electrolytes; clearing toxins; regulating blood pressure; and regulating hormones, such as erythropoietin; and activation of vitamin D.
- The GFR is regarded as the best overall measure of the kidney's ability to carry out these numerous functions. An estimate of the GFR is used clinically to determine the degree of kidney impairment and to track the progression of the disease.
- The GFR, however, does not reveal the source of the kidney disease. This is accomplished by urinalysis, measurement of urine protein excretion, kidney imaging, and, if necessary, kidney biopsy.

**Clinical assessment** can be used to assess the function of the kidneys. This is because a person with abnormally functioning kidneys may have symptoms that develop. For example, a person with chronic kidney disease may develop oedema due to failure of the kidneys to regulate water balance. They may develop evidence of chronic kidney disease, that can be used to assess its severity, for example high blood pressure, osteoporosis or anaemia. If the kidneys are unable to excrete urea, a person may develop a widespread itch or confusion.

**URINE TESTS:**

Part of the assessment of kidney function includes the measurement of urine and its contents. Abnormal kidney function may cause too much or too little urine to be produced. The ability of the kidneys to filter protein is often measured, as urine albumin or urine protein levels,measured either at a single instance or, because of variation throughout the day, as 24-hour urine tests.

**BLOOD TESTS**

- Blood tests are also used to assess kidney function. These include tests that are intended to directly measure the function of the kidneys, as well as tests that assess the function of the kidneys by looking for evidence of problems associated with abnormal function. One of the measures of kidney function is the glomerular filtration rate (GFR).
- Other tests that can assess the function of the kidneys include assessment of electrolyte levels such as potassium and phosphate, assessment of acid-base status by the measurement of bicarbonate levels from a vein, and assessment of the full blood count for anaemia.

**GLOMERULAR FILTRATION RATE (GFR)**

- The glomerular filtration rate (GFR) describes the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time.
- GFR is equal to the renal clearance ratio when any solute is freely filtered and is neither reabsorbed nor secreted by the kidneys.
- The rate therefore measured is the quantity of the substance in the urine that originated from a calculable volume of blood.
- Relating this principle to the below equation – for the substance used, the product of urine concentration and urine flow equals the mass of substance excreted during the time that urine has been collected.
- This mass equals the mass filtered at the glomerulus as nothing is added or removed in the nephron.

- Dividing this mass by the plasma concentration gives the volume of plasma which the mass must have originally come from, and thus the volume of plasma fluid that has entered Bowman's capsule within the aforementioned period of time.

**MEDICAL IMAGING**

The kidney function can also be assessed with medical imaging. Some forms of imaging, such as kidney ultrasound or CT scans, may assess kidney function by indicating chronic disease that can impact function, by showing a small or shrivelled kidney. Other tests, such as nuclear medicine tests, directly assess the function of the kidney by measuring the perfusion and excretion of radioactive substances through the kidneys.

- A decreased renal function can be caused by many types of kidney disease. Upon presentation of decreased renal function, it is recommended to perform a history and physical examination, as well as performing a renal ultrasound and a urinalysis.
- The most relevant items in the history are medications, edema, nocturia , gross hematuria, family history of kidney disease, diabetes and polyuria.
- The most important items in a physical examination are signs of vasculitis, lupus erythematosus, diabetes, endocarditis and hypertension.

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EVALUATION AND MANAGEMENT OF INTESTINAL FUNCTION

Acute intestinal obstruction occurs when there is an interruption in the forward flow of intestinal contents.

This interruption can occur at any point along the length of the gastrointestinal tract, and clinical symptoms often vary based on the level of obstruction. Intestinal obstruction is most commonly caused by intra-abdominal adhesions, malignancy, or intestinal herniation. The clinical presentation generally includes nausea and emesis, colicky abdominal pain, and a failure to pass flatus or bowel movements. The classic physical examination findings of abdominal distension, tympany to percussion, and high-pitched bowel sounds suggest the diagnosis.

**Lab tests**

- **Fecal occult blood test.** A fecal occult blood test checks for hidden (occult) blood in the stool. It involves placing a very small amount of stool on a special card. The stool is then tested in the healthcare provider's office or sent to a lab.

- **Stool culture.** A stool culture checks for the presence of abnormal bacteria in the digestive tract that may cause diarrhea and other problems. A small sample of stool is collected and sent to a lab by your healthcare provider's office. In 2 or 3 days, the test will show whether abnormal bacteria are present.

**Imaging tests**

- **Barium beefsteak meal.** During this test, the patient eats a meal containing barium (a metallic, chalky liquid used to coat the inside of organs so that they will show up on an X-ray). This allows the radiologist to watch the stomach as it digests the meal. The amount of time it takes for the barium meal to be digested and leave the stomach gives the healthcare provider an idea of how well the stomach is working and helps to find emptying problems that may not show up on the liquid barium X-ray.

- **Colorectal transit study.** This test shows how well food moves through the colon. The patient swallows capsules containing small markers which are visible on X-ray. The patient follows a high-fiber diet during the course of the test. The movement of the markers through the colon is monitored with abdominal X-rays taken several times 3 to 7 days after the capsule is swallowed.

- **Computed tomography scan (CT or CAT scan).** This is an imaging test that uses X-rays and a computer to make detailed images of the body. A CT scan shows details of the bones, muscles, fat, and organs. CT scans are more detailed than general X-rays.

- **Defecography.** Defecography is an X-ray of the anorectal area that evaluates completeness of stool elimination, identifies anorectal abnormalities, and evaluates rectal muscle contractions and relaxation. During the exam, the patient's rectum is filled with a soft paste that is the same consistency as stool. The patient then sits on a toilet positioned inside an X-ray machine, and squeezes and relaxes the anus to expel the solution. The radiologist studies the X-rays to determine if anorectal problems happened while the patient was emptying the paste from the rectum.

- **Lower GI (gastrointestinal) series (also called barium enema).** A lower GI series is a test that examines the rectum, the large intestine, and the lower part of the small intestine. Barium is given into the rectum as an enema. An X-ray of the abdomen shows strictures (narrowed areas), obstructions (blockages), and other problems.

- **Magnetic resonance imaging (MRI).** MRI is a diagnostic test that uses a combination of large magnets, radiofrequencies, and a computer to produce detailed images of organs and structures within the body. The patient lies on a bed that moves into the cylindrical MRI machine. The machine takes a series of pictures of the inside of the

body using a magnetic field and radio waves. The computer enhances the pictures produced. The test is painless, and does not involve exposure to radiation. Because the MRI machine is like a tunnel, some people are claustrophobic or unable to hold still during the test. They may be given a sedative to help them relax. Metal objects cannot be present in the MRI room, so people with pacemakers or metal clips or rods inside the body cannot have this test done. All jewelry must be removed before the test.

- **Magnetic resonance cholangiopancreatography (MRCP).** This test uses magnetic resonance imaging (MRI) to view the bile ducts. The machine uses radio waves and magnets to scan internal tissues and organs.

- **Oropharyngeal motility (swallowing) study.** This is a study in which the patient is given small amounts of a liquid containing barium to drink with a bottle, spoon, or cup. A series of X-rays is taken to evaluate what happens as the liquid is swallowed.

- **Radioisotope gastric-emptying scan.** During this test, the patient eats food containing a radioisotope, which is a slightly radioactive substance that will show up on a scan. The dosage of radiation from the radioisotope is very small and not harmful, but allows the radiologist to see the food in the stomach and how quickly it leaves the stomach, while the patient lies under a machine.

- **Ultrasound.** Ultrasound is a diagnostic imaging technique that uses high-frequency sound waves and a computer to create images of blood vessels, tissues, and organs. Ultrasounds are used to view internal organs as they function, and to assess blood flow through various vessels. Gel is applied to the area of the body being studied, such as the abdomen, and a wand called a transducer is placed on the skin. The transducer sends sound waves into the body that bounce off organs and return to the ultrasound machine, producing an image on the monitor. A picture or videotape of the test is also made so it can be reviewed in the future.

- **Upper GI (gastrointestinal) series (also called barium swallow).** Upper GI series is a diagnostic test that examines the organs of the upper part of the digestive system: the esophagus, stomach, and duodenum (the first section of the small intestine). Barium is swallowed and X-rays are then taken to evaluate the digestive organs.

**Endoscopic procedures**

- **Colonoscopy.** Colonoscopy is a procedure that allows the healthcare provider to view the entire length of the large intestine (colon). It can often help identify abnormal growths, inflamed tissue, ulcers, and bleeding. It involves inserting a colonoscope, a long, flexible, lighted tube, in through the rectum up into the colon.

The colonoscope allows the healthcare provider to see the lining of the colon, remove tissue for further exam, and possibly treat some problems that are discovered.

- **Endoscopic retrograde cholangiopancreatography (ERCP).** ERCP is a procedure that allows the healthcare provider to diagnose and treat problems in the liver, gallbladder, bile ducts, and pancreas. The procedure combines X-ray and the use of an endoscope. This is a long, flexible, lighted tube. The scope is guided through the patient's mouth and throat, then through the esophagus, stomach, and duodenum (the first part of the small intestine). The healthcare provider can examine the inside of these organs and detect any abnormalities. A tube is then passed through the scope, and a dye is injected that will allow the internal organs to appear on an X-ray.

- **Esophagogastroduodenoscopy (also called EGD or upper endoscopy).** An EGD (upper endoscopy) is a procedure that allows the healthcare provider to examine the inside of the esophagus, stomach, and duodenum with an endoscope. This is guided into the mouth and throat, then into the esophagus, stomach, and duodenum. The endoscope allows the healthcare provider to view the inside of this area of the body, as well as to insert instruments through the scope for the removal of a sample of tissue for biopsy (if necessary).

- **Sigmoidoscopy.** A sigmoidoscopy is a diagnostic procedure that allows the healthcare provider to examine the inside of a portion of the large intestine, and is helpful in identifying the causes of diarrhea, abdominal pain, constipation, abnormal growths, and bleeding. A short, flexible, lighted tube, called a sigmoidoscope, is inserted into the intestine through the rectum. The scope blows air into the intestine to inflate it and make viewing the inside easier.

**Other procedures**

- **Anorectal manometry.** This test helps determine the strength of the muscles in the rectum and anus. These muscles normally tighten to hold in a bowel movement and relax when a bowel movement is passed. Anorectal manometry is helpful in evaluating anorectal malformations and Hirschsprung disease, among other problems. A small tube is placed into the rectum to measure the pressures exerted by the sphincter muscles that ring the canal.

- **Esophageal manometry.** This test helps determine the strength of the muscles in the esophagus. It is useful in evaluating gastroesophageal reflux and swallowing abnormalities. A small tube is guided into the nostril, then passed into the throat,

and finally into the esophagus. The pressure the esophageal muscles produce at rest is then measured.

- **Capsule endoscopy.** A capsule endoscopy helps healthcare providers examine the small intestine, because traditional procedures, such as an upper endoscopy or colonoscopy, cannot reach this part of the bowel. This procedure is helpful in identifying causes of bleeding, detecting polyps, inflammatory bowel disease, ulcers, and tumors of the small intestine. A sensor device is placed on a patient's abdomen and a PillCam is swallowed. The PillCam passes naturally through the digestive tract while transmitting video images to a data recorder. The data recorder is secured to a patient's waist by a belt for 8 hours. Images of the small bowel are downloaded onto a computer from the data recorder. The images are reviewed by a healthcare provider on a computer screen. Normally, the PillCam passes through the colon and is eliminated in the stool within 24 hours.

- **Gastric manometry.** This test measures electrical and muscular activity in the stomach. The healthcare provider passes a thin tube down the patient's throat into the stomach. This tube contains a wire that takes measurements of the electrical and muscular activity of the stomach as it digests foods and liquids. This helps show how the stomach is working, and if there is any delay in digestion.

- **Magnetic resonance cholangiopancreatography (MRCP).** This test uses magnetic resonance imaging (MRI) to obtain pictures of the bile ducts. The machine uses radio waves and magnets to scan internal organs and tissues.

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**DIAGONSTIC ENZYMOLOGY**

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical importance. Enzymes in the circulation are divided into two Eroups - plasma functional and plasma non-functional.

**l. Plasma specific or Plasma functional enzymes.**

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin etc. lmpairment in liver function or genetic disorders often leads to a fall in the activities of plasma functional enzymes e.g' deficiency of ceruloplasmin in Wilson's disease.

**2, On-plasma specific or plasma non-functional enzymes:**

These enzymes are either totally absent or oresent at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as secretory enzymes. All the other plasma enzymes associated with metabolism of the cell are collectivefy referred to as consfitutive enzymes (e.g. lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatinephosphokinase). Estimation of the activities of non-plasma specific enzymes is very important for the diagnosis and prognosis of several diseases.

The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum enzymes are conveniently used as markers to detect the cellular damage which ultimately helps in the diagnosis of diseases.

**A brief account of selected diagnostic enzymes is discussed**

**Amylase:** The activity of serum amylase is increased in acute pancreatitis (normal 80-180 Somogyi units/dl). The peak value is observed within 8-12 hours after the onset of disease which returns to normal by 3rd or 4th day. Elevated activity of amylase is also found in urine of the patients of acute pancreatitis. Serum amylase is also important for the diagnosis of chronic pancreatitis, acute parotitis (mumps) and obstruction of pancreatic duct.

**Alanine transaminase (ALT/SGPT) :** SCPT is elevated in acute hepatitis of viral or toxic origin, jaundice and cirrhosis of liver (normal 3- 40, lU/l).

**Aspartate transaminase (AST/SGOT):** SCOT activity in serum is increased in myocardial infarction and also in liver diseases (normal 4-4.5IU/I).It may be noted that SCPT is more specific for the diagnosis of liver diseases while SCOT is for heart diseases. This is mainly because of their cellular distribution - SCPT is a cytosomal enzyme while SCOT is found in cytosol and mitochondria.

**Alkaline phosphatase (ALP) :** lt is elevated in certain bone and liver diseases (normal 3-1 3 KA units/dl). ALP is useful for the diagnosis of rickets, hyperparathyroidism, carcinoma of bone, and obstructive jaundice.

**Acid phosphatase (ACP) :** lt is increased in the cancer of prostate gland (normal 0.5-4 KA units/dl). The tartarate labile ACP (normal.1〈 KA units/dl). ) is useful for the diagnosis and pi-ognosis of prostate cancers i.e. ACP is a good tumor marker.

**Lactate dehydrogenase (LDH):** LDH is useful for the diagnosis of myocardial infarction, infective hepatitis, leukemia and muscular dystrophy (serum LDH normal 50-200 lull). LDH has five isoenzymes, the details of which are described later.

**Creatine kinase (CK) .** It is elevated in myocardial infarction (early detection) and muscular dystrophy (normal 10-50 lUL). CK has three isoenzyme.

**LACTATE DEHYDROGENASE (LDH):**

- LDH whose systematic name is L-lactateNAD+ oxidoreductase catalyses the interconversion of lactate and pyruvate .
- LDH has five distinct isoenzymes LDHt, LDH2, LDH3, LDHa and LDH5. They can be separated by electrophoresis (cellulose or starch gel or agarose gel). LDHI has more positive charge and fastest in electrophoretic mobility while LDH5 is the slowest.

**Structure of LDH isoenzymes :**

LDH is an oligomeric (tetrameric) enzyme made up of four polypeptide subunits.

- Two types of subunits namely M (for muscle) and H (for heart) are produced by different genes. M-subunit is basic while H subunit is, acidic. The isoenzymes contain either one or both the subunits giving LDH1to LDH5.
- LDHl (H1) is predominantly found in heart muscle and is inhibited by pyruvate - the substrate.
- Hence, pyruvate is not converted to lactate in cardiac muscle but is converted to acetyl CoA which enters citric acid cycle. LDH5 (M+) is mostly present in skeletal muscle and the inhibition of this enzyme by pyruvate is minimal, hence pyruvate is converted to lactate.
- Further, LDH5 has low K. (high affinity) while LDHl has high Km (low affinity) or pyruvate.
- The differential catalytic activities of LDHl and LDH5 in heart and skeletal muscle, respectively, are well suited for the aerobic (presence of oxygen) and anaerobic (absence of oxygen) conditions, prevailing in these tissues.

**Diagnostic importance of LDH:**

- lsoenzymes of LDH have immense value in the diagnosis of heart and liver related disorders. .
- In healthy individuals, the activity of LDH2 is higher than that of LDHl in serum. In the case of myocardial infarction, LDHI is much greater than LDH2 and this happens within 12 to 24 hours after infarction. Increased activity of LDH5.
- In serum is an indicator of liver diseases. LDH activity in the RBC is 80-100 times more than that in the serum. Hence for estimation of LDH or its isoenzymes, serum should be totally free from hemolysis or else false positive results will be obtained.

**Creatine kinase (CK) or creatine phosphokinase :**

- Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the inter-conversion of phosphocreatine (or creatine phosphate) to creatine.


- CPK2 (MB) is almost undetectable in serum with less than 2%of total CpK. After the myocardial infarction (Ml), withirr the first 6-18 hours, CPK2 increases in the serum to as high as 20%(against 2% normal). CpK isoenzyme , not elevated in skeletal muscle disorders. Therefore, estimation of the enzyme CpK (MB) is the earliest reliable indication of myocardial infarction.

**TRANSAMINASES**

These are enzymes involved in the trasfer of amino from an amino acid to a keto acid.

- Two aminotransferases are in use in diagnostic enzymology.

They are:

- Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT).

**ALANINE TRANSAMINASE (ALT)**

- Alanine transaminase (ALT), also known as alanine aminotransferase, is an enzyme that's mainly found in your liver, though it exists in other parts of your body.
- An enzyme is a type of protein in a cell that acts as a catalyst and allows certain bodily processes to happen. There are thousands of enzymes throughout your body that have important functions.
- An alanine transaminase (ALT) blood test measures the amount of ALT in your blood. ALT levels in your blood can increase when your liver is damaged, so healthcare providers often use an ALT blood test to help assess the health of your liver.

**DIFFERENCE**

- Aspartate transferase (AST) is another enzyme that's commonly measured along with AST in a liver function panel or comprehensive metabolic panel. Both of these enzymes can leak into your bloodstream when certain cells in your body are damaged.
- AST and ALT are both commonly considered liver enzymes, but there are greater amounts of AST in other parts of your body,

such as your heart, skeletal muscles and pancreas. Because of this, ALT is considered to be more directly tied to your liver health, but healthcare providers use both measurements to assess the health of your liver.

High levels of ALT in your blood can be due to damage or injury to the cells in your liver. An increased ALT level may indicate the following conditions:

- Alcohol-induced liver injury.
- Fatty liver disease (too much fat in your liver).
- Hepatitis (liver inflammation).
- Cirrhosis (scarring of the liver).
- Taking medications that are toxic to your liver.
- Liver tumor or liver cancer.
- Liver ischemia (not enough blood flow to your liver, which leads to death of liver tissue).
- Hemochromatosis (having too much iron in your body).
- Mononucleosis ("mono").
- Certain genetic conditions can affect your liver.

Although it's less common, elevated ALT levels can also indicate injury to cells in other parts of your body, since ALT isn't solely found in your liver.

- It's important to know that having a high ALT test result doesn't necessarily mean you have a medical condition. Less than 5% of people with elevated ALT levels have severe liver conditions. Other factors can affect your ALT levels. Your provider will take into consideration several factors, including other blood test results and your medical history, when analyzing your results.
- Having a lower than normal ALT result is uncommon and usually isn't a cause for concern. However, a lower than normal ALT level could indicate a vitamin B6 deficiency or chronic kidney disease.
- If your ALT result is lower than what's considered normal, your healthcare provider will likely have you retake the test or undergo further testing to make sure nothing is causing your low level.

Normal findings

- Adult/child: 4-36 units/L at 37°C, or 4-36 units/L (SI units)
- Elderly: May be slightly higher than an adult

- Infant: May be twofold the level of an adult

**ASPARTATE TRANSAMINASE: (SGOT/GOT):**

Aspartate Aminotransferase (AST), also referred to as glutamate oxaloacetate transaminase (GOT), is one of a group of enzymes which catalyzes the interconversion of amino acids and α-keto acids by transfer of amino groups.

Both AST and alanine aminotransferase (ALT) are normally found in most body fluids, but not in urine except in instances of kidney lesions.

The greatest concentrations of AST are found in heart, liver, muscle, and kidney tissues. Damage to these tissues can greatly elevate serum AST levels. AST is mostly used in the evaluation of liver disease.

Elevated levels are found with acute myocardial infarction, severe angina, hepatitis, and liver necrosis, cancer of the liver, alcoholism, musculoskeletal disease, recent convulsions, heat stroke, severe burns, acute pancreatitis, strenuous exercise, toxic shock syndrome, cerebral infarction, trauma, and intramuscular injection, among others.

Depressed levels are seen in uremia, vitamin B deficiency, and with the administration of some drugs.

**PRINCIPLES OF PROCEDURE**

AST present in the sample catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate, in the presence of pyridoxal-5'- phosphate, forming oxaloacetate and L-glutamate.

Oxaloacetate in the presence of NADH and malate dehydrogenase (MDH) is reduced to L-malate. In this reaction, the NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD by colorimetrically.

(Also known as Serum Glutamate Oxaloacetate Transaminase) Both the enzymes are widely distributed in the body tissues such as heart, liver, skeletal muscle, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST level.

- Causes of rise in plasma AST
- In vitro hemolysis.
- Circulatory failure with shock and hypoxia·
- Myocardial infarction
- Acute viral or toxic hepatitis·
- Cirrhosis·
- Cholestatic jaundice·

- Skeletal muscle disease·
- Severe hemolytic anemia·
- After surgery.
- ALT is increased in hepatocellular injury in dog and cat. It is not useful in evaluating chronic liver disease. ALT may also be elevated in corticosteriod treatment. This enzyme is not useful in evaluating hepatic disease in horse, cow, sheep, goat and pig. Elevation of AST is more specific than that of ALT in evaluating hepatic disorders in large animals.

**ALDOLASE**

- A (ALDOA, or ALDA), also known as fructose-bisphosphate aldolase, is an enzyme that in humans is encoded by the ALDOA gene on chromosome 16.
- The protein encoded by this gene is a glycolytic enzyme that catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP).
- Three aldolase isozymes (A, B, and C), encoded by three different genes, are differentially expressed during development.
- Aldolase A is found in the developing embryo and is produced in even greater amounts in adult muscle.
- Aldolase A expression is repressed in adult liver, kidney and intestine and similar to aldolase C levels in brain and other nervous tissue
- Aldolase A deficiency has been associated with myopathy and hemolytic anemia.

**FUNCTION**

- ALDOA is a key enzyme in the fourth step of glycolysis, as well as in the reverse pathway gluconeogenesis.
- It catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehydes-3-phosphate and dihydroxyacetone phosphate by aldol cleavage of the C3–C4 bond. As a result, it is a crucial player in ATP biosynthesis.
- ALDOA also contributes to other "moonlighting" functions such as muscle maintenance, regulation of cell shape and motility, striated muscle contraction, actin cytoskeleton organization, and regulation of cell proliferation.

- ALDOA likely regulates actin cytoskeleton remodeling through interacting with cytohesin-2 (ARNO) and Arf6.]
- ALDOA is ubiquitously expressed in most tissues, though it is predominantly expressed in developing embryo and adult muscle.
- ALDOA is regulated by the energy metabolism substrates glucose, lactate, and glutamine.
- In human mast cells (MCs), ALDOA has been observed to undergo post-translational regulation by protein tyrosine nitration, which may alter its relative affinity for FBP and/or IP3. This change then affects IP3 and PLC signaling cascades in IgE-dependent responses.[
- Aldolase a (ALDOA) is highly expressed in multiple cancers, including lung squamous cell carcinoma (LSCC), renal cancer, and hepatocellular carcinoma. It is proposed that ALDOA overexpression enhances glycolysis in these tumor cells, promoting their growth.
- In LSCC, its upregulation correlates with metastasis and poor prognosis, while its downregulation reduces tumor cell motility and tumorigenesis. Thus, ALDOA could be a potential LSCC biomarker and therapeutic drug target.
- Aldolase A deficiency is a rare, autosomal recessive disorder that is linked to hemolysis and accompanied by weakness, muscle pain, and myopathy.