1. **write about the sources, activation, biochemical functions, deficiency disease and its detection of thiamine (E-Aug 07)**

**Sources:**

Plant sources like Cereals (outer layer), pulses, oil seeds, nut and yeast.

Animal sources like organ meats, pork, milk etc.

**Active form of Thiamine:**

The co-enzyme form of vitamin is TPP (thiamine pyrophosphate). It is synthesized by phosphorylation of thiamine.

\[ \text{Thiamine} + \text{ATP} \rightarrow \text{TPP} + \text{AMP} \]

**Functions:**

The Thiamine pyrophosphate is acts as Co – carboxylase, and involved in oxidative decarboxylation reactions, & transketolase reactions.

\[ \text{Pyruvate} \xrightarrow{\text{PDH - complex}} \text{acetyl co-A} \]

\[ \text{TTP} \quad \text{CO}_2 \quad \text{NAD} \quad \text{NADH+H} \]

\[ \text{α – keto glutarate} \xrightarrow{\text{α - KDH}} \text{succinyl co -A} \]

\[ \text{TTP} \quad \text{CO}_2 \quad \text{NAD} \quad \text{NADH+H} \]

\[ \text{α – keto amino acids} \xrightarrow{\text{α - KADH}} \text{respected thioesters} \]

\[ \text{TTP} \quad \text{CO}_2 \]

Involved in HMP – shunt for the synthesis of pentoses, and NADPH.

**Deficiency:**

The deficiency of thiamine leads to disease called beri – beri, its features are depending on its type as follows,

**Wet beri-beri:**

- It related to edema of face, trunk, and serous cavities.
- Breathless ness palpitation, swollen calf muscles, elevated systolic pressure, fast and bouncing pulse is seen.
- The heart becomes weak.
Dry beri-beri:
- It is not related to edema, and mostly related to degeneration of nervous system (peripheral neuritis).
- Muscles are weak and unable to movement and patients are become bedridden.

Infantile beri-beri:
The child has symptoms like sleeplessness, restlessness, vomiting, convulsions, and death.

Detection of Thiamine in biological samples:
Assessment of Trasketolase activity in serum is the key indicator of activity TPP
2. Write in detail about glucose homeostasis in the human organism and add a note on its biomedical importance (E-Mar 2002)

**Blood glucose homeostasis:**

Blood glucose is maintained at certain normal range. This normal is for optimal utilization of glucose by the body. In our body, certain tissues like brain, retina, testes etc solely dependent on glucose for their energy and they need glucose at certain concentration. If any alteration in the glucose levels leads to alterations of glucose utilization, which leads to impairment of cell functions.

**Sources of blood glucose:**

Dietary starch – which is degraded to glucose in the intestine and absorbed in to blood.

Gluconeogenesis: glucose is formed from non carbohydrates.

Glycogenolysis: the glycogen present in liver and muscle break down to glucose.

**Utilization of glucose:**

Glucose is used as main energy source by all cells.

Excess glucose is converted to glycogen in both liver and muscle.

Glucose is utilized for synthesis of non-essential amino acids and fat.

Glucose is utilized for synthesis of aminosugars.

**Excretion of glucose:**

The kidney is the major organs which regulate the glucose excretion.

Glucose is excreted through urine if blood glucose is more than 180 mg/dL, this is called as renal threshold level.

The maximum Reabsorption of glucose by renal tubules is 350mg/minute.

In normal healthy persons there is no excretion of glucose in urine.

**Blood glucose regulating during fed state:** following a meal, glucose levels are increased in circulation. The high concentration of these glucose is regulated via two process –

Action of glucokinase: GK specifically found in liver and is having high Km, it means which have low affinity to the substrate, but during postprandial, glucose levels are high and this is enough concentration to bind with enzyme. Hence, immediately after meals the glucose is acted upon by GK in liver and consumed.
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Action of insulin: high concentration of glucose in blood will stimulates the production of insulin from pancreas. Uptake of glucose by most of extrahepatic tissues except brain is dependent on insulin.

**Blood glucose regulation during fasting:**

After meal, about 2.5 hrs the blood glucose levels are regulated to normal range. After another 3 hrs later the glucose is supplied through glycogenolysis, and there after gluconeogenesis.

**Regulation of Glucose by Hormones:**

The various hormones play significant role in regulation of blood glucose concentrations, they are as follows –

1. **Insulin:**

   It is 51 amino acid peptide produced from β-cells of pancreas. Insulin is a hypoglycemic hormone. Insulin lowers the blood glucose by means of following mechanisms

   - Insulin stimulates glycolysis, glycogenesis, HMP shunt, fat synthesis.
   - Insulin suppresses gluconeogenesis, glycogenolysis.

2. **Glucagon:**

   - It is produced from α-cells of pancreas, it functions are agonist to the insulin actions.
   - It stimulates gluconeogenesis, glycogenolysis.

3. **Epinephrine, thyroxine, glucocorticoids:**

   - These hormones are hyperglycemic in nature.
   - They stimulate gluconeogenesis, and glycogenolysis.
   - Glucocorticoids stimulates protein metabolism.

4. **Growth hormone:**

   - It inhibits the glucose utilization by cells.
   - It stimulates protein synthesis.
3. Write short notes on
   a. Biochemical function of vitamin D (E-Apr 2001, SN-Feb 11)

Biochemical functions:

Vitamin D is act as steroid hormone. It binds to specific nuclear proteins and induces the synthesis of mRNA for specific proteins for instance Calmodulin (calbindin), which will leads to biological action

Effect on intestine:

It induces the synthesis of calmdulin from intestinal cells

Calmodulin helps in the absorption of calcium from intestine

Effects on bone:

Calcitriol stimulates the activity of osteoblasts

Osteoblasts are helps in mineralization of bone, briefly they secrete the ALP, and ALP in turn increases the ionic concentration of phosphate.

Effects on kidney:

Increases the reabsorption of calcium and phosphorous from renal tubules
b. Absorption of lipids (SN-Apr 2001, aug 10, aug 08)

Absorption of long chain FA –

The products of lipid digestion namely 2-monoglycerids, LCFA, cholesterol, PLs and lysoPLs are emulsified and formed into mixed micelle by the help of bile salts

This micelle essential for the absorption of other fat soluble compounds such as fat soluble vitamins and steroid drugs etc.

So formed micelle water soluble and they are passively absorbed into mucosal cells

Inside the mucosal cell the LFA re-esterified to form TAG

Free fatty acids are activated into fatty acyl CoA by the enzyme CoA synthase

Two acyl CoA molecules react with monoacyl glycerol to form TAG

**Formation of Chylomicrons:**

TAG, CE, PL and apoB&A are incorporated into chylomicrons

Chylomicrons transported through lacteals into the thoracic duct and then into lymph circulation
c. Excretion of bilirubin and clinical importance of bilirubin estimations (aug 10)

The water soluble conjugated bilirubin is excreted into the bile by an active process and this occurs against a concentration gradient. This is rate limiting step in heme catabolism.

From bile it reaches intestine and decomposed by intestinal bacteria and vonveted into unconjugated reduced urobilinogen. It is further reduced to stercobilingen and it is excreted through feces.

About 20% of urobilinogen reabsorbed from the intestine and returned to liver by portal blood and UBG is again reexcrede via enterohepatic circulation.

Finally both UBG and SBG oxidized to urobilin and stercobilin by atmospheric O2. Both these present in urine as well as feces.

Bilirubin estimation:

Van Den Bergh Test:

Serum bilirubin is estimated by Van den Bergh test. Normal serum does not give positive Van den Bergh reaction.

Principle: diazotised sulfanilic acid reacts with bilirubin to form a purple colour complex called as azobilirubin.

Reagents:
- Sulfanilic acid in HCL
- Sodium nitrate

Types of Van den Bergh reaction and their significance

1. Indirect van den Bergh test: Unconjugated bilirubin in blood gives positive test. This test helps in diagnosing pre-hepatic and hepatic jaundice.
2. Direct van den Bergh test: conjugated bilirubin present in the blood give immediate positive test. This test helps in detection of post hepatic jaundice.

Biphassic test: when both conjugated and unconjugated bilirubin present in higher amount in the sample, a purple colour produced immediate and the colour is intensified on adding alcohol. This is specific for hepatocellular jaundice.
d. Coupling of oxidative phosphorylation, uncouplers and their importance (SN, Mar 02, Aug 08, aug 09, aug 11)

Oxidative phosphorylation:

It is the richest source of energy production in aerobic organisms, the free energy to drive this process is comes from ETC as reducing equivalents using molecular O2 within mitochondria.

The system in mitochondria that couples respiration to the generation of the high energy intermediate, ATP is termed as coupling of oxidative phosphorylation.

The mitochondria contain the series of catalysts known as the respiratory chain that collect and transport reducing equivalent and direct them to their final reaction with O2 to form water. And it also contains machinery for trapping the liberated free energy as high energy phosphate

Mechanism of Oxidative Phosphorylation:

Chemiosmotic theory:

Peter Mitchell in 1961 proposed a theory to explain oxidative phosphorylation.

Generation of proton gradient and synthesis of ATP:

Mitochondria have an outer membrane that is permeable to most metabolites but the inner membrane is selectively permeable and which is the basis of generation of proton gradient. The complexes I, III and IV expel protons form inside to outside of the mitochondria membrane. Inner membrane semipermeable to protons. This causes the electrochemical potential difference across the membrane, once established it inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the ATP synthase. This in turn depends on availability of ADP and Pi.

Uncouplers:

Uncouplers are amphipathic and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. Therefore the oxidation can proceed without phosphorylation.

Uncouplers dissociate oxidation in the respiratory chain from phosphorylation.

These compounds are toxic in vivo, causing respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or Pi.

Eg: The uncoupler that has been used most frequently is 2,4-dinitrophenol, but other compounds act in a similar manner
**Atractyloside** inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion.

The antibiotic **oligomycin** completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP synthase.

**Thermogenin (or the uncoupling protein)** is a physiological uncoupler found in brown adipose tissue that functions to generate body heat, particularly for the newborn and during hibernation in animals.

**Significance of uncoupling:**

The cold habitat animals contain brown adipose tissue, which is rich in electron carriers and they are specialized to carry out an oxidation uncoupled form phosphorylation. This is essential to control body temperature.
e. Biochemical changes in von Gierke's disease and their relation to enzyme deficiency (SN-Apr 2001)

- It is also called as Glycogen storage disease I and it is most common attack 1 in 1 Lack births.
- Glucose 6-phosphatase enzyme is deficient in patients. Hence the glucose cannot be released from liver during overnight fasting and leads to hypoglycaemia
- G-6-P may divert to glycogen synthesis. Therefore, the accumulation of large amount of glycogen in liver leads to enlargement of liver and cirrhosis. Due to this children may die in early age
- The excess G-6-P is then diverted to HMP shunt with increasing production of pentoses and nucleotides
- The more Nucleotides and the large amount of formation of Uric Acids, which is characterized as hyperuricemia
- Other symptoms include, hyperlipidemia, lactic acidosis and ketosis
f. Role of LDL receptors in metabolism of LDL and the disease caused by its defect

Structure of LDL receptor:

LDL receptor also called “apoB, E” receptor, since it is specific for apo B100 and E. It is look like pits and occurs on the cell surface. These pits are coated with a protein called Clathrin on the cytosolic side of the cell membrane. The glycoprotein receptor spans the membrane, the B-100 binding region being at the exposed amino terminal end.

**LDL metabolism:** LDL cholesterol after binding to LDL receptors is taken up intact by endocytosis. The apoprotein and cholesteryl ester are then hydrolyzed in the lysosomes, and cholesterol is translocated into the cell. The receptors are recycled to the cell surface. This influx of cholesterol inhibits in a coordinated manner HMG-CoA synthase, HMG-CoA reductase, and, therefore, cholesterol synthesis; stimulates ACAT activity; and down-regulates synthesis of the LDL receptor. Thus, the number of LDL receptors on the cell surface is regulated by the cholesterol requirement for membranes, steroid hormones, or bile acid synthesis.

LDL receptors is absent is familial hypercholesterolemia, which is characterized by high plasma cholesterol levels.

| Familial hypercholesterolemia (type Ia) | Defective LDL receptors or mutation in ligand region of apo B-100. | Elevated LDL levels and hypercholesterolemia, resulting in atherosclerosis and coronary disease. |
g. Definition expression and significance of Km value (SN, Mar 2002)

**Km-Michaelis menten constant:**

According to Michaelis theory, the formation of enzyme substrate complex is a reversible while the breakdown of complex to enzyme and product is irreversible.

\[ v_i = \frac{V_{\text{max}}[S]}{K_m + [S]} \]

Km value is substrate concentration at half maximal velocity, means 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration.

Km is independent of enzyme concentration. If enzyme concentration is double, the Vmax will be double, but ½ Vmax will remain same. In other words irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration.

**Significance of Km:**

Km is constant and characteristic feature of a particular enzyme for a specific substrate.

Km denotes the affinity of enzyme for substrate. The lesser the km the more affinity for enzyme to substrate and vice versa.

**Kd dissociation constant**

The affinity of an enzyme towards its substrate is inversely related to the dissociation constant.

\[ K_d = E+S \quad \text{ES complex} \quad E+P \]

\[ K_d = k_2/k_1 \quad \text{and} \quad K_m = k_2+k_3/k_1 \]

Therefore, the smaller the tendency for the dissociation of the complex, the greater is the affinity of the enzyme for the substrate.
h. Role of liver in integration of metabolism during post prandial state

For several hours after a meal, while the products of digestion are being absorbed, there is an abundant supply of metabolic fuels. Under these conditions, glucose is the major fuel for oxidation in most tissues; this is observed as an increase in the respiratory quotient (theratio of carbon dioxide produced to oxygen consumed) from about 0.8 in the starved state to near 1.

Glucose uptake into muscle and adipose tissue is controlled by insulin, which is secreted by the B islet cells of the pancreas in response to an increased concentration of glucose in the portal blood. An early response to insulin in muscle and adipose tissue is the migration of glucose transporter vesicles to the cell surface, exposing active glucose transporters (GLUT 4). These insulin-sensitive tissues will only take up glucose from the bloodstream to any significant extent in the presence of the hormone. As insulin secretion falls in the starved state, so the transporters are internalized again, reducing glucose uptake.

The uptake of glucose into the liver is independent of insulin, but liver has an isoenzyme of hexokinase (glucokinase) with a high $K_m$, so that as the concentration of glucose entering the liver increases, so does the rate of synthesis of glucose 6-phosphate. This is in excess of the liver’s requirement for energy and is used mainly for synthesis of glycogen. In both liver and skeletal muscle, insulin acts to stimulate glycogen synthase and inhibit glycogen phosphorylase. Some of the glucose entering the liver may also be used for lipogenesis and synthesis of triacylglycerol. In adipose tissue, insulin stimulates glucose uptake, its conversion to fatty acids, and their esterification; and inhibits intracellular lipolysis and the release of free fatty acids. The products of lipid digestion enter the circulation as triacylglycerol-rich chylomicrons. In adipose tissue and skeletal muscle, lipoprotein lipase is activated in response to insulin; the resultant free fatty acids are largely taken up to form triacylglycerol reserves, while the glycerol remains in the bloodstream and is taken up by the liver and used for glycogen synthesis or lipogenesis. Free fatty acids remaining in the bloodstream are taken up by the liver and reesterified.

The lipid-depleted chylomicron remnants are also cleared by the liver, and surplus liver triacylglycerol—including that from lipogenesis—is exported in very low density lipoprotein. Under normal feeding patterns the rate of tissue protein catabolism is more or less constant throughout the day; it is only in cachexia that there is an increased rate of protein catabolism. There is net protein catabolism in the postabsorptive phase of the feeding cycle and net protein synthesis in the absorptive phase, when the rate of synthesis increases by about 20–25%. The increased rate of protein synthesis is, again, a response to insulin action. Protein synthesis is an energy-expensive process, accounting for up to almost 20% of energy expenditure in the fed state, when there is ample supply of amino acids from the diet, but under 9% in the starved state.
i. Caloric requirement and its recommended distribution among nutrients in an adult male (SN-Apr 2001)