Lectures of Biochemistry III-4th class

1st lecture Introduction of Metabolism

Metabolism is the set of life-sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

Metabolism is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism. Metabolism can be conveniently divided into two categories:

- Catabolism - the breakdown of molecules to obtain energy
- Anabolism - the synthesis of all compounds needed by the cells

Anabolism is the set of constructive metabolic processes where the energy released by catabolism is used to synthesize complex molecules. In general, the complex molecules that make up cellular structures are constructed step-by-step from small and simple precursors. Anabolism involves three basic

stages. Firstly, the production of precursors such as aminoacids, monosaccharides,isoprenoids and nucleotides, secondly, their activation into reactive forms using energy from ATP, and thirdly, the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids.

Catabolic reactions are used to capture and save energy from nutrients, as well as to degrade larger molecules into smaller, molecular raw materials for reuse by the cell. The energy is stored in the form of energy-rich ATP, whichpowers the reactions of anabolism. The useful energy of ATP is stored in theform of a high-energy bond between the second and third phosphate groups of ATP. The cell makes ATP by adding a phosphate group to the molecule adenosinediphosphate (ADP). Therefore, ATP is the major chemical link between the energy-yielding reactions of catabolism, and the energy-requiring reactions of anabolism.

The food we eat, (carbohydrates, lipids, and proteins), are our only source of energy for doing the biological work of cells.

Three major metabolic uses for nutrients:

1. used immediately for energy for active processes

2. Synthesized into structural or functional molecules

3. Synthesized as fat or glycogen for later use as energy

Pathway	Function
glycolysis, citric acid cycle	complete degradation of glucose for ATP
respiratory chain	
hexose monophosphate shunt	degradation of glucose for regeneration of NADPH
glycogen synthesis and degradation	short-term glucose storage
gluconeogenesis	synthesis of glucose from amino acids, lactate, or acetone

OXIDATION-REDUCTION REACTIONS

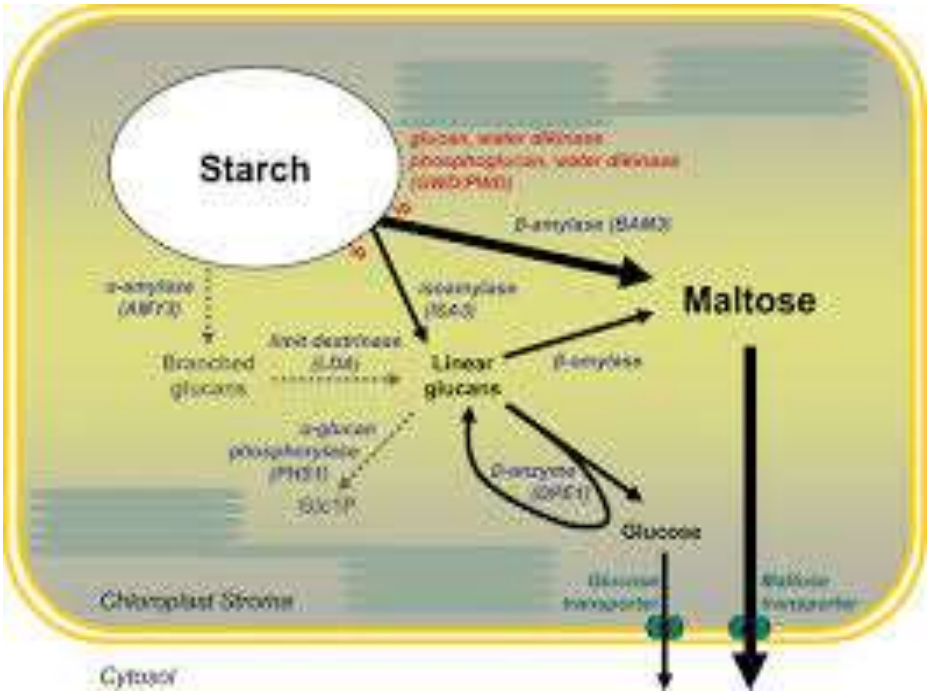
Oxidation = the removal of electrons from a molecule and results in a decrease in the energy content of the molecule. Because most biological reactions involve the loss of hydrogen atoms, they are called dehydrogenation reactions.

Reduction = the opposite of oxidation; the addition of electrons to a molecule, and results in an increase in the energy content of the molecule.

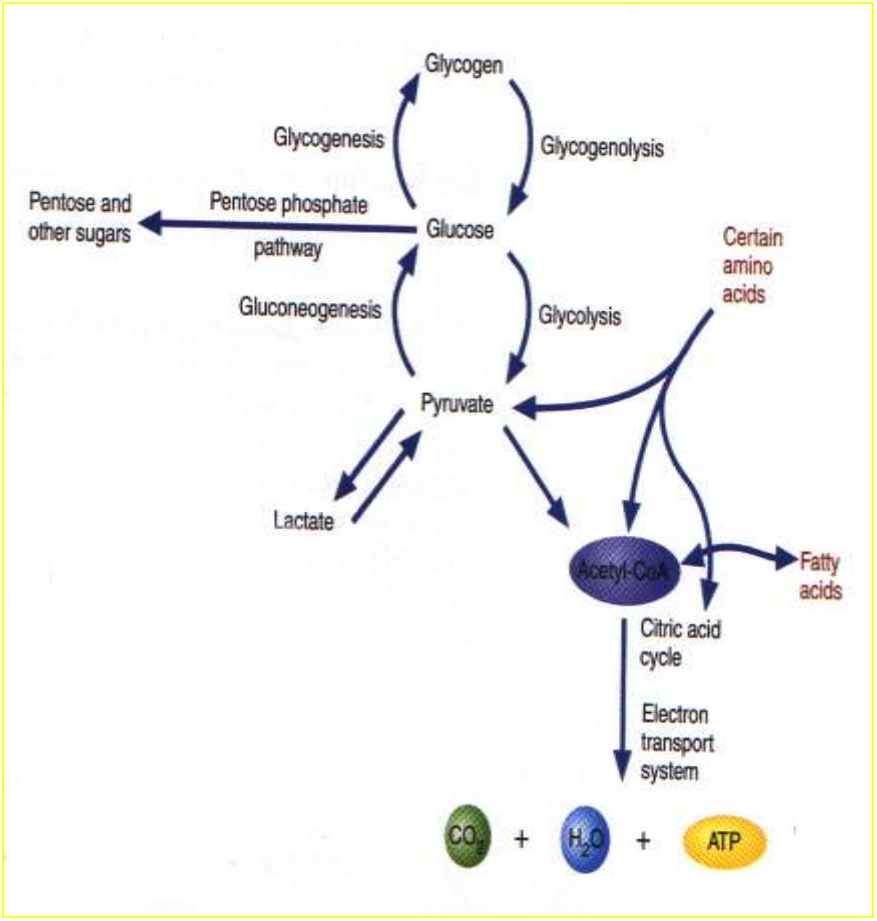
An important point to remember in Oxidation-Reduction reactions is that oxidation is usually an energy-releasing reaction.

2nd lecture CARBOHYDRATE METABOLISM

During digestion, polysaccharides are converted to monosaccharide (primarily glucose), which are absorbed and transported to the liver via the hepatic portal veins.



Digestion of carbohydrates



Carbohydrates pathways

Glucose is the body's preferred source for synthesizing ATP.

If cells require immediate energy, glucose is oxidized by the cells to produce ATP.

Glucose can also be used to form amino acids, which then can be incorporated into proteins.

Excess glucose can be stored by the liver and skeletal muscle as glycogen (how animals store carbohydrate)in a process called glycogenesis.

If glycogen storage areas are filled up ,liver cells and fat cells convert glucose to glycerol and fatty acids that can be used for synthesis of triglycerides in a process called lipogenesis.

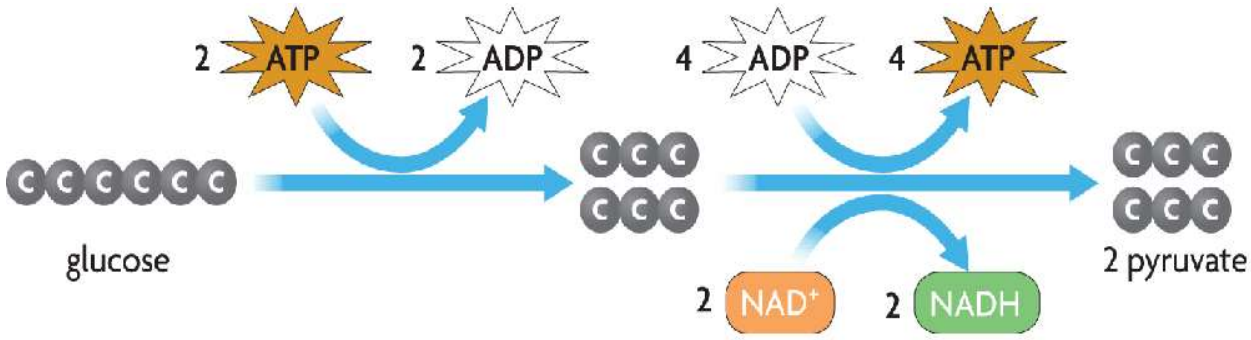
Glucose Catabolism:

Glycolysis is an oxidative specific pathway by which one mole of glucose is enzymatically split into two moles of pyruvate. It occurs in cytosol of all cells of the body. The principle function of glycolysis is the generation of ATP. Glycolysis also provides precursors for fatty acids biosynthesis, for the synthesis of amino acids and pentoses. Anaerobic glycolysis is a process that functions in the absence of oxygen, the final product of anaerobic glycolysis is lactate .In some cell types anaerobic glycolysis generates all of the cell's ATP requirements (in RBC which lack mitochondria) or at least a portion of all ATP requirements (in skeletal muscles at the onset of exercise and during intensive exercise and in tissues like lymphocytes, white blood cells, the kidney medulla). Aerobic glycolysis is a process that functions when oxygen is available, the final product of aerobic glycolysis is pyruvate .Glycolysis can be divided into two stages:

1) the conversion of glucose into 2 triose phosphates: dihydroxyacetone phosphate and glyceraldehyde 3-phosphate . This stage involves a series of reactions that require two molecules of ATP for each molecule of glucose that is split. Dihydroxyacetone phosphate is reversibly converted into glyceraldehyde-3-phosphate . Thus in this stage one mole of glucose is split into two moles of glyceraldehyde 3- phosphate.

2) the conversion of 2 moles of glyceraldehyde 3-phosphate into 2 moles of pyruvate (steps: 6 - 10)). Pay attention that the number of moles of all metabolites in these steps (6 - 10) is multiplied by coefficient 2. During this stage ATP, NADH, pyruvate are produced.

The complete oxidation of glucose to CO_2, H_2O results in large amounts of energy (ATP) and occurs in successive stages: glycolysis, formation of acetyl CoA, the tricarboxylic acid cycle and the electron transport system.



Glycolysis reactions use two ATP molecules, but produce four, resulting in a net gain of two ATP.

Anaerobic glycolysis Enzymes

1 – hexokinase / glucokinase (in liver) 2 – phosphoglucose isomerase 3 – phosphofructokinase 4 – aldolase 5 – triose phosphate isomerase 6 - glyceraldehyde 3-phosphate dehydrogenase 7 – phosphoglycerate kinase 8 – phosphoglyceromutase 9 – enolase 11 – lactate dehydrogenase 10 –pyruvate kinase

Aerobic glycolysis - Complete oxidation of glucose. Enzymes:

1 – hexokinase / glucokinase (in liver) 2 - phosphoglucose isomerase 3 – phosphofructokinase 1 4 – aldolase 5 - triose phosphate isomerase 6 - glyceraldehyde 3-phosphate dehydrogenase 7 – phosphoglycerate kinase 8 – phosphoglyceromutase 9 – enolase 10 – pyruvate kinase

Formation of Acetyl Coenzyme A:

1. Pyruvic acid is prepared for entrance into the tricarboxylic acid cycle (Kreb's cycle)by conversion to a two-carbon compound (acetyl group) followed by the addition of coenzyme A (CoA) to form acetyl coenzyme A (acetyl CoA).

2. Coenzyme A is derived from pantothenic acid, a B vitamin.

Interaction of glycolysis and gluconeogenesis: The Cori Cycle - The Cori Cycle operates during exercise, when aerobic metabolism in muscle cannot keep up with energy needs. - Glucose synthesized in liver and transported to muscle and blood. - A highly exercising muscle generates a lot of NADH from glycolysis but without oxygen there is no way to regenerate NAD+ from the NADH (need NAD+ !) - Lactic acidosis can and would result from insufficient oxygen (an increase in lactic acid and decrease in blood pH) - So, the NADH is reoxidized by reduction of pyruvate to lactate by enzyme lactate dehydrogenase; Results in replenishment of NAD+ for glycolysis, Lactate is transported through the bloodstream to the liver o Lactate is oxidized to pyruvate in the liver .Liver lactate dehydrogenase reconverts lactate to pyruvate since has high NAD+ /NADH ratio

Pyruvate is used to remake glucose by gluconeogenesis - Glucose is transported back to the muscles via the bloodstream

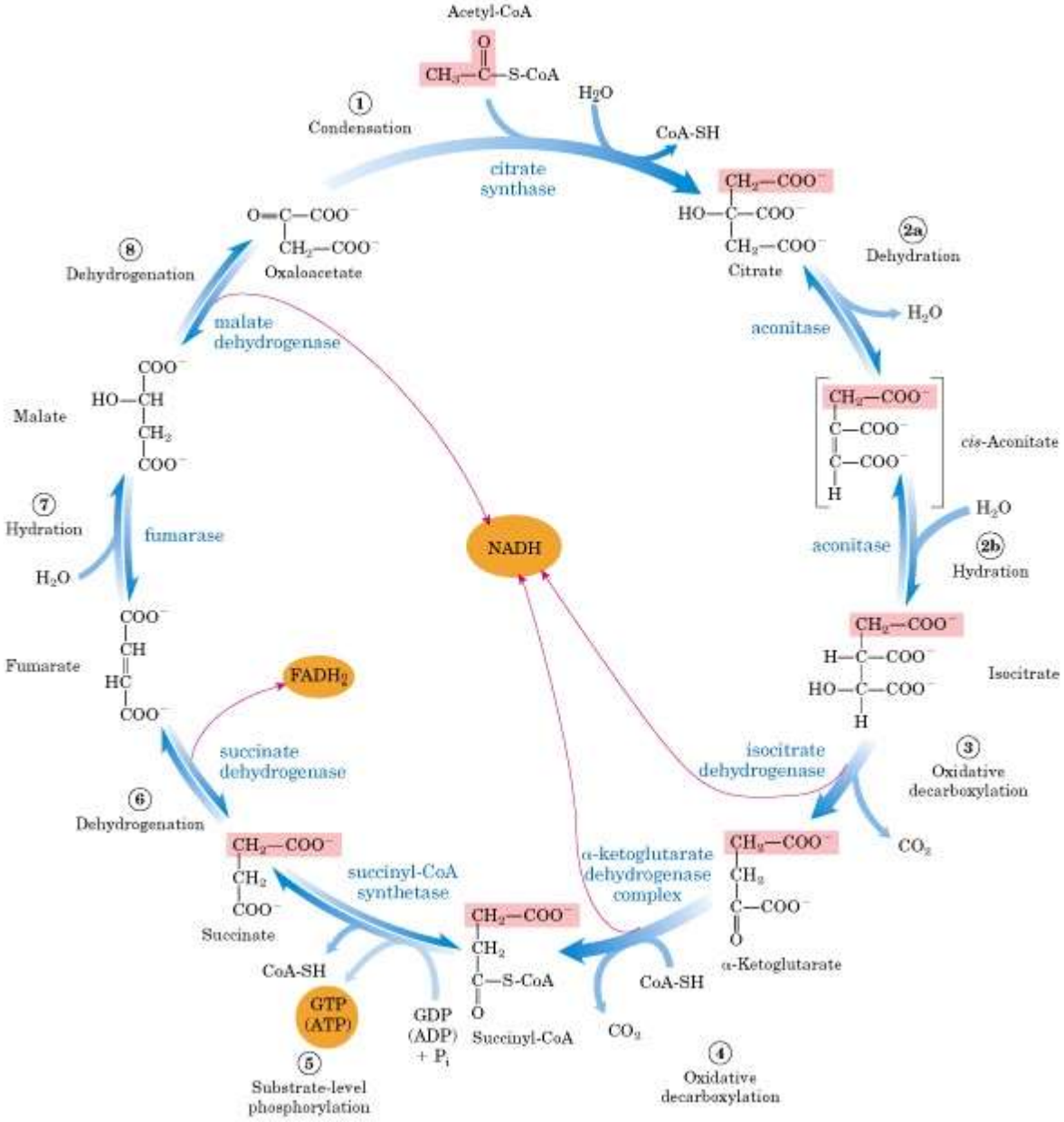
ETHANOL METABOLISM: - Metabolism of ethanol alters the NAD+ /NADH ratio in cells - Primary site of ethanol metabolism is LIVER - Some in stomach, kidneys, bone marrow - Ethanol à Acetate by TWO enzymes in liver

1. Alcohol Dehydrogenase (cytosol)

2. Aldehyde Dehydrogenase (mitochondrial matrix)

3rd lecture

Tricarboxylic Acid Cycle (TCA):



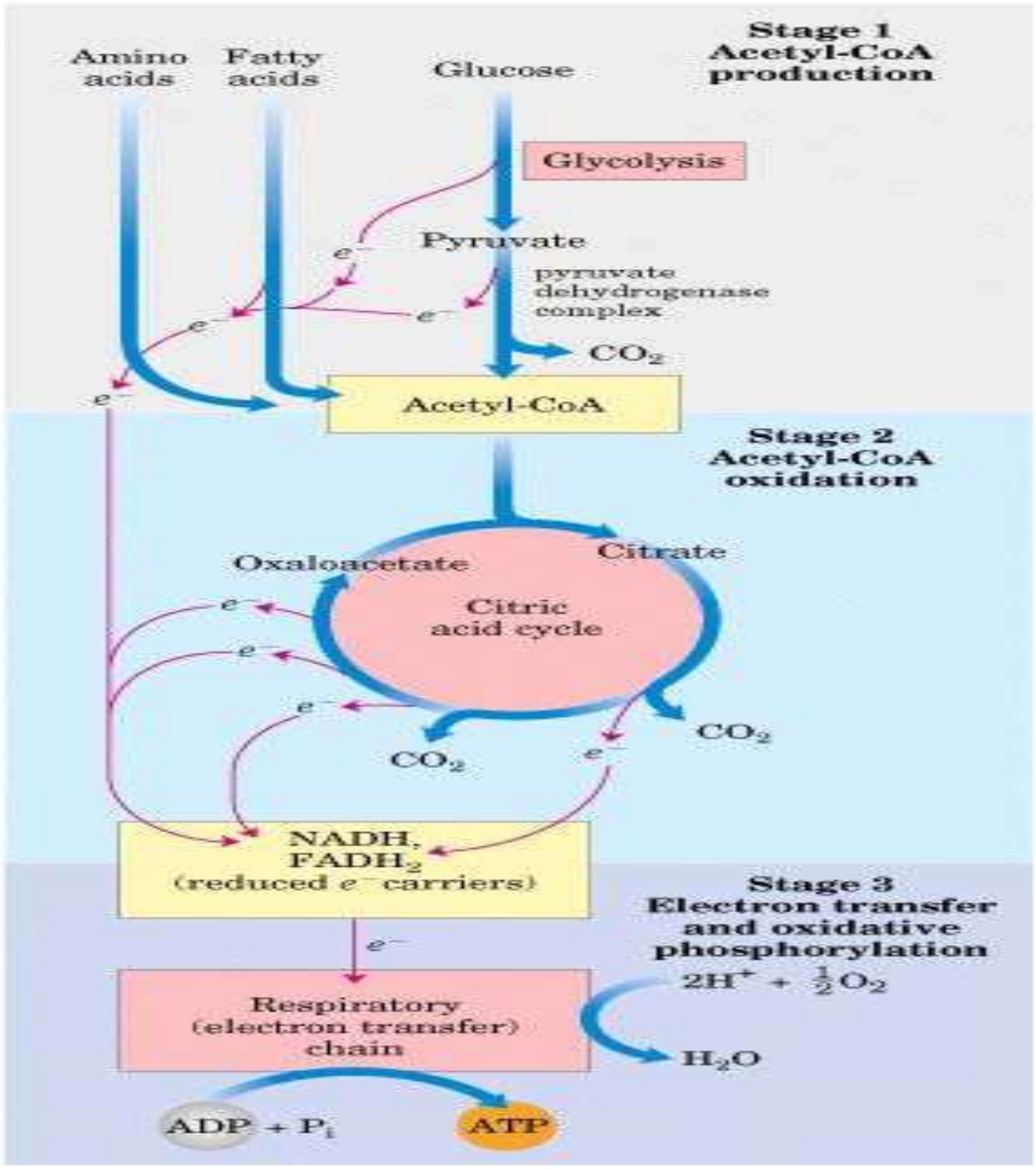
1. Also called the Kreb's cycle or Citric Acid Cycle.

2. The TCA is a series of biochemical reactions that occurs in the matrix of mitochondria.

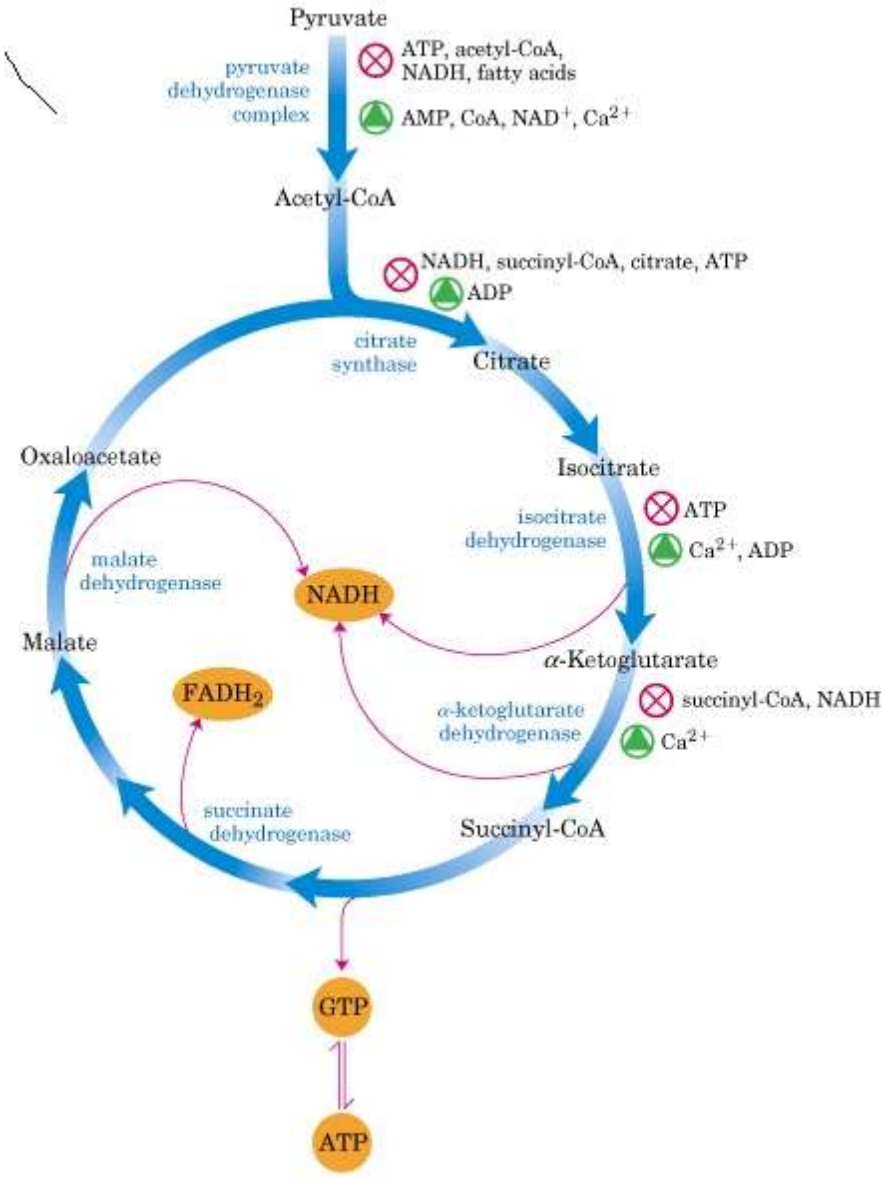
3. A large amount of chemical potential energy stored in intermediate substances derived from pyruvic acid is released step by step.

4. The TCA cycle involves decarboxylations, oxidations and reductions of various organic acids.

5. For every two molecules of acetyl CoA that enters the TCA cycle, 6 H+, 6 NADH and 2 $FADH_2$ are produced by oxidation-reduction reactions and two molecules of ATP are generated.


Stage 1
Acetyl-CoA
production
Amino acids
Fatty acids
Glucose
Glycolysis
Pyruvate
pyruvate dehydrogenase complex
CO_2
Acetyl-CoA
Stage 2
Acetyl-CoA
oxidation
Oxaloacetate
Citrate
Citric acid cycle
CO_2
CO_2
NADH, $FADH_2$
(reduced e^- carriers)
Stage 3
Electron transfer and oxidative phosphorylation
$2H^+ + \frac{1}{2}O_2$
H_2O
Respiratory (electron transfer) chain
ADP + P_i
ATP

Regulation of CAC:
Rate controlling enzymes:
Citrate synthatase
Isocitrate dehydrogenase
・- keoglutaratedehydrogenase

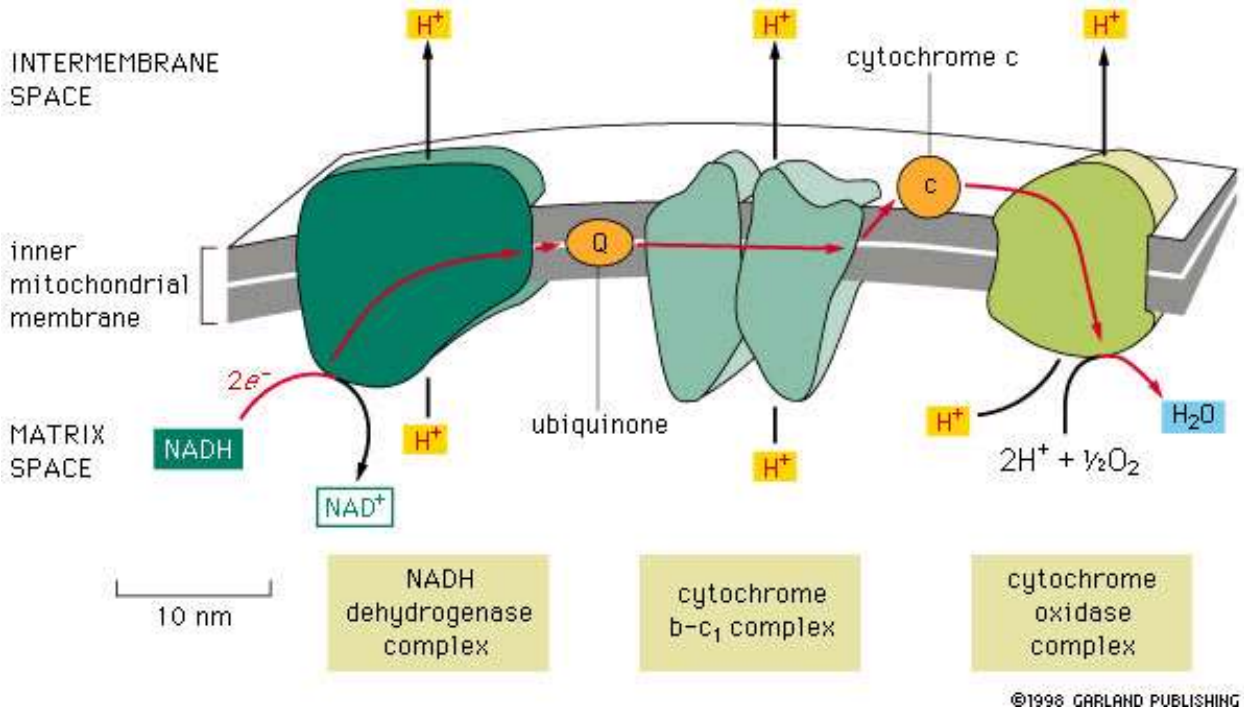


4th lecture

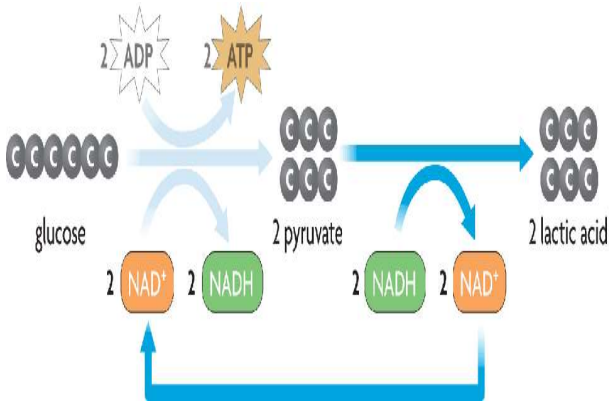
Electron Transport System:

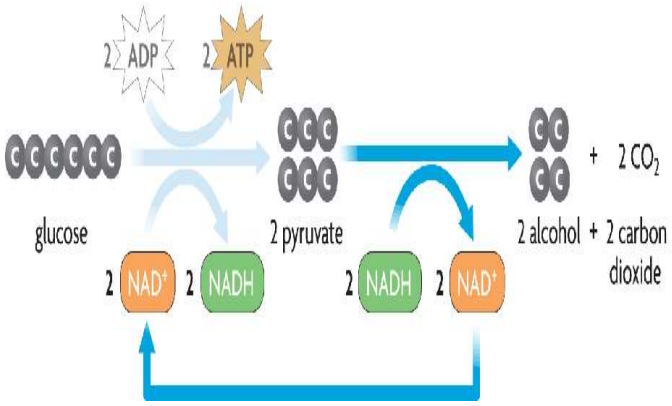
1. Involves a sequence of electron carrier molecules on the inner mitochondrial membrane, capable of a series of oxidation-reduction reactions.

a) As electrons are passed through the chain, there is a step-like release of energy from the electrons which is used for the generation of ATP.



b) in aerobic cellular respiration, the last electron receptor of the chain is molecular oxygen (O2).





Lactic Acid Fermentation **Alcoholic Fermentation**

2. The process involves a series of oxidation-reduction reactions in which the energy in NADH and FADH2 is liberated and transferred to ATP for storage.

a) This mechanism of ATP generation links chemical reactions (electrons passing along the electron chain) with pumping of hydrogen ion (H+).

b) This process is called chemiosis.

Summary of Aerobic Cellular Respiration:

C6H12O6 + 6 O2 >>>>>> 6 CO2 + 6 H2O = 36 ATP

Note: Carbohydrate loading is the practice of eating large amounts of carbohydrates prior to an athletic endurance event such as a marathon run. This is done to fill up the carbohydrate (glycogen) storage areas and provide additional energy for the marathon race. Carbohydrates are the main source of energy for humans.

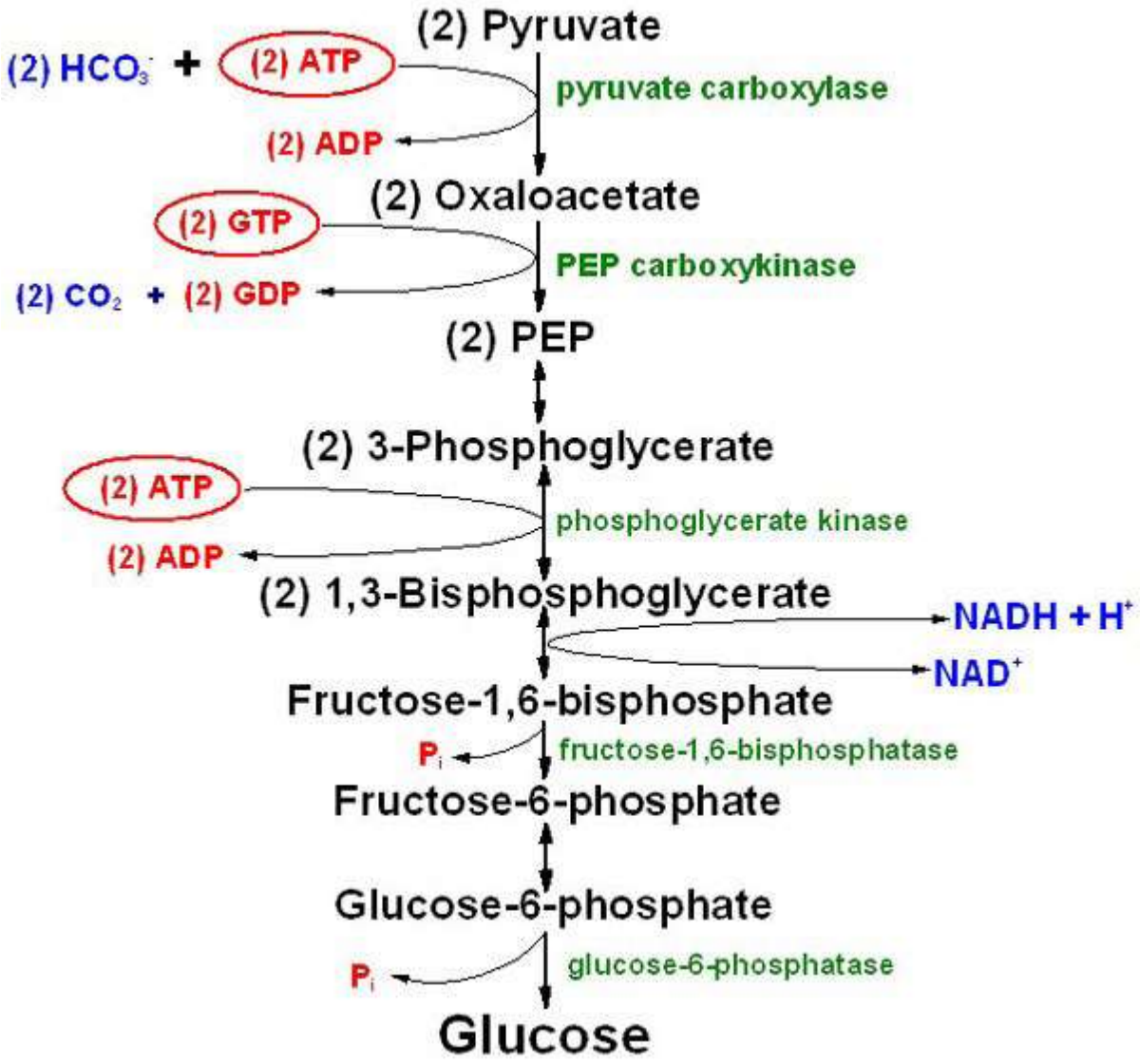
5th lecture

GLUCOSE ANABOLISM

The conversion of glucose to glycogen for storage in the liver and skeletal muscle is called glycogenesis. The process is stimulated by insulin.

The conversion of glycogen back into glucose is called glycogenolysis. This process occurs between meals and is stimulated by glucagon and epinephrine.

Gluconeogenesis is the conversion of protein or fat molecules into glucose. Glycerol from fat can be converted to glyceraldehyde-3-phosphate and some amino acids may be converted to pyruvic acid. Both of these compounds can enter the TCA cycle to provide energy.



Gluconeogenesis is the synthesis of glucose from noncarbohydrate precursors. It occurs in fasting conditions and maintains normal blood glucose level. (Remember that glucose is universal fuel for human cells; if blood glucose decreases, tissues that depend on glucose would suffer from a lack of energy). Carbon sources for gluconeogenesis depend on physiological states in humans. In fasting the breakdown of adipose triacylglycerol releases glycerol that serves as a source of

carbon in gluconeogenesis. During starvation the major precursors of glucose formation are amino acids obtained by degradation of muscle protein and glycerol. Lactate produced by exercising muscle and red blood cells serves as a source of carbon in gluconeogenesis during exercise. Liver, kidney and intestine contain glucose-6- phosphatase which catalyzes the production of free glucose. The liver is responsible for 85% - 95% of glucose production, the kidney cortex and epithelial cells of the small intestine also contributes to glucose formation. The normal fasting glucose range is 80- 100 mg/dL (3.5 -5.5 mM/l).

Gluconeogenesis and glycolysis differ at only three points. They involve conversion of pyruvate to phosphoenolpyruvate (it occurs in two steps and is catalyzed by two enzymes instead of the single enzyme used for glycolysis), removing phosphate from fructose 1,6-bisphosphate to form fructose 6-phosphate and removing phosphate from glucose 6-phosphate to form glucose. Thus, glucose is not generated by reactions which are simply reversals of glycolysis .Pyruvate (produced from lactate, alanine and other aminoacids) is first converted to oxaloacetate .by pyruvate carboxylase, a mitochondrial enzyme that requires biotin and ATP. Oxaloacetate is converted to malate or aspartate, which travels to cytosol and is reconverted to oxaloacetate. The conversion of oxaloacetate to phosphoenolpyruvate is catalyzed by phosphoenolpyruvate carboxykinase, a cytosolic enzyme that requires GTP. Fructose-6-bisphosphatase converts fructose

1,6-bisphosphate to fructose-6-phosphate releasing inorganic phosphate. Glucose-6-phosphatase releases inorganic phosphate converting glucose 6-phosphate to free glucose, which enters the blood.

Pentose phosphate pathway (PPP) is an alternative pathway of carbohydrate metabolism. PPP oxidizes glucose-6-phosphate to intermediates of glycolytic pathway (fructose 6-phosphate and glyceraldehyde 3-phosphate), generating NADPH and ribose 5- phosphate. The NADPH is utilized for reductive pathways, such as fatty acid biosynthesis, detoxification of drugs by monoaminooxidases and the glutathione defense system against injury by reactive oxygen species .Ribose 5-phosphate is required for nucleotides synthesis and synthesis of nucleic acids. PPP has two phases. Initially there is oxidative phase that requires 3 enzymes, 2 of which NADP+ specific dehydrogenases. Glucose-6-phosphate dehydrogenase catalyzes the rate limiting reaction of the pathway. The cellular concentration of NADP+ is the major controlling factor; its availability regulates the rate limiting reaction. The reactions of nonoxidative phase are reversible. They can serve for hexoses generation from pentose.

6th lecture

LIPID METABOLISM

Most proteins are transported in the blood in combination with proteins as lipoproteins. There are 4 classes of lipoproteins:

1. chylomicrons

2. VLDL's (very low density lipoproteins)

3. LDL's (low density lipoproteins)

4. HDL's (high density lipoproteins)

Chylomicrons:

Form in small intestinal mucosal cells and contain dietary lipids. They enter villi lacteals, are carried into the systemic circulation into adipose tissue where their triglyceride fatty acids are released and stored in the adipocytes and used by muscle cells for ATP production.

VLDL's:

Are transport vehicles that carry triglycerides synthesized in hapatocytes to adipocytes for storage.

LDL's:

Carry about 75% of total blood cholesterol and deliver it to cells throughout the body. When present in excessive numbers, LDL's deposit cholesterol in and around smooth muscle fibers in arteries.

HDL's:

Remove excess cholesterol from body cells and transport it to the liver for elimination.

NOTE:

There are two sources of cholesterol in the body: food we eat, and liver synthesis.

For adults, desirable levels of blood cholesterol are under 200 mg/dL for total cholesterol; LDL under 130 mg/dL; and HDL over 40 mg/dL. Normally, triglycerides are in the range of 10-190 mg/dL.

Exercise, diet and drugs may be used to reduce blood cholesterol levels.

Fate of Lipids:

Some lipid may be oxidized to produce ATP, where each unit of lipid produces TWICE the amount of ATP as an equivalent unit of carbohydrate.

Some lipids are stored in adipose tissue.

Other lipids are used as structural molecules or to synthesize essential molecules. Examples include phospholipids of cell membranes, lipoproteins that transport cholesterol, and cholesterol used to synthesize bile salts and steroid hormones.

7th lecture

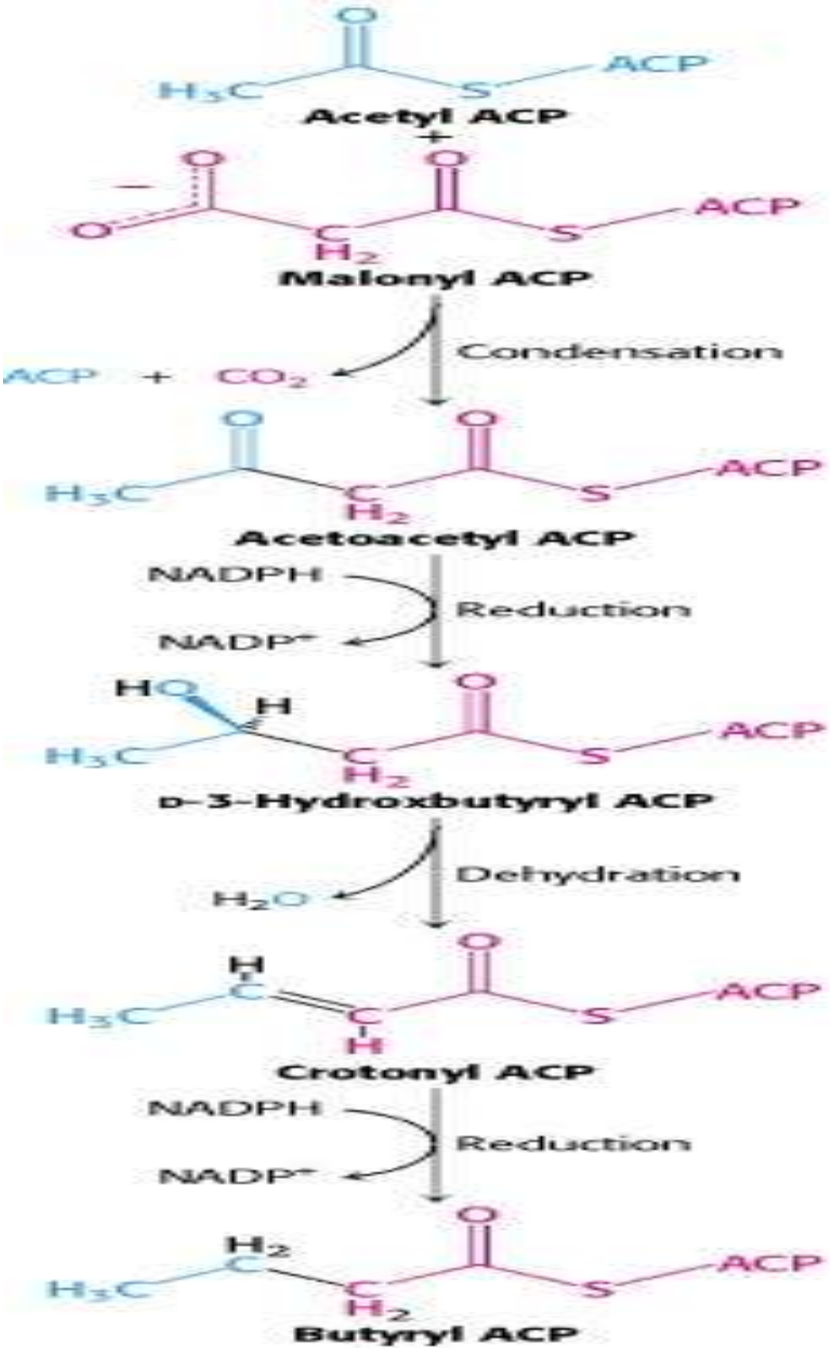
Triglyceride Storage:

Triglycerides are stored in adipose tissue, mostly in the subcutaneous layer.

Adipose cells contain lipases that hydrolyze fats into glycerol and fatty acids.

LIPID ANABOLISM: Lipogenesis

Fatty Acid Synthesis. Fatty acids are synthesized by the repetition of the following reaction sequence: condensation, reduction, dehydration, and reduction. The intermediates shown here are produced in the first round of synthesis.



Lipogenesis = the conversion of glucose or amino acids into lipids.

Fatty acid Synthesis Synthesis of FA through condensation of C2 (C3-CO2) units - reversal of β-oxidation Cytosolic, NADPH <-> mitochondrial, FAD, NAD Difference in stereochemistry C3 unit for growth (malonyl-CoA) <-> C2 for oxidation (acetyl-CoA) Growing chain esterified to acyl-carrier protein (ACP) Esterified to phosphopantetheine group as in CoA which itself is bound to a Ser on ACP ACP synthase transfers phosphopantetheine to apo-ACP to form a holo-ACP

The Stoichiometry of Fatty Acid Synthesis

The stoichiometry of the synthesis of palmitate is

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ \text{H}^+ \longrightarrow \text{palmitate} + 7\ \text{CO}_2 + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O}$$

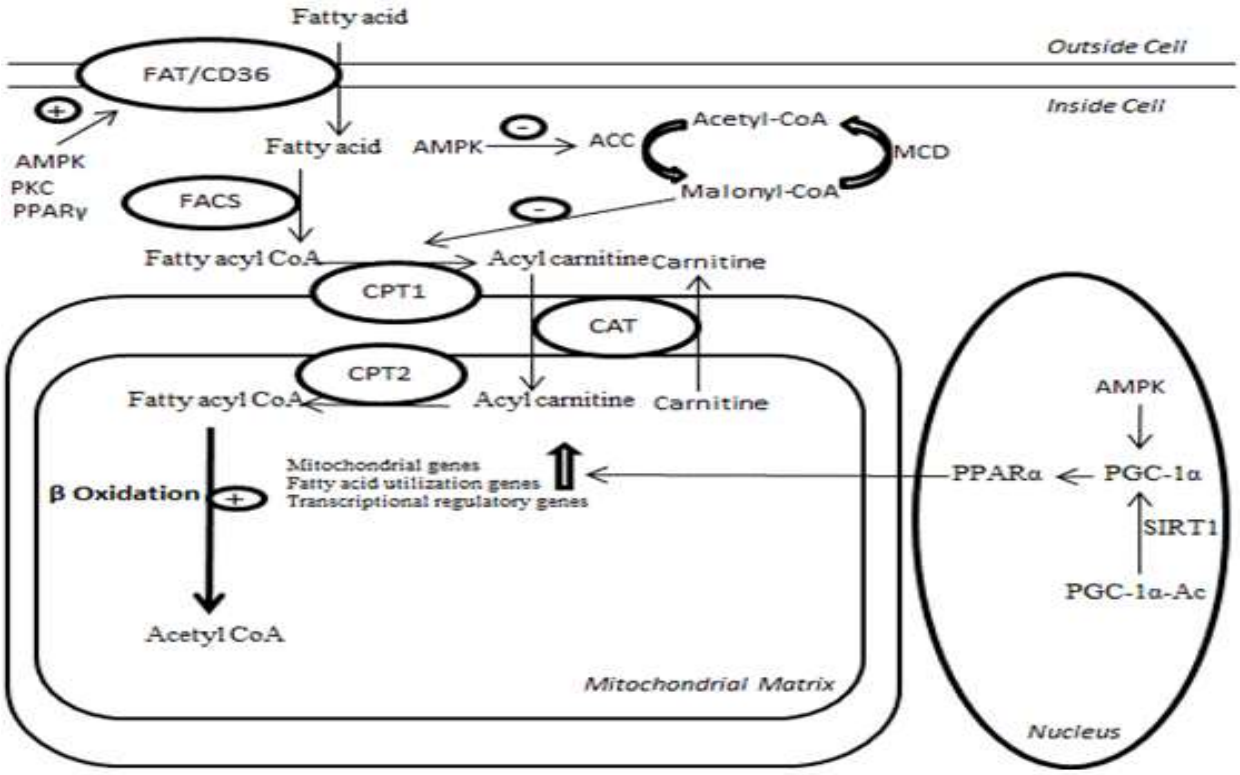
The equation for the synthesis of the malonyl CoA used in the preceding reaction is

$$7\ \text{Acetyl CoA} + 7\ \text{CO}_2 + 7\ \text{ATP} \longrightarrow 7\ \text{malonyl CoA} + 7\ \text{ADP} + 7\ \text{P}_i + 14\ \text{H}^+$$

Hence, the overall stoichiometry for the synthesis of palmitate is

$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ \text{H}^+ \longrightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O} + 7\ \text{ADP} + 7\ \text{P}_i$$

LIPID CATABOLISM: Lipolysis



Lipolysis = triglycerides are split into fatty acids and glycerol.

As a part of normal fatty acid catabolism, ketone bodies are formed.

An excess of ketone bodies (ketosis), may cause acidosis or abnormally low blood pH.

Each round of β-oxidation produces: 1 NADH -> 3 ATP 1 FADH2 -> 2 ATP 1 acetyl-CoA -> TCA (1 GTP, 3 NADH, 1 FADH2) (

Ketogenesis

3 step reaction: 1. Condesation of 2 acetylCoA -> acetoacetyl-CoA (reversal of thiolase rxt) 2. Addition of third acetylCoA 3. Cleavage by HMG-CoA lyase Ketosis: Spontaneous decarboxylation of acetoacetate to CO2 and acetone breath (more fuel than used)

Regulation of fatty acid metabolism Differences in energy needs: - between resting and activated muscle 100x - feed <-> fasting - Breakdown of glycogen and fatty acids concern the whole organism - organs and tissues connected by blood stream, coordination - Blood glucose levels sensed by pancreatic α cells, glucose down -> secrete glucagon -> glycogen degradation, - β cells, glucose up -> insulin -> glucose uptake, FS synthesis - These hormones also control fatty acid synthesis <-> β oxidation Short term regulation regulates catalytic activities of key enzymes in minutes or less: substrate availability allosteric interactions Covalent modification -> ACC (activated by citrate, inhibited by palmitoyl-CoA, inactivated by phosphorylation) Long term regulation amount of enzyme present, within hours or days -> ACC

8th lecture

PROTEIN METABOLISM

During digestion, proteins are hydrolyzed into amino acids, which are then absorbed by the capillaries of villi and enter the liver via the hepatic portal vein.

Amino acids, under the influence of human growth hormone and insulin, enter the body cells by active transport.

Inside cells, amino acids are synthesized into protein that function as enzymes, transport molecules, antibodies, clotting chemicals, hormones, contractile elements in muscle fibers and structural elements such as hair. They may also be stored as fat or glycogen or used for energy.

Protein Catabolism:

Before amino acids can be catabolized, they must be converted to substances that can enter the TCA cycle. These conversions involve deamination, decarboxylation, and hydrogenation.

Amino acids can be converted into glucose, fatty acids and ketone bodies.

Protein Anabolism:

Involves the formation of peptide bonds between amino acids to produce new proteins.

Protein synthesis is stimulated by human growth hormone, thyroxine, and insulin.

Protein synthesis is carried out on the ribosomes of almost every cell in the body, directed by the cells' DNA and RNA.

Of the 20 amino acids in your body, 10 are referred to as "essential" amino acids. These amino acids cannot be synthesized by the human body from molecules present within the body. Foods containing these amino acids are "essential" for human growth and must be part of the diet.

Nonessentail amino acids CAN be synthesized by body cells by a process called transamination. Once the appropriate essential and nonessential amino acids are present in cells, protein synthesis occurs rapidly.