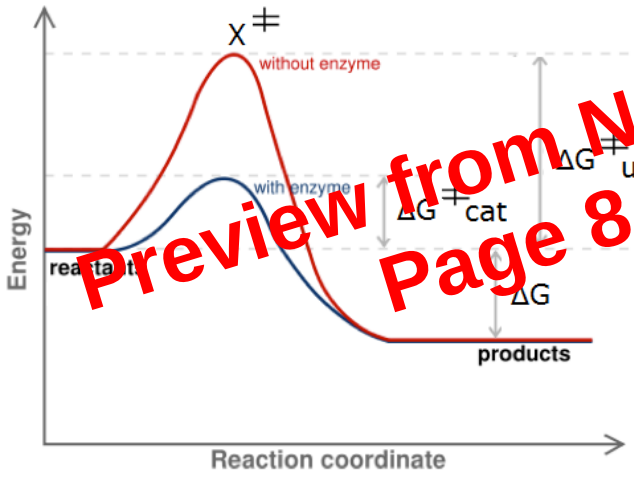
Mechanism of enzyme action:

$S \rightarrow X^{\ddagger} \rightarrow P$

A chemical reaction of S → P goes through a transition state, $X^{\ddagger}$ which has a higher free energy than either S or P. $X^{\ddagger}$ is an intermediate state; it is neither S nor P. $\Delta G^{\ddagger}$ (activation energy barrier) is the difference in free energy between S and $X^{\ddagger}$.

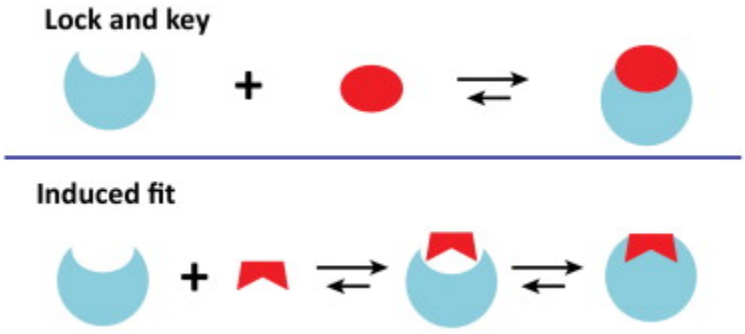
$$\Delta G^{\ddagger} = G_X - G_S$$

Rate of reaction is proportional to the number of molecules with free energy ≥ $\Delta G^{\ddagger}$. Enzymes increase the rate of reaction by providing an alternative pathway in which $\Delta G^{\ddagger}$ is lower than otherwise would be.



ΔG is the difference in free energy between S and P.

Also, **'lock and key'** and **'induced fit'** hypothesis of enzyme action:



Active site and substrate are complementary- in terms of geometry i.e. structure or electronically i.e chemical affinity

Substrate induces conformational change in active site- this changes the amino acids/other groups in the enzyme

usually dissociation of the cleaved peptide). Proenzymes include digestive enzymes pepsinogen, trypsinogen and procarboxypeptidase synthesized in the pancreas. Pepsinogen is activated to pepsin by HCL, which cleaves it from its N-terminal, then cleaves its inhibitor peptide.

(2) Covalent modification

Involves phosphorylation and dephosphorylation, catalyzed by protein kinase and protein phosphatase, respectively e.g. glycogen phosphorylase is activated by phosphorylation, while glycogen synthase**, pyruvate dehydrogenase**, acetyl CoA carboxylase, HMG-CoA reductase etc. are inactived by phosphorylation.

(3) Allosteric effects

Allosteric effectors bind to an allosteric site on the enzyme or a regulatory subunit. This changes enzyme shape, therefore enzyme activity. Allosteric effectors act as activators or inhibitors. Usually they are multimeric e.g. **pyruvate dehydrogenase** is a multimer, inhibited by allosteric effectors NADH and acetyl CoA, while **glycogen phosphorylase** is a dimer, inhibited by allosteric effectors ATP and glucose 6 phosphate and activated by allosteric effectors AMP.

Control of metabolic pathways:

Involves:

(1) Feedback control, involving rate limiting enzymes. Usually, metabolic pathways are branched, with first steps synthesizing several metabolites. Feedback control occurs in branching sites.

(2) Compartmentation of metabolic pathways in different cell compartments, ensures control, through transport control.

10. Overview of Intermediary Metabolism. Metabolic Pathways: Catabolic and Anabolic Processes. The High Energy Bond. The Role of ATP

- Biological Turnover is a system of processes which characterise living organisms
- It involves 3 steps:

Nutrition --------> Metabolism --------> Excretion of the end products

- Small molecules obtained from digestion circulate in blood and enter tissues
- The molecules get taken up by cells and metabolise --> metabolites
- Metabolites take part in catabolic and anabolic processes

Metabolic Functions

- Energy maintenance
- Transformation of food into metabolites (which serve as substrates for cellular processes)
- Synthesis & degradation of proteins, nucleic acids, carbohydrates and lipids – structural metabolites
- Synthesis of enzymes, hormones, mediators, growth factors etc – molecules with specific function

Metabolism can be divided into Catabolism and Anabolism

Catabolism

- Refers to conversion of large complex molecules into simple metabolites
- Some of these reactions produce ATP from degradation of energy-rich fuel molecules
- Catabolism takes place in 3 stages:
    1. Degradation of macromolecules:
       Proteins ---> 20 AA
       Carbs ---> Glucose
       Lipids ---> FA + glycerol
    2. Degradation of main organic molecules to simple molecules:
       Pyruvate and Acetyl Co-A
    3. Metabolism of 2 carbons (acetyl CoA fragments) are converted to CO_2 in TCA with reduction of NAD and FAD
       NADH and $FADH_2$ are involved in electron transport chain for oxidative phosphorylation which produces the main cell fuel – ATP
- Catabolic pathways are typically oxidative and require oxidized coenzymes such as NAD+.

- Allows molecules in the diet or nutrient molecules stored in cells to be converted into building blocks needed for synthesis of complex molecules.

Anabolism

- Refers to the conversion of small molecules into large ones during biosynthesis
- Anabolic reactions require energy (endergonic), derived from ATP → ADP + inorganic phosphate (Pi)
    1. Synthesis of the main organic compounds (aa, bases, aceyl-CoA, metabolites of Kreb's Cycle)
    2. Synthesis of macromolecules and complex compounds from the main organic molecules.

The High Energy Bond

- Energy rich compounds comprise 5 kinds of high energy bonds

Type of Bond	Compound	ΔG kJ/mol
Phosphoanhydride	**ATP**	**30.5**
Enol Phosphate	**PEP**	**61**
Acyl Phosphate	**1,3-Bisphosphoglycerate**	**49**
Guanidine Phosphate	**Phosphocreatine**	**43**
Thioester	**Acetyl-CoA**	**41**

11. Mitochondrial Oxidation. Structure of Electron Transfer Chain. The coupling processes of Oxidation and Phosphorylation. Regulation of ETC: Inhibitors, Uncoupling agents. Thermogenesis.

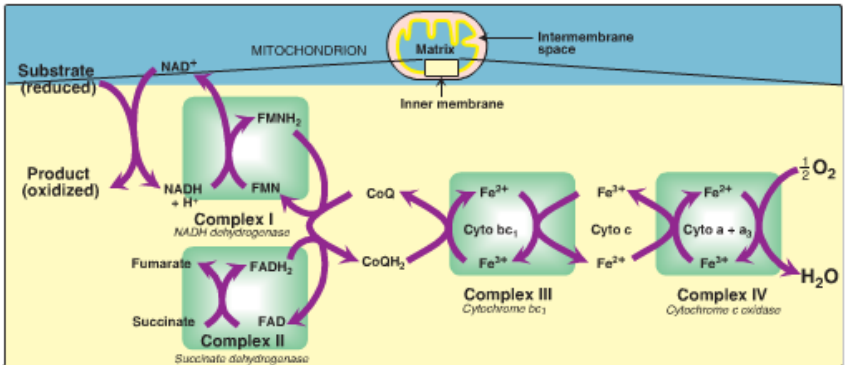
Mitochondrial Oxidation. Structure of ETC

- Consists of 4 Complexes
    1. NADH Dehydrogenase
    2. Succinate Dehydrogenase
    3. Cytochrome C Oxireductase
    4. Cytochrome Oxidase
- The mitochondrion is the powerhouse of the cell, where most of the energy is produced.
- Processes that take place in the mitochondria:
    - Kreb's Cycle
    - Oxidative Phosphorylation
    - B-Oxidation
- The ETC collects the reducing equivalents from all processes and direct them to their final reaction with Oxygen.
- Creates ATP via ATP synthase (complex 5)
- Uses Electron Carriers: NADH & $FADH_2$ which donate electrons
- Donated electrons pass through the chain
- Electrons will be reduced to form H_2O
- H^+ ions are pumped out

Reactions

Order of the complexes approximates the increase in standard reduction potential.

1. Complex 1: NADH + H^+ → NAD^+
2. Complex 1 has a tightly bound molecule of FLAVIN MONO NUCLETOTIDE
3. FMN is a coenzyme that helps transferring hydrogen atoms to CoQ
4. Complex 1 splits H atoms from NADH, transports them to outer membrane and splits them into 2 p^+ & 2 e^-
5. Complex 2: FAD → $FADH_2$ → CoQ
6. Coenzyme Q aka Ubiquinone (mobile protein) transfers electrons to complex 3
7. Coenzyme Q will interact w/- Complex 3
8. Complex 3 has 3 parts
    i. Cytochrome B
    ii. Iron sulphur proteins
    iii. Cytochrome c1
9. Complex 3 transfers electrons to cytochrome C (mobile protein)
10. Cytochrome C will bind to Complex 4
11. Complex 4 will reduce O_2 to H_2O



12. Dietary Carbohydrates. Digestion and Absorption of Carbohydrates. Glucose transport.

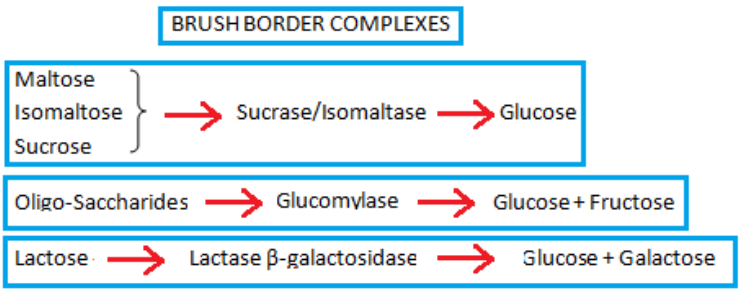
- Most abundant organic molecule in nature (60% poly, 35% di, 5% mono)
- We need approx 200g of carbs per day.
- Normal glucose 4-6mmol/L
- Digestion function is to form monosaccharides from poly/di sachs.

Digestion

1) Starts in the mouth. Salivary glands secrete α-amylase (aka ptyalin) which starts breakdown of starch and glycogen. Enzyme = endo-glycosidase. This enzyme is removed once arrival to the stomach as the acid dentures it.
2) Continues in small intestine. Pancreatic α-amylase takes over as pH in small intestine is slightly alkaline. Cl^- activates both types of amylase. Starch is hydrolysed to maltose, isomaltose and glucose.

Enzymes

Maltase, Isomaltase, Sucrase, Trehalase and lactase are anchored to the plasma membrane of the brush border of cells of the SI.



∴ Major monosaccharides which result from digestion include glucose, galactose and fructose.

Lactose intolerance:

1^o – Defective/Lack- Defective lactase enzyme at birth (rare). Late onset is when there is a loss of the lactose enzyme and appears gradually in teens/ adults.

2^o – Acquired – Consequence of damage to the brush border (tropical sprue/ celiac sprue

Glucose Transporters – Each of the transporters has different affinities for glucose and the other hexoses, which largely dictates their function.

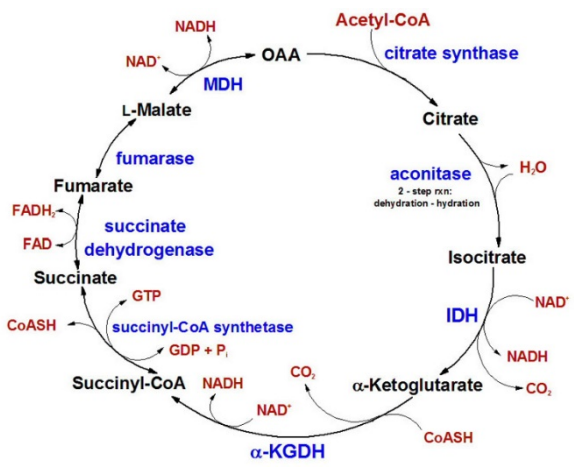
Transporter	Major Sites of Expression	Characteristics
SGLUT 1	Intestinal mucosa, kidney tubules.	Co-transports 1 molecule of glucose/galactose, with 2 Na^+ ions. NOT fructose.
GLUT-1	Erythrocytes, brain, fetal tissues.	Transports glucose & galactose. NOT fructose.
GLUT-2	Liver, pancreatic β cell, SI, kidney	Transports glucose/galactose/fructose. Glucose sensor in pancreatic β cells.
GLUT-3	Brain/placenta/testes	Transports glucose/galactose. NOT fructose. 1^o transporter for neurons.
GLUT-4	Skeletal muscle/ cardiac muscle/ adipocytes	Insulin dependent.
GLUT-5	SI, sperm	Transports fructose. NOT glucose/galactose.

Glut 1,3,4 have high affinity for glucose. Indicates that they are functioning at maximal rate under physiological concentrations of glucose.

Glut 2 has low affinity for glucose. It allows it to change transport rate in proportion to the increasing glucose concentration that occurs after ingestion of a carb-rich meal.

Topic 15 | Citric Acid Cycle. Regulation. Energetic and metabolic balance. Relationship with other metabolic pathways.

The citric acid cycle / Tricarboxylic acid cycle / Krebs cycle occurs in the mitochondria of cells. The main function of this cycle is oxidation of acetyl-coA and production of NADH and FADH for the respiratory chain.



The Krebs cycle is catalytic. The oxaloacetate acts as a catalyst (Mediates the process and is regenerated at the end of the cycle.) During each completion of the cycle, 1 acetyl-coA is destroyed. (1 molecule of Oxaloacetate mediates the oxidation of numerous acetyl-coA molecules.

The enzymes of the Krebs cycle are compartmented in the Mitochondria. There are 8 reactions which begin and end with the same product.

Initiation: A reaction between acetyl-coA and oxaloacetate to produce a 6-carbon citrate.

The net reaction of the Krebs cycle:

$CH_3CO\text{-}CoA + 3NAD + FAD + ADP + Pi + 2H_2O \rightarrow 2CO_2 + 3NADH_2 + FADH_2 + ATP + CoASH$

Reactions

1. **Acetyl-CoA + Oxaloacetate + Water → Citrate**
   Beginning of the Krebs cycle; Catalysed by Citrate Synthase, this is an irreversible reaction regulated by negative feedback control. OA binds to the active site of Citrate synthase causing conformational changes which expose the residues which then bind to the Acetyl-CoA to form Citryl-CoA which is hydrolysed by Water to Citrate and CoA. The product, citrate is an enzyme inhibitor.

2. **Citrate – Water □ [Cis-aconitate] + water □ Isocitrate**
   The aconitase reaction: Aconitase catalyses the elimination of water from Citrate to form Cis-aconitate intermediate, then water binds asymmetrically to this intermediate to produce Isocitrate. The isomerisation is an endergonic and reversible process. (Fluoracetate inhibits aconitase activity.)

3. **Isocitrate □ Ketoglutarate + Carbon dioxide**
   This is catalysed by Isocitrate-dehydrogenase. Oxidative decarboxylation coupled to *the reduction of NAD to $NADH_2$.* Decarboxylation is exergonic and promotes the overall reaction. <u>This reaction is the main regulatory step in the cycle.</u> (Apart from the Isocitrate-dehydrogenase' catabolic role in the Krebs cycle, mitochondrial and cytosolic IDH play an important role in cellular defence against oxidative damage as a source of NADPH.)

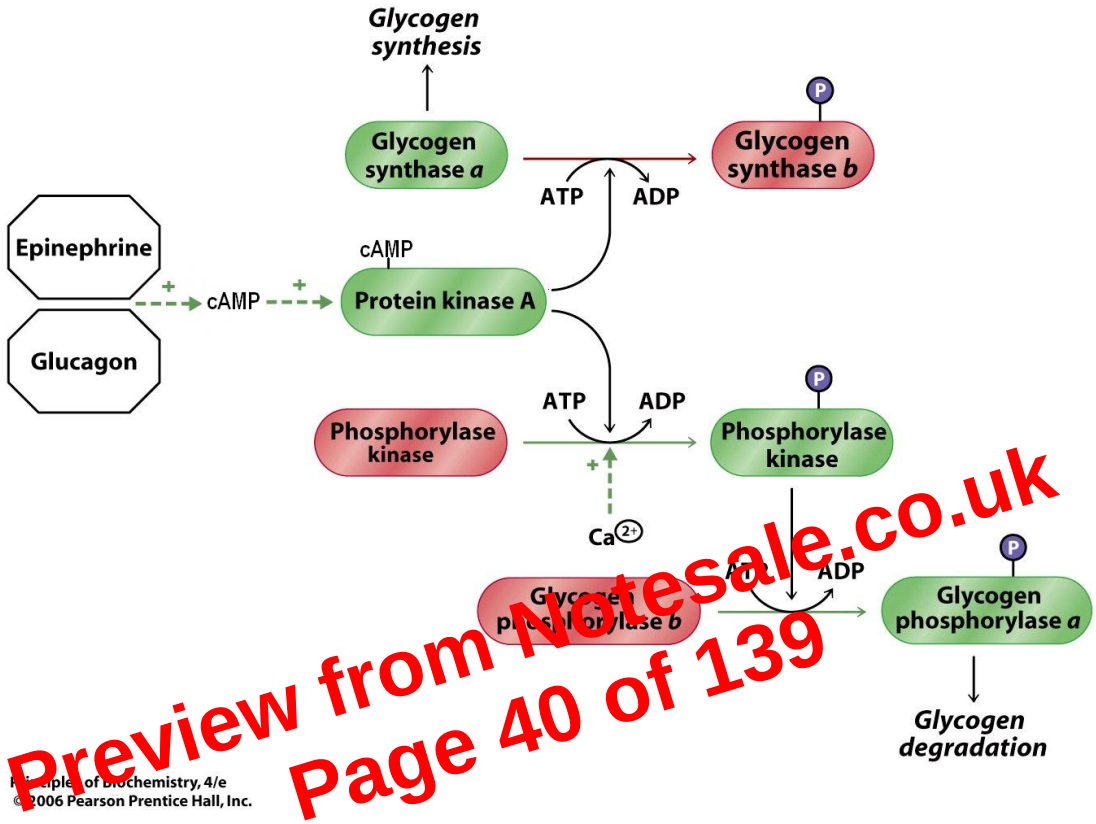
4. **Ketoglutarate □ Succinyl-CoA + Carbon dioxide**
   The oxidative decarboxylation of α-Ketoglutarate to succinyl-coA is catalysed by the α-Ketoglutarate dehydrogenase complex which is a large multi-subunit enzyme complex composed of 3 enzymes; α-Ketoglutarate dehydrogenase/decarboxylase, trans-succinylase and lipoamide dehydrogenase. There are also 5 coenzymes required for the overall reaction; thiamine pyrophosphotase, lipoic acid, CoASH, FAD and NAD+.
   The decarboxylation and oxidation of the keto group releases a huge amount of energy, which is conserved in the reduction state of NADH and in the thioester bond of succinyl-CoA. The lesser amount of energy in the thioester bond is used for the generation of GTP in the next step of the cycle…

5. **Succinyl-CoA □ Succinate**
   This reaction is catalysed by succinate thiokinase, and generates GTP from GDP and Pi. This reaction is an example of substrate level phosphorylation. First the Succinyl-CoA reacts with Pi to produce Succinyl-phosphate which transfers a phosphate group to succinate thiokinase which transfers it to GDP to produce GTP.

of hormones: glucagon, epinephrine, cortisol and insulin

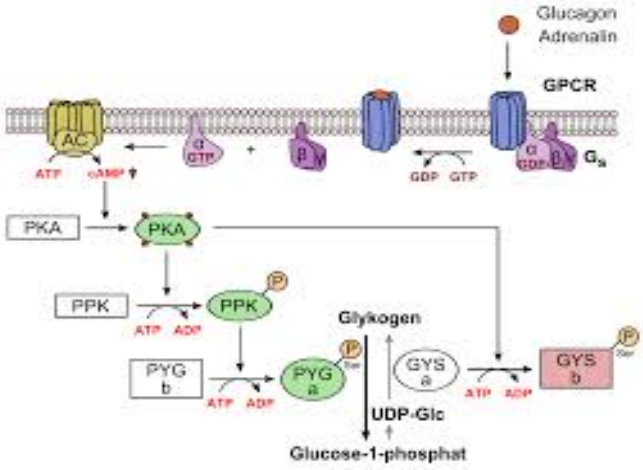
- **GLUCAGON AND EPINEPHRINE act as extracellular regulators:** their mechanism of action includes second messengers cAMP, IP_3 and DAG

- Cells possess different adrenergic receptors: $\alpha_{1,\,2}$, $\beta_{1,\,2,\,3}$. The α_2 and $\beta_{1,\,2,\,3}$ receptors mediate physiological effects through cAMP. First messenger is the hormone molecule (does no penetrate cell membrane).



Adenylate-cyclase mechanism

- **Epinephrine binds β receptor** which induces a change in the GPCR which in turn activates adenylate cyclase, producing cAMP



- cAMP activates PKA in cytosol. cAMP may be degraded by phosphodiesterase, which is activated by insulin

- When **epinephrine binds α_2 adrenoceptors**, adenylate cyclase is inhibited, which results in the activation of glycogen synthase and inactivation of glycogen phosphorylase a. Thus glycogen synthesis will be initiated.

- Epinephrine on α_1: phospholipase C is activated, which catalyses the hydrolysis of membrane phospholipid phosphatidylinositol-diphosphate into 2 products; inositol triphosphate IP_3 and diacylglycerol DAG

- IP_3 opens calcium ion channels in the ER, thus increasing its concentration in the cytoplasm, where calcium binds to calmodulin. The calcium-calmodulin complex activates phosphorylase kinase. DAG activates PK-C

Pathology of Glycogen Metabolism

- **Type I**: Glycogen storage disease type I (GSD I) or von Gierke's disease, is the most common of the glycogen storage diseases. This genetic disease results from deficiency of the enzyme glucose-6-phosphatase

- The deficiency impairs the ability of the liver to produce free glucose from glycogen and from gluconeogenesis. Since these are the two principal metabolic mechanisms by which the liver supplies glucose to the rest of the body during periods of fasting, it causes severe hypoglycaemia and results in increased glycogen storage in liver and kidneys. This can lead to enlargement of both. Both organs function normally in childhood, but are susceptible to a variety of problems in adult years

- **Type II**: Glycogen storage disease type II (also called Pompe disease /ˈpɒmpə/ or acid maltase deficiency) is an autosomal recessive metabolic disorder which damages muscle and nerve cells throughout the body. It is caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase enzyme. It is the only glycogen storage disease with a defect in lysosomal metabolism, and the first glycogen storage disease to be identified, in 1932 by the Dutch pathologist J. C. Pompe.

- The build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system

- **Type V**: Glycogen storage disease type V (GSD-V) is a metabolic disorder, more specifically a glycogen storage disease, caused by a deficiency of myophosphorylase. Its incidence is approximately the same as glycogen storage disease type I.

- GSD type V is also known as McArdle disease or muscle phosphorylase (myophosphorylase) deficiency.

23. Regulation of Carbohydrate Metabolism. Control of Blood Glucose Levels in Different Conditions: Well Fed, Fasting, Prolonged Starvation. Metabolic Disturbance in Diabetes Mellitus.

Regulation of Carbohydrate Metabolism

- Glucoregulation is the maintenance of steady levels of glucose in the body; it is part of homeostasis, and so keeps a constant internal environment around cells in the body.
- Some tissues only metabolise glucose:
    - CNS, PNS, erythrocytes, eyes
    - They metabolise glucose at a constant rate

Control of Insulin and Glucagon Secretion

- Summarised in the table below:

FACTOR	Insulin	Glucagon
Nutrients		
glucose conc. rise by 5mM	+	-
glucose conc. drop by 5mM	-	+
rise in amino acid conc.	+	+
rise in fatty acid conc.	+	0
Hormones/ Neurotransmitters		
GIT	+	0
adrenaline	-	+
noradrenaline	-	+

Actions of Insulin and Glucagon

- Insulin:
    - Signal of feeding
    - Target tissues: liver, adipose, skeletal muscle
    - Affects metabolism of: carbs, fats, proteins
    - Actions: anabolic
- Glucagon:
    - Signal of fasting
    - Target tissues: liver, adipose
    - Affects metabolism of: carbs, fats

2. Activates glycogen synthase and inactivates glycogen phosphorylase (liver and muscle)

3. Stimulates storage of excess fuel as fat

    a. Insulin stimulates glucose-6-P to pyruvate via effect on phosphofructokinase 1

    b. Acetyl CoA fuels FA synthesis. FAs made in liver are converted to TAGs and transported in VLDLs to fat cells

    c. In fat cells, insulin stimulates TAG synthesis (lipoprotein lipase). Note- stored fat derived from glucose

Glucagon:

1. Increases blood glucose levels by: signalling that blood glucose levels are low; even in absence of physical activity or stress, several hours after food intake blood glucose falls to below 4.mM

2. Stimulating glycogen breakdown in liver (activates glycogen phosphorylase and inactivates glycogen synthase)

3. Inhibits liver glycolysis and stimulates gluconeogenesis (by lowering levels of fructose-2,6-bisphosphate and inhibiting pyruvate kinase

4. Glucagon acts on liver to maintain blood glucose levels. Adrenaline acts on muscle (glycogenolysis), liver (glycogenolysis) and adipose tissue (lipolysis)

Control of Blood Glucose Level in Different Conditions

- Well-fed state: shortly after eating, the blood glucose level may rise, in non-diabetics, temporarily up to 7.8 mmol/L (140 mg/dL) or slightly more

- Fasting: normal blood glucose level (tested while fasting) for non-diabetics, should be between 3.9 and 5.5 mmol/L (70 to 100 mg/dL). The mean normal blood glucose level in humans is about 5.5 mmol/L (100 mg/dL)

- Prolonged starvation: blood glucose levels will remain relatively stable during prolonged starvation. After the exhaustion of the glycogen reserve, and for the next 2–3 days, fatty acids are the principal metabolic fuel. At first, the brain continues to use glucose, because, if a non-brain tissue is using fatty acids as its metabolic fuel, the use of glucose in the same tissue is switched off. Thus, when fatty acids are being broken down for energy, all of the remaining glucose is made available for use by the brain

Diabetes

Diabetes mellitus, often simply referred to as diabetes—is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

There are three main types of diabetes:

- Core consisting of a droplet of TAGs and/or cholesterol esters (CEs)
- Surface monolayer of phospholipid, unesterified cholesterol and specific proteins (apolipoporteins)

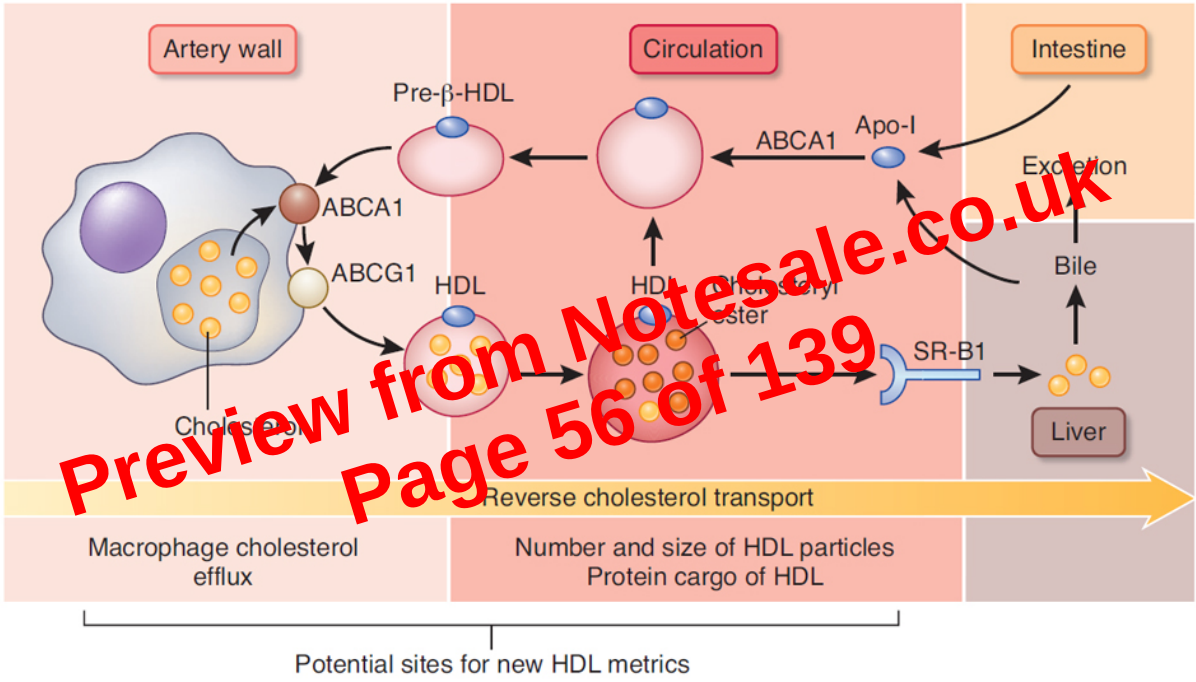
- Lipoproteins differ in content of protein and lipids, and classified depending on their density:
  - CM: largest, low density. Major associated apolipoprotein is apoB48

- CMs secreted by the intestinal epithelial cells into the lymphatic system by the process of exocytosis. Enter blood via thoracic duct

- Transport of dietary lipids in the blood performed by CMs

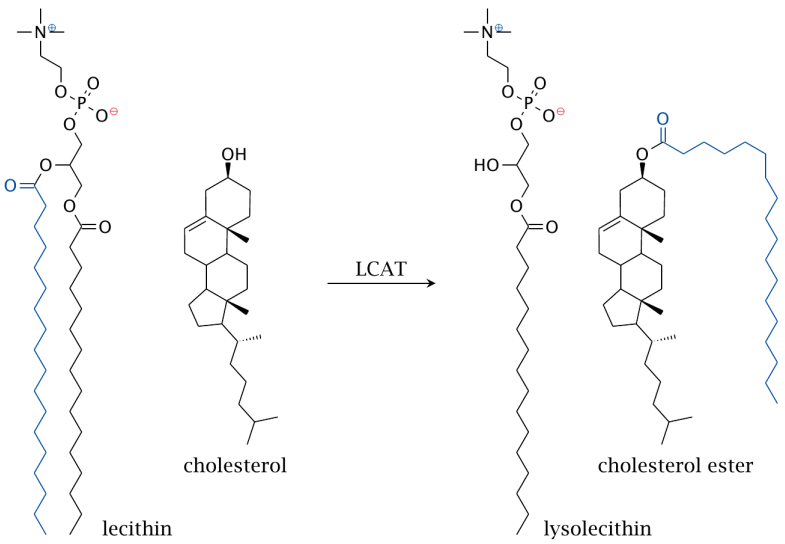
- This esterification prevents the diffusion of cholesterol from HDL to the plasma membrane, amplifying cholesterol efflux

- CE rich HDL can return to the liver through 3 distinct pathways:
  - Through action of CE transfer protein CETP, cholesterol rich proteins exchange triglycerides with CE from CE rich HDL. After the cholesterol becomes associated with HDL, LCAT catalyses the conversion of cholesterol to CE
  - Through selective uptake of CE rich HDL, mediated by hepatic receptor SRB1 scavenger receptor. SRB1 mediated uptake becomes more efficient through activity of hepatic lipase.
  - The enzyme is able to remodel HDL through the hydrolysis of surface phospholipids, thus allowing CE to flow from the lipoprotein core to the plasma membrane



LCAT Reaction

- The HDL particle contains the enzyme lecithin cholesterol acyltransferase or LCAT for short, which converts cholesterol to cholesterol esters.
- The LCAT reaction occurs at the surface of HDL particles. The transfer of one acyl chain from a lecithin (phosphatidylcholine)



27. Metabolism of TAGs. Regulation of Lipolysis and Lipogenesis.

Degradation of triacylglycerols - lipolysis. Participation of adipolytic lipase, hepatic lipase, lipiproteinlipase and digestive enzymes. Synthesis of triacylglycerols - lipogenesis. General synthetic route, the synthesis in adipose tissue and the intestinal cells. Regulation of lipolysis and lipogenesis. Rapid hormonal control by phosphorylation / dephosphorylation of enzymes, slow hormonal control. Role of the liver in the metabolism of triacylglycerols and the role of nutrition. Metabolism of glycerol. Thermogenesis – shivering and non-shivering, futile "substrate cycles" uncoupling of oxidation and phosphorylation in the respiratory chain. Natural uncoupling agents - fatty acids, bilirubin, thyroid hormones, UCP. Role of brown adipose tissue thermogenesis

Within all cell types, even those of the brain, triacylglycerols are stored as cytoplasmic 'lipid droplets' (also termed 'fat globules', 'oil bodies', 'lipid particles', 'adiposomes', etc.) enclosed by a monolayer of phospholipids and hydrophobic proteins, such as the perilipins in adipose tissue or oleosins in seeds. These lipid droplets are now treated as distinctive organelles, with their own characteristic metabolic pathways and associated enzymes – no longer boring blobs of fat. They are not unique to animals and plants as Mycobacteria and yeasts have similar lipid inclusions.

The lipid serves as a store of energy, which can be released rapidly on demand, and as a reserve of essential fatty acids and precursors for eicosanoids. However, lipid droplets may also serve as a protective agency to remove any excess of biologically active and potentially harmful lipids such as free fatty acids, diacylglycerols, cholesterol (as cholesterol esters), retinol esters and coenzyme A esters.

Two main biosynthetic pathways are known, the sn-glycerol-3-phosphate pathway, which predominates in liver and adipose tissue, and a monoacylglycerol pathway in the intestines

Degradation of TAGs

- Lipolysis or hydrolysis performed by specific lipases. Main lipases: LPL, pancreatic lipase, adipolytic and liver lipase.
- LPL: water soluble enzyme that hydrolyses TAGs in CM and VLDL, into free FA and glycerol. Attached to the luminal surface of endothelial cells in capillaries
- Pancreatic lipase is a TAG lipase. It is the primary enzyme that hydrolyses dietary fat; converting TAG substrates if ingested oils to MAGs and free FAs
- Hepatic TAG lipase expressed in liver and adrenal glands. Converts IDL to LDL
- Adipolytic lipase hydrolyses TAG to DAG and DAG to MAG

28. β-Oxidation of FAs with an Even Number of Carbon Atoms. Regulation.

Sources of fatty acids in the body. Transport of fatty acids into the serum. β-oxidation. Activation of fatty acids. Transport of activated fatty acids across the mitochondrial membrane - carnitine shuttle. Enzymes of the oxidation of fatty acids with an even number of carbon atoms. Energetic balance. Regulation of β -oxidation-regulatory responses. Pathology - deficiency of acyl-CoA dehydrogenase.

Fatty acids divided into 3 classes: short chain (2 to 4 carbons); medium chain (6 to 10 carbons) and long chain (more than 12 carbons).

Most FAs present in human cells are long chain. Lipids are mobilised from adipose tissue as free FAs attached to serum albumin. The FAs form complexes with albumin in the blood, and are taken up by muscle, kidney and other tissues; where ATP is generated by their oxidation to carbon dioxide and water.

After dissociation of FA-albumin complex at the plasma membrane, FAs bind to FA binding protein (FABP), which acts as a transmembrane cotransporter with sodium ions or FAT/CD36. On entering the cytosol, free FAs are bound by a FABP.

B-oxidation of fatty acyl CoA occurs in mitochondria. FAs are oxidised into activated 2C molecules; acetyl CoA

Activation and Membrane Transport

- Free fatty acids cannot penetrate any biological membrane due to their negative charge. Free fatty acids must cross the cell membrane through specific transport proteins, such as the SLC27 family fatty acid transport protein. Once in the cytosol, the following processes bring fatty acids into the mitochondrial matrix so that beta-oxidation can take place.

- Long-chain-fatty-acid—CoA ligase catalyses the reaction between a fatty acid with ATP to give a fatty acyl adenylate, plus inorganic pyrophosphate, which then reacts with free coenzyme A to give a fatty acyl-CoA ester and AMP.

- If the fatty acyl-CoA has a long chain, then the carnitine shuttle must be utilized:

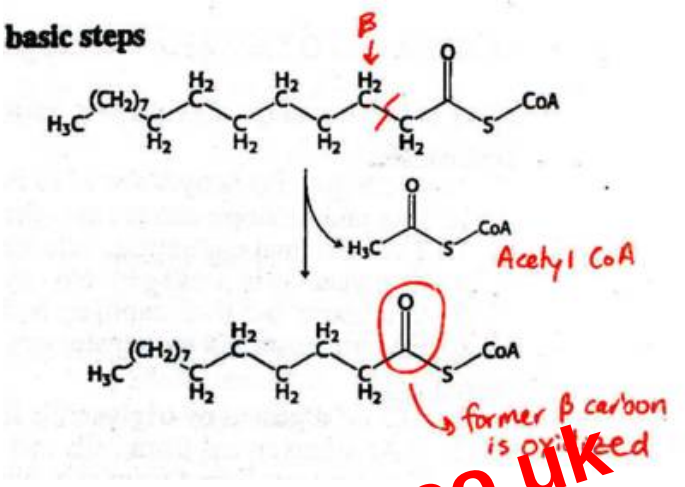
    - Acyl-CoA is transferred to the hydroxyl group of carnitine by carnitine palmitoyltransferase I, located on the cytosolic faces of the outer and inner mitochondrial membranes.

    - Acyl-carnitine is shuttled inside by a carnitine-acylcarnitine translocase, as a carnitine is shuttled outside.

- Acyl-carnitine is converted back to acyl-CoA by carnitine palmitoyltransferase II, located on the interior face of the inner mitochondrial membrane. The liberated carnitine is shuttled back to the cytosol, as an acyl-carnitine is shuttled into the matrix.

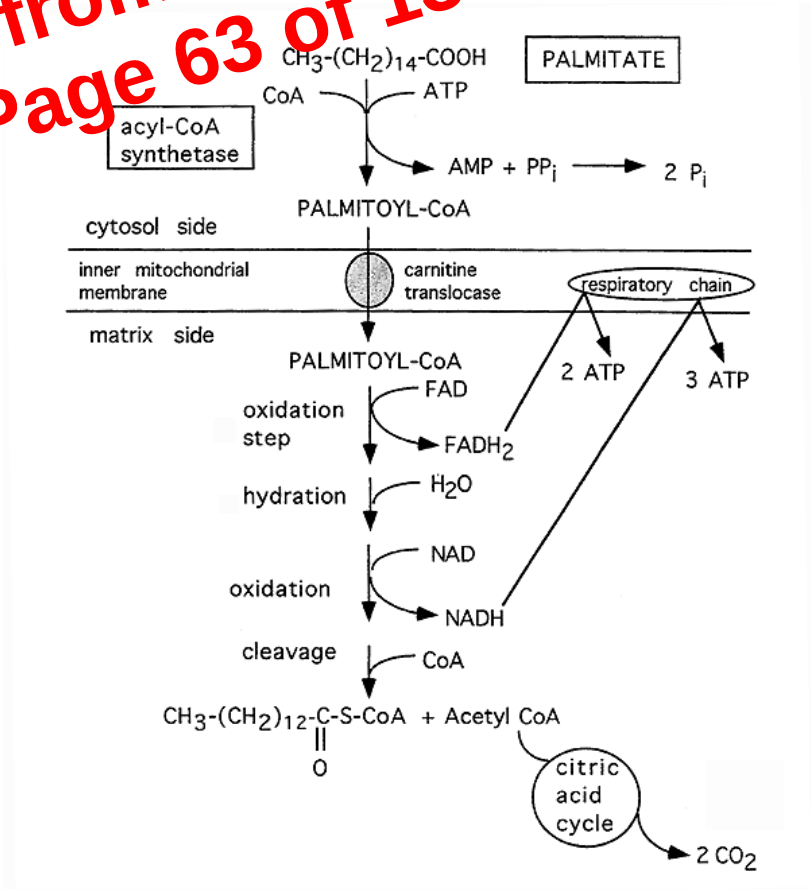
- If the fatty acyl-CoA contains a short chain, these short-chain fatty acids can simply diffuse through the inner mitochondrial membrane.

General Mechanism

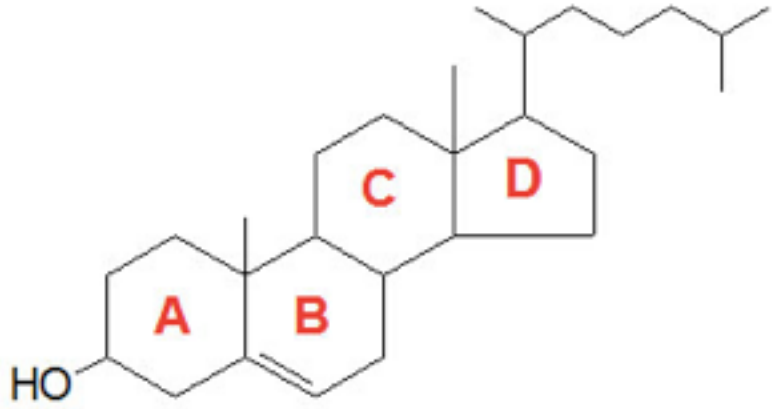


Once the fatty acid is inside the mitochondrial matrix, beta-oxidation occurs by cleaving two carbons every cycle to form acetyl-CoA. The process consists of 4 steps.

1. A long-chain fatty acid is dehydrogenated to create a trans double bond between C2 and C3. This is catalysed by acyl CoA dehydrogenase to produce trans-delta 2-enoyl CoA. It uses FAD as an electron acceptor and it is reduced to FADH2.
2. Trans-delta2-enoyl CoA is hydrated at the double bond to produce L-B-hydroxyacyl CoA by enoyl-CoA hydratase.
3. L-B-hydroxyacyl CoA is dehydrogenated again to create B-ketoacyl CoA by B-hydroxyacyl CoA dehydrogenase. This enzyme uses NAD as an electron acceptor.
4. Thiolysis occurs between C2 and C3 (alpha and beta carbons) of B-ketoacyl CoA. Thiolase enzyme catalyses the reaction when a new

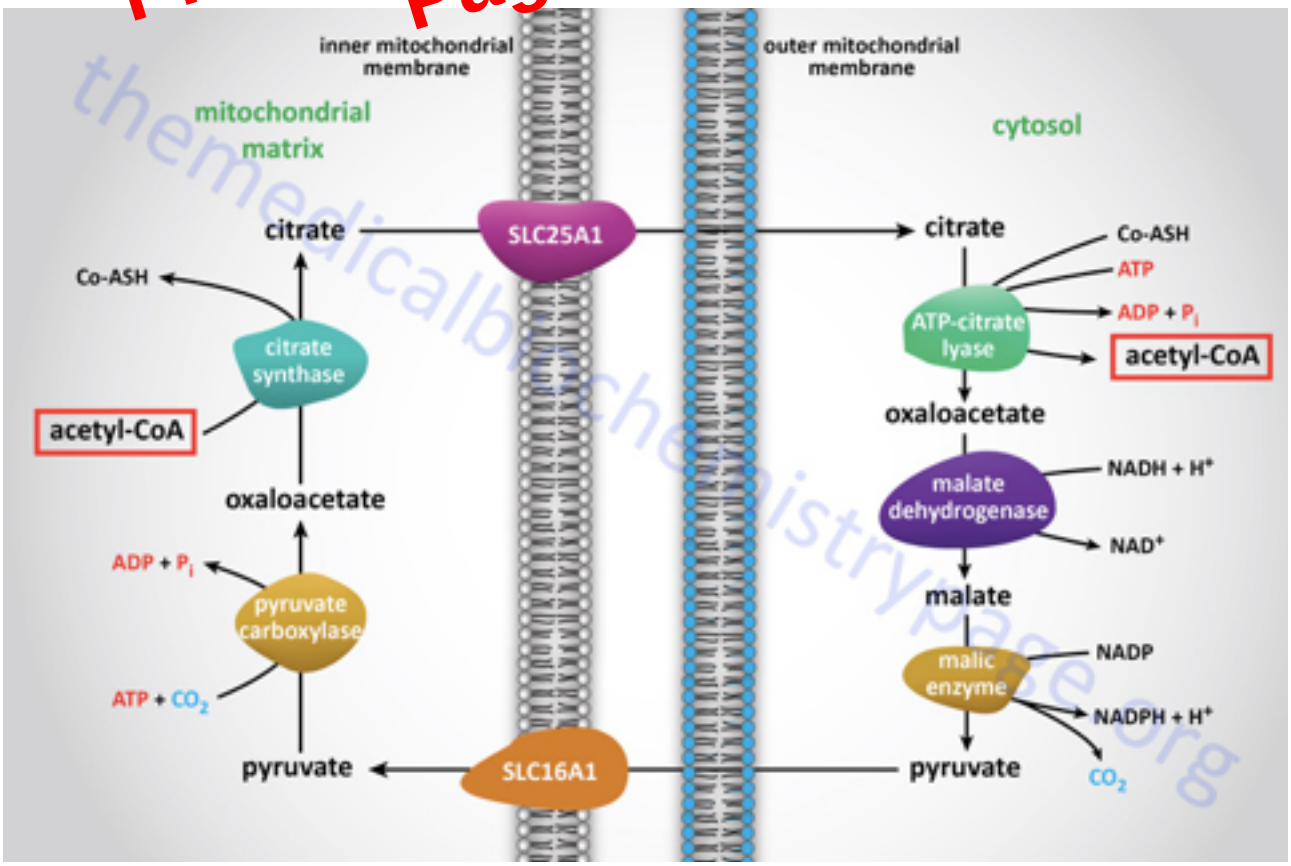


34. Cholesterol Synthesis. Main Metabolites. Regulation.



Slightly less than half of the cholesterol in the body derives from biosynthesis de novo. Biosynthesis in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. Cholesterol synthesis occurs in the cytoplasm and microsomes (ER) from the two-carbon acetate group of acetyl-CoA.

The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (e.g., fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis (see the Figure below). Acetyl-CoA can also be synthesized from cytosolic acetate derived from cytoplasmic oxidation of ethanol which is initiated by cytoplasmic alcohol dehydrogenase (ADH3). All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor. The isoprenoid intermediates of cholesterol biosynthesis can be diverted to other synthesis reactions, such as those for dolichol (used in the synthesis of N-linked glycoproteins, coenzyme Q (of the oxidative phosphorylation pathway) or the side chain of heme-a. Additionally, these intermediates are used in the lipid modification of some proteins.



Normal healthy adults synthesize cholesterol at a rate of approximately 1g/day and consume approximately 0.3g/day. A relatively constant level of cholesterol in the blood (150–200 mg/dL) is maintained primarily by controlling the level of de novo synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of the steroid hormones and bile acids. The greatest proportion of cholesterol is used in bile acid synthesis.

The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms:

1. Regulation of HMGR activity and levels

2. Regulation of excess intracellular free cholesterol through the activity of acyl-CoA:cholesterol acyltransferase, ACAT

3. Regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport.

Regulation of HMGR activity is the primary means for controlling the level of cholesterol biosynthesis. The enzyme is controlled by four distinct mechanisms: feed-back inhibition, control of gene expression, rate of enzyme degradation and phosphorylation-dephosphorylation.

The first three control mechanisms are exerted by cholesterol itself. Cholesterol acts as a feed-back inhibitor of pre-existing HMGR as well as inducing rapid degradation of the enzyme. The latter is the result of cholesterol-induced polyubiquitination of HMGR and its degradation in the proteosome (see proteolytic degradation below). This ability of cholesterol is a consequence of the sterol sensing domain, SSD of HMGR. In addition, when cholesterol is in excess the amount of mRNA for HMGR is reduced as a result of decreased expression of the gene. The mechanism by which cholesterol (and other sterols) affect the transcription of the HMGR gene is described below under regulation of sterol content.

Regulation of HMGR through covalent modification occurs as a result of phosphorylation and dephosphorylation. The enzyme is most active in its unmodified form. Phosphorylation of the enzyme decreases its activity. HMGR is phosphorylated by AMP-activated protein kinase, AMPK (this is not the same as cAMP dependent protein kinase, PKA). AMPK itself is activated via phosphorylation. Phosphorylation of AMPK is catalyzed by at least 2 enzymes. The primary kinase sensitive to rising AMP levels is LKB1. LKB1 was first identified as a gene in humans carrying an autosomal dominant mutation in Peutz-Jeghers syndrome, PJS. LKB1 is also found mutated in lung adenocarcinomas. The second AMPK phosphorylating enzyme is calmodulin-dependent protein kinase kinase-beta (CaMKKβ). CaMKKβ induces phosphorylation of AMPK in response to increases in intracellular Ca2+ as a result of muscle contraction. Visit AMPK: The Master Metabolic Regulator for more detailed information on the role of AMPK in regulating metabolism.

Two different sets of compounds are produced - one set alpha-hydroxyl group at positions 3,7 and 12 and produces chalk acid series of bile salts.

Remaining 5-carbon fragment contains a carboxyl group.

The first and rate limiting step in the conversion of cholesterol to bile salts is catalysed by cholesterol 7 alpha-hydroxylase (alpha position are those under the steroid ring). The reaction involves NADPH, molecular oxygen and the cytochrome P-450.

They are derived from primary bile salts by the action of enteric bacteria. pK of the bile acids is about 6. Hence, in the contents of the intestinal lumen which is normally pH6 about half the molecules are present in the protonated form and half in the ionised.

Excretion of Cholesterol - cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. Three different mechanisms can form these; autoxidation, secondary oxidation to lipid peroxidation, and cholesterol-metabolizing enzyme oxidation. Additional roles for oxysterols in human physiology include their: participation in bile acid biosynthesis, function as transport forms of cholesterol, and regulation of gene transcription.

In biochemical experiments radiolabelled forms of cholesterol, such as tritiated-cholesterol are used. These derivatives undergo degradation upon storage and it is essential to purify cholesterol prior to use. Cholesterol can be purified using small Sephadex LH-20 columns.

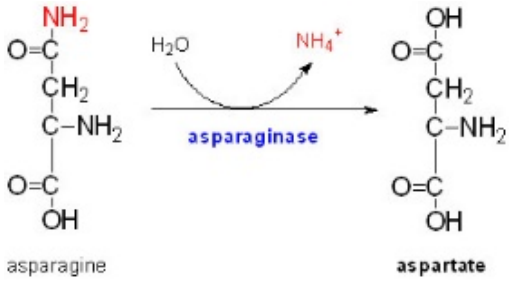
Cholesterol is oxidized by the liver into a variety of bile acids. These, in turn, are conjugated with glycine, taurine, glucuronic acid, or sulfate. A mixture of conjugated and nonconjugated bile acids, along with cholesterol itself, is excreted from the liver into the bile. Approximately 95% of the bile acids are reabsorbed from the intestines, and the remainder are lost in the faeces. The excretion and reabsorption of bile acids forms the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallises and is the major constituent of most gallstones. Although, less commonly, bilirubin gallstones also occur, but less frequently. Every day, up to 1 g of cholesterol enters the colon. This cholesterol originates from the diet, bile, and desquamated intestinal cells, and can be metabolised by the colonic bacteria. Cholesterol is converted mainly into coprostanol, a non-absorbable sterol that is excreted in the faeces. A cholesterol-reducing bacterium origin has been isolated from human faeces.

Although cholesterol is a steroid generally associated with mammals, the human pathogen Mycobacterium tuberculosis is able to completely degrade this molecule and contains a large number of genes that are regulated by its presence. Many of these cholesterol-regulated genes are homologues of fatty acid β-oxidation genes, but have evolved in such a way as to bind large steroid substrates like cholesterol.

According to the lipid hypothesis, abnormal cholesterol levels (hypercholesterolemia) — or, more properly, higher concentrations of LDL particles and lower concentrations of functional HDL particles — are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease. Since higher blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the HDL particles, LDL particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL, which can remove cholesterol from cells and atheroma, offer protection and are sometimes referred to as "good cholesterol". These balances are mostly genetically determined, but can be changed by body build, medications, food choices, and other factors.

Asparaginase catalyses the hydrolysis of asparagine to yield aspartate and ammonia:

Asparagine + H2O → Aspartate + Ammonia



For both reactions, asparaginase and glutaminase are exergonic.

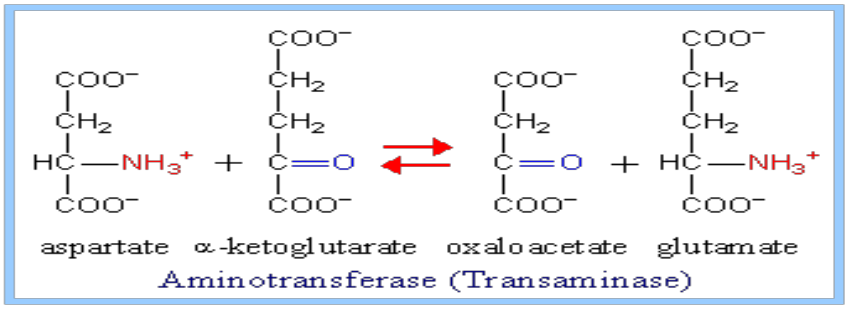
Asparaginase is used in the treatment of cancer0 For example, in some form of leukaemia. Asparaginase decreases the level of asparagine-essential for tissue growth. This helps to impair tumour expansion.

Glutamine nitrogen is derived mainly from the BCAA. Free ammonia in skeletal muscle arises principally from the deamination of glutamate by glutamate dehydrogenase, or from the purine nucleotide cycle

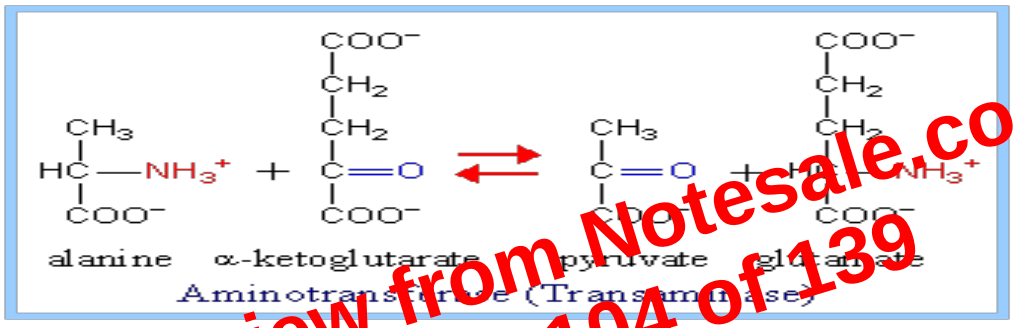
43.Catabolism of carbon skeletons of amino acids. Glucogenic and ketogenic amino acids.

Surplus amino acids are used as metabolic fuel. The major site of degradation of amino acids (AA) is the liver, although muscles readily degrade branched-chain AA (Leu, Ile and Val). This is then oxidatively deaminated to yield ammonium ions (NH_4^+). Aminotransferases or transaminases catalyse the transfer of amino groups of a variety of AA . Examples :

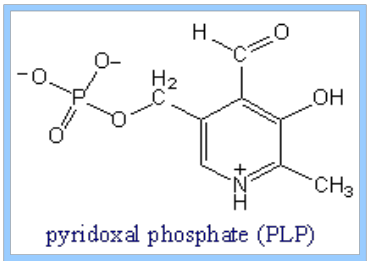
1. Aspartate aminotransferase is a very important enzyme that catalyzes the transfer of aspartate to α-ketoglutarate.



2. Alanine aminotransferase reaction



These reactions are reversible so can be used to synthesize AA from α-ketoacids. The nitrogen atom in glutamate is converted into free ammonium ion by oxidative deamination. This reaction is catalysed by glutamate dehydrogenase, which can utilize either NAD+ or NADP+ to carry out dehydrogenation of the C-N bond, followed by hydrolysis of the resulting Schiff-base intermediate. This reaction is close to equilibrium in the liver, and the direction is determined by the conc. Of the reactants and products. Its usually driven forward by the rapid removal of NH_4^+ produced from the hydrolysis of the Schiff base intermediate. Mechanism of formation of this intermediate: Pyridoxal phosphate (PLP) is a prosthetic group present in all aminotransferases, derived from pyridoxine (vit b6). PLP includes a pyridine ring that's slightly basic, attached to an OH group which is slightly acidic. Therefore, derivates of PLP can form a stable tautomeric form where N is protonated and so +vely charged, whereas the OH loses a H+ becoming –vely charged, forming a phenolate.



The most important functional group on PLP is the aldehyde, which forms covalent Schiff-base intermediates with amino acid substrates. Even in the absence of substrate, this group usually forms Schiff-base linkage with the ε- amino group of a specific lysine

The most common manner of biotransformation is **oxidation** of xenobiotic. Oxidation (or rather oxygenation) reactions are catalysed by enzymes **oxygenases** – the most important are so called **mixed-function oxygenases.** This system may be found on **cytosolic side of endoplasmic reticulum** and consists of three components

1) Flavoprotein – NADPH cytochrome P450 reductase

2) Hemoprotein – cytochrome P450

3) Lipid component – phosphatidylcholine

Because of presence of cytochrome P450 this system is also called **enzyme system of cytochrome P450 – CYP**.

MFO have both oxidase and oxygenase ability, i.e. in reaction the substrate is oxygenated and NADPH is oxidised. Reductive cleavage of oxygen molecule O_2 takes place, thus one atom of oxygen is incorporated into the substrate molecule, and the second atom of oxygen is released as water.

$$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + R\text{-}OH$$

$$Fe^{3+}\text{-P-450-RH} + e^- (NADPH) \rightarrow Fe^{2+}\text{-P-450-RH}$$

$$O_2\text{-}Fe^{2+}\text{-P-450-RH} + e^- (NADPH) \rightarrow Fe^{3+}\text{-P-450} + R\text{-}OH + H_2O$$

RH – substrate (xenobiotic)

Enzyme system of cytochrome P450 is huge **heme protein family** (heme binds O_2). It includes approximately 70 isoenzymes that perform not only xenobiotics detoxification, but metabolic processes of endogenous molecules: **steroid hormones production, bile acids production, eicosanoid production, unsaturated fatty acids production, etc.** Many types of cytochrome P450 are found in the liver, in the adrenal cortex (steroid hormone synthesis) and in other organs.

There are three typical features for this system: **wide substrate specificity, inducibility** and **important gene polymorphism**.

In long-term load by particular compound there is in a period of few days **induction of synthesis of enzymes** of endoplasmic reticulum. Induction of synthesis of particular enzymes that metabolise that particular compound (hence this **enzyme is inducible**). This leads to **faster biotransformation** of all substances metabolised by this particular enzyme (i.e. faster biotransformation of even different compounds than that one that caused induction). This is basis of many **drug interactions** – when administration of particular drug is able to cause change in metabolism of simultaneously administered drugs. Described effect dissipates when inducing substances is removed.

Important gene polymorphism is typical for enzymes of cytochrome P450 system. There are persons with **high activity** of particular enzyme, therefore they are able to metabolise particular substances quickly (**quick metabolisers)** and at the same time there are persons with **low activity** of the same enzyme – **slow metabolizers**.

Next enzymes that are important in biotransformation:

1) Catalase – it catalyses alcohol and several amines oxidation; its substrate is H_2O_2.

2) Dehydrogenases:

a) alcohol dehydrogenase (ADH) is localised in hepatocyte cytoplasm. This enzyme catalyses oxidation of alcohols to aldehydes. ADH is inducible enzyme – it is induced by ethanol.

b) aldehyde dehydrogenase (ALDH) is localised in hepatocyte mitochondria.

Result of the first phase

First phase may have these results:

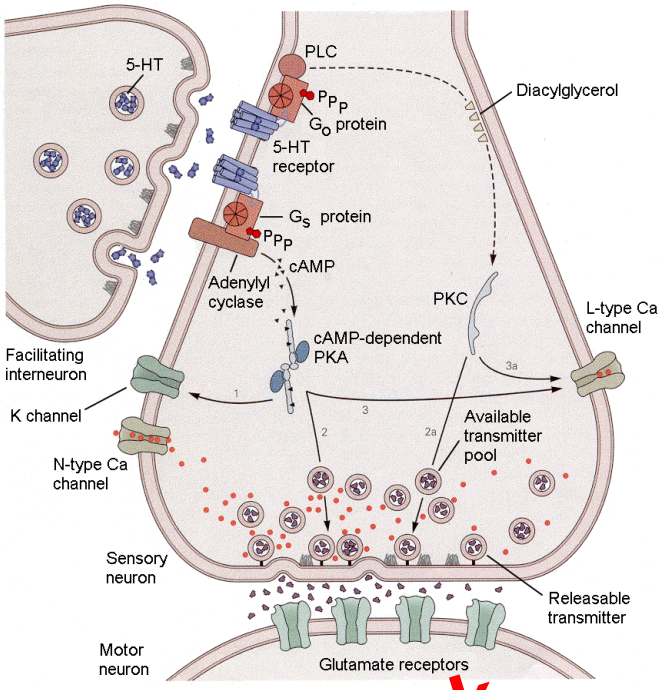
1) **increased polarity of xenobiotic**

2) **inactivation of xenobiotic (detoxification)**

3) **bioactivation of xenobiotic** (pharmaceuticals X carcinogens) – there is potential danger of damage

Second (conjugation, synthetic) phase – conjugation

1. The facilitator neuron releases modulator which binds to the pre-synaptic neuron.
2. Increase in cAMP levels
3. K^+ channels close-prolonged action potential
4. This allows the Ca^{2+} levels to rise
5. Causes exocytosis of neurotransmitters
6. Neuron transmitters bind to post-synaptic neuron (motor neuron)
7. Generates an action potential which is conducted along the axon.



original notes.

70. Biochemistry of Connective Tissue. Collagens. Structure of glycosaminoglycans.

Connective tissue proteins.

Collagen and elastin are the main proteins of skin, connective tissue, sclera and cornea of the eye, and blood vessel walls. They have structural functions in the body. They exhibit special mechanical properties resulting from unique structure obtained by combining specific amino acids into regular elements.

IV = basal lamina
I, II, III = most abundant

Collagen

Collagen is the most abundant protein in the human body. There are nineteen types of collagen. Although these molecules are found throughout the body, their types and organization differs in a particular organ. Collagen of bone occurs as fibers arranged at an angle to each other to resist mechanical shear from any direction. In other tissues, collagen may be bundled in tight parallel fibers that provide great strength, as in tendons. In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering.

Collagen structure. Collagen molecules possess a special triple-helical structure. Collagen molecules consist of three polypeptides, called Œ-chains, which wrap around each other in a triple helix; forming a rope-like structure. The three polypeptide chains are stabilized by hydrogen bonds. The elongated triple-helical structure places amino acid side chains on the outside of the molecule. This allows interaction between triple-helical molecules that leads to aggregation of collagen monomers into long fibers

The cc-chains of different collagen types vary in the amino acid content but are almost the same size, approximately 1000 amino acids in length. The primary structure of collagen is unusual, most of the molecule can be regarded as a polypeptide which sequence can be represented as (Gly-X Y)333 where X is frequently proline and Y is often hydroxyproline. The most common collagen. type I, contains two chains called U], and one chain called ct2.

Collagen contains hydroxyproline and hydroxylysine, which are. not present in most other proteins. These residues result from the hydroxylation of certain proline and lysine residues after their incorporation into polypeptide chains (posttranslational modification). Hydroxyproline is important in stabilizing the triple-helical structure of collagen.

The hydroxyl group of the hydroxylysine residues of collagen may be glycosylated. Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation.

Biosynthesis of collagen takes place in fibroblasts (or in the related osteoblasts of bone and chondroblasts of cartilage). Collagen is one of many proteins that normally functions outside of cells. Like proteins