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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – Intermediary Metabolism – SBC3101

I. Lipid Metabolism

Fatty acid oxidation - α, β, ω oxidation. Biosynthesis of saturated and unsaturated fatty acids. Metabolism of cholesterol, triglycerides. Biosynthesis of steroid hormones.

Fatty acid oxidation:

Fatty acids are the storage form of energy in living systems. The are constantly being synthesized and degraded in adipocytes (cells which store lipids). The adipocytes are generally yellow to brown in colour due to the excess number of mitochondria present in them. Mitochondria plays a vital role in the metabolism of lipids and hence a large number of them are found in the adipocytes. The metabolism of fatty acids occurs intensively in hepatocytes (liver cells) and also in other adipocytes. Although lipid catabolism yields a lot a energy comparable to carbohydrates, it also yields ketone bodies which are toxic and hence lipid remain only as storage form of energy.

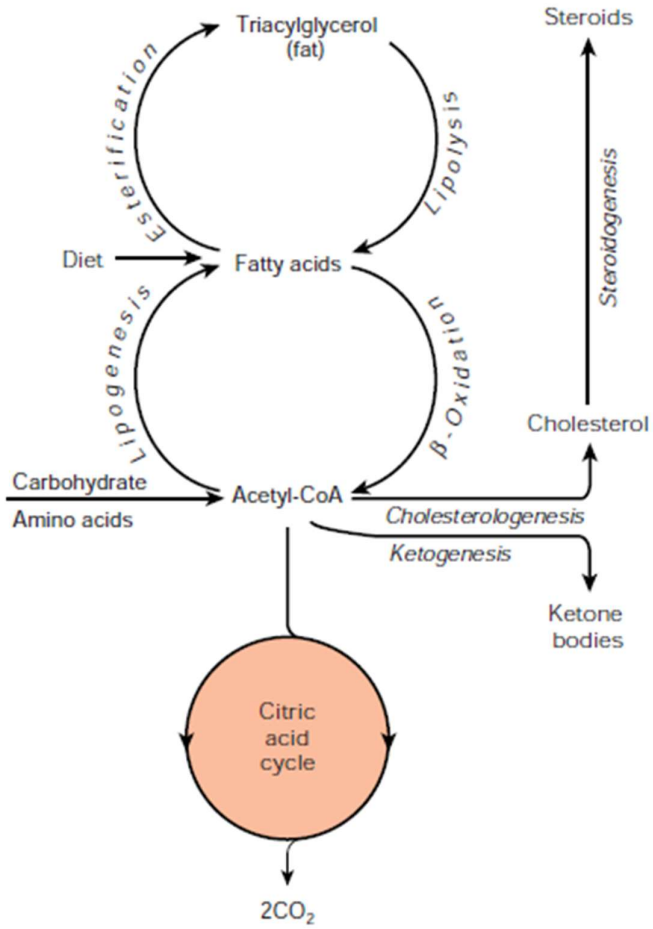
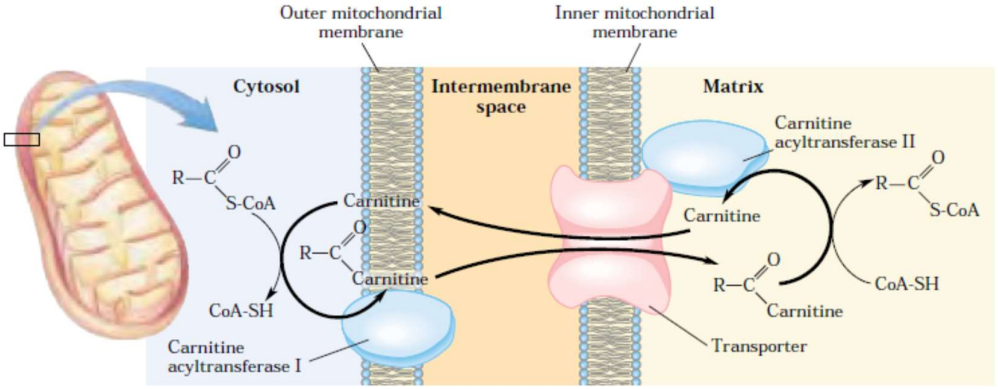


Fig.1. Overview of fatty acid metabolism showing the major pathways and end products. The ketone bodies are acetoacetate, 3-hydroxybutyrate, and acetone.

Fatty acid degradation: β-oxidation

After uptake by the cell, fatty acids are activated by conversion into their CoA derivatives acyl CoA is formed. This uses up two energy-rich anhydride bonds of ATP per fatty acid. For channeling into the mitochondria, the acyl residues are first transferred to *carnitine* and then transported across the inner membrane as acyl carnitine.



The degradation of the fatty acids occurs in the mitochondrial matrix through an oxidative cycle in which C2 units are successively cleaved off as acetyl CoA (*activated acetic acid*). Before the release of the acetyl groups, each CH_2 group at C-3 of the acyl residue (the β-C atom) is oxidized to the keto group hence the term β-oxidation for this metabolic pathway. Both spatially and functionally, it is closely linked to the tricarboxylic acid cycle and to the respiratory chain.

[1] The first step is dehydrogenation of acyl CoA at C-2 and C-3. This yields an unsaturated β2-enoyl-CoA derivative with a *trans*-configured double bond. The two hydrogen atoms are initially transferred from FAD-containing *acyl CoA dehydrogenase* to the electron-transferring flavoprotein (ETF). *ETF dehydrogenase* passes them on from ETF to ubiquinone (coenzyme Q), a component of the *respiratory chain*.

Other FAD-containing mitochondrial dehydrogenases are also able to supply the respiratory chain with electrons in this fashion.

There are three *isoenzymes* of *acyl CoA dehydrogenase* that are specialized for long-chain fatty acids (12–18 C atoms), medium-chain fatty acids (4–14), and short chain fatty acids (4–8).

[2] The next step in fatty acid degradation is the addition of a water molecule to the double bond of the enoyl CoA (*hydration*), with formation of β-hydroxyacyl CoA.

[3] In the next reaction, the OH group at C-3 is oxidized to a carbonyl group (*dehydrogenation*). This gives rise to β-ketoacyl CoA, and the reduction equivalents are transferred to NAD+, which also passes them on to the *respiratory chain.*

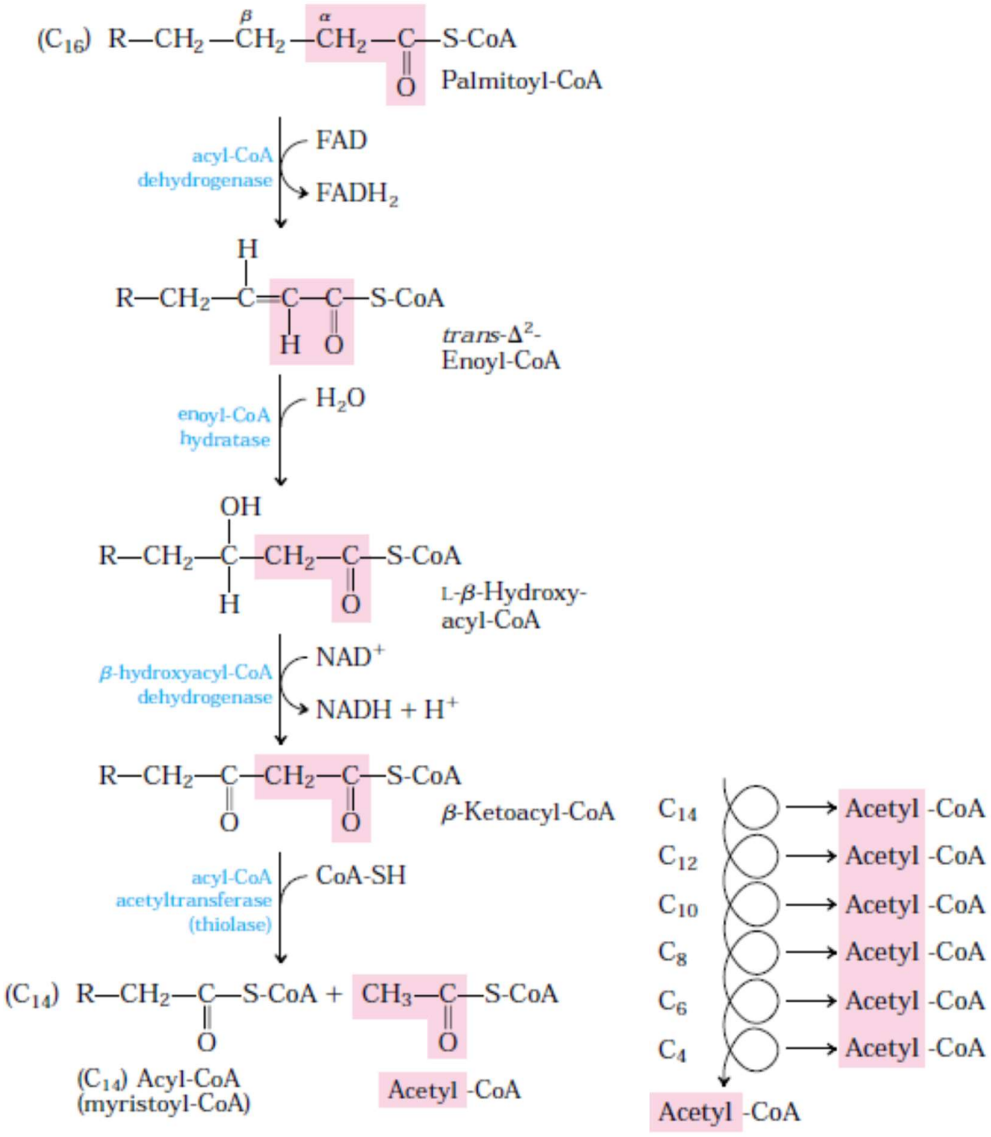
[4] β-Ketoacyl-CoA is now broken down by an *acyl transferase* into acetyl CoA and an acyl CoA shortened by 2 C atoms (*"thioclastic cleavage"*).

Several cycles are required for complete degradation of long-chain fatty acids eight cycles in the case of stearyl-CoA (C18:0), for example. The acetyl CoA formed can then undergo further metabolism in the *tricarboxylic acid cycle*, or can be used for biosynthesis. When there is an excess of acetyl CoA, the liver can also form ketone bodies.

When oxidative degradation is complete, one molecule of palmitic acid supplies around 106 molecules of ATP, corresponding to an energy of 3300 kJ mol–1. This high energy yield makes fats an ideal form of storage for metabolic energy. Hibernating animals such as polar bears can meet their own energy requirements for up to 6 months solely by fat degradation, while at the same time producing the vital water they need via the respiratory chain ("respiratory water").

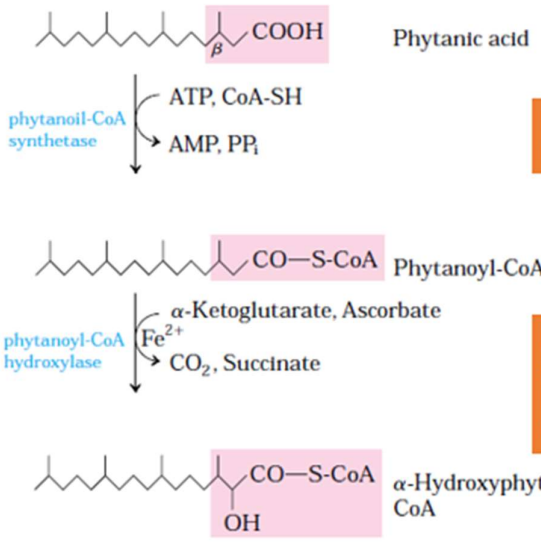
The overall equation for the oxidation of palmitoyl-CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylations, is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7O_2 + 28P_i + 28\text{ADP} \longrightarrow 8\ \text{acetyl-CoA} + 28\text{ATP} + 7H_2O$$



α oxidation of fatty acids

- Alpha – oxidation is defined as the oxidation of fatty acid (methyl group at beta carbon) with the removal of one carbon unit adjacent to the α-carbon from the carboxylic end.
- The carbon unit is removed in the form of CO_2.
- Alpha oxidation occurs in those fatty acids that have a methyl group ($-CH_3$) at the beta-carbon, which blocks beta oxidation.
- Peroxisomes are the cellular sites for α-oxidation.
- **Substrate:** Phytanic acid, which is present in milk or derived from phytol present in chlorophyll and animal fat.
- Phytanic acid is a 20-carbon, branched-chain fatty acid.



Activation of phytanic acid

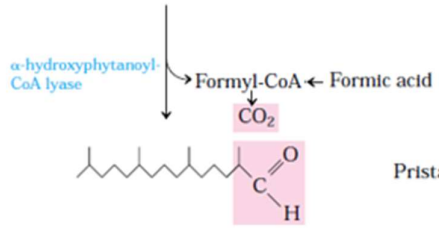
Phytanic acid is first attached to CoA to form phytanoyl-CoA.

Hydroxylation

Phytanoyl-CoA is oxidized by phytanoyl-CoA dioxygenase, in a process using $Fe^{2+}$and O_2, to yield 2-hydroxyphytanoyl-CoA.

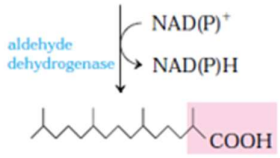
Removal of formyl CoA (CO_2)

2-hydroxyphytanoyl-CoA is cleaved by 2-hydroxyphytanoyl-CoA lyase in a TPP-dependent reaction to form pristanal and formyl-CoA (in turn later broken down into formate and eventually CO_2).



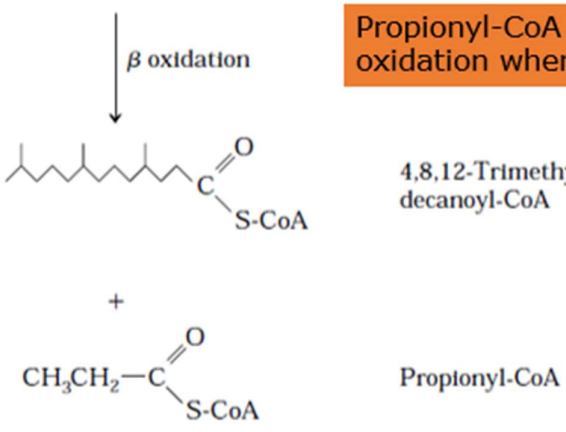
Oxidation of Pristanal

Pristanal is oxidized by aldehyde dehydrogenase to form pristanic acid (which can then undergo beta-oxidation).



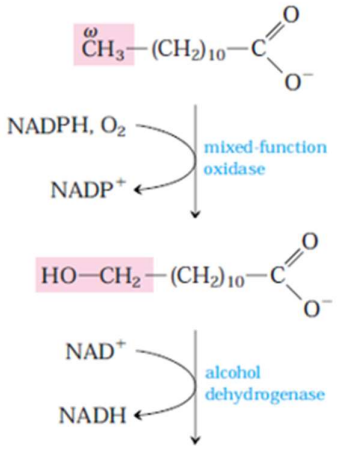
Beta-oxidation of pristanic acid

Propionyl-CoA is released as a result of beta oxidation when the beta carbon is substituted.



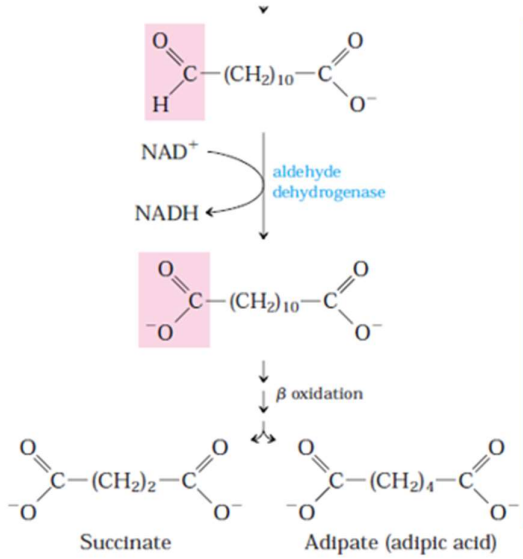
ω-oxidation of fatty acid

- Omega oxidation (ω-oxidation) is a process of fatty acid metabolism in some species of animals.
- It is an alternative pathway to beta oxidation that, instead of involving the β carbon, involves the oxidation of the ω carbon (the carbon most distant from the carboxyl group of the fatty acid).
- The ω (omega)-carbon (the methyl carbon) of fatty acids is oxidized to a carboxyl group in the endoplasmic reticulum.
- The process is normally a minor catabolic pathway for medium-chain fatty acids (10-12 carbon atoms), but becomes more important when β oxidation is defective (because of mutation or a carnitine deficiency, for example).



•The first step introduces a hydroxyl group onto the ω-carbon.

- The oxygen for this group comes from molecular oxygen (O_2) in a complex reaction that involves cytochrome P450 and the electron donor NADPH.
- Reactions of this type are catalyzed by mixed function oxidases.



•Two more enzymes now act on the ω carbon:

- Alcohol dehydrogenase oxidizes the hydroxyl group to an aldehyde, and
- Aldehyde dehydrogenase oxidizes the aldehyde group to a carboxylic acid, producing a fatty acid with a carboxyl group at each end.

•At this point, either end can be attached to coenzyme A, or the molecule can enter the mitochondrion and undergo β-oxidation by the normal route.

•In each pass through the oxidation pathway, the "double-ended" fatty acid yields dicarboxylic acids such as succinic acid, which can enter the citric acid cycle, and adipic acid.

- In vertebrates, the enzymes for ω oxidation are located in the smooth ER of liver and kidney cells, instead of in the mitochondria as with β-oxidation.
- **Substrate:** Medium to Long chain fatty acids (Fatty acid with 10-12 carbon atoms).
- **End Product:** Dicarboxylic acids are produced.

Significance of ω-oxidation

- It is a subsidiary pathway for β-oxidation of fatty acids when β-oxidation is blocked.
- It is observed that ω- and (ω-1)-oxidation of fatty acids are related to energy metabolism in some laboratory animals such as musk shrews and Mongolian gerbils.
- Studies confirm that ω- and (ω-1)-oxidation of fatty acids play crucial roles in the production of insect pheromones of honeybees and in the formation of biopolyesters of higher plants.
- Many studies also have demonstrated that the ω-oxidation serves to provide succinyl-CoA for the citric acid cycle and for gluconeogenesis under conditions of starvation and diabetes.

Fatty acid synthesis

In the vertebrates, biosynthesis of fatty acids is catalyzed by *fatty acid synthase,* a multifunctional enzyme. Located in the cytoplasm, the enzyme requires acetyl CoA as a starter molecule. In a cyclic reaction, the acetyl residue is elongated by one C2 unit at a time for seven cycles. NADPH + H^+ is used as a reducing agent in the process. The end product of the reaction is the saturated C16 acid, *palmitic acid.*

A. Fatty acid synthase

Fatty acid synthase in vertebrates consists of two identical peptide chains i. e., it is a homodimer. Each of the two peptide chains, which are shown here as hemispheres, catalyzes all seven of the partial reactions required to synthesize palmitate. The spatial compression of several successive reactions into a single multifunctional enzyme has advantages in comparison with separate enzymes.

Competing reactions are prevented, the individual reactions proceed in a coordinated way as if on a production line, and due to low diffusion losses they are particularly efficient. Each subunit of the enzyme binds acetyl residues as thioesters at two different SH groups: at one peripheral *cysteine residue* (CysSH) and one central *4_-phosphopantetheine group* (Pan-SH). Pan-SH, which is very similar to coenzyme A, is covalently bound to a protein segment of the synthase known as the *acyl-carrier protein* (ACP).

This part functions like a long arm that passes the substrate from one reaction center to the next. The two subunits of fatty acid synthase cooperate in this process; the enzyme is therefore only capable of functioning as a dimer. Spatially, the enzyme activities are arranged into three different domains.

Domain 1 catalyzes the entry of the substrates acetyl CoA and malonyl CoA by *[ACP]-S acetyltransferase* and *[ACP]-S malonyl transferase* and subsequent condensation of the two partners by *3-oxoacyl-[ACP]-synthase*.
Domain 2 catalyzes the conversion of the 3-oxo group to a CH2 group by *3-oxoacyl-[ACP]-reductase*, *3-hydroxyacyl-[ACP]-dehydratase*, and *enoyl-[ACP]-reductase*.
Finally, domain 3 serves to release the finished product by *acyl-[ACP]-hydrolase* after seven steps of chain elongation.

B. Reactions of fatty acid synthase
The key enzyme in fatty acid synthesis is acetyl CoA carboxylase, which precedes the synthase and supplies the malonyl-CoA required for elongation. Like all carboxylases, the enzyme contains covalently bound *biotin* as a prosthetic group and is hormone dependently *inactivated* by phosphorylation or *activated* by dephosphorylation. The precursor *citrate* is an allosteric activator, while *palmitoyl-CoA* inhibits the end product of the synthesis pathway.

[1] The first cycle (n = 1) starts with the transfer of an acetyl residue from acetyl CoA to the peripheral cysteine residue (Cys-SH). At the same time,
[2] a malonyl residue is transferred from malonyl CoA to 4-phosphopantetheine (Pan-SH).
[3] By condensation of the acetyl residue or (in later cycles) the acyl residue with the malonyl group, with simultaneous decarboxylation, the chain is elongated.

[4]–[6] The following three reactions (reduction of the 3-oxo group, dehydrogenation of the 3-hydroxyl derivative, and renewed reduction of it) correspond in principle to a reversal of β-oxidation, but they are catalyzed by other enzymes and use NADPH + H^+ instead of NADH + H^+ for reduction.

They lead to an acyl residue bound at Pan-SH with 2n + 2 C atoms (n = the number of the cycle). Finally, depending on the length of the product, The acyl residue is transferred back to the peripheral cysteine, so that the next cycle can begin again with renewed loading of the ACP with a malonyl residue, or:

[7] After seven cycles, the completed palmitic acid is hydrolytically released.

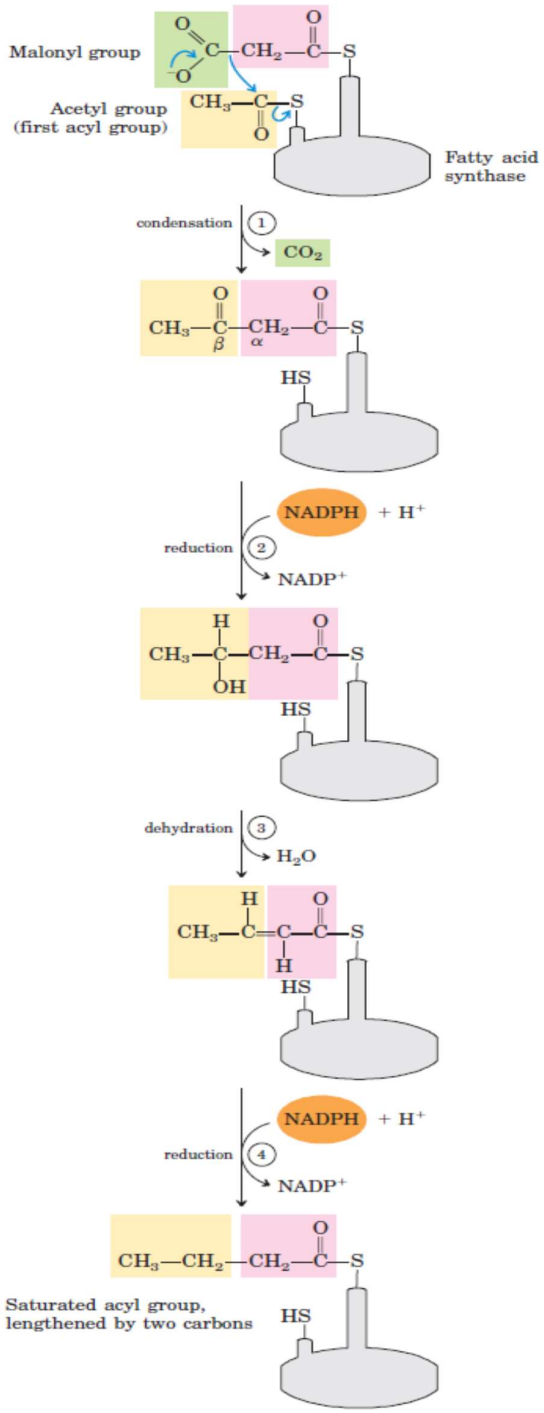
In all, one acetyl-CoA and seven malonyl-CoA are converted with the help of 14 NADPH + H^+ into one palmitic acid, 7 CO_2, 6 H_2O, 8 CoA and 14 NADP+. Acetyl CoA carboxylase also uses up seven ATP.

Addition of two carbons to a growing fatty acyl chain: a four-step sequence. Each malonyl group and acetyl (or longer acyl) group is activated by a thioester that links it to fatty acid synthase, a multienzyme complex.

1 Condensation of an activated acyl group (an acetyl group from acetyl-CoA is the first acyl group) and two carbons derived from malonyl-CoA, with elimination of CO_2 from the malonyl group, extends the acyl chain by two carbons. The mechanism of the first step of this reaction is given to illustrate the role of decarboxylation in facilitating condensation. The *β*-keto product of this condensation is then reduced in three more steps nearly identical to the reactions of *β* oxidation, but in the reverse sequence.

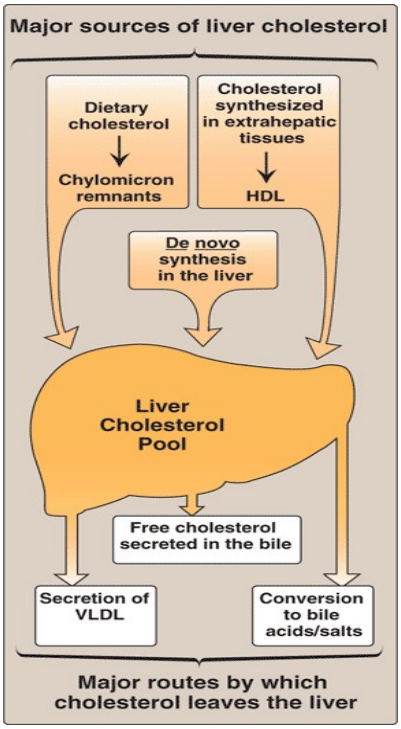
2. the *β*-keto group is reduced to an alcohol,

3. elimination of H_2O creates a double bond, and 4 the double bond is reduced to form the corresponding saturated fatty acyl group.


Malonyl group
Acetyl group
(first acyl group)
Fatty acid
synthase
condensation
1
CO2
HS
NADPH
+ H+
reduction
2
NADP+
dehydration
3
H2O
NADPH
+ H+
reduction
4
NADP+
Saturated acyl group,
lengthened by two carbons
HS

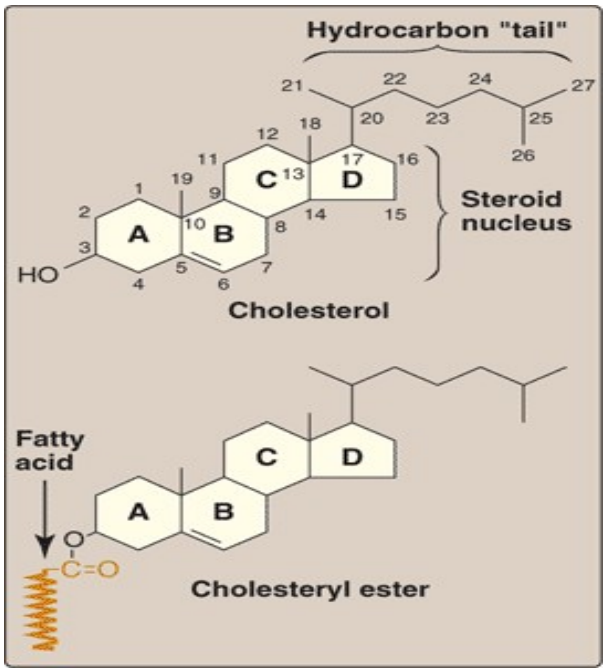
Biosynthesis of Cholesterol:

- Most important animal steroid
- Maintains membrane fluidity
- Insulating effect on nerve fibres
- Cholesterol is the parent molecule for
  - Bile acids and bile salts
  - Steroid hormones
  - Vitamin D_3
- Cholesterol is a light yellow crystalline solid
- It is a 27 Carbon compound
- Contains cyclopentano perhydro phenanthrene ring
- One hydroxyl group (OH) at 3rd position
- Double bond between carbons 5 & 6
- 8 carbon side chain at 17th carbon
- Normal blood level of cholesterol is 150 – 250 mg/dl



Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and, in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. It is therefore of critical importance that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis.

Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings (A, B, C, and D, called the “steroid nucleus”), and it has an eight-carbon, branched hydrocarbon chain attached to C-17 of the D ring. Ring A has a hydroxyl group at C-3, and ring B has a double bond between C-5 and C-6.



Cholesterol is one of the isoprenoids, synthesis of which starts from **acetyl CoA**. In a long and complex reaction chain, the C27 sterol is built up from C2 components. The biosynthesis of cholesterol can be divided into four sections.

1. In the first (**1**), **mevalonate,** a C6 compound, arises from three molecules of **acetyl CoA**.
2. In the second part (**2**), mevalonate is converted into **isopentenyl diphosphate,** the "active isoprene."
3. In the third part (**3**), six of these C5 molecules are linked to produce **squalene,** a C30 compound.
4. Finally, squalene undergoes cyclization, with three C atoms being removed, to yield cholesterol (**4**). The illustration only shows the most important intermediates in biosynthesis.

(1) Formation of mevalonate. The conversion of acetyl CoA to acetoacetyl CoA and then to *3-hydroxy-3-methylglutaryl CoA* (3-HMG CoA) corresponds to the biosynthetic pathway for *ketone bodies* (details on p. 312). In this case, however, the synthesis occurs not in the mitochondria as in ketone body synthesis, but in the smooth endoplasmic reticulum. In the next step, the 3-HMG group is cleaved from the CoA and at the same time reduced to mevalonate with the help of NADPH+H+. *3-HMG CoA reductase* is the *key enzyme* in cholesterol biosynthesis. It is regulated by *repression* of transcription (effectors: oxysterols such as

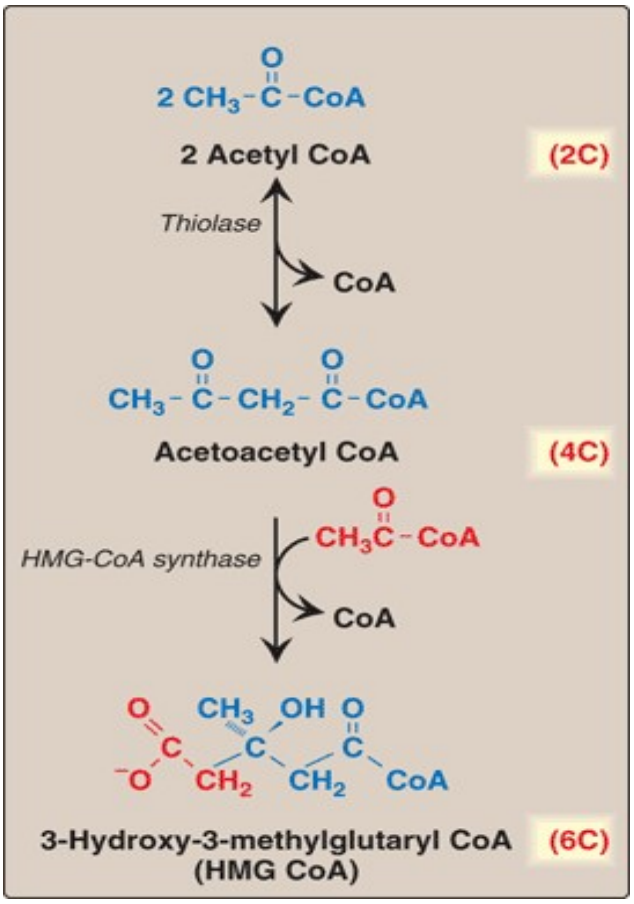
cholesterol) and by *interconversion* (effectors: hormones). Insulin and thyroxine stimulate the enzyme and glucagon inhibits it by cAMP-dependent phosphorylation. A large supply of cholesterol from food also inhibits 3-HMG-CoA reductase.

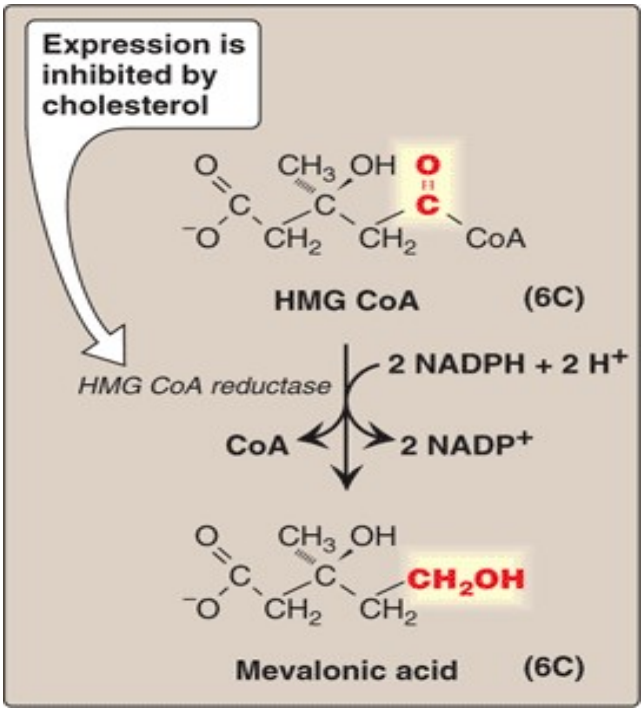
(2) Formation of isopentenyl diphosphate.

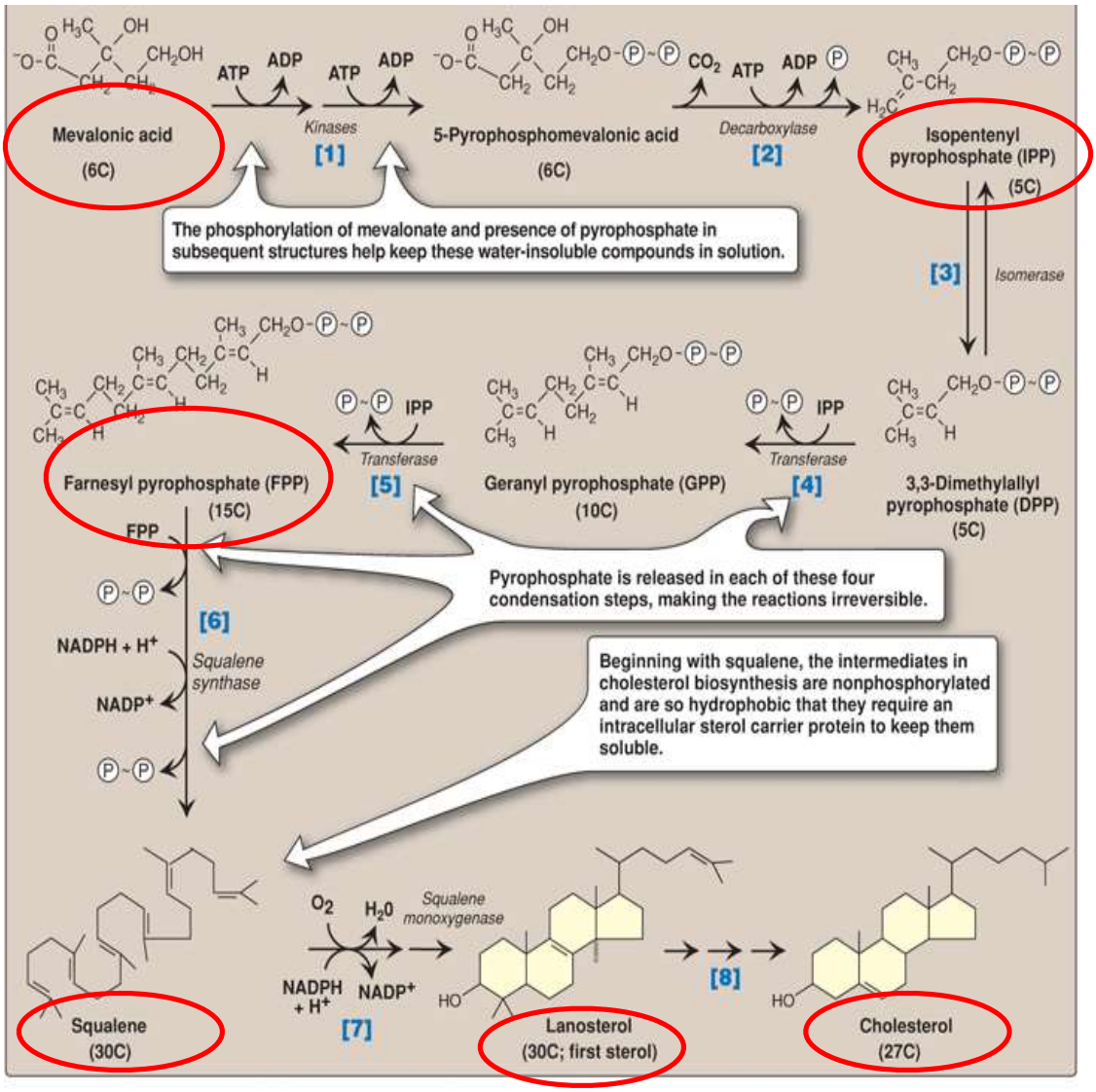
After phosphorylation, mevalonate is decarboxylated to *isopentenyl diphosphate,* with consumption of ATP. This is the component from which all of the isoprenoids are built.

(3) Formation of squalene. Isopentenyl diphosphate undergoes isomerization to form dimethylallyl diphosphate. The two C5 molecules condense to yield geranyl diphosphate, and the addition of another isopentenyl diphosphate produces farnesyl diphosphate. This can then undergo dimerization, in a *head-to-head reaction,* to yield squalene. Farnesyl diphosphate is also the starting point for other polyisoprenoids, such as dolichol and ubiquinone.

(4) Formation of cholesterol. Squalene, a linear isoprenoid, is cyclized, with O2 being consumed, to form lanosterol, a C30 sterol. Three methyl groups are cleaved from this in the subsequent reaction steps, to yield the end product cholesterol. Some of these reactions are catalyzed by *cytochrome P450 systems*. The endergonic biosynthetic pathway described above is located entirely in the *smooth endoplasmic reticulum.* The energy needed comes from the CoA derivatives used and from ATP. The reducing agent in the formation of mevalonate and squalene, as well as in the final steps of cholesterol biosynthesis, is NADPH+H+. The division of the intermediates of the reaction pathway into three groups is characteristic: CoA compounds, diphosphates, and highly lipophilic, poorly soluble compounds (squalene to cholesterol), which are bound to *sterol carriers* in the cell.


2 CH3-C-CoA
2 Acetyl CoA
(2C)
Thiolase
CoA
CH3-C-CH2-C-CoA
Acetoacetyl CoA
(4C)
HMG-CoA synthase
CH3C-CoA
CoA
3-Hydroxy-3-methylglutaryl CoA
(HMG CoA)
(6C)


Expression is inhibited by cholesterol
HMG CoA
(6C)
HMG CoA reductase
2 NADPH + 2 H+
CoA
2 NADP+
Mevalonic acid
(6C)



Degradation of Cholesterol

The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in humans. Rather, the intact sterol nucleus is eliminated from the body by conversion to bile acids and bile salts, which are excreted in the feces, and by secretion of cholesterol into the bile, which transports it to the intestine for elimination. Some of the cholesterol in the intestine is modified by bacteria before excretion. The primary compounds made are the isomers coprostanol and cholestanol, which are reduced derivatives of cholesterol. Together with cholesterol, these compounds make up the bulk of neutral fecal sterols.

Bile Acids and Bile Salts

Bile consists of a watery mixture of organic and inorganic compounds. Phosphatidylcholine (lecithin) and bile salts (conjugated bile acids) are quantitatively the most important organic components of bile. Bile can either pass directly from the liver where it is synthesized into the duodenum through the common bile duct, or be stored in the gallbladder when not immediately needed for digestion.

Steroid Hormones

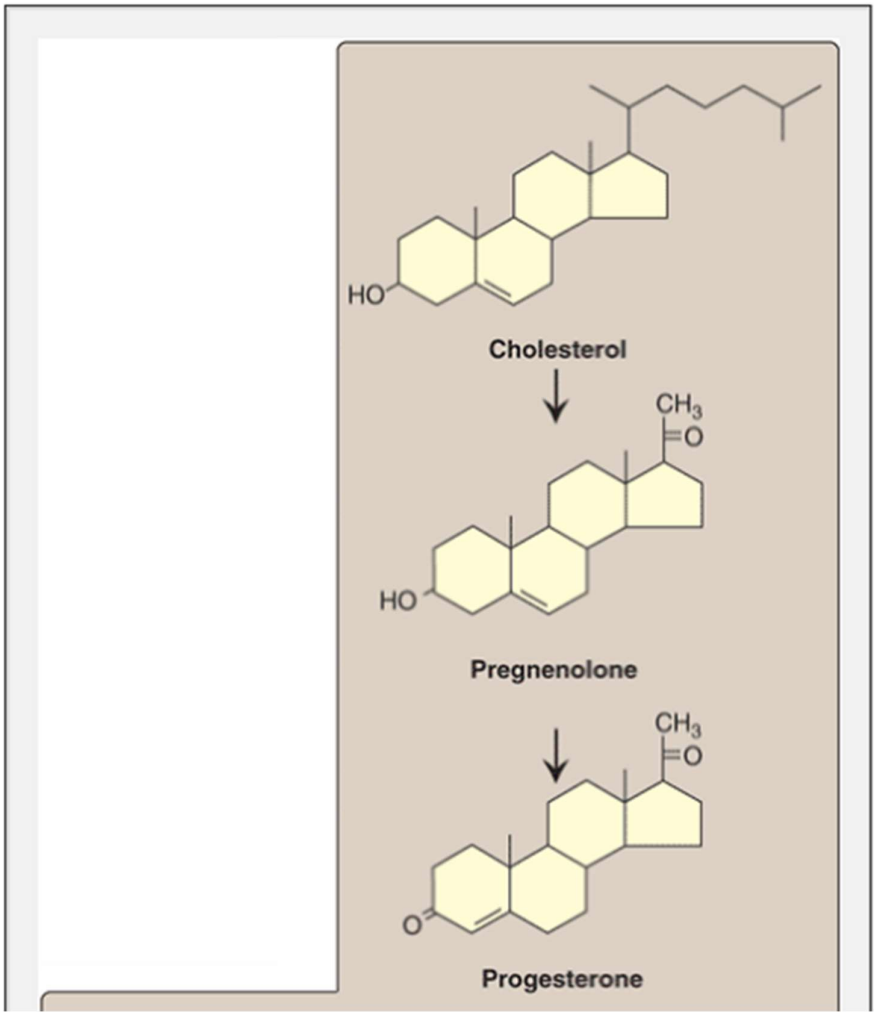
Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (for example, cortisol), mineralocorticoids (for example, aldosterone), and sex hormones—androgens, estrogens, and progestins. [Note: Glucocorticoids and mineralocorticoids are collectively called corticosteroids.] Synthesis and secretion occur in the adrenal cortex (cortisol, aldosterone, and androgens), ovaries and placenta (estrogens and progestins), and testes (testosterone).

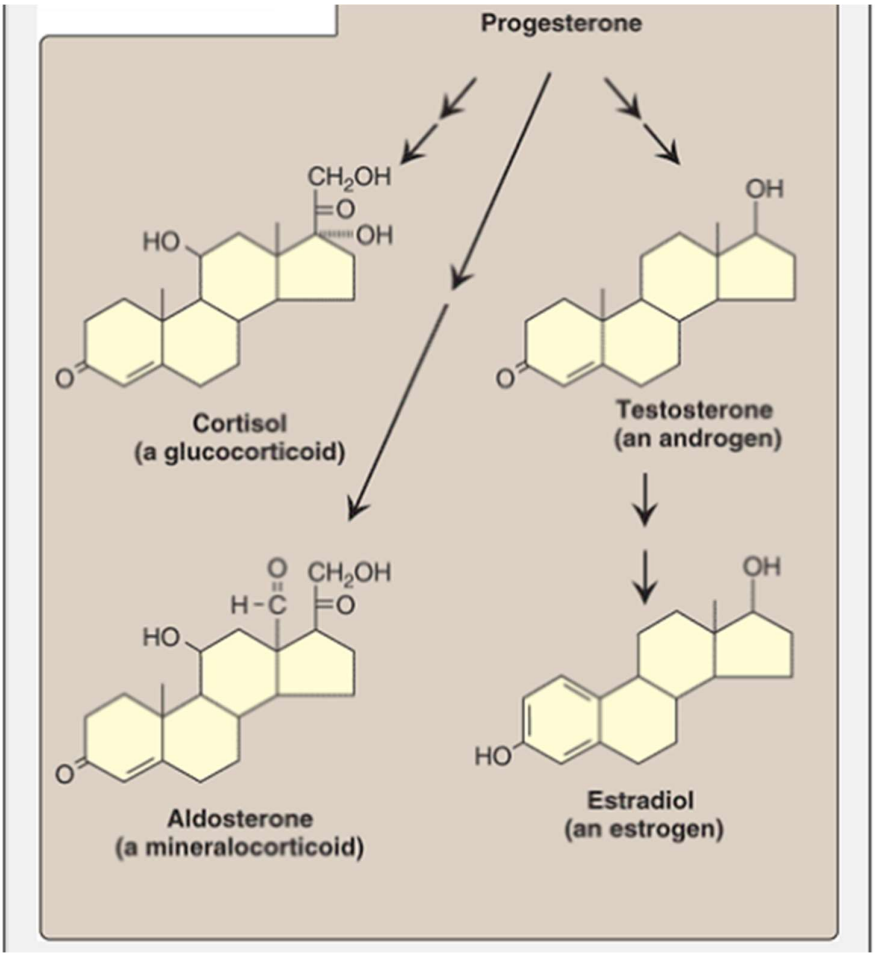
Steroid hormones are transported by the blood from their sites of synthesis to their target organs. Because of their hydrophobicity, they must be complexed with a plasma protein. Plasma albumin can act as a nonspecific carrier, and does carry aldosterone. However, specific steroid-carrier plasma proteins bind the steroid hormones more tightly than does albumin, for example, corticosteroid-binding globulin (transcortin) is responsible for transporting cortisol, and sex hormone–binding protein transports sex steroids. A number of genetic diseases are caused by deficiencies in specific steps in the biosynthesis of steroid hormones.

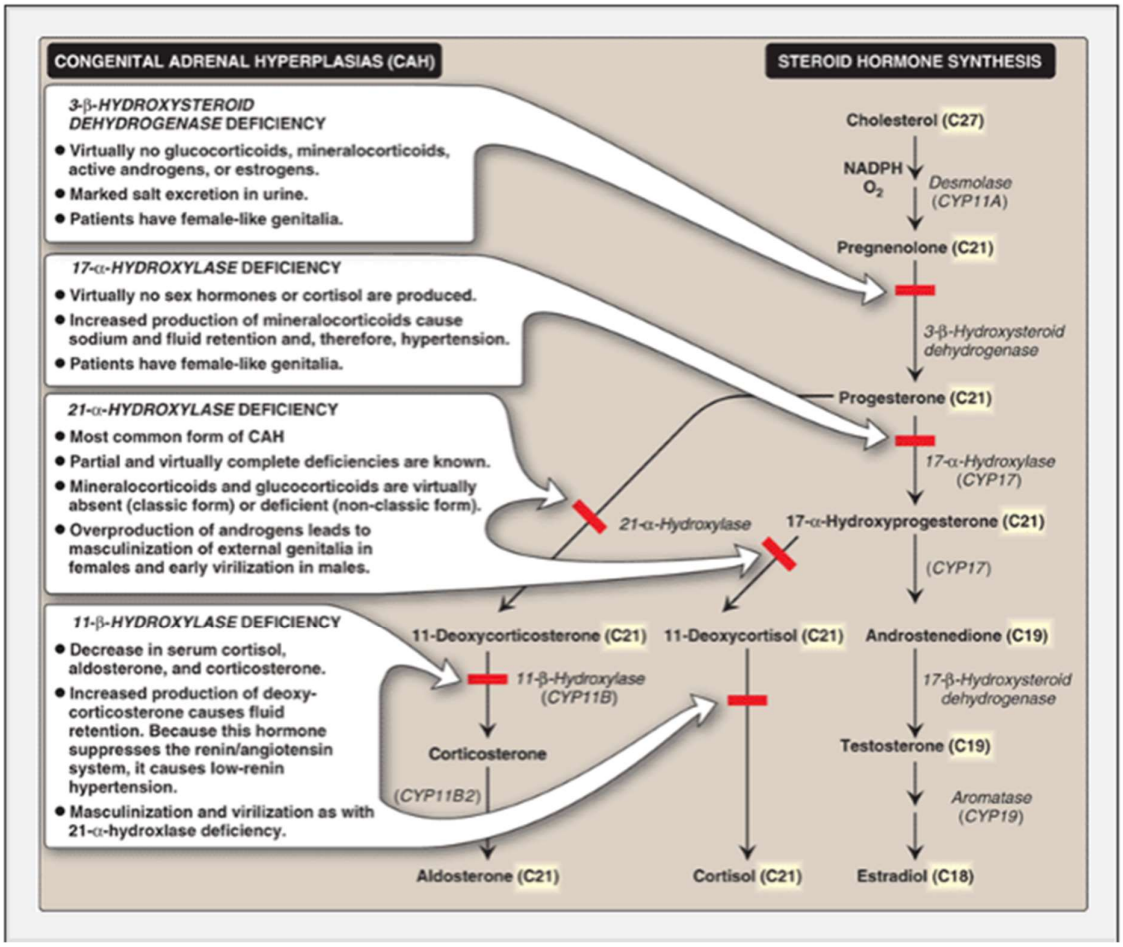
Synthesis involves shortening the hydrocarbon chain of cholesterol, and hydroxylation of the steroid nucleus. The initial and rate-limiting reaction converts cholesterol to the 21-carbon pregnenolone. It is catalyzed by the cholesterol side-chain cleavage enzyme complex (desmolase)—a CYP mixed function oxidase of the inner mitochondrial membrane. NADPH and molecular oxygen are required for the reaction. The cholesterol substrate can be newly synthesized, taken up from lipoproteins, or released from cholesteryl esters stored in the cytosol of steroidogenic tissues.

An important control point is the movement of cholesterol into mitochondria. This process is mediated by StAR (steroidogenic acute regulatory protein). [Note: Steroid hormone synthesis consumes little cholesterol as compared with that required for bile acid synthesis.]

Pregnenolone is the parent compound for all steroid hormones. Pregnenolone is oxidized and then isomerized to progesterone, a progestin, which is further modified to the other steroid hormones by hydroxylation reactions that occur in the ER and mitochondria. Like desmolase, the enzymes are CYP proteins. A defect in the activity or amount of an enzyme in this pathway can lead to a deficiency in the synthesis of hormones beyond the affected step, and to an excess in the hormones or metabolites before that step. Because all members of the pathway have potent biologic activity, serious metabolic imbalances occur if enzyme deficiencies are present. Collectively these disorders are known as the congenital adrenal hyperplasias.




Progesterone
CH2OH
O
HO
OH
O
Cortisol
(a glucocorticoid)
OH
O
Testosterone
(an androgen)
O
CH2OH
H-C
O
HO
O
Aldosterone
(a mineralocorticoid)
OH
HO
Estradiol
(an estrogen)


CONGENITAL ADRENAL HYPERPLASIAS (CAH)
STEROID HORMONE SYNTHESIS
3-β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY
Virtually no glucocorticoids, mineralocorticoids, active androgens, or estrogens.
Marked salt excretion in urine.
Patients have female-like genitalia.
17-α-HYDROXYLASE DEFICIENCY
Virtually no sex hormones or cortisol are produced.
Increased production of mineralocorticoids cause sodium and fluid retention and, therefore, hypertension.
Patients have female-like genitalia.
21-α-HYDROXYLASE DEFICIENCY
Most common form of CAH
Partial and virtually complete deficiencies are known.
Mineralocorticoids and glucocorticoids are virtually absent (classic form) or deficient (non-classic form).
Overproduction of androgens leads to masculinization of external genitalia in females and early virilization in males.
11-β-HYDROXYLASE DEFICIENCY
Decrease in serum cortisol, aldosterone, and corticosterone.
Increased production of deoxycorticosterone causes fluid retention. Because this hormone suppresses the renin/angiotensin system, it causes low-renin hypertension.
Masculinization and virilization as with 21-α-hydroxlase deficiency.
Cholesterol (C27)
NADPH
O2
Desmolase (CYP11A)
Pregnenolone (C21)
3-β-Hydroxysteroid dehydrogenase
Progesterone (C21)
17-α-Hydroxylase (CYP17)
21-α-Hydroxylase
17-α-Hydroxyprogesterone (C21)
(CYP17)
11-Deoxycorticosterone (C21)
11-Deoxycortisol (C21)
Androstenedione (C19)
11-β-Hydroxylase (CYP11B)
17-β-Hydroxysteroid dehydrogenase
Corticosterone
(CYP11B2)
Testosterone (C19)
Aromatase (CYP19)
Aldosterone (C21)
Cortisol (C21)
Estradiol (C18)

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – II – Intermediary Metabolism – SBC3101

Protein Metabolism

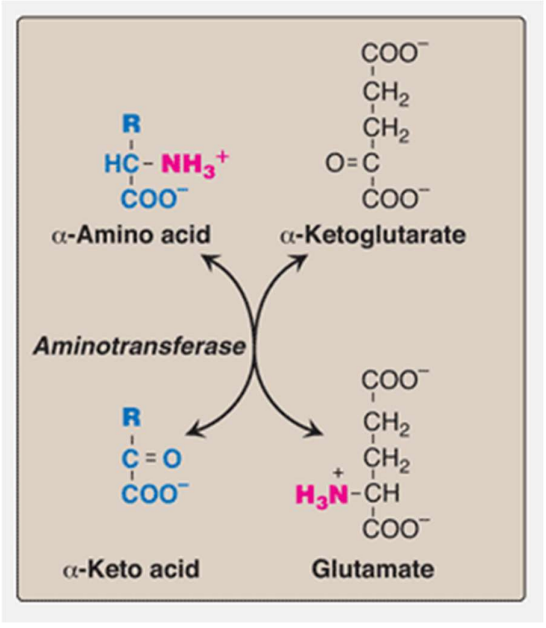
Ketogenic and Glucogenic amino acids metabolism. Deamination, Transamination and Decarboxylation, Urea cycle.

Quantitatively, proteins are the most important group of endogenous macromolecules. A person weighing 70 kg contains about 10 kg protein, with most of it located in muscle. By comparison, the proportion made up by other nitrogen containing compounds is minor. The organism's nitrogen balance is therefore primarily determined by protein metabolism.

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid, and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted, with the carbon skeletons being metabolized.

Transamination: the funneling of amino groups to glutamate:

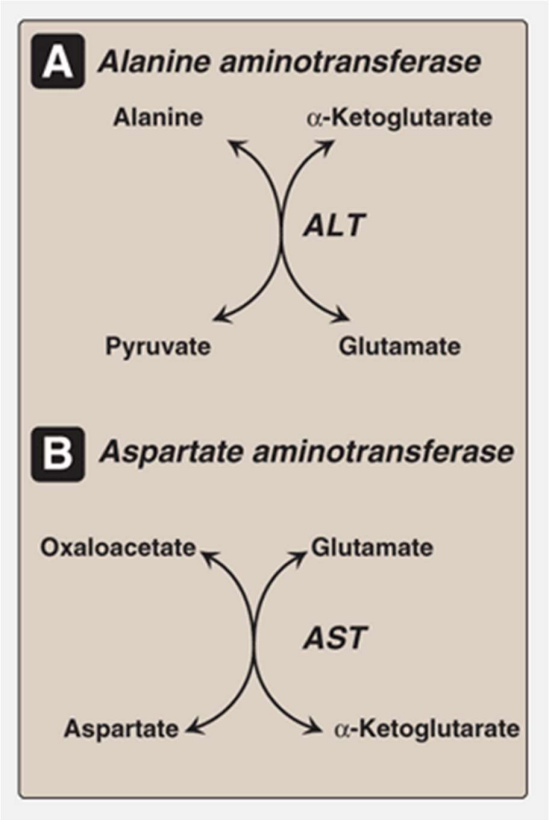
The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate.



The products are an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from other amino acids, thus becoming glutamate. Glutamate produced by transamination can

be oxidatively deaminated, or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases (formerly called transaminases). These enzymes are found in the cytosol and mitochondria of cells throughout the body—especially those of the liver, kidney, intestine, and muscle. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism. [Note: These two amino acids lose their α-amino groups by deamination].

Each aminotransferase is specific for one or, at most, a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. The two most important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase AST,)



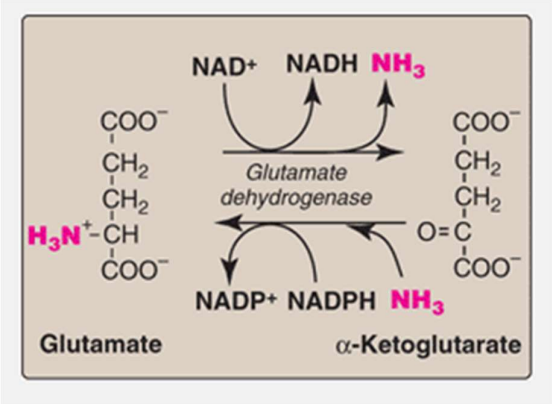
Alanine aminotransferase (ALT): Formerly called glutamate-pyruvate transaminase, ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily

reversible. However, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. Thus, glutamate, in effect, acts as a "collector" of nitrogen from alanine.

Aspartate aminotransferase (AST): AST formerly called glutamate-oxaloacetate transaminase, AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle. [Note: The AST reaction is also reversible.]

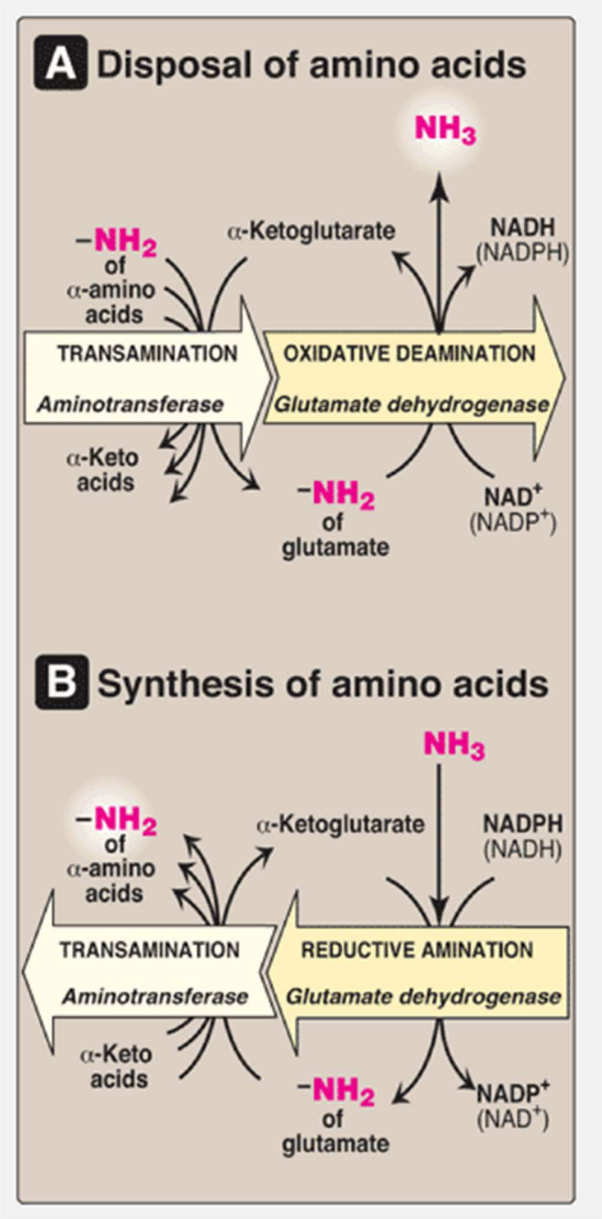
Glutamate dehydrogenase: the oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as free ammonia. These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathway of energy metabolism, and ammonia, which is a source of nitrogen in urea synthesis.



Glutamate dehydrogenase: As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination—a reaction catalyzed by glutamate dehydrogenase. Therefore, the sequential action of transamination (resulting in the collection of amino groups from other amino acids onto α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia, and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia.

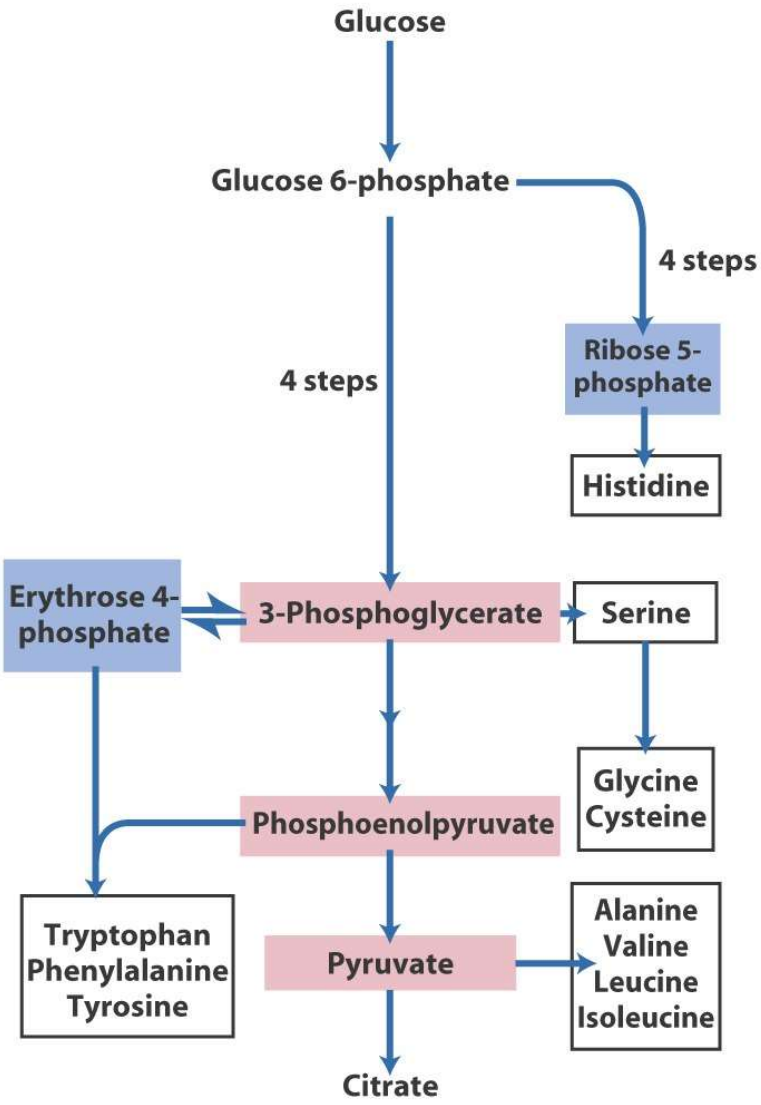


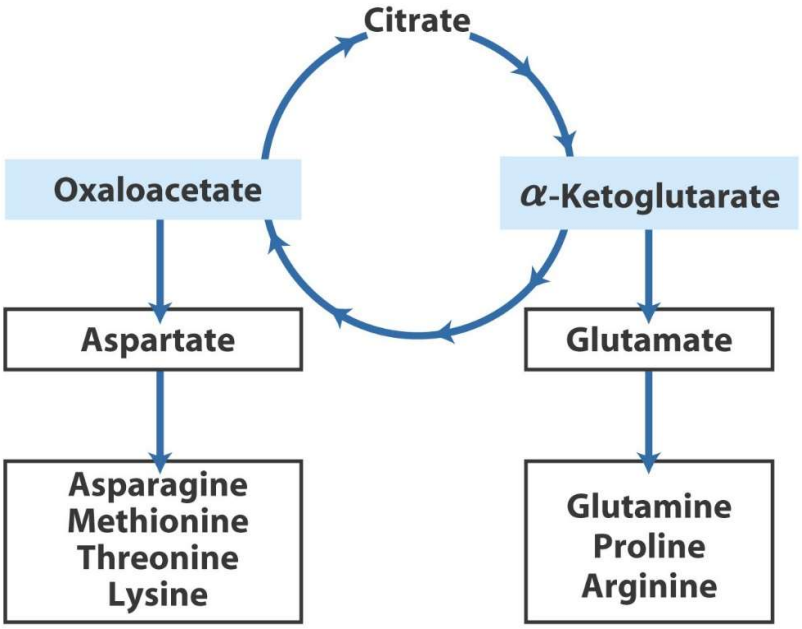
Decarboxylation:

Several amino acids are broken down by *decarboxylation.* This reaction gives rise to what are known as biogenic amines, which have various functions. Some of them are **components of biomolecules**, such as *ethanolamine* in phospholipids. *Cysteamine* and gamma-*alanine* are

components of coenzyme A and of pantetheine. Other amines function as signaling substances. An important **neurotransmitter** derived from glutamate is γ-aminobutyrate (GABA). The transmitter *dopamine* is also a precursor for the catecholamines epinephrine and norepinephrine. The biogenic amine *serotonin*, a substance that has many effects, is synthesized from tryptophan via the intermediate 5-hydroxytryptophan. Monamines are inactivated into aldehydes by *amine oxidase* (monoamine oxidase, "MAO") with deamination and simultaneous oxidation. MAO inhibitors therefore play an important role in pharmacological interventions in neurotransmitter metabolism.

Biosynthesis of Amino Acids



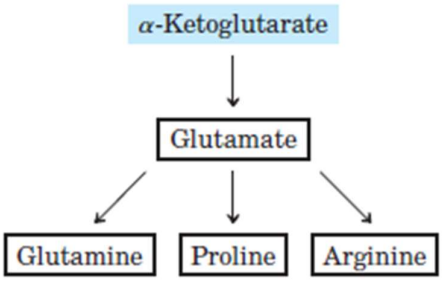


Biosynthetic Families

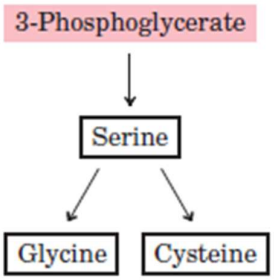
Metabolic Precursors	**Amino Acids**
alpha-ketoglutarate	Glutamate Glutamine Proline Arginine
3-Phosphoglycerate	Serine Glycine Cysteine
Oxaloacetate	Aspartate Asparagine Methionine Threonine Lysine
Pyruvate	Alanine Valine Leucine Isoleucine

Phosphoenolpyruvate and erythrose 4-phosphate	Tryptophan Phenlyalanine Tyrosine
Ribose 5-phosphate	Histidine

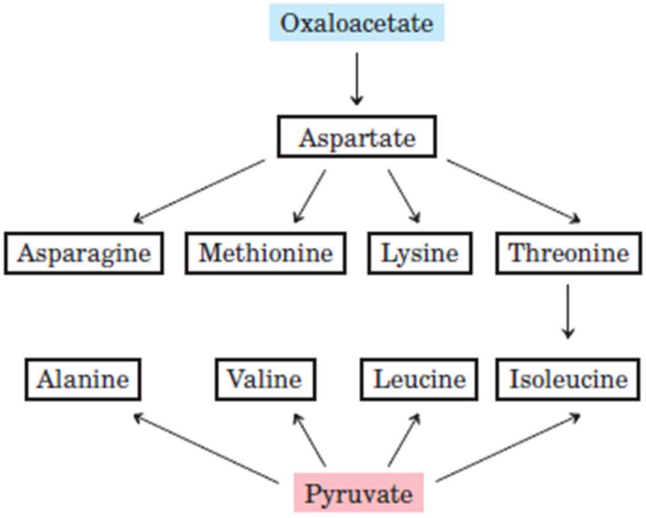
Alpha-**Ketoglutarate Gives Rise to Glutamate, Glutamine, Proline, and Arginine**



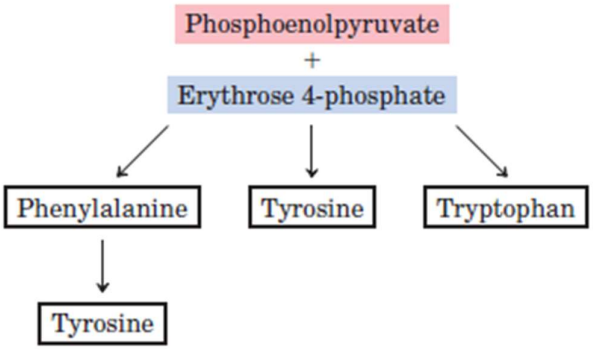
Serine, Glycine, and Cysteine Are Derived from 3-Phosphoglycerate



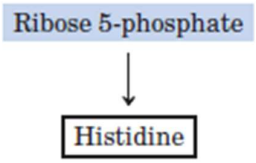
Three Nonessential and Six Essential Amino Acids Are Synthesized from Oxaloacetate and Pyruvate



Chorismate Is a Key Intermediate in the Synthesis of Tryptophan, Phenylalanine, and Tyrosine



Histidine Biosynthesis Uses Precursors of Purine Biosynthesis



Urea cycle

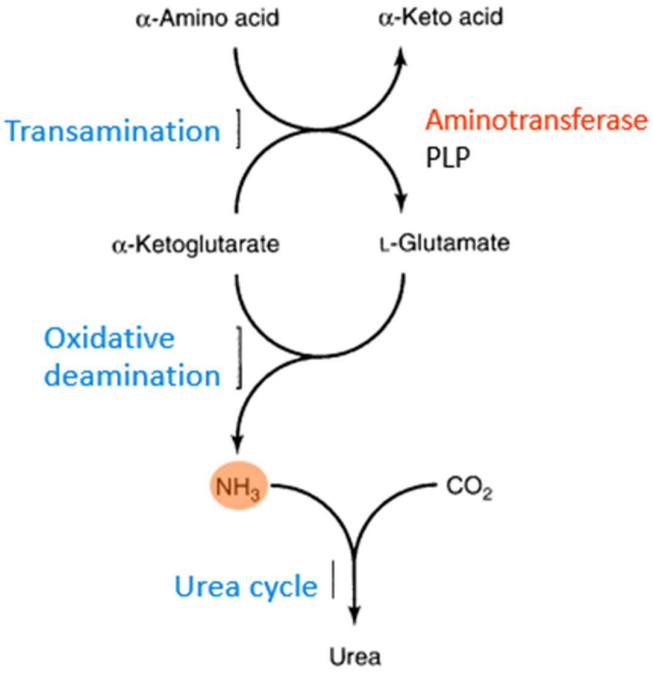
If not reused for the synthesis of new amino acids or other nitrogenous products, amino groups are channeled into a single excretory end product. Most aquatic species, such as the bony fishes, are **ammonotelic,** excreting amino nitrogen as ammonia. The toxic ammonia is simply diluted in the surrounding water. Terrestrial animals require pathways for nitrogen excretion that minimize toxicity and water loss. Most terrestrial animals are **ureotelic,** excreting amino nitrogen in the form of urea; birds and reptiles are **uricotelic,** excreting amino nitrogen as uric acid. Plants recycle virtually all amino groups, and nitrogen excretion occurs only under very unusual circumstances.

In ureotelic organisms, the ammonia deposited in the mitochondria of hepatocytes is converted to urea in the **urea cycle.** This pathway was discovered in 1932 by Hans Krebs.

Ammonia originates in the catabolism of amino acids that are primarily produced by the degradation of proteins – dietary as well as existing within the cell:

- digestive enzymes
- proteins released by digestion of cells sloughed-off the walls of the GIT
- muscle proteins
- hemoglobin
- intracellular proteins (damaged, unnecessary)

Ammonia is toxic, especially for the CNS, because it reacts with α-ketoglutarate, thus making it limiting for the TCA cycle ⇒ decrease in the ATP level. Liver damage or metabolic disorders associated with elevated ammonia can lead to tremor, slurred speech, blurred vision, coma, and death. Normal conc. of ammonia in blood: 30-60 µM.



Urea ($H_2N–CO–NH_2$) is the diamide of carbonic acid. In contrast to ammonia, it is **neutral** and therefore relatively **non-toxic**. The reason for the lack of basicity is the molecule's mesomeric characteristics. The free electron pairs of the two nitrogen atoms are *delocalized* over the whole structure, and are therefore no longer able to bind protons. As a small, uncharged molecule, urea is able to cross biological membranes easily. In addition, it is easily transported in the blood and excreted in the urine. Urea is produced **only in the liver**, in a cyclic sequence of reactions (the **urea cycle**) that starts in the mitochondria and continues in the cytoplasm. The two nitrogen atoms are derived from **$NH4^+$**. The keto group comes from **hydrogen carbonate** (HCO_3^-), or CO2 that is in equilibrium with HCO_3^-.

[1] In the first step, **carbamoyl phosphate** is formed in the mitochondria from hydrogen carbonate (HCO_3^-) and NH_4^+, with two ATP molecules being consumed. In this compound, the carbamoyl residue ($–O–CO–NH_2$) is at a high chemical potential. In hepatic mitochondria, enzyme [1] makes up about 20% of the matrix proteins.

[2] In the next step, the carbamoyl residue is transferred to the non-proteinogenic amino acid **ornithine**, converting it into **citrulline**, which is also non-proteinogenic. This is passed into the cytoplasm via a transporter.

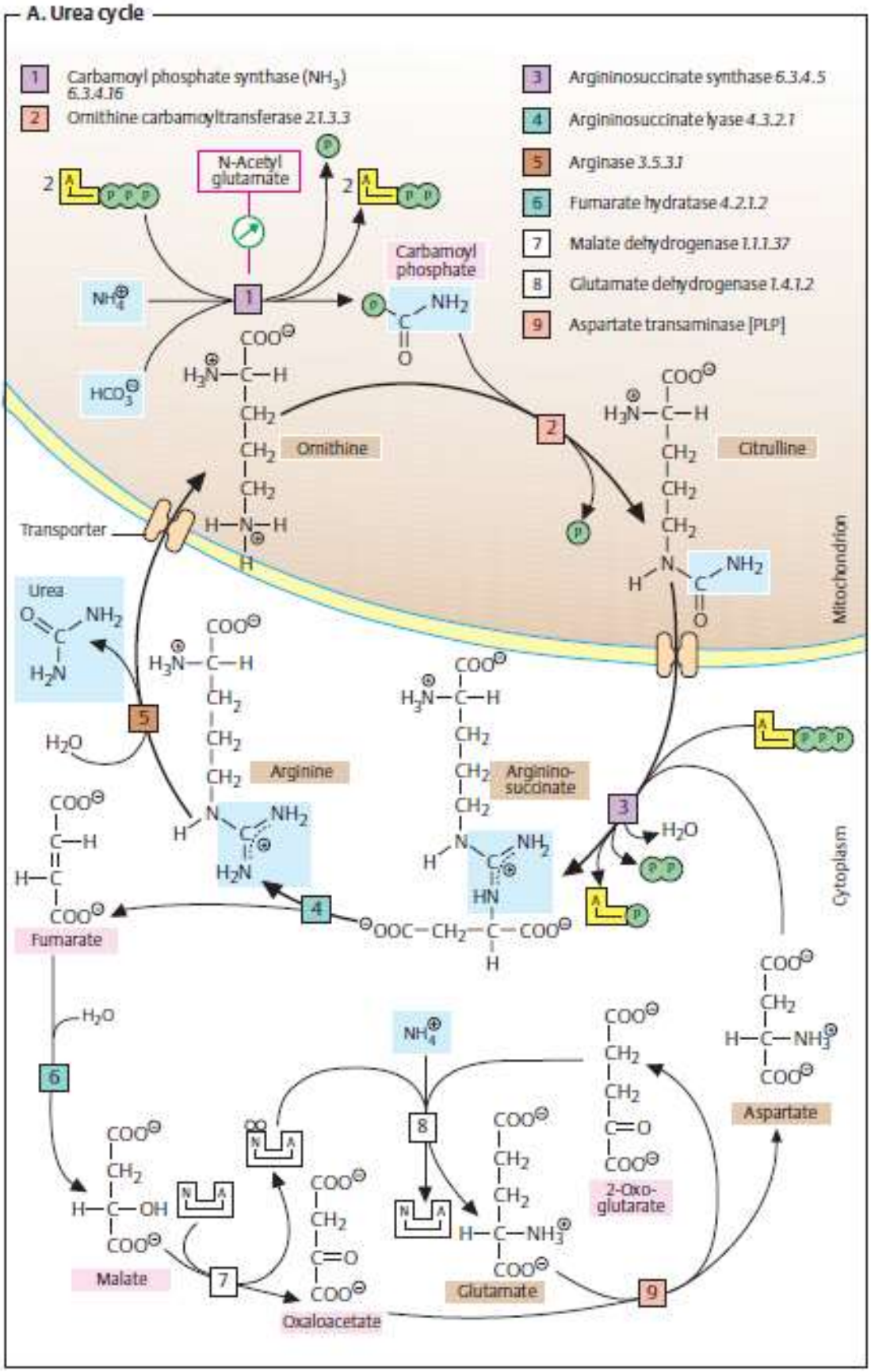
[3] The second NH_2 group of the later urea molecule is provided by **aspartate**, which condenses with citrulline into **argininosuccinate**. ATP is cleaved into AMP and diphosphate (PPi) for this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, diphosphate is removed from the equilibrium by hydrolysis.

[4] Cleavage of fumarate from argininosuccinate leads to the proteinogenic amino acid **arginine**, which is synthesized in this way in animal metabolism.

[5] In the final step, isourea is released from the guanidinium group of the arginine by hydrolysis (not shown), and is immediately rearranged into **urea**. In addition, ornithine is regenerated and returns via the ornithine transporter into the mitochondria, where it becomes available for the cycle once again.

The **fumarate** produced in step [4] is converted via malate to oxaloacetate [6, 7], from which **aspartate** is formed again by transamination [9]. The glutamate required for reaction [9] is derived from the glutamate dehydrogenase reaction [8], which fixes the second $NH4^+$ in an organic bond. Reactions [6] and [7] also occur in the tricarboxylic acid cycle. However, in urea formation they take place in the cytoplasm, where the appropriate isoenzymes are available.

The rate of urea formation is mainly controlled by reaction [1]. *N*-**acetyl glutamate**, as an allosteric effector, activates *carbamoylphosphate synthase.* In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.


A. Urea cycle
1 Carbamoyl phosphate synthase (NH_3) 6.3.4.16
2 Ornithine carbamoyltransferase 2.1.3.3
3 Argininosuccinate synthase 6.3.4.5
4 Argininosuccinate lyase 4.3.2.1
5 Arginase 3.5.3.1
6 Fumarate hydratase 4.2.1.2
7 Malate dehydrogenase 1.1.1.37
8 Glutamate dehydrogenase 1.4.1.2
9 Aspartate transaminase [PLP]
N-Acetyl glutamate
Carbamoyl phosphate
Ornithine
Citrulline
Transporter
Mitochondrion
Urea
Arginine
Arginino-succinate
Fumarate
Cytoplasm
Malate
Oxaloacetate
Glutamate
2-Oxo-glutarate
Aspartate

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UNIT – III – Intermediary Metabolism – SBC3101

Nucleotide Metabolism

Biosynthesis of purine and pyrimidines – de novo pathway and salvage pathway. Formation of deoxy nucleotides and its regulation. Degradation of purine and pyrimidine nucleotides.

Nucleotides play a variety of important roles in all cells. They are the precursors of DNA and RNA. They are essential carriers of chemical energy—a role primarily of ATP and to some extent GTP. They are components of the cofactors NAD, FAD, *S*-adenosylmethionine, and coenzyme A, as well as of activated biosynthetic intermediates such as UDP-glucose and CDP-diacylglycerol. Some, such as cAMP and cGMP, are also cellular second messengers.

Two types of pathways lead to nucleotides: the **de novo pathways** and the **salvage pathways.** De novo synthesis of nucleotides begins with their metabolic precursors: amino acids, ribose 5-phosphate, CO2, and NH3. Salvage pathways recycle the free bases and nucleosides released from nucleic acid breakdown. Both types of pathways are important in cellular metabolism.

De novo biosynthesis of Purines:

In the first committed step of the pathway, an amino group donated by glutamine is attached at C-1 of PRPP. The resulting **5-phosphoribosylamine** is highly unstable, with a half-life of 30 seconds at pH 7.5. The purine ring is subsequently built up on this structure. The pathway described here is identical in all organisms, with the exception of one step that differs in higher eukaryotes as noted below.

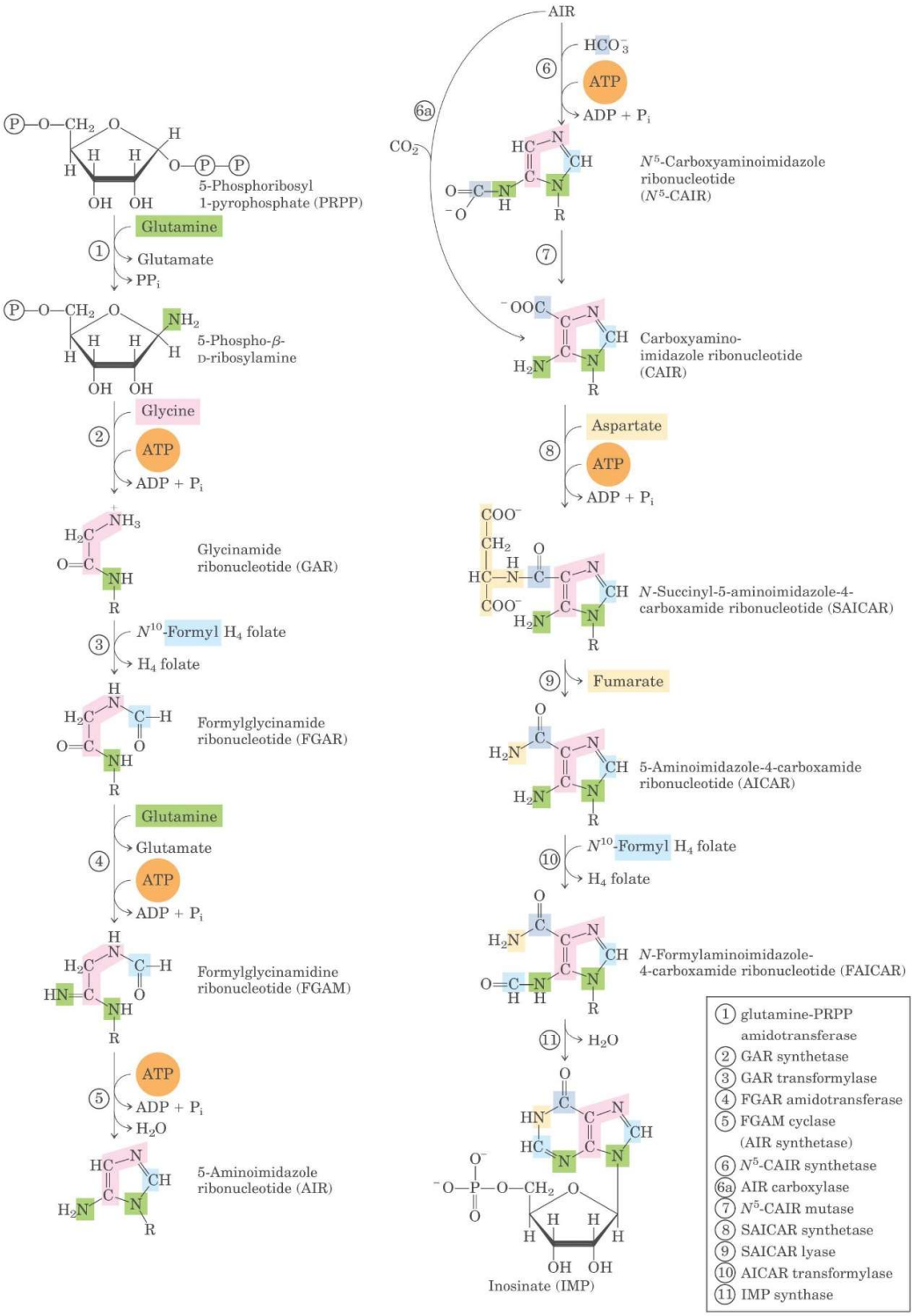
The second step is the addition of three atoms from glycine (step 2). An ATP is consumed to activate the glycine carboxyl group (in the form of an acyl phosphate) for this condensation reaction. The added glycine amino group is then formylated by *N*10-formyltetrahydrofolate (step 3), and a nitrogen is contributed by glutamine (step 4), before dehydration and ring closure yield the five-membered imidazole ring of the purine nucleus, as 5-aminoimidazole ribonucleotide (AIR; step 5).

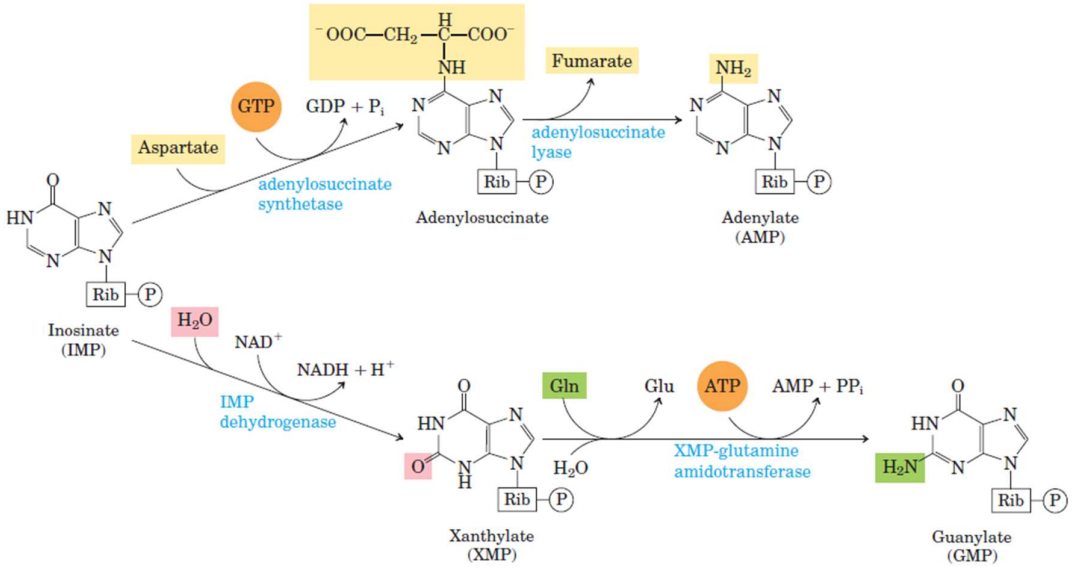
At this point, three of the six atoms needed for the second ring in the purine structure are in place. To complete the process, a carboxyl group is first added (step 6). This carboxylation is unusual in that it does not require biotin, but instead uses the bicarbonate generally present in aqueous solutions. A rearrangement transfers the carboxylate from the exocyclic amino group to position 4 of the imidazole ring (step 7). Steps 6 and 7 are found only in bacteria and fungi.

In higher eukaryotes, including humans, the 5-aminoimidazole ribonucleotide product of step 5 is carboxylated directly to carboxyaminoimidazole ribonucleotide in one step instead of two (step 6a). The enzyme catalyzing this reaction is AIR carboxylase. Aspartate now donates its amino group in two steps (8 and 9): formation of an amide bond, followed by elimination of the carbon skeleton of aspartate (as fumarate). Recall that aspartate plays an analogous role in two steps of the urea cycle. The final carbon is contributed by *N*10-formyltetrahydrofolate (step 10), and a second ring closure takes place to yield the second fused ring of the purine nucleus (step 11).

The first intermediate with a complete purine ring is **inosinate (IMP).** As in the tryptophan and histidine biosynthetic pathways, the enzymes of IMP synthesis appear to be organized as large, multienzyme complexes in the cell. Once again, evidence comes from the existence of single polypeptides with several functions, some catalyzing nonsequential steps in the pathway. In eukaryotic cells ranging from yeast to fruit flies to chickens, steps 1 , 3 , and 5 catalyzed by a multifunctional protein. An additional multifunctional protein catalyzes steps 10 and 11. In humans, a multifunctional enzyme combines the activities of AIR carboxylase and SAICAR synthetase (steps 6a and 8).

In bacteria, these activities are found on separate proteins, but a large noncovalent complex may exist in these cells. The channeling of reaction intermediates from one enzyme to the next permitted by these complexes is probably especially important for unstable intermediates such as 5-phosphoribosylamine. Conversion of inosinate to adenylate requires the insertion of an amino group derived from aspartate; this takes place in two reactions similar to those used to introduce N-1 of the purine ring (steps 8 and 9). A crucial difference is that GTP rather than ATP is the source of the high-energy phosphate in synthesizing adenylosuccinate. Guanylate is formed by the NAD_-requiring oxidation of inosinate at C-2, followed by addition of an amino group derived from glutamine. ATP is cleaved to AMP and PPi in the final step.


5-Phosphoribosyl 1-pyrophosphate (PRPP)
Glutamine
Glutamate
PPi
5-Phospho-β-D-ribosylamine
Glycine
ATP
ADP + Pi
Glycinamide ribonucleotide (GAR)
N10-Formyl H4 folate
H4 folate
Formylglycinamide ribonucleotide (FGAR)
Glutamine
Glutamate
ATP
ADP + Pi
Formylglycinamidine ribonucleotide (FGAM)
ATP
ADP + Pi
H2O
5-Aminoimidazole ribonucleotide (AIR)
AIR
HCO3−
ATP
ADP + Pi
CO2
N5-Carboxyaminoimidazole ribonucleotide (N5-CAIR)
Carboxyamino-imidazole ribonucleotide (CAIR)
Aspartate
ATP
ADP + Pi
N-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
Fumarate
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-Formyl H4 folate
H4 folate
N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
H2O
Inosinate (IMP)
1 glutamine-PRPP amidotransferase
2 GAR synthetase
3 GAR transformylase
4 FGAR amidotransferase
5 FGAM cyclase (AIR synthetase)
6 N5-CAIR synthetase
6a AIR carboxylase
7 N5-CAIR mutase
8 SAICAR synthetase
9 SAICAR lyase
10 AICAR transformylase
11 IMP synthase



De novo synthesis of Pyrimidine nucleotides:

The common pyrimidine ribonucleotides are cytidine 5_-monophosphate (CMP; cytidylate) and uridine 5_-monophosphate (UMP; uridylate), which contain the pyrimidines cytosine and uracil. De novo pyrimidine nucleotide biosynthesis proceeds in a somewhat different manner from purine nucleotide synthesis; the six-membered pyrimidine ring is made first and then attached to ribose 5-phosphate.

Required in this process is carbamoyl phosphate, also an intermediate in the urea cycle. However, in animals the carbamoyl phosphate required in urea synthesis is made in mitochondria by carbamoyl phosphate synthetase I, whereas the carbamoyl phosphate required in pyrimidine biosynthesis is made in the cytosol by a different form of the enzyme, **carbamoyl phosphate synthetase II.**

In bacteria, a single enzyme supplies carbamoyl phosphate for the synthesis of arginine and pyrimidines. Carbamoyl phosphate reacts with aspartate to yield *N*-carbamoylaspartate in the first committed step of pyrimidine biosynthesis. This reaction is catalyzed by **aspartate transcarbamoylase.** In bacteria, this step is highly regulated, and bacterial aspartate transcarbamoylase is one of the most thoroughly studied allosteric enzymes (see below). By removal of water from *N*-carbamoylaspartate, a reaction catalyzed by **dihydroorotase,** the pyrimidine ring is closed to form L-dihydroorotate. This compound is oxidized to the pyrimidine derivative orotate, a reaction in which NAD_ is the ultimate electron acceptor.

In eukaryotes, the first three enzymes in this pathway—carbamoyl phosphate synthetase II, aspartate transcarbamoylase, and dihydroorotase— are part of a single trifunctional protein. The protein, known by the acronym CAD, contains three identical polypeptide chains, each with active sites for all three reactions. This suggests that large, multienzyme complexes may be the rule in this pathway. Once orotate is formed, the ribose 5-phosphate side chain, provided once again by PRPP, is attached to yield orotidylate. Orotidylate is then decarboxylated to uridylate, which is phosphorylated to UTP. CTP is formed from UTP by the action of **cytidylate synthetase,** by way of an acyl phosphate intermediate (consuming one ATP). The nitrogen donor is normally glutamine, although the cytidylate synthetases in many species can use NH_4^- directly.

Nucleoside Monophosphates Are Converted to Nucleoside Triphosphates

Nucleotides to be used in biosynthesis are generally converted to nucleoside triphosphates. The conversion pathways are common to all cells. Phosphorylation of AMP to ADP is promoted by **adenylate kinase,** in the reaction.

$$ATP + AMP \rightleftharpoons 2ADP$$

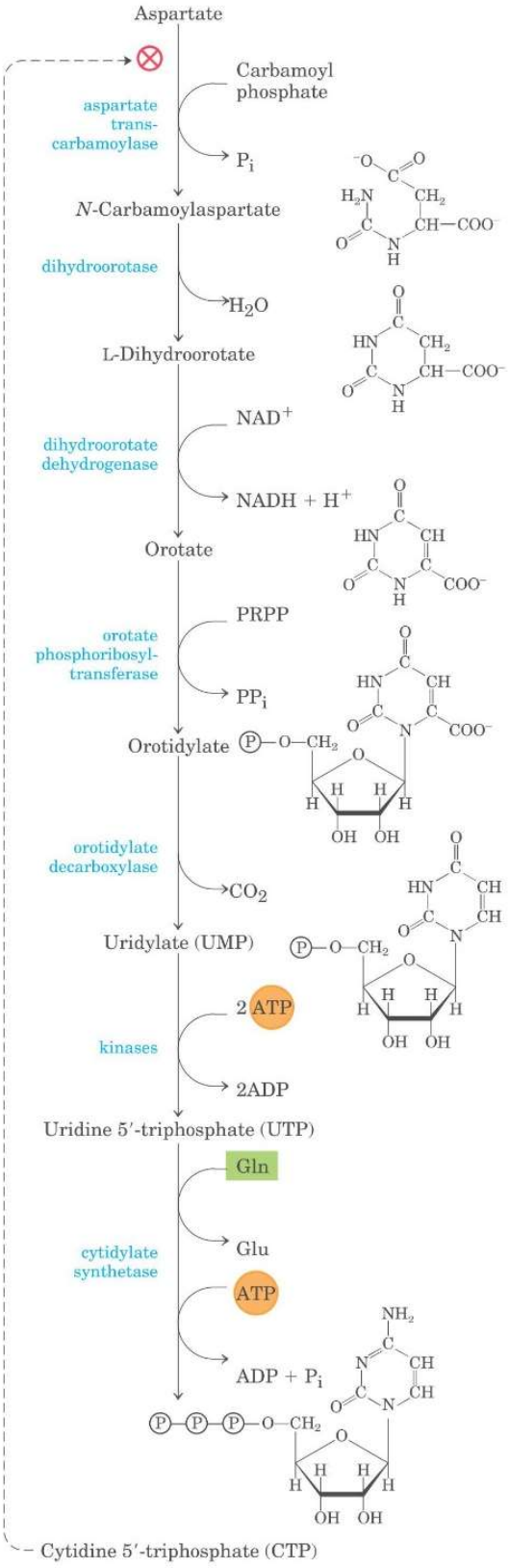
The ADP so formed is phosphorylated to ATP by the glycolytic enzymes or through oxidative phosphorylation. ATP also brings about the formation of other nucleoside diphosphates by the action of a class of enzymes called **nucleoside monophosphate kinases.** These enzymes, which are generally specific for a particular base but nonspecific for the sugar (ribose or deoxyribose), catalyze the reaction

$$ATP + NMP \rightleftharpoons ADP + NDP$$

The efficient cellular systems for rephosphorylating ADP to ATP tend to pull this reaction in the direction of products. Nucleoside diphosphates are converted to triphosphates by the action of a ubiquitous enzyme, **nucleoside diphosphate kinase,** which catalyzes the reaction

$$NTP_D + NDP_A \rightleftharpoons NDP_D + NTP_A$$

This enzyme is notable in that it is not specific for the base (purines or pyrimidines) or the sugar (ribose or deoxyribose). This nonspecificity applies to both phosphate acceptor (A) and donor (D), although the donor (NTP_D) is almost invariably ATP, because it is present in higher concentration than other nucleoside triphosphates under aerobic conditions.


Aspartate
Carbamoyl phosphate
aspartate transcarbamoylase
P_i
N-Carbamoylaspartate
dihydroorotase
H_2O
L-Dihydroorotate
NAD^+
dihydroorotate dehydrogenase
$NADH + H^+$
Orotate
PRPP
orotate phosphoribosyltransferase
PP_i
Orotidylate
orotidylate decarboxylase
CO_2
Uridylate (UMP)
2 ATP
kinases
2ADP
Uridine 5′-triphosphate (UTP)
Gln
cytidylate synthetase
Glu
ATP
$ADP + P_i$
Cytidine 5′-triphosphate (CTP)

Ribonucleotides Are the Precursors of Deoxyribonucleotides

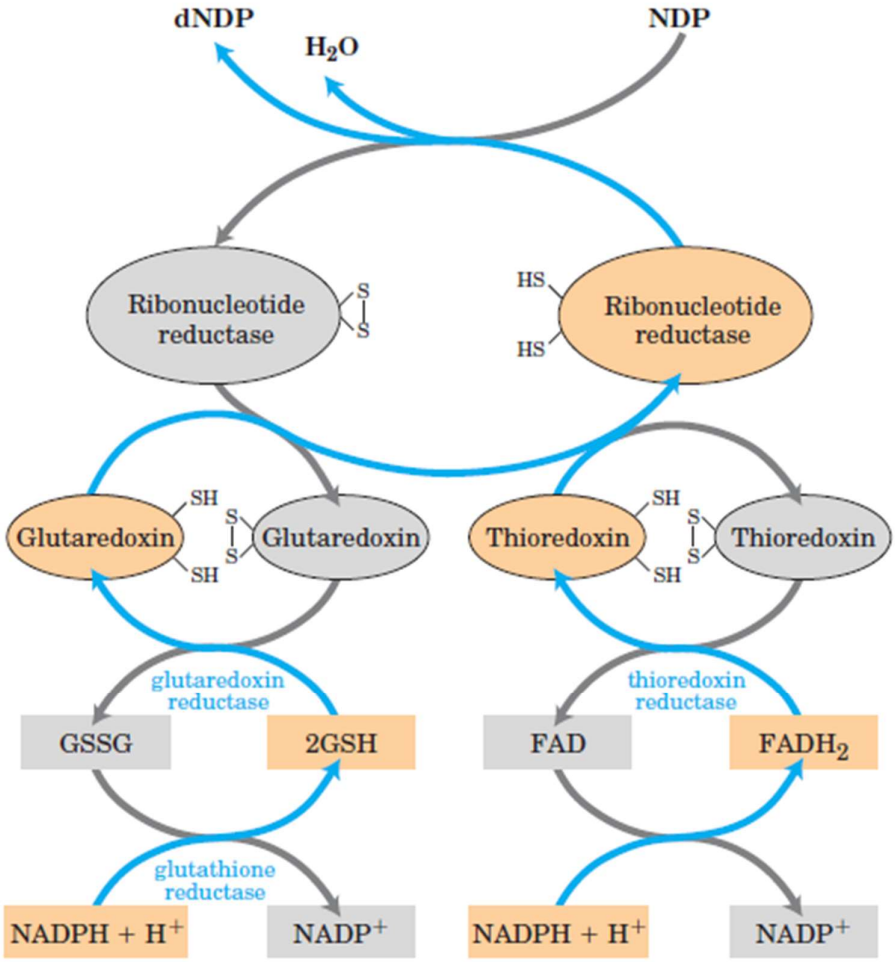
Deoxyribonucleotides, the building blocks of DNA, are derived from the corresponding ribonucleotides by direct reduction at the 2-carbon atom of the D-ribose to form the 2_-deoxy derivative. For example, adenosine diphosphate (ADP) is reduced to 2_-deoxyadenosine diphosphate (dADP), and GDP is reduced to dGDP. This reaction is somewhat unusual in that the reduction occurs at a nonactivated carbon; no closely analogous chemical reactions are known. The reaction is catalyzed by **ribonucleotide reductase,** best characterized in *E.coli,* in which its substrates are ribonucleoside diphosphates.

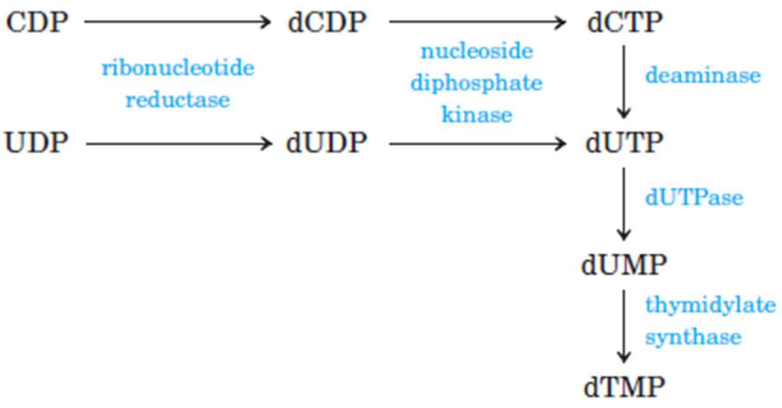
The reduction of the D-ribose portion of a ribonucleoside diphosphate to 2_-deoxy-D-ribose requires a pair of hydrogen atoms, which are ultimately donated by NADPH via an intermediate hydrogen-carrying protein, **thioredoxin.** This ubiquitous protein serves a similar redox function in photosynthesis and other processes. Thioredoxin has pairs of OSH groups that carry hydrogen atoms from NADPH to the ribonucleoside diphosphate.

Its oxidized (disulfide) form is reduced by NADPH in a reaction catalyzed by **thioredoxin reductase**, and reduced thioredoxin is then used by ribonucleotide reductase to reduce the nucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs). A second source of reducing equivalents for ribonucleotide reductase is glutathione (GSH). Glutathione serves as the reductant for a protein closely related to thioredoxin, **glutaredoxin,** which then transfers the reducing power to ribonucleotide reductase

Thymidylate Is Derived from dCDP and dUMP

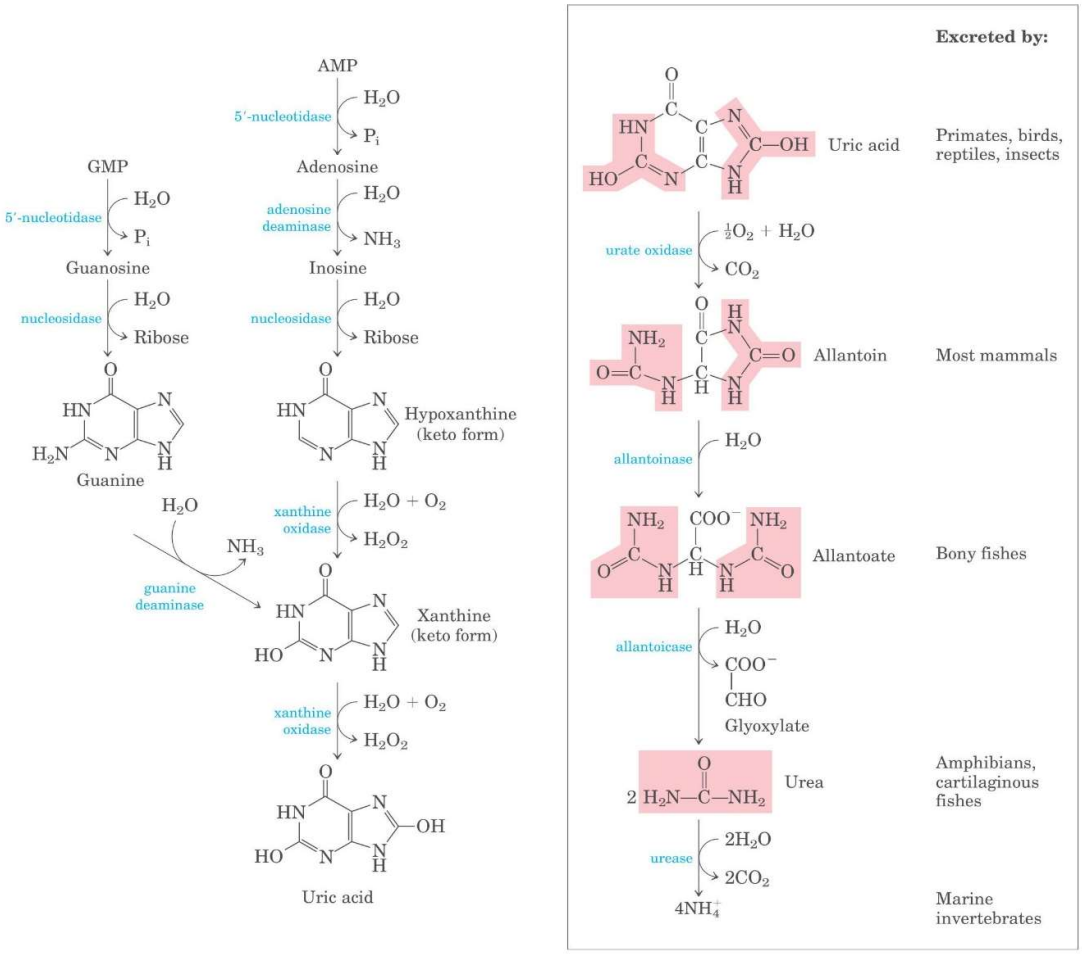
DNA contains thymine rather than uracil, and the de novo pathway to thymine involves only deoxyribonucleotides. The immediate precursor of thymidylate (dTMP) is dUMP. In bacteria, the pathway to dUMP begins with formation of dUTP, either by deamination of dCTP or by phosphorylation of dUDP. The dUTP is converted to dUMP by a dUTPase. The latter reaction must be efficient to keep dUTP pools low and prevent incorporation of uridylate into DNA.


dNDP
H_2O
NDP
Ribonucleotide reductase
S
S
HS
HS
Ribonucleotide reductase
Glutaredoxin
SH
S
S
SH
Glutaredoxin
Thioredoxin
SH
S
S
SH
Thioredoxin
glutaredoxin reductase
thioredoxin reductase
GSSG
2GSH
FAD
$FADH_2$
glutathione reductase
$NADPH + H^+$
$NADP^+$
$NADPH + H^+$
$NADP^+$
(a)
(b)


CDP
dCDP
dCTP
ribonucleotide reductase
nucleoside diphosphate kinase
deaminase
UDP
dUDP
dUTP
dUTPase
dUMP
thymidylate synthase
dTMP

Degradation of Purines and Pyrimidines Produces Uric Acid and Urea

Purine nucleotides are degraded by a pathway in which they lose their phosphate through the action of **5_-nucleotidase**. Adenylate yields adenosine, which is deaminated to inosine by **adenosine deaminase,** and inosine is hydrolyzed to hypoxanthine (its purine base) and D-ribose. Hypoxanthine is oxidized successively to xanthine and then uric acid by **xanthine oxidase,** a flavoenzyme with an atom of molybdenum and four iron-sulfur centers in its prosthetic group. Molecular oxygen is the electron acceptor in this complex reaction.

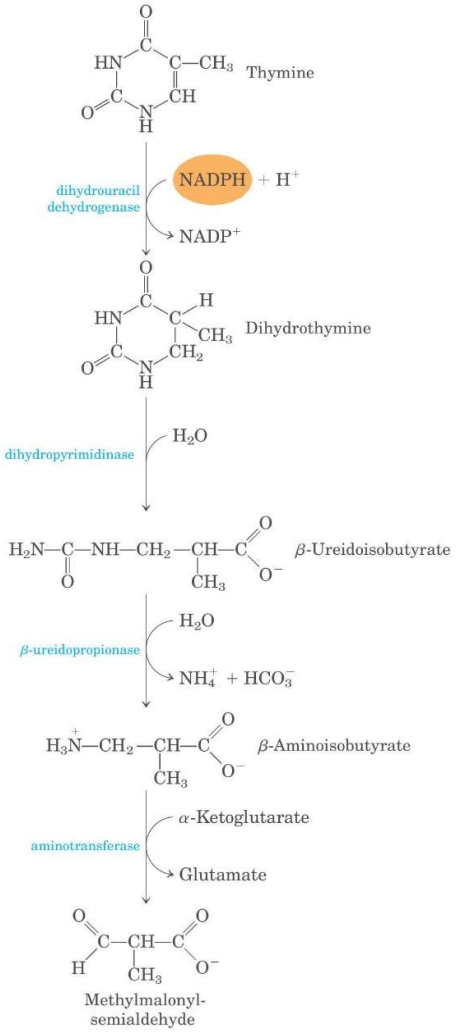


GMP catabolism also yields uric acid as end product. GMP is first hydrolyzed to guanosine, which is then cleaved to free guanine. Guanine undergoes hydrolytic removal of its amino group to yield xanthine, which is converted to uric acid by xanthine oxidase. Uric acid is the

excreted end product of purine catabolism in primates, birds, and some other animals. A healthy adult human excretes uric acid at a rate of about 0.6 g/24 h; the excreted product arises in part from ingested purines and in part from turnover of the purine nucleotides of nucleic acids. In most mammals and many other vertebrates, uric acid is further degraded to **allantoin** by the action of **urate oxidase.** In other organisms the pathway is further extended. The pathways for degradation of pyrimidines generally lead to NH4 _ production and thus to urea synthesis.

Purine and Pyrimidine Bases Are Recycled by Salvage Pathways

Free purine and pyrimidine bases are constantly released in cells during the metabolic degradation of nucleotides. Free purines are in large part salvaged and reused to make nucleotides, in a pathway much simpler than the de novo synthesis of purine nucleotides described earlier.



One of the primary salvage pathways consists of a single reaction catalyzed by **adenosine phosphoribosyltransferase,** in which free adenine reacts with PRPP to yield the corresponding adenine nucleotide:

$$\text{Adenine} + \text{PRPP} \longrightarrow \text{AMP} + \text{PP}_i$$

Free guanine and hypoxanthine (the deamination product of adenine) are salvaged in the same way by **hypoxanthine-guanine phosphoribosyltransferase.** A similar salvage pathway exists for pyrimidine bases in microorganisms, and possibly in mammals.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV – Intermediary Metabolism – SBC3101

Disorders of Metabolism

Disorders of carbohydrate metabolism, lipid metabolism, protein metabolism and nucleotide metabolism. Treatment of metabolic disorders.

Many childhood conditions are caused by gene mutations that encode specific proteins. These mutations can result in the alteration of primary protein structure or the amount of protein synthesized. The functional ability of protein, whether it is an enzyme, receptors, transport vehicle, membrane, or structural element, may be relatively or seriously compromised. These hereditary biochemical disorders are collectively termed as **''Inborn errors of metabolism''**

Disorders of carbohydrate metabolism:

Lactose is a disaccharide sugar composed of galactose and glucose that is found in milk. Lactose can not be absorbed by the intestine and needs to be split in the small intestine into galactose and glucose by the enzyme called lactase; unabsorbed lactose can cause abdominal pain, bloating, diarrhea, gas, and nausea.

In most mammals, production of lactase diminishes after infants are weaned from maternal milk. However, 5% to 90% of the human population possess an advantageous autosomal mutation in which lactase production persists after infancy. The geographic distribution of lactase persistence is concordant with areas of high milk intake. Lactase non-persistence is common in tropical and subtropical countries. Individuals with lactase non-persistency may experience nausea, bloating and diarrhea after ingesting dairy.

Galactosemia, the inability to metabolize galactose in liver cells, is the most common monogenic disorder of carbohydrate metabolism, affecting 1 in every 55,000 newborns. When galactose in the body is not broken down, it accumulates in tissues. The most common signs are failure to thrive, hepatic insufficiency, cataracts and developmental delay. Long term disabilities include poor growth, mental retardation, and ovarian failure in females. Galactosemia is caused by mutations in the gene that makes the enzyme galactose-1-phosphate uridylyltransferase.

Fructose malabsorption is a digestive disorder in which absorption of fructose is impaired by deficient fructose carriers in the small intestine's enterocytes. Three autosomal recessive disorders impair fructose metabolism in liver cells. The most common is caused by mutations in the gene encoding hepatic fructokinase, an enzyme that catalyzes the first step in the

metabolism of dietary fructose. Inactivation of the hepatic fructokinase results in asymptomatic fructosuria.

Hereditary fructose intolerance (HFI) results in poor feeding, failure to thrive, chronic liver disease and chronic kidney disease, and death. HFI is caused by a deficiency of fructose 1,6-biphosphate aldolase in the liver, kidney cortex and small intestine. Infants and adults are asymptomatic unless they ingest fructose or sucrose.

Deficiency of hepatic fructose 1,6-biphosphate (FBPase) causes impaired gluconeogenesis, hypoglycemia and severe metabolic acidemia. If patients are adequately supported beyond childhood, growth and development appear to be normal. Essential fructosuria is a clinically benign condition characterized by the incomplete metabolism of fructose in the liver, leading to its excretion in urine.

Glycogen Storage Diseases

Glycogen storage diseases are a group of inherited conditions characterized by tissue deposits of glycogen that are abnormal in amount or structure. Glycogen is found principally in liver and muscle, and is synthesized from glucose by different enzymes from those which mediate glycogenolysis.

Features of glycogen storage diseases

Type	Principle tissues involved	Enzyme deficiency	Features
I (von Gierke)	Liver	Glucose-6-phosphatase	Hypoglycaemia, lactic acidosis, hepatomegaly, hyperuricaemia
II (Pompe)	Heart, liver, muscle	Lysosomal (1-4) glucosidase	Muscle weakness, cardiac hypertrophy, normoglycaemia
III (Cori)	Liver, muscle	Debranching enzyme	Hypoglycaemia, hepatomegaly
IV	Liver	Branching enzyme	Cirrhosis
V (McArdle)	Muscle	Phosphorylase	Muscle fatigue after exercise, myoglobinuria
VI (Hers)	Liver, muscle	Phosphorylase	Hepatomegaly
VII	Muscle	Phosphofructokinase	Muscle fatigue after exercise, myoglobinuria
IX	Liver, muscle	Phosphorylase kinase	Hepatomegaly

Disorders of lipid metabolism:

Classification:

1. Disorders of F.A-oxidation:-

A. Defects in Beta-oxidation:-

a) Sudden infant death syndrome(SIDS)

- Unexpected death of healthy infants, usually overnight.
- Disorder due to blockage in beta-oxidation.
- Real cause of SIDS is not known.
- SIDS is due to deficiency of medium chain acyl-CoA dehydrogenase

b) Zellweger's Syndrome.

Also called *Hepato renal syndrome*

Rare inherited disorder

Due to the absence of peroxisomes and its enzymes in all tissues, fail to oxidize long chain FA in peroxisomes.

- As a result there is accumulation of FA C26 – C38 chain length in brain tissues and other tissues like Liver and Kidney.
- Also called *Hepato renal syndrome*

c) Carnitine deficiency.

It occurs in

a)In newborns:- Specially premature infants, owing to inadequate synthesis or renal leakage.

b) In adults:-

- Can occur in hemodialysis.
- Patients with organic acidurias, carnitine is lost in urine being conjugated with organic acid.

Clinical features:- Hypoglycemia.

Treatment:- oral therapy with Carnitine.

d) Carnitine palmitoyl transferese deficiency.

Features:

a) Hepatic deficiency of the enzyme results in *hypoglycemia* and *low plasma ketone bodies*.

b) Muscular deficiency of these enzyme produces impaired FA-oxidation which results in *recurrent muscle weakness* and *myoglobinuria.*

e) Jamaican Vomiting Sickness:-

- Caused by eating the unripe akee fruits, which contains a toxin, ***hypoglycin*** that inactivates medium and short chain acyl-coA dehydrogenase.
- Inhibits β-oxidation.

Clinical Features:- Hypoglycemia with excretion of medium and short chain mono and dicarboxylic acids.

B. Defect in Alpha-oxidation:-

Refsum's disease.

Enzyme deficiency:- "Phytanate alpha-oxidase''.

Inheritance:- autosomal recessive.

Age:- from childhood to adult life.

Biochemical Defect:-

- Accumulates in tissues & blood.
- Blood shows increase up to 20% of the total FA.

2) Lipid Storage diseases:-

Also known as sphingolipidoses. Genetically acquired. Group of inherited diseases that are caused by a genetic defect in the catabolism of lipids containing sphingosine. They are part of a larger group of lysosomal disorders.

A. Niemann-pick disease

- Caused by deficiency of *sphingomyelinase.*
- Principal storage substance: sphingomyelin which accumulates in reticuloendothelial cells.
- Liver and spleen enlargement, mental retardation.

B. Gaucher's disease

- Caused by a deficiency of *lysosomal glucocerebrosidase.*
- Increase content of glucocerebroside in the spleen and liver.
- Erosion of long bones and pelvis.
- Enzyme replacement therapy is available for the Type I disease (Imiglucerase or Cerezyme).
    - Also miglustat (Zavesca) – an oral drug which inhibits the enzyme glucosylceramide synthase, an essential enzyme for the synthesis of most glycosphingolipids.

C. Krabbe's disease

- Also known as *globoid leukodystrophy.*
- Caused by a deficiency in the *lysosomal enzyme galactocerebrosidase*
- Increased amount of galactocerebroside in the white matter of the brain.

D. Tay- sachs disease

- A fatal disease which is due to the deficiency of *hexosaminidase -A* activity.
- Accumulation of ganglioside GM2 in the brain of infants.
- Mental retardation, blindness, inability to swallow.
- A "cherry red " spot develops on the macula (back of the the eyes).
- Tay-Sachs children usually die by age 5 and often sooner.

E. Fabry's disease

- Caused by deficient in lysosomal α-galactosidase.
- Accumulation of ceramide trihexoside in kidneys of these patients.
- Sometimes referred to as ceramide trihexosidase.
- Skin rash, kidney failure, pains in the lower extremities.
- Now treated with enzyme replacement therapy:
    - agalsidase beta (Fabrazyme).

3) Disorders associated with Lipoprotein Metabolism

A. Hyper-Lipoproteinaemias :-

a) Type-I: familial lipoprotein lipase deficiency.

- Rare disorder characterized by hypertriglyceridaemia & hyperchylomicronaemia.
- VLDL(Pre-beta Lipoproteins) also increased.
- Alpha Lipoprotein(HDL) & Beta-Lipoproteins(LDL) is decreased.
- Inheritance:- Autosomal recessive.
- Enzyme deficiency:- "Lipoprotein lipase"

b) Type-II: familial hypercholesterolaemia.

- Common disorder.
- Characterized by:-
    - Increased Total Cholesterol & HDL.
    - May be high TG & VLDL.
- Inheritance:- Autosomal dominant
- Frequency:- 1:500(0.2%)
- Metabolic defect:- No enzyme deficiency but defect of LDL receptors.
- Clinical features:-Atherosclerosis, CAD,Corneal arcus & Tuberous xanthoma.
- Management:-
    - Low cholesterol diet decreased intake of saturated fat.
    - Give PUFA & drug like statins.

c) Type-III: familial dys-beta Lipoproteinaemia.

- Synonyms:- ***Broad beta disease & Remnant removal disease.***
- Characterised by:-
    - Increased LDL & VLDL.
    - Rise in IDL
    - Hypercholesterolemia & hypertriglyceridaemia
- Inheritance:- Autosomal dominant
- Frequency:- 1:5000(0.02%)
- Metabolic defect:-
    - Increased apo-E & apo-B
    - Conversion of normal VLDL to IDL & its degradation without conversion of LDL.
    - Defect is in “Remnant” metabolism

d) Type-IV: familial hypertriglyceridaemia.

- Characterised by:-
    - Increased TG & VLDL.
    - Cholesterol may be normal or increased.

- Decreased HDL & LDL.

- Inheritance:- Autosomal dominant
- Metabolic defects:-over production of VLDL & Apo-CII
- Clinical features:- Associated with diabetes mellitus, IHD & Obesity.
- Management:-
  - Reduction of weight.
  - Restriction of Carbohydrate & chol.
  - Hypolipidaemic drugs.

e) Type-V: Combined hyperlipidaemias.

- Hypercholesterolemia & hypertriglyceridaemia.
- Decreased HDL & LDL.
- Inheritance:- Autosomal dominant
- Metabolic defects:-Secondary to other causes
- Clinical features:-
  - Manifested only in adulthood.
  - Xanthomas.
  - Abnormal glucose tolerance.
  - Frequency Associated with diabetes mellitus & Obesity.
- Management:-
  - Reduction of weight.
  - High PUFA intake & Hypocholipidemic drugs.

B. Hypolipo-proteinaemias:-

a) A-beta- Lipoproteinaemia.

- Rare inherited disorder.
- Characterized by:-
  - Decreased plasma cholesterol due to absence of LDL.
  - Low TG.
  - No Chylomicrons & VLDL formed.
- Clinical features:-

- Malabsorption.
- Mental & physical retardation.
- Acanthocytosis.

- Metabolic defect:- Defect in *"Synthesis of apo-B"* leading to gross deficiency of apo-B resulting to deficiency of lipoproteins containing apo-B i.e mainly Chylomicrons, VLDL & LDL.

b) Familial alpha-Lipoprotein deficiency (Tangier's disease).

- Also called Tangier's disease
- Characterized by:-
  - Deficiency of HDL.
- In homozygous patients plasma HDL may be nearly completed absent.
- Inheritance:- Autosomal recessive
- Metabolic defect:-
  - Reduction in apo-AI & apo-AII
  - Leading to accumulation of cholesteryl esters in diff. tissues.
- Clinical features-
  - Increased risk of CAD.
  - Adenoids.

Disorders of Protein Metabolism:

Protein contains carbon, hydrogen, oxygen and nitrogen as the major components while sulfur and phosphorus are minor components. Nitrogen is characteristics of proteins. On an average, the nitrogen content of ordinary proteins is 16% by weight. All proteins are polymers of amino acids. Amino acids are linked by protein. A moderately active man consuming about 300g carbohydrates, 100g fats and 100g proteins daily must excrete about 16.5g of nitrogen daily. 95% is eliminated by the kidneys and the remaining 5% for the most part as nitrogen in the feces.

Inborn errors of urea cycle :- These are divided in to five types.

1.Hyperammonemia type-I

A familiar disorder, enzyme deficiency carbamoyl phosphate synthase 1, produces Hyperammonemia and symptoms of ammonia toxicity.

2.Hyperammonemia type-II

X-linked inheritance. Enzyme deficiency ornithine transcarbamoylase, produces Hyperammonemia and symptoms of ammonia toxicity.

3. Citrullinemia.

It is an autosomal recessive disorder.

• Enzyme deficiency is Argininosuccinate synthatase.

• Clinically :- Presents with, produces Hyperammonemia and symptoms of ammonia toxicity, and mental retardation.

• Urine:- large quantities of citrulline are excreted in urine (1-2g/dl).

• Feeding arginine in the patients enhance citrulline excretion.

4. Argininosuccinic aciduria.

- Autosomal recessive disorder.
  - Enzyme deficiency Argininosuccinase.

- Clinically :- Hyperammonemia, ammonia toxicity and mental retardation.
- The enzyme deficiency has been identified in brain, liver, kidney and RBC.

5.Hyper argininemia.

• Enzyme deficiency is Arginase.

• Defect in liver and RBC.
• Clinically:- Hyperammonemia.
• Urine :- increased urinary excretion of lysine, cystine, ornithine and Arginine.
• Low protein diet result in lowering of plasma ammonia levels and disappearance of urinary lysinecystinuria pattern.

Disorders of Aromatic amino acids { Phe, Tyr & Trp } :-

1.Phenylketonuria.

• Deficiency of the enzyme phenylalanine hydroxylase.

• In some patients dihydrobiopterin reductase deficiency, neurological symptoms appear.

• Frequency is 1 in 10,000 births.

• Introduction of better diagnostic facilities showed that the incidence is as high as 1 in 1,500 births (WHO-2003).

Biochemical abnormalities :-

- Phenyl alanine could not be converted to tyrosine.
- So phenylalanine accumulates in blood.
- So alternate minor pathways are opened, phenyl ketone, phenyl lactate, phenyl acetate are excreted in urine.

• Clinical conditions :-

- Mental retardation
- Failure to walk/talk.
- Failure of growth.

• This maybe because phenyalanine interferes with neurotransmitter synthesis.

• The child often has hypopigmentation explained by the inhibition of tyrosinase.

• Phenyllactic acid in sweet may lead to moucy body odur.

2.Alkaptonuria.

Alkaptonuria is an autosomal recessive condition with an incidence of 1 in 2,50,000 births.

• The metabolic defect is the deficiency of homogentisate oxidase. This results in excretion of homogentisic acid in urine.

• Homogentisic acid 4-maleyl aceto acetate

• The only abnormality is the blackening of urine on standing.

• The homogentsic acid is oxidised by polyphenyl oxidase to bezoquinine acetate.

• It is then polymerized to black colored alkapton bodies.

• Black pigments are deposited over the connective tissue including joint cavities to produce arthritis.

• No specific treatment is required.

• But low protein with phenylalanine less than 500mg/day.

3.Tyrosinemia.

• It is due to deficiency of phenylacetoacetate hydrolase.

• Symptoms :- the first six months of life and death occurs rapidly.

• Cabbage like odour and hypoglycemia are seen.

• Urine contains tyrosine, p-hydroxy phenyl pyruvic acid and phenyl lactic acid; and serum shows tyrosine and methionine.

4.Albinism.

• It is an autosomal recessive disease with an incidence of 1 in 20,000 births.

• Defect is tyrosinase enzyme leads complete absence of melanin synthesis.

• The ocular fundus is hypopigmented and iris may be grey or red. They will be associated photophobia and decreased visual acuity.

• The skin has low pigmentation and so skin is sensitive to UV rays.

• The hair is also white.

• Tyrosine DOPA

5.Hartnup's disease.

- It is a hereditary disorder of tryptophan metabolism the clinical symptoms include dermatitis and ataxia.
- The pellagra like symptoms are due to the deficiency of niacin derived from tryptophan.
- The diagnosis is based on aminoaciduria and increased excretion of indole compounds detected by the *Obermeyer test.*
- Hartnup's is characterized by low plasma level of tryptophan and other neutral amino acids and their elevated urinary excretion.

Maple Syrup urine disease:

- The urine of effected individuals smells like maple syrup or burnt sugar.
- Enzyme defect is *α-keto acid dehydrogenase,* which causes a blockade in conversion of α-keto acid to the respective acyl CoA thioesters.
- Elevated levels of branched aa & their ketoacids in plasma & urine, so known as branched chain ketonuria.

Disorders of nucleotide metabolism:

- NUCLEIC ACID METABOLISM is the process by which nucleic acids are synthesized and degraded.
- Nucleic acids are polymers of nucleotides
- Nucleotides can be separated into purines and pyrimidines.
- Nucleotides metabolic pathways involved in the biosynthesis, catabolism, salvage are may

subjected to different kind of disturbances- this may lead to different type of disorders.

Disorders are classified as:

- Purine metabolism disorder
- Pyrimidine metabolism disorders

Purine metabolism disorders

1. Hyperuricemia
   - Hyperuricemia is an excess of uric acid in blood.
   - Normal uric acid level are

   Female = 2.4-6.0 mg/dL

   Male = 3.4-7.0 mg/dL

   (normal value will vary from laboratory to laboratory)
   - Uric acid is the end product of purine metabolism in humans.
   - Human beings have high levels of uric acid because of deficiency of hepatic enzyme uricase
   - Approximately 2/3 rd of the total body urate is produced endogenously, while remaining is accounted by dietary purines
   - Purine breaks down into uric acid
   - Increased levels of uric acid from excess purines may accumulate in tissues and form crystals
2. Hypouricemia
   - Hypouricemia is arbitrarily defined as a serum urate concentration of less than 2 mg/mL
   - It occurs in about 2% of hospitalized patients & less than 0.5% of the normal population.

Causes :-

- Decreased uric acid production
- Uric acid oxidation due to the treatment with uricase
- Decreased renal tubular reabsorption due to inherited or acquired disorders in nucleic acid metabolism.

3. Gout

• Also known as monosodium urate crystal deposition disease or podagra

• It is characterized biochemically by extracellular fluid urate saturation, which is reflected in blood by hyperuricemia, which serum urate concentration exceeding 6.8mg/mL

Clinical manifestations of gout may include

- Recurrent attacks of acute inflammatory arthritis
- A chronic arthropathy
- Accumulation of crystals in the form of tophaceous deposits
- Uric acid nephrolithiasis

3 stages of gout

- First stage

• In first stage have high uric acid level in blood

• Uric acid level may stay the same but no symptoms

• Some people may have kidney stone before having their attack of gout

- Second stage

• Uric acid crystals begin to form, usually in big toe

• Patient begin to have gout attack

• After an attack the affected joints feels normal

• Later attack may be more severe, last longer, and involves more than one joints

- Third stage

• Symptoms may never go away

• May affect more than one joints

• Gritty nodules called “tophi” may form under the skin

Causes:-

• Gout is caused by hyperuricemia

4. Lesch-Nyhan syndrome

- It is an over production of hyperuricemia
- characterized by frequent episodes of uric acid lithiasis & bizarre syndrome
- Reflect the defect in HGPRT purine salvage

5. Von Gierke disease

• It is also known as glucose-6-phospate deficiency

• Purine overproduction and hyperuricemia in Von - Gierk disease occur secondary to enhanced generation of PRPP precursor ribose-5-phosphate.

• An associated lactic acidosis elevates the renal threshold for urate, elevate total body urate.

6. Purine nucleoside deficiency

• It is associated with a severe deficiency of T cells but apparently normal B cell function

• Immune dysfunctions appers to result from the accumulation of dGTP & dATP, which inhibit ribonucleotide reductase and there by deplete cells of DNA precursors.

Pyrimidine catabolism disorders

• Orotic Acidurias

• It accompanies Reye syndrome

• Probably is a consequence of the inability of mitochondria to utilize carbonyl phosphate, which then become available to cytosolic overproduction of orotic acid.

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UNIT – V – Intermediary Metabolism – SBC3101

Integration of Metabolism

Metabolic effects of hormones (Insulin and Glucagon), The Feed / fast cycle, Diabetes mellitus, obesity. Role of minerals and vitamins in metabolism.

Four major organs play a dominant role in fuel metabolism: liver, adipose, muscle, and brain. These tissues contain unique sets of enzymes, such that each organ is specialized for the storage, use, and generation of specific fuels. These tissues do not function in isolation, but rather form part of a community in which one tissue may provide substrates to another, or process compounds produced by other organs. Communication between tissues is mediated by the nervous system, by the availability of circulating substrates, and by variation in the levels of plasma hormones. The integration of energy metabolism is controlled primarily by the actions of two peptide hormones: insulin and glucagon, with the catecholamines epinephrine and norepinephrine playing a supporting role. Changes in the circulating levels of these hormones allow the body to store energy when food is available in abundance, or to make stored energy available, for example, during "survival crises," such as famine, severe injury, and "fight-or-flight" situations. This chapter describes the structure, secretion, and metabolic effects of the two hormones that most profoundly affect energy metabolism.

Metabolic effects of hormones:

The liver plays a major role in glucose homeostasis in the organism. If glucose deficiency arises, the liver releases glucose into the blood, and when blood sugar levels are high, it takes glucose up from the blood and converts it into different metabolites. Several hormones from both groups are involved in controlling these processes. **Glycogen** is the form in which glucose is stored in the liver and muscles. The rate of glycogen synthesis is determined by *glycogen synthase*, while its breakdown is catalyzed by *glycogen phosphorylase*.

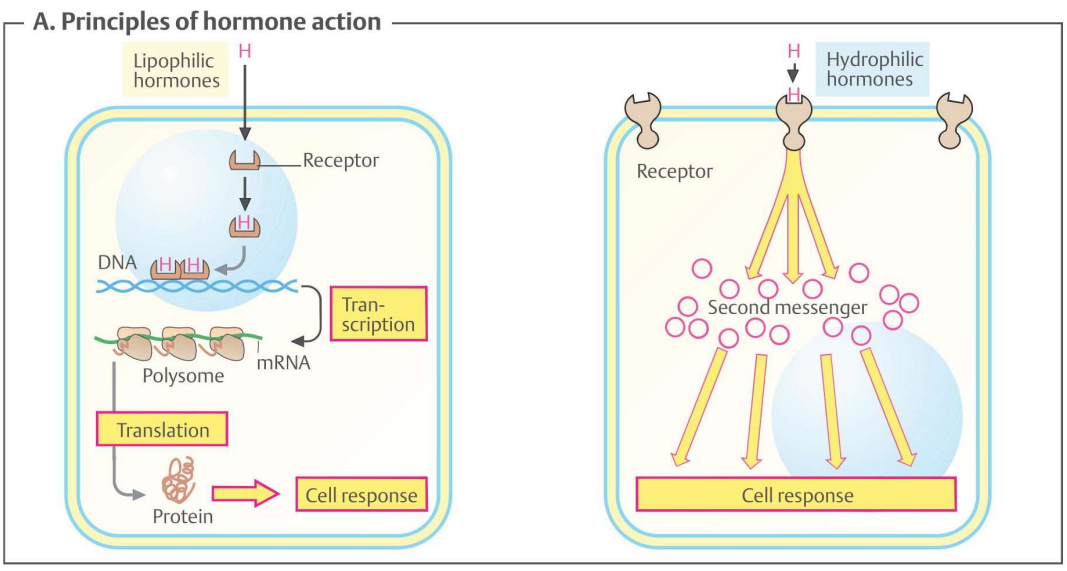
Regulation by interconversion: . If the blood glucose level falls, the peptide hormone **glucagon** is released. This activates glycogen breakdown, releasing glucose, and at the same time inhibits glycogen synthesis. Glucagon binds to receptors in the plasma membrane and, with mediation by a G-protein, activates the enzyme *adenylate cyclase*, which forms the *second messenger* 3,5_-cyclo-AMP (**cAMP**) from ATP. cAMP binds to another enzyme, *protein kinase A* (PK-A), and activates it. PK-A has several points of attack. Through *phosphorylation,*

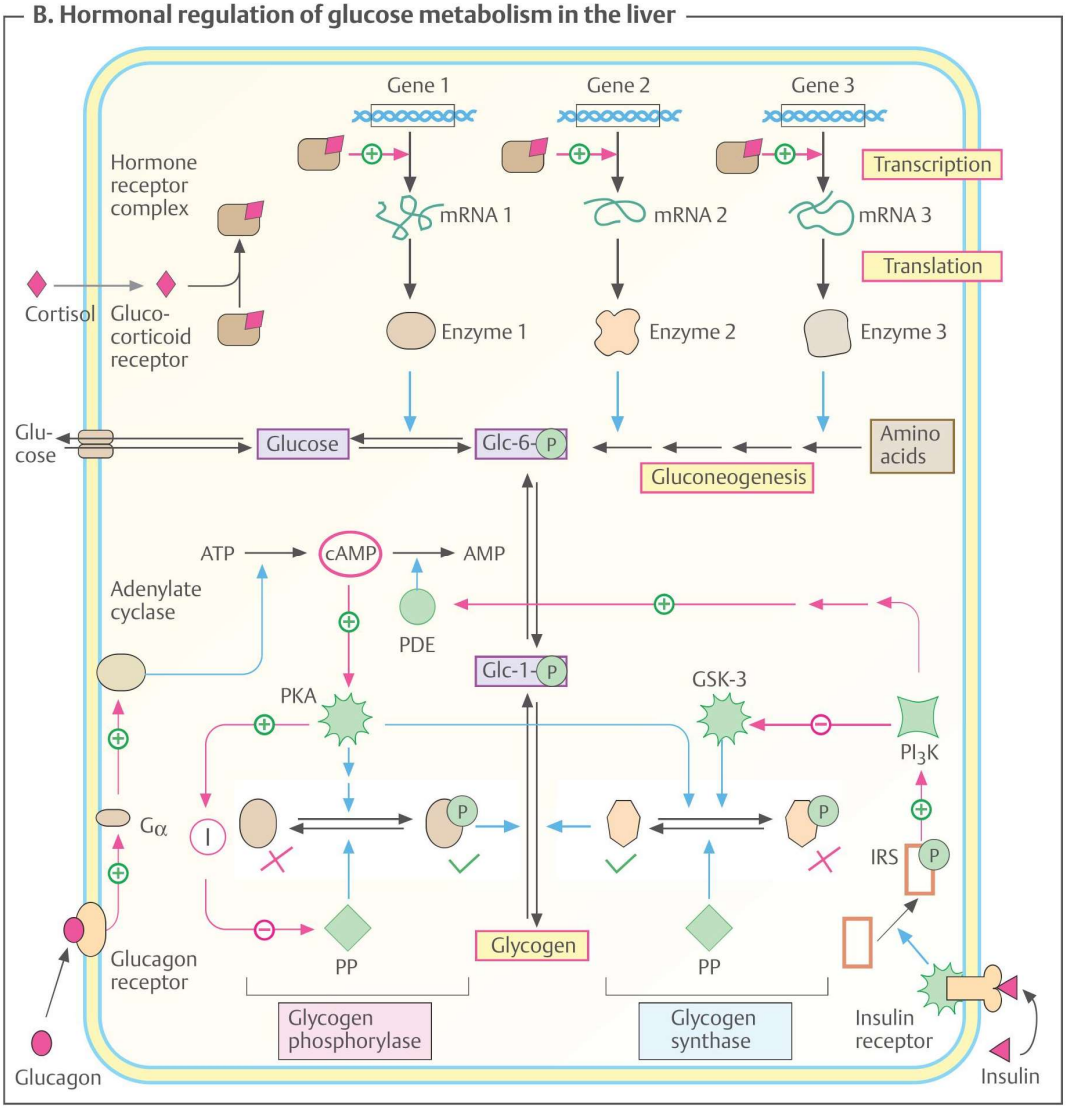
it converts the active form of *glycogen synthase* into the inactive form, thereby terminating the synthesis of glycogen.

Secondly, it activates another protein kinase, which ultimately converts the inactive form of *glycogen phosphorylase* into the active form through phosphorylation. The active phosphorylase releases glucose 1-phosphate from glycogen, which after conversion into glucose 6-phosphate supplies free glucose. In addition, via an inhibitor (I) of protein phosphatase (PP), active PK-A inhibits inactivation of glycogenphosphorylase. When the cAMP level falls again, *phosphoprotein phosphatases* become active, which dephosphorylate the various phosphoproteins in the cascade described, and thereby arrest glycogen breakdown and re-start glycogen synthesis.

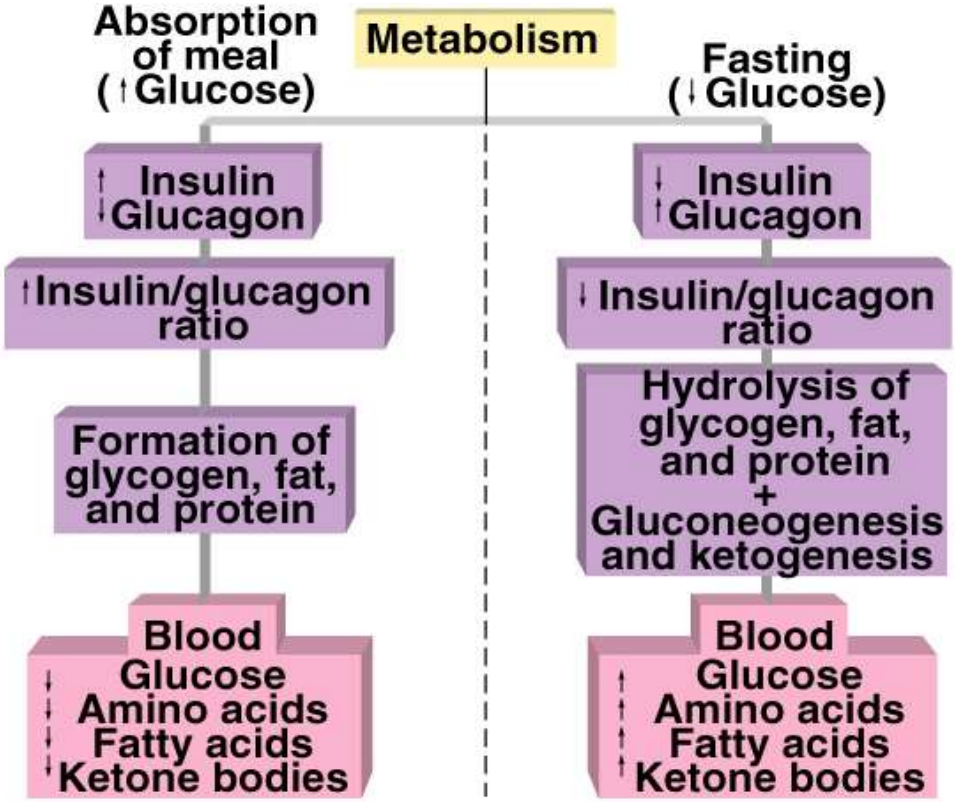
Activation and inactivation of proteins through phosphorylation or dephosphorylation is referred to as **interconversion**. In contrast to glucagon, the peptide hormone **insulin** increases glycogen synthesis and inhibits glycogen breakdown. Via several intermediates, it inhibits protein kinase GSK-3 and thereby prevents inactivation of glycogen synthase. In addition, insulin reduces the cAMP level by activating *cAMP phosphodiesterase* (PDE).

Regulation by transcriptional control : If the liver's glycogen reserves have been exhausted, the steroid hormone **cortisol** maintains glucose release by initiating the conversion of amino acids into glucose (*gluconeogenesis*). In the cell nucleus, the complex of cortisol and its receptor binds to the promoter regions of various key enzymes of gluconeogenesis and leads to their transcription. The active enzymes are produced through translation of the mRNA formed.


A. Principles of hormone action
Lipophilic hormones
H
Receptor
DNA
Tran-scription
mRNA
Polysome
Translation
Protein
Cell response
Hydrophilic hormones
Receptor
Second messenger
Cell response


B. Hormonal regulation of glucose metabolism in the liver
Gene 1
Gene 2
Gene 3
Transcription
Translation
Hormone receptor complex
mRNA 1
mRNA 2
mRNA 3
Cortisol
Gluco-corticoid receptor
Enzyme 1
Enzyme 2
Enzyme 3
Glu-cose
Glucose
Glc-6-P
Amino acids
Gluconeogenesis
ATP
cAMP
AMP
Adenylate cyclase
PDE
Glc-1-P
PKA
GSK-3
PI3K
Gα
I
P
IRS
Glucagon receptor
PP
Glycogen
Glycogen phosphorylase
Glycogen synthase
Insulin receptor
Glucagon
Insulin

Feed / Fast cycle:



Enzymic Changes in the Fed State

The flow of intermediates through metabolic pathways is controlled by four mechanisms:

1) the availability of substrates
2) allosteric regulation of enzymes
3) covalent modification of enzymes
4) induction-repression of enzyme synthesis.

Each mechanism operates on a different timescale and allows the body to adapt to a wide variety of physiologic situations. In the fed state, these regulatory mechanisms ensure that available nutrients are captured as glycogen, TAG, and protein.

1. Allosteric changes usually involve rate-determining reactions. For example,

- glycolysis in the liver is stimulated following a meal by an increase in fructose 2,6-bisphosphate—an allosteric activator of phosphofructokinase-1.

Gluconeogenesis is inhibited by fructose 2,6-bisphosphate, an inhibitor of fructose 1,6-bisphosphatase.

2. Regulation of enzymes by covalent modification

- Many enzymes are regulated by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme.
- In the fed state, most of the enzymes regulated by these covalent modifications are in the dephosphorylated form and are active.
- Three exceptions are:
  - glycogen phosphorylase kinase
  - glycogen phosphorylase and
  - hormone-sensitive lipase of adipose tissue,

which are inactive in their dephosphorylated state.

3. Induction and repression of enzyme synthesis

- Induction or repression of protein synthesis leads to changes in the total population of active sites, rather than influencing the efficiency of existing enzyme molecules.
- Enzymes subject to regulation of synthesis are often those that are needed at only one stage of development or under selected physiologic conditions.
- For example, in the fed state:

elevated insulin levels result in an increase in the synthesis of key enzymes, such as acetyl coenzyme (CoA) carboxylase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase involved in anabolic metabolism.

Fasting state:

- Fasting may result from an inability to obtain food, from the desire to lose weight rapidly, or in clinical situations in which an individual cannot eat, for example, because of trauma, surgery, neoplasm, or burns.
- In the absence of food, plasma levels of glucose, amino acids, and TAG fall, triggering a decline in insulin secretion and an increase in glucagon release.
- The decreased insulin to glucagon ratio, and the decreased availability of circulating substrates, makes the period of nutrient deprivation a catabolic period characterized by degradation of: TAG, glycogen, and protein.
- This triggers an exchange of substrates between liver, adipose tissue, muscle, and brain that is guided by two priorities:

  1) the need to maintain adequate plasma levels of glucose to sustain energy metabolism of the brain, red blood cells, and other glucose-requiring tissues, and

  2) the need to mobilize fatty acids from adipose tissue, and the synthesis and release of ketone bodies from the liver, to supply energy to all other tissues.

Diabetes mellitus:

Diabetes mellitus is a very common metabolic disease that is caused by absolute or relative insulin deficiency. The lack of this peptide hormone mainly affects carbohydrate and lipid metabolism. Diabetes mellitus occurs in two forms. In **type 1** diabetes (insulin- dependent diabetes mellitus, IDDM), the insulin-forming cells are destroyed in young individuals by an autoimmune reaction. The less severe **type 2** diabetes (noninsulin- dependent diabetes mellitus, NIDDM) usually has its first onset in elderly individuals. The causes have not yet been explained in detail in this type.

A. Insulin biosynthesis _

Insulin is produced by the B cells of the *islets of Langerhans* in the pancreas. As is usual with secretory proteins, the hormone's precursor (*preproinsulin*) carries a signal peptide that directs the peptide chain to the interior of the endoplasmic reticulum. *Proinsulin* is produced in the ER by cleavage of the signal peptide and formation of disulfide bonds. Proinsulin passes to the Golgi apparatus, where it is packed into vesicles—the β-*granules.* After cleavage of the *C peptide*, *mature insulin* is formed in the β-granules and is stored in the form of zinc-containing hexamers until secretion.

B. Effects of insulin deficiency _

In simplified terms, they can be described as *stimulation of glucose utilization* and *inhibition of gluconeogenesis*. In addition, the transport of glucose from the blood into most tissues is also insulin-dependent (exceptions to this include the liver, CNS, and erythrocytes). The **lipid metabolism** of adipose tissue is also influenced by the hormone. In these cells, insulin stimulates the reorganization of glucose into fatty acids. This is mainly based on activation of *acetyl CoA carboxylase* and increased availability of NADPH+H+ due to increased PPP activity. On the other hand, insulin also inhibits the degradation of fat by hormone sensitive lipases and prevents the breakdown of muscle protein. Particularly noticeable is the increase in the glucose concentration in the blood, from 5 mM to 9 mM (90 mg dL–1) or more (**hyperglycemia,** elevated blood glucose level). In *muscle* and *adipose tissue* – the two most important glucose consumers—glucose uptake and glucose utilization are impaired by insulin deficiency. Glucose utilization in the *liver* is also reduced. At the same time, gluconeogenesis is stimulated, partly due to increased proteolysis in the muscles. This increases the blood sugar level still further. When the capacity of the *kidneys* to resorb glucose is exceeded (at plasma concentrations of 9 mM or more), glucose is excreted in the urine (**glucosuria**).

The increased degradation of fat that occurs in insulin deficiency also has serious effects. Some of the fatty acids that accumulate in large quantities are taken up by the liver and used for lipoprotein synthesis **(hyperlipidemia)**, and the rest are broken down into acetyl CoA. As the tricarboxylic acid cycle is not capable of taking up such large quantities of acetyl CoA, the excess is used to form **ketone bodies** (*acetoacetate* and *hydroxybutyrate)*. As H+ ions are released in this process, diabetics not receiving adequate treatment can suffer severe **metabolic acidosis** (diabetic coma). The *acetone* that is also formed gives these patients' breath a characteristic odor. In addition, large amounts of ketone body anions appear in the urine (**ketonuria**).

Diabetes mellitus can have serious secondary effects. A constantly raised blood sugar level can lead in the long term to changes in the blood vessels (diabetic angiopathy), kidney damage (nephropathy) and damage to the nervous system (neuropathy), as well as to cataracts in the eyes.

Obesity:

Obesity is a complex disease involving an excessive amount of body fat. Obesity isn't just a cosmetic concern. It is a medical problem that increases your risk of other diseases and health problems, such as heart disease, diabetes, high blood pressure and certain cancers.

There are many reasons why some people have difficulty avoiding obesity. Usually, obesity results from a combination of inherited factors, combined with the environment and personal diet and exercise choices.

The good news is that even modest weight loss can improve or prevent the health problems associated with obesity. Dietary changes, increased physical activity and behavior changes can help you lose weight. Prescription medications and weight-loss procedures are additional options for treating obesity.

Symptoms

Obesity is diagnosed when your body mass index (BMI) is 30 or higher. To determine your body mass index, divide your weight in pounds by your height in inches squared and multiply by 703. Or divide your weight in kilograms by your height in meters squared.

BMI	Weight status
Below 18.5	Underweight
18.5-24.9	Normal
25.0-29.9	Overweight
30.0 and higher	Obesity

For most people, BMI provides a reasonable estimate of body fat. However, BMI doesn't directly measure body fat, so some people, such as muscular athletes, may have a BMI in the obesity category even though they don't have excess body fat.

Causes

Although there are genetic, behavioral, metabolic and hormonal influences on body weight, obesity occurs when you take in more calories than you burn through exercise and normal daily activities. Your body stores these excess calories as fat.

Most Americans' diets are too high in calories — often from fast food and high-calorie beverages. People with obesity might eat more calories before feeling full, feel hungry sooner, or eat more due to stress or anxiety.

Risk factors

Obesity usually results from a combination of causes and contributing factors:

Family inheritance and influences

The genes you inherit from your parents may affect the amount of body fat you store, and where that fat is distributed. Genetics may also play a role in how efficiently your body converts food into energy, how your body regulates your appetite and how your body burns calories during exercise.

Obesity tends to run in families. That's not just because of the genes they share. Family members also tend to share similar eating and activity habits.

Lifestyle choices

- **Unhealthy diet.** A diet that's high in calories, lacking in fruits and vegetables, full of fast food, and laden with high-calorie beverages and oversized portions contributes to weight gain.
- **Liquid calories.** People can drink many calories without feeling full, especially calories from alcohol. Other high-calorie beverages, such as sugared soft drinks, can contribute to significant weight gain.
- **Inactivity.** If you have a sedentary lifestyle, you can easily take in more calories every day than you burn through exercise and routine daily activities. Looking at computer, tablet and phone screens is a sedentary activity. The number of hours you spend in front of a screen is highly associated with weight gain.

Certain diseases and medications

In some people, obesity can be traced to a medical cause, such as Prader-Willi syndrome, Cushing syndrome and other conditions. Medical problems, such as arthritis, also can lead to decreased activity, which may result in weight gain.

Some medications can lead to weight gain if you don't compensate through diet or activity. These medications include some antidepressants, anti-seizure medications, diabetes medications, antipsychotic medications, steroids and beta blockers.

Social and economic issues

Social and economic factors are linked to obesity. Avoiding obesity is difficult if you don't have safe areas to walk or exercise. Similarly, you may not have been taught healthy ways of cooking, or you may not have access to healthier foods. In addition, the people you spend time with may influence your weight — you're more likely to develop obesity if you have friends or relatives with obesity.

Age

Obesity can occur at any age, even in young children. But as you age, hormonal changes and a less active lifestyle increase your risk of obesity. In addition, the amount of muscle in your body tends to decrease with age. Generally, lower muscle mass leads to a decrease in metabolism. These changes also reduce calorie needs, and can make it harder to keep off excess weight. If you don't consciously control what you eat and become more physically active as you age, you'll likely gain weight.

Other factors

- **Pregnancy.** Weight gain is common during pregnancy. Some women find this weight difficult to lose after the baby is born. This weight gain may contribute to the development of obesity in women. Breast-feeding may be the best option to lose the weight gained during pregnancy.
- **Quitting smoking.** Quitting smoking is often associated with weight gain. And for some, it can lead to enough weight gain to qualify as obesity. Often, this happens as people use food to cope with smoking withdrawal. In the long run, however, quitting smoking is still a greater benefit to your health than is continuing to smoke. Your doctor can help you prevent weight gain after quitting smoking.
- **Lack of sleep.** Not getting enough sleep or getting too much sleep can cause changes in hormones that increase your appetite. You may also crave foods high in calories and carbohydrates, which can contribute to weight gain.

- **Stress.** Many external factors that affect your mood and well-being may contribute to obesity. People often seek more high-calorie food when experiencing stressful situations.
- **Microbiome.** Your gut bacteria are affected by what you eat and may contribute to weight gain or difficulty losing weight.
- **Previous attempts to lose weight.** Previous attempts of weight loss followed by rapid weight regain may contribute to further weight gain. This phenomenon, sometimes called yo-yo dieting, can slow your metabolism.

Even if you have one or more of these risk factors, it doesn't mean that you're destined to develop obesity. You can counteract most risk factors through diet, physical activity and exercise, and behavior changes.

Complications

People with obesity are more likely to develop a number of potentially serious health problems, including:

- **Heart disease and strokes.** Obesity makes you more likely to have high blood pressure and abnormal cholesterol levels, which are risk factors for heart disease and strokes.
- **Type 2 diabetes.** Obesity can affect the way your body uses insulin to control blood sugar levels. This raises your risk of insulin resistance and diabetes.
- **Certain cancers.** Obesity may increase your risk of cancer of the uterus, cervix, endometrium, ovary, breast, colon, rectum, esophagus, liver, gallbladder, pancreas, kidney and prostate.
- **Digestive problems.** Obesity increases the likelihood that you'll develop heartburn, gallbladder disease and liver problems.
- **Gynecological and sexual problems.** Obesity may cause infertility and irregular periods in women. Obesity also can cause erectile dysfunction in men.

- **Sleep apnea.** People with obesity are more likely to have sleep apnea, a potentially serious disorder in which breathing repeatedly stops and starts during sleep.
- **Osteoarthritis.** Obesity increases the stress placed on weight-bearing joints, in addition to promoting inflammation within the body. These factors may lead to complications such as osteoarthritis.
- **Severe COVID-19 symptoms.** Obesity increases the risk of developing severe symptoms if you become infected with the virus that causes coronavirus disease 2019 (COVID-19). People who have severe cases of COVID-19 may require treatment in intensive care units or even mechanical assistance to breathe.

Vitamins and Minerals:

The term micronutrients is used to describe vitamins and minerals in general. Macronutrients, on the other hand, include proteins, fats and carbohydrates. Your body needs smaller amounts of micronutrients relative to macronutrients. That's why they're labeled "micro." Humans must obtain micronutrients from food since your body cannot produce vitamins and minerals — for the most part. That's why they're also referred to as essential nutrients.

Vitamins are organic compounds made by plants and animals which can be broken down by heat, acid or air. On the other hand, minerals are inorganic, exist in soil or water and cannot be broken down. When you eat, you consume the vitamins that plants and animals created or the minerals they absorbed.

The micronutrient content of each food is different, so it's best to eat a variety of foods to get enough vitamins and minerals. An adequate intake of all micronutrients is necessary for optimal health, as each vitamin and mineral has a specific role in your body. Vitamins and minerals are vital for growth, immune function, brain development and many other important functions.

Types and Functions of Micronutrients

Vitamins and minerals can be divided into four categories: water-soluble vitamins, fat-soluble vitamins, macrominerals and trace minerals. Regardless of type, vitamins and minerals are absorbed in similar ways in your body and interact in many processes.

Water-Soluble Vitamins

Most vitamins dissolve in water and are therefore known as water-soluble. They're not easily stored in your body and get flushed out with urine when consumed in excess. While each water-soluble vitamin has a unique role, their functions are related. For example, most B vitamins act as coenzymes that help trigger important chemical reactions. A lot of these reactions are necessary for energy production.

The water-soluble vitamins — with some of their functions — are:

- **Vitamin B1 (thiamine):** Helps convert nutrients into energy
- **Vitamin B2 (riboflavin):** Necessary for energy production, cell function and fat metabolism (
- **Vitamin B3 (niacin):** Drives the production of energy from food
- **Vitamin B5 (pantothenic acid):** Necessary for fatty acid synthesis
- **Vitamin B6 (pyridoxine):** Helps your body release sugar from stored carbohydrates for energy and create red blood cells
- **Vitamin B7 (biotin):** Plays a role in the metabolism of fatty acids, amino acids and glucose
- **Vitamin B9 (folate):** Important for proper cell division
- **Vitamin B12 (cobalamin):** Necessary for red blood cell formation and proper nervous system and brain function
- **Vitamin C (ascorbic acid):** Required for the creation of neurotransmitters and collagen, the main protein in your skin

As you can see, water-soluble vitamins play an important role in producing energy but also have several other functions. Since these vitamins are not stored in your body, it's important to get enough of them from food.

Sources and Recommended Dietary Allowances (RDAs) or Adequate Intakes (AIs) of water-soluble vitamins are

Nutrient	**Sources**	**RDA or AI (adults > 19 years)**
Vitamin B1 (thiamine)	Whole grains, meat, fish	1.1–1.2 mg
Vitamin B2 (riboflavin)	Organ meats, eggs, milk	1.1–1.3 mg
Vitamin B3 (niacin)	Meat, salmon, leafy greens, beans	14–16 mg
Vitamin B5 (pantothenic acid)	Organ meats, mushrooms, tuna, avocado	5 mg
Vitamin B6 (pyridoxine)	Fish, milk, carrots, potatoes	1.3 mg
Vitamin B7 (biotin)	Eggs, almonds, spinach, sweet potatoes	30 mcg
Vitamin B9 (folate)	Beef, liver, black-eyed peas, spinach, asparagus	400 mg
Vitamin B12 (cobalamin)	Clams, fish, meat	2.4 mcg
Vitamin C (ascorbic acid)	Citrus fruits, bell peppers, Brussels sprouts	75–90 mg

Fat-Soluble Vitamins

Fat-soluble vitamins do not dissolve in water. They're best absorbed when consumed alongside a source of fat. After consumption, fat-soluble vitamins are stored in your liver and fatty tissues for future use.

The names and functions of fat-soluble vitamins are:

- **Vitamin A:** Necessary for proper vision and organ function
- **Vitamin D:** Promotes proper immune function and assists in calcium absorption and bone growth
- **Vitamin E:** Assists immune function and acts as an antioxidant that protects cells from damage
- **Vitamin K:** Required for blood clotting and proper bone development

Sources and recommended intakes of fat-soluble vitamins are

Nutrient	**Sources**	**RDA or AI (adults > 19 years)**
Vitamin A	Retinol (liver, dairy, fish), carotenoids (sweet potatoes, carrots, spinach)	700–900 mcg
Vitamin D	Sunlight, fish oil, milk	600–800 IU
Vitamin E	Sunflower seeds, wheat germ, almonds	15 mg
Vitamin K	Leafy greens, soybeans, pumpkin	90–120 mcg

Macrominerals

Macrominerals are needed in larger amounts than trace minerals in order to perform their specific roles in your body.

The macrominerals and some of their functions are:

- **Calcium:** Necessary for proper structure and function of bones and teeth. Assists in muscle function and blood vessel contraction
- **Phosphorus:** Part of bone and cell membrane structure
- **Magnesium:** Assists with over 300 enzyme reactions, including regulation of blood pressure
- **Sodium:** Electrolyte that aids fluid balance and maintenance of blood pressure
- **Chloride:** Often found in combination with sodium. Helps maintain fluid balance and is used to make digestive juices
- **Potassium:** Electrolyte that maintains fluid status in cells and helps with nerve transmission and muscle function
- **Sulfur:** Part of every living tissue and contained in the amino acids methionine and cysteine.

Sources and recommended intakes of the macrominerals are:

Nutrient	Sources	RDA or AI (adults > 19 years)
Calcium	Milk products, leafy greens, broccoli	2,000–2,500 mg
Phosphorus	Salmon, yogurt, turkey	700 mg
Magnesium	Almonds, cashews, black beans	310–420 mg
Sodium	Salt, processed foods, canned soup	2,300 mg

Chloride	Seaweed, salt, celery	1,800–2,300 mg
Potassium	Lentils, acorn squash, bananas	4,700 mg
Sulfur	Garlic, onions, Brussels sprouts, eggs, mineral water	None established

Trace Minerals

Trace minerals are needed in smaller amounts than macrominerals but still enable important functions in your body.

The trace minerals and some of their functions are:

- **Iron:** Helps provide oxygen to muscles and assists in the creation of certain hormones
- **Manganese:** Assists in carbohydrate, amino acid and cholesterol metabolism
- **Copper:** Required for connective tissue formation, as well as normal brain and nervous system function
- **Zinc:** Necessary for normal growth, immune function and wound healing
- **Iodine:** Assists in thyroid regulation
- **Fluoride:** Necessary for the development of bones and teeth
- **Selenium:** Important for thyroid health, reproduction and defense against oxidative damage

Sources and recommended intakes of trace minerals are:

Nutrient	**Sources**	**RDA or AI (adults > 19 years)**
Iron	Oysters, white beans, spinach	8–18 mg

Manganese	Pineapple, pecans, peanuts	1.8–2.3 mg
Copper	Liver, crabs, cashews	900 mcg
Zinc	Oysters, crab, chickpeas	8–11 mg
Iodine	Seaweed, cod, yogurt	150 mcg
Fluoride	Fruit juice, water, crab	3–4 mg
Selenium	Brazil nuts, sardines, ham	55 mcg

Health Benefits of Micronutrients

All micronutrients are extremely important for the proper functioning of your body. Consuming an adequate amount of the different vitamins and minerals is key to optimal health and may even help fight disease. This is because micronutrients are part of nearly every process in your body. Moreover, certain vitamins and minerals can act as antioxidants.

Antioxidants may protect against cell damage that has been associated with certain diseases, including cancer, Alzheimer's and heart disease. For example, research has linked an adequate dietary intake of vitamins A and C with a lower risk of some types of cancer.

Getting enough of some vitamins may also help prevent Alzheimer's disease. A review of seven studies found that adequate dietary intake of vitamins E, C and A is associated with a 24%, 17% and 12% reduced risk of developing Alzheimer's, respectively. Certain minerals may also play a role in preventing and fighting disease.

Research has linked low blood levels of selenium to a higher risk of heart disease. A review of observational studies found that the risk of heart disease decreased by 24% when blood concentrations of selenium increased by 50%. Additionally, a review of 22 studies noticed that adequate calcium intake decreases the risk of death from heart disease and all other causes .

These studies suggest that consuming enough of all micronutrients — especially those with antioxidant properties — provides ample health benefits. However, it's unclear whether consuming more than the recommended amounts of certain micronutrients — either from foods or supplements — offers additional benefits.

Micronutrient Deficiencies and Toxicities

Micronutrients are needed in specific amounts to perform their unique functions in your body. Getting too much or too little of a vitamin or mineral can lead to negative side effects.

Deficiencies

Most healthy adults can get an adequate amount of micronutrients from a balanced diet, but there are some common nutrient deficiencies that affect certain populations.

These include:

- **Vitamin D:** Approximately 77% of Americans are deficient in vitamin D, mostly due to lack of sun exposure.
- **Vitamin B12:** Vegans and vegetarians may develop vitamin B12 deficiency from refraining from animal products. Elderly individuals are also at risk due to decreased absorption with age .
- **Vitamin A:** The diets of women and children in developing countries often lack adequate vitamin A.
- **Iron:** Deficiency of this mineral is common among preschool children, menstruating women and vegans.
- **Calcium:** Close to 22% and 10% of men and women over 50, respectively, don't get enough calcium.

The signs, symptoms and long-term effects of these deficiencies depend on each nutrient but can be detrimental to the proper functioning of your body and optimal health.

Toxicities

Micronutrient toxicities are less common than deficiencies.

They are most likely to occur with large doses of the fat-soluble vitamins A, D, E and K since these nutrients can be stored in your liver and fatty tissues. They cannot be excreted from your body like water-soluble vitamins. A micronutrient toxicity usually develops from supplementing with excess amounts — rarely from food sources. Signs and symptoms of toxicity vary depending on the nutrient.

It's important to note that excessive consumption of certain nutrients can still be dangerous even if it does not lead to overt toxicity symptoms.One study examined over 18,000 people with a high risk of lung cancer due to past smoking or asbestos exposure. The intervention group received two types of vitamin A — 30 mg of beta-carotene and 25,000 IU of retinyl palmitate a day.

The trial was halted ahead of schedule when the intervention group showed 28% more cases of lung cancer and a 17% greater incidence of death over 11 years compared to the control group.

Micronutrient Supplements

The safest and most effective way to get adequate vitamin and mineral intake appears to be from food sources. More research is needed to fully understand the long-term effects of toxicities and supplements. However, people at risk of specific nutrient deficiencies may benefit from taking supplements under the supervision of a doctor.