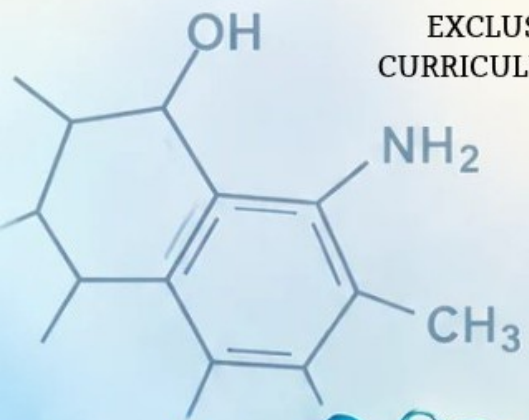
The Textbook of
BIOCHEMISTRY

EXCLUSIVELY DESIGNED AS PER NEP 2020
CURRICULUM, NEW SYLLABUS BY PCI NEW DELHI



Dr. Jiten Mishra
Dr. T.Deborah Paripuranam
Dr. Jaganmai G
Dr. Sanjeev Kumar
Mrs. Hemlata Mahant
Dr. Shakeel Ahmed Adhoni

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PREFACE

Pharmaceutical Biochemistry: Principles, Metabolism and Clinical Applications has been developed to provide pharmacy students with a clear, systematic, and comprehensive understanding of fundamental biochemical concepts and their relevance to pharmaceutical and clinical sciences. The book has been structured in accordance with the academic requirements of the B.Pharm curriculum and covers essential areas including enzymology, nutrition, biomolecules, bioenergetics, metabolism, and clinical biochemistry.

The contents have been presented in a simple and organised manner to facilitate effective learning and conceptual understanding. Appropriate biochemical reactions, equations, tables, illustrations, and examples have been incorporated wherever necessary to explain important concepts and their practical significance.

We hope that this book will help students strengthen their understanding of pharmaceutical biochemistry and develop a sound foundation for advanced study. It is intended to serve as a useful academic resource for B.Pharm students as well as for learners seeking a better understanding of the biochemical basis of pharmaceutical and clinical sciences.

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ACKNOWLEDGEMENT

We express our sincere gratitude to all those who have contributed, directly or indirectly, to the successful completion of this book, *Pharmaceutical Biochemistry: Principles, Metabolism and Clinical Applications*. We are especially grateful to our respected teachers, academic colleagues, and subject experts for their valuable guidance, thoughtful suggestions, and continuous encouragement throughout the preparation of this work.

We extend our sincere appreciation to the faculty members and students of Pharmacy, whose academic requirements and learning perspectives inspired us to present the concepts of biochemistry, enzymology, metabolism, nutrition, and clinical biochemistry in a clear, systematic, and application-oriented manner.

We are deeply thankful to our family members, friends, and well-wishers for their constant support, patience, and motivation during the development of this book. We also acknowledge the efforts of everyone involved in its preparation, review, editing, and publication.

It is our sincere hope that this book will serve as a useful academic resource for pharmacy students and assist them in developing a sound understanding of biochemical principles and their pharmaceutical and clinical applications.

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Unit 1…

ENZYMOLOGY, NUTRITION AND CLINICAL BIOCHEMISTRY

The study of enzymes is a vital area within biochemistry that encompasses the structure, properties, classification, mechanism, kinetics and regulation of enzymes. Since enzymes control biochemical reactions taking place in the body, they are very important in relation to both physiological processes and diseases. Sound knowledge about enzymology forms the basis of studies in the areas of metabolism, nutrition, clinical biochemistry and pharmacology. From the perspective of pharmacy, enzymes are of special significance due to their involvement in drug metabolism, use as drugs/drug targets and as biochemical indicators of organ function. Therefore, the syllabus prescribed for B.Pharm includes enzyme properties, nomenclature, IUB classification, enzyme kinetics, inhibitors and regulation.

1.1 INTRODUCTION TO ENZYMES

Life involves the constant conductance of thousands of chemical reactions. These include digestion of food nutrients, oxidation of glucose for energy production, synthesis of proteins and nucleic acids, breakdown of unnecessary chemicals, and replacement of cellular components. While some of these reactions are thermodynamically feasible, they will occur too slowly in living things without the help of biological catalysts. Enzymes render these reactions fast enough to keep an organism alive.

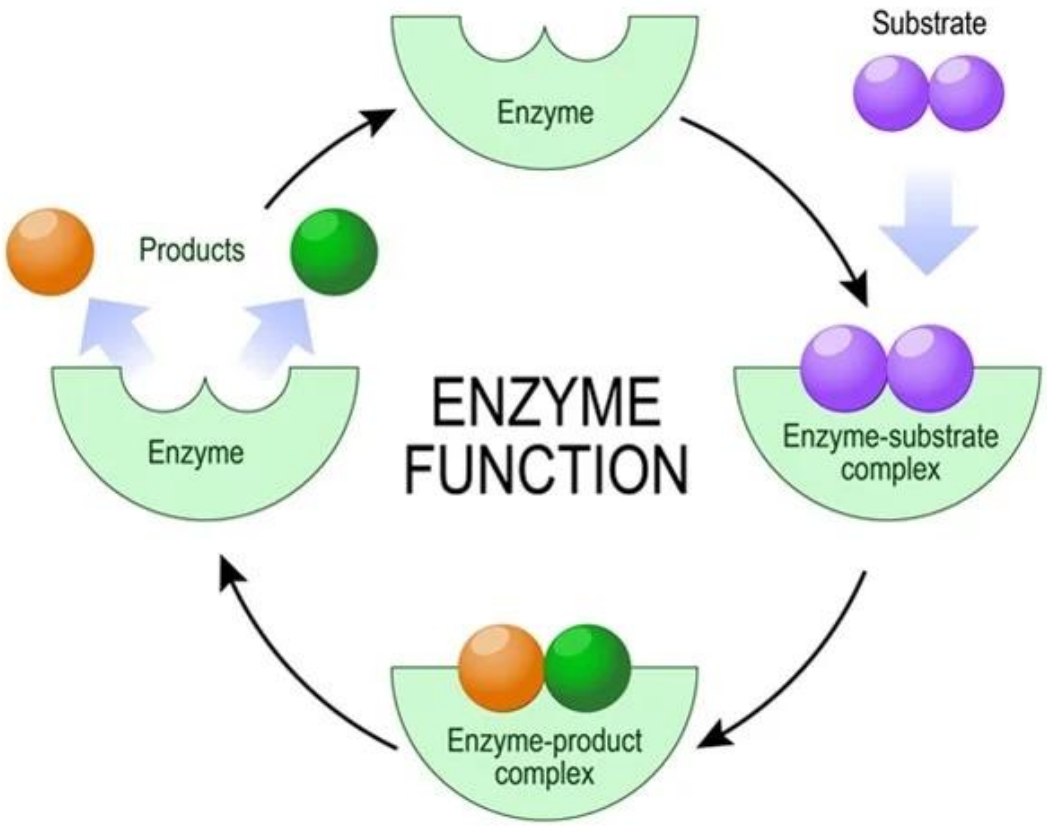


Figure 1: Mechanism of Enzyme–Substrate Interaction and Product Formation

(**Image Source**: https://www.news-medical.net/life-sciences/What-is-an-Enzyme-Cofactor.aspx)

An enzyme is the biological catalyst which speeds up biochemical reactions without itself being used up in the course of reactions. Enzymes are mainly globular proteins that have a definite three-dimensional structure. There are also some RNA molecules which act as catalysts called ribozymes.

Enzymes do not facilitate impossible reactions. Rather, they speed up possible reactions through the provision of a different pathway of reactions that has a lower energy of activation. When diagnosing purine metabolism disorders, biochemical evaluation of serum urate is helpful; however, hyperuricemia is not always indicative of clinical gout.

$$\boxed{\Delta G = G_{\text{products}} - G_{\text{reactants}}}$$

However, the change in Gibbs free energy is represented by ΔG. The speed at which equilibrium is reached is accelerated by enzymes, although neither ΔG nor the location of equilibrium are impacted.

1.1.1 Enzyme–Substrate Interaction

An enzyme reacts with a substance known as a substrate. A particular three-dimensional area where the substrate binds is known as the active site of an enzyme. This creates a complex between the enzyme and the substrate. The enzyme-substrate combination undergoes chemical reactions that change the substrate into a product. After the product has left, the enzyme is ready to start a new cycle of reactions.

The process can be represented as:

$$\boxed{E + S \rightleftharpoons ES \rightarrow E + P}.$$

The active site consists of amino acid residues, which have been arranged such that substrate binding and catalysis is facilitated. The interactions that occur between the enzyme and the substrate could include hydrogen bonding, electrostatic attraction, and hydrophobic interactions, amongst others.

Two popular theories exist for explaining how substrate binding is done. The lock and key theory states that the substrate and the active site are highly complementary. The induced fit theory states that substrate binding can alter an enzyme's conformation.

1.1.2 Components of Enzyme Systems

Every enzyme is not made of just a single protein. Some enzymes need other substances like metals and other organic compounds to become fully active.

Table 1: Important Terms and Components Associated with Enzymes

Term	Meaning	Biochemical Importance
Enzyme	Biological catalyst	Accelerates biochemical reactions
Substrate	Molecule acted upon by an enzyme	Undergoes conversion into product
Active site	Region where substrate binding and catalysis occur	Determines catalytic characteristics
Apoenzyme	Protein component requiring an additional factor for full activity	Provides the protein framework of the enzyme
Cofactor	Non-protein component required by some enzymes	Assists catalytic function

Coenzyme	Organic non-protein molecule involved in catalysis	Transfers electrons, atoms or functional groups
Prosthetic group	Tightly bound non-protein component	Remains closely associated with the enzyme
Holoenzyme	Complete catalytically active enzyme	Contains the necessary protein and additional component

The relationship between an apoenzyme and its required cofactor may be represented simply as:

$$\text{Apoenzyme} + \text{Cofactor} \rightarrow \text{Holoenzyme}$$

Enzymes do not merely serve as catalysts for individual chemical reactions. Rather, they form part of a catalytic system where cell metabolism is coordinated.

1.2 PROPERTIES OF ENZYMES

There are some specific features of enzymes that make them unique and appropriate for biological systems. These are the following: efficient catalysis, specificity, activity at physiological conditions, dependence on environment and regulation.

1.2.1 Catalytic Efficiency

Enzymes are very efficient biological catalysts. The amount of the enzyme required for catalyzing a huge amount of substrate is relatively smaller since the enzyme is reused after each reaction.

Hence, the reaction may be described as:

$$E + S \rightarrow ES \rightarrow E + P$$

The newly produced enzyme can combine with another substrate and continue the process.

1.2.2 Specificity of Enzymes

Specificity is the characteristic by which an enzyme recognizes certain substrates and catalyzes specific reactions. This trait ensures that chemical reactions do not take place in a disorderly manner and enables each metabolic pathway to function independently.

This quality of enzymes is mainly attributed to their active sites.

Table 2: Major Types of Enzyme Specificity

Type	Characteristics	Example
Absolute specificity	Enzyme acts predominantly on one substrate	Urease acts on urea
Group specificity	Enzyme acts on structurally related compounds or a particular functional group	Peptidases act on peptide-containing substrates
Bond specificity	Enzyme recognizes a particular type of bond	Lipases hydrolyse ester bonds in lipids
Stereospecificity	Enzyme distinguishes between stereoisomeric forms	L-amino acid oxidase preferentially acts on L-amino acids

Specifity is very crucial in pharmaceutical biochemistry since both endogenous compounds and drugs are susceptible to enzyme catalysis.

1.2.3 Enzymes Remain Unchanged After the Overall Reaction

Enzymes play a temporary role in catalysis but are regenerated at the end of the process. They can thus be used repeatedly in future catalysis reactions. However, enzymes can become inactive due to denaturation, breakdown, and chemical modification.

1.2.4 Effect of Temperature

In terms of enzyme activity, temperature is a major factor. Higher temperatures cause molecules to travel faster and collide more frequently, which speeds up reactions. On the other hand, if the temperature is too high, the enzyme's structural hydrogen bonds might be broken. Denature occurs when heat causes structural changes in the enzyme. Enzymes become inactive when they denature because they lose their active site.

1.2.5 Effect of pH

The efficiency of enzymes is also affected by pH. An increase or decrease in hydrogen ions affects the ionization of the amino acids responsible for binding the substrate or catalyzing the reaction. A significant change in pH may also affect the structure of the enzyme.

In general, enzymes have a certain pH at which they are most efficient.

1.2.6 Effect of Substrate Concentration

At first, the reaction velocity increases with increasing substrate concentration when the number of enzymes in the experiment remains constant. This is because an abundance of enzyme-substrate complexes can be produced when the concentration of substrate is low, as the enzyme's active sites will remain empty.

However, when there is high substrate concentration, all the active sites will already be filled up and the enzyme will get to its saturation point.

1.2.7 Effect of Enzyme Concentration

In the presence of sufficient substrate, increased enzyme concentration results in more active sites available for catalysis. Under appropriate conditions, reaction rate is proportional to enzyme concentration:

$$\boxed{v \propto [E]}$$

where $v$is reaction velocity and $[E]$represents enzyme concentration.

1.2.8 Reversibility

There are many reactions catalyzed by enzymes that are reversible. The direction at which a reversible reaction occurs is dependent on various things like the concentration of substrates and products among other thermodynamic conditions.

Reversible reaction can be represented as:

$$\boxed{A + B \rightleftharpoons C + D}$$

Enzymes catalyse reactions both forward and backward, but they do not affect the equilibrium constant.

1.2.9 Regulation

Enzymes are important points of control in metabolic processes. There are various ways to control the action of enzymes; such as changes in enzyme levels, allosteric control, covalent modification, and substrate availability. The B.Pharm course requires special emphasis on the regulation of enzyme synthesis, inhibition, and allosteric control.

1.3 NOMENCLATURE OF ENZYMES

The huge number of enzymes that exist in living organisms demands a systematic approach for naming and classification of enzymes. Initially, enzymes were given common names. Some of these names like pepsin and trypsin have been retained for their convenience.

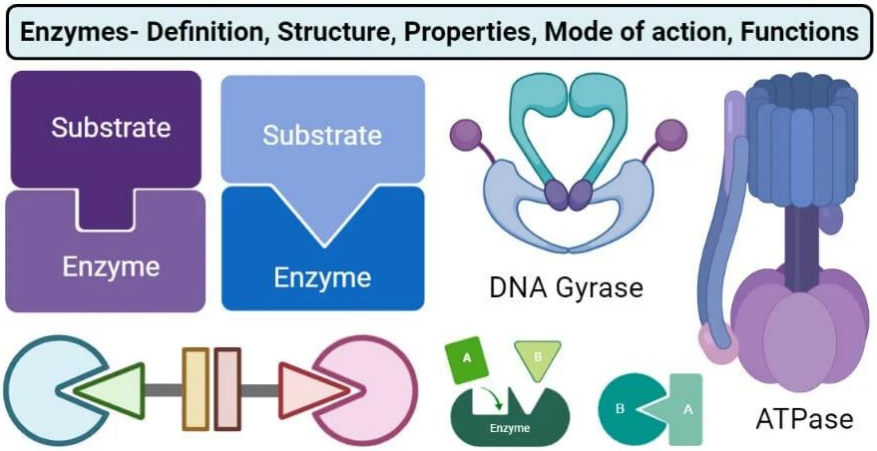


Figure 2: Structure, Substrate Interaction and Functional Diversity of Enzymes

(Image Source: https://microbenotes.com/enzymes/)

In modern times, the term used for naming enzymes always ends with the suffix '-ase'. The name usually refers to the nature of the substrate acted upon by the enzyme or the kind of reaction it catalyzes. For example, urease works on urea; lipase works on lipids, and lactase works on lactose.

Consequently, enzyme classification can be considered under traditional names, substrate names, reaction names and systematic names.

1.3.1 Substrate-Based Nomenclature

The substrate name is combined with the suffix "-ase." For example:

Urea → Urease

Lactose → Lactase

Lipid → Lipase

This method is simple but does not always provide sufficient information about the reaction being catalysed.

1.3.2 Reaction-Based Nomenclature

Other enzymes are classified based on the kind of chemical reaction they facilitate. Oxidases facilitate oxidation reactions, while dehydrogenases facilitate reduction-oxidation reactions that involve reducing agents. Kinases facilitate phosphorylation reactions, while decarboxylases remove carboxyl groups in the form of carbon dioxide.

1.3.3 Systematic Nomenclature

The use of systematic enzyme names ensures a better description of the enzymes in relation to their substrates and reactions. This is what systematic nomenclature is based on.

Table 3: Examples of Enzyme Nomenclature and Functions

Enzyme	Substrate/Reaction	Main Function
Urease	Urea	Catalyses hydrolysis of urea
Lactase	Lactose	Hydrolyses lactose
Lipase	Lipids	Catalyses lipid hydrolysis
Protease	Proteins/peptides	Hydrolyses peptide bonds
Phosphatase	Phosphorylated compounds	Removes phosphate groups by hydrolysis
Kinase	Phosphate-transfer reaction	Transfers phosphate groups, commonly from ATP
Dehydrogenase	Oxidation-reduction reaction	Transfers reducing equivalents
Decarboxylase	Carboxyl-containing substrate	Removes a carboxyl group as CO_2

1.4 IUB CLASSIFICATION OF ENZYMES

Classification of enzymes is needed since enzymes may vary in terms of substrate specificity, mechanism of reaction and their biological functions. International enzyme classification classifies enzymes mainly based on the reactions that the enzymes catalyze. The syllabus attached clearly states IUB classification of enzymes.

- An EC number may be given to any classified enzyme.
- This numerical system gives biochemical identity of the enzyme independent of its popular names.
- There are seven major classes of enzymes in modern EC classification.

1.4.1 Oxidoreductases

Oxidoreductases are enzymes that facilitate the passage of electrons or reducing agents between molecules, a process known as oxidation reduction. Dehydrogenases and oxidases are two important types of oxidoreductases.

The generalized reaction is:

$$\boxed{A_{\text{red}} + B_{\text{ox}} \rightleftharpoons A_{\text{ox}} + B_{\text{red}}}$$

1.4.2 Transferases

Functional groups can be transferred between molecules with the help of transferases. Chemical groups such as phosphates, amino acids, methyl groups, or any other type of group can be transferred in this way.

The reaction can be generalized as:

$$\boxed{A - X + B \rightarrow A + B - X}$$

Kinases and transaminases are important examples.

1.4.3 Hydrolases

Hydrolases act by breaking down bonds using water molecules. There are many digestive enzymes that fall into this category.

$$\boxed{A - B + H_2O \rightarrow A - H + B - OH}$$

Proteases, lipases and phosphatases are representative hydrolases.

1.4.4 Lyases

The enzymes lyases catalyze reactions involving cleavage and formation that do not depend on hydrolysis or oxidation and reduction. The decarboxylases are good examples of this group of enzymes.

1.4.5 Isomerases

Isomerases catalyse rearrangements within a molecule, converting one isomer into another:

$$\boxed{A \rightarrow \text{isomer of } A}$$

Racemases and epimerases are examples.

1.4.6 Ligases

Ligases facilitate the ligation of two molecules together, often accompanied by the use of energy. DNA ligase is a good example from biology.

A simplified equation is:

$$\boxed{A + B + \text{ATP} \rightarrow A - B + \text{products of ATP utilization}}$$

1.4.7 Translocases

Translocases catalyze transport of ions or molecules across membrane boundaries or their compartmentalization in the membrane. These are necessary for maintaining ionic gradients and transportation of materials between cellular organelles.

1.5 COENZYMES AND THEIR BIOLOGICAL IMPORTANCE

There are many enzymes that cannot catalyze their reactions using only the protein part of the enzyme. There are other non-protein components required. If the component required by the enzyme is an organic compound that takes part in the process of catalysis, then it is called a coenzyme.

Coenzymes normally act as carriers. In different reactions, they carry electrons, hydrogen molecules, acyl groups, amino groups or one carbon group. Coenzyme gets modified temporarily in one reaction and regenerates in another reaction. An enzyme that requires a coenzyme can be written as:

$$\boxed{\text{Apoenzyme} + \text{Coenzyme} \rightleftharpoons \text{Active enzyme system}}$$

Many coenzymes are derived from vitamins, especially those that are water-soluble, such as the B-complex vitamins. Therefore, vitamins have other biochemical roles apart from being essential nutrients. Vitamin deficiency may interfere with the efficiency of enzyme-mediated reactions and disrupt the normal metabolic processes. This subject is part of the prescribed syllabus.

Table 4: Major Coenzymes, Vitamin Sources and Functions

Coenzyme	Vitamin Relationship	Major Function	Important Metabolic Role
$NAD^+/NADP^+$	Niacin (B3)	Transfer of reducing equivalents	Oxidation-reduction metabolism
FAD/FMN	Riboflavin (B2)	Electron transfer	Oxidative metabolic reactions
Coenzyme A	Pantothenic acid (B5)	Acyl-group transfer	Fatty-acid and energy metabolism
TPP	Thiamine (B1)	Carbon-unit transfer/decarboxylation	Carbohydrate metabolism
PLP	Vitamin B6	Amino-group chemistry	Amino-acid metabolism
THF	Folate (B9)	One-carbon transfer	Nucleotide and amino-acid metabolism
Biotin	Vitamin B7	CO_2 transfer	Carboxylation reactions

1.5.1 NAD^+ and $NADP^+$

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are produced from niacin. They are crucial to redox processes because they are both electron donors and acceptors.

The equation can be written as:

$$\boxed{NAD^+ + 2e^- + H^+ \rightarrow NADH}$$

While NADPH is essential for reduction events in biosynthesis and cell defence, NAD-related activities are important in metabolic pathways that generate energy.

1.5.2 FAD and FMN

FAD and FMN are both derivatives of riboflavin. They participate in oxidation-reduction processes and are key constituents of various flavoproteins.

A reduced form can be illustrated by the following reaction:

$$\boxed{FAD + 2H \rightarrow FADH_2}$$

1.5.3 Coenzyme A

Coenzyme A (CoA) is primarily involved in the transfer of acyl groups and is produced from pantothenic acid. Among these molecules, acetyl-CoA stands out due to the crucial function it plays in metabolic processes.

1.5.4 Pyridoxal Phosphate

The physiologically active form of vitamin B6, pyridoxal phosphate (PLP), is a vital enzyme cofactor that is crucial to amino acid metabolism, especially in transamination and decarboxylation reactions.

1.5.5 Tetrahydrofolate

Tetrahydrofolate (THF) is the biologically active form of folate and functions as a key cofactor in one-carbon transfer reactions. It plays an essential role in nucleotide biosynthesis and amino acid metabolism, thereby contributing significantly to cellular growth and metabolic processes.

1.6 ENZYME KINETICS

Enzyme kinetics refers to the quantitative study of the rates of enzyme-catalyzed reactions and the factors that influence these rates. It involves evaluating reaction velocity in relation to variables such as substrate and enzyme concentrations, as well as the presence of inhibitors and other regulatory molecules.

A comprehensive understanding of enzyme kinetics is essential for elucidating enzyme function and determining how enzymes interact with different substrates under varying biochemical conditions. It also provides valuable insights into the mechanisms of enzyme inhibition and the effects of specific substances on enzymatic activity. Thus, enzyme kinetics serves as an important foundation for investigating biochemical pathways and regulating enzyme-mediated proces

In an enzyme-catalysed reaction:

$$\boxed{E + SESE + P}$$

1.6.1 Reaction Velocity

Reaction velocity refers to the rate at which substrate molecules are converted into products during an enzyme-catalyzed reaction. It can also be expressed as the rate of product formation over a specific

period of time. Thus, reaction velocity provides a quantitative measure of the progress and rate of an enzymatic reaction:

$$\boxed{v = \frac{d[P]}{dt}}$$

or the rate of substrate disappearance:

$$\boxed{v = -\frac{d[S]}{dt}}$$

Initial velocity (v_0) is commonly measured before substantial substrate depletion or product accumulation occurs.

1.6.2 Effect of Substrate Concentration on Velocity

A significant percentage of the enzyme's active sites are left empty at low substrate concentrations. Reaction velocity rises in proportion to the frequency of enzyme–substrate complex formation, which increases with substrate concentration.

Substrate molecules gradually occupy more enzyme active sites as the concentration of substrate rises. The enzyme eventually gets close to a saturation stage, where the majority of its active sites are occupied. At this point, the reaction velocity is getting close to its maximum value; additional increases in substrate concentration only slightly raise the response velocity. The foundation for comprehending enzyme saturation kinetics is this distinctive link between substrate concentration and reaction velocity.

1.6.3 Michaelis–Menten Equation and Plot

A basic mathematical framework for characterising the kinetics of straightforward enzyme-catalyzed processes is provided by the Michaelis–Menten relationship. Under specific experimental settings, it determines the link between the initial reaction velocity and substrate concentration. Important kinetic parameters, such as the maximal reaction velocity (Vmax) and the Michaelis constant (Km), which aid in describing an enzyme's catalytic characteristics and substrate affinity, are frequently found using the Michaelis–Menten equation.

The equation is:

$$\boxed{v = \frac{V_{\max}[S]}{K_m + [S]}}$$

Derivation of the Michaelis–Menten Relationship

Consider:

$$E + SESE + P$$

For the enzyme–substrate complex under the steady-state approximation:

$$\text{Rate of ES formation} = \text{Rate of ES breakdown}$$

Therefore:

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

he Michaelis constant is defined as:

$$\boxed{K_m = \frac{k_{-1} + k_2}{k_1}}$$

Thus:

$$K_m = \frac{[E][S]}{[ES]}$$

The total enzyme concentration is:

$$[E]_T = [E] + [ES]$$

The initial reaction velocity is:

$$v = k_2[ES]$$

When essentially all available enzyme is present as the enzyme–substrate complex:

$$V_{\max} = k_2[E]_T$$

Combining these relationships gives the Michaelis–Menten equation:

$$\boxed{v = \frac{V_{\max}[S]}{K_m + [S]}}$$

Relationship Between Km and Vmax

When:

$$[S] = K_m$$

substitution into the Michaelis–Menten equation gives:

$$v = \frac{V_{\max}K_m}{K_m + K_m}$$

$$v = \frac{V_{\max}K_m}{2K_m}$$

Therefore:

$$\boxed{v = \frac{V_{\max}}{2}}$$

Consequently, Km represents the concentration of the substrate at which the initial reaction rate is one-half of Vmax.

- **Importance of Km**

Km represents an important kinetic constant, since it determines the concentration of the substrate at which half maximal rate occurs in accordance with the Michaelis–Menten model. The lower the value of Km, the lower will be the concentration of the substrate necessary to reach the half-maximal rate; vice versa.

It is not always advisable to consider Km as a general index of affinity between the enzyme and substrate because its value is related to the kinetic mechanism and corresponding rate constants.

Importance of Vmax

Vmax is the maximum reaction rate approached at saturation of the enzyme with substrate.

For a simple system:

$$\boxed{V_{\max} = k_{cat}[E]_T}$$

where the maximum number of substrate molecules converted to products per enzyme active site per unit of time when the substrate concentration is saturating is known as the turnover number, or kcat.

At Different Substrate Concentrations

The Michaelis-Menten equation can also be rewritten for limiting cases.

When:

$$[S] \ll K_m$$

then $K_m + [S] \approx K_m$, and:

$$\boxed{v \approx \frac{V_{\max}}{K_m}[S]}$$

Thus, velocity is approximately proportional to substrate concentration.

When:

$$[S] \gg K_m$$

then $K_m + [S] \approx [S]$, and:

$$\boxed{v \approx V_{\max}}$$

This stage marks the point at which the enzyme is saturated by substrate; hence, further increments in the substrate concentration do not result in a significant increment in velocity.

This graph is included among the topics highlighted in the attached syllabus.

1.6.4 Lineweaver–Burk Plot

The Lineweaver-Burk equation, often known as the double reciprocal of the Michaelis-Menten equation, linearises the velocity and substrate concentration graph's hyperbola.

Beginning with:

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

Taking the reciprocal of both sides:

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max}[S]}$$

Separating the terms gives:

$$\boxed{\frac{1}{v} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}}$$

This corresponds to the equation for a straight line:

$$\boxed{y = mx + c}$$

Therefore:

$$y = \frac{1}{v}$$

$$x = \frac{1}{[S]}$$

$$\boxed{\text{Slope} = \frac{K_m}{V_{\max}}}$$

$$\boxed{\text{Y-intercept} = \frac{1}{V_{\max}}}$$

and:

$$\boxed{\text{X-intercept} = -\frac{1}{K_m}}$$

Lineweaver–Burk diagram has been conventionally employed for estimating kinetic constants and also for demonstrating the impact of inhibitors on enzymes. Since the use of reciprocals in a plot attaches a lot of importance to data points with low substrate concentrations, current practice of kinetic studies involves estimation of parameters through nonlinear fitting of the Michaelis–Menten equation. However, the Lineweaver-Burk plot is still useful for educational and theoretical purposes, and has been specified in the curriculum.

❖ **Comparison of Michaelis–Menten and Lineweaver–Burk Relationships**

While both equations refer to the same kinetic equation, they differ in how they portray this information. The Michaelis-Menten equation directly correlates reaction velocity with substrate concentration, while the Lineweaver-Burk equation takes reciprocals to create a linear equation.

The most important equations to recall are:

$$\boxed{E + S \rightleftharpoons ES \rightarrow E + P}$$

$$\boxed{v = \frac{V_{\max}[S]}{K_m + [S]}}$$

At:

$$\boxed{[S] = K_m}$$

then:

$$\boxed{v = \frac{V_{\max}}{2}}$$

and the double-reciprocal equation is:

$$\boxed{\frac{1}{v} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}}$$

All these form the quantitative base necessary for comprehension of the upcoming topics in this unit, which include inhibitors of enzymes, enzyme control, influencing factors on enzyme activity, and clinical application of enzymes.

1.7 ENZYME INHIBITION

Enzyme inhibition involves a reduction or inhibition of enzyme activity by a molecule called an enzyme inhibitor. The inhibitor acts against the catalytic ability of the enzyme through binding to either the free enzyme, enzyme-substrate complex or modification of a functional group within the enzyme. Consequently, the production of products is reduced.

Enzyme inhibition is a significant phenomenon in biochemistry, pharmacology, toxicology and clinical medicine. There are various drugs that exert their action by inhibition of certain enzymes, as well as some toxic agents that inhibit enzymes involved in normal cellular metabolism. The recommended B.Pharm syllabus includes enzyme inhibitors as part of enzymology with examples.

A normal enzyme catalyzed reaction can be expressed as:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

In the presence of an inhibitor I, additional complexes may be formed:

$$E + I \rightleftharpoons EI$$

or

$$ES + I \rightleftharpoons ESI$$

These interactions may reduce or completely prevent normal catalysis.

Enzyme inhibition is broadly classified into:

$$\boxed{\text{Enzyme Inhibition} = \text{Reversible Inhibition} + \text{Irreversible Inhibition}}$$

1.7.1 Types of Enzyme Inhibitors

- **Reversible Inhibition**

Reversible inhibitors interact with enzymes in such a manner that they can be dissociated. The removal of these inhibitors or their dilution can revive enzyme activity. Types include competitive, non-competitive, uncompetitive and mixed inhibition.

Table 5: Comparison of Major Types of Enzyme Inhibition

Type	Binding Pattern	Effect on Apparent K_m	Effect on $V_{\max}$	Effect of Increasing Substrate
Competitive	Binds mainly to free enzyme	Increases	Unchanged	Can overcome inhibition
Pure non-competitive	Binds E and ES equally	Unchanged	Decreases	Cannot overcome
Uncompetitive	Binds only to ES	Decreases	Decreases	Cannot overcome
Mixed	Binds both E and ES unequally	May increase or decrease	Decreases	Cannot completely overcome
Irreversible	Persistent binding or chemical modification	Not described by a simple reversible pattern	Effective activity decreases	Cannot generally overcome

❖ **Competitive Inhibition**

In competitive inhibition, the inhibitor competes with the substrate for access to the enzyme. In the classical form, both interact with the substrate-binding region.

$$E + S \rightleftharpoons ES$$
$$E + I \rightleftharpoons EI$$

The kinetic equation is:

$$v = \frac{V_{\max}[S]}{\alpha K_m + [S]}$$

where:

$$\alpha = 1 + \frac{[I]}{K_i}$$

Thus:

$$K_m^{app} = \alpha K_m$$

while:

$$V_{\max}^{app} = V_{\max}$$

Therefore, competitive inhibition causes:

$$\boxed{K_m \uparrow,\ V_{\max} \text{ unchanged}}$$

Increasing substrate concentration can reduce the effect of competitive inhibition.

- **Non-Competitive Inhibition**

For simple non-competitive inhibition, the binding site for the inhibitor is not the same as that for the substrate and the interaction is similar whether or not the enzyme-substrate complex is formed.

$$E + I \rightleftharpoons EI$$
$$ES + I \rightleftharpoons ESI$$

In this case:

$$\boxed{V_{\max} \downarrow}$$

while:

$$\boxed{K_m \text{ remains unchanged}}$$

Increasing substrate concentration does not restore the original maximum velocity.

- **Uncompetitive Inhibition**

In uncompetitive inhibition, the inhibitor binds only to the enzyme–substrate complex:

$$ES + I \rightleftharpoons ESI$$

The rate equation can be written as:

$$v = \frac{V_{\max}[S]}{K_m + \alpha'[S]}$$

where:

$$\alpha' = 1 + \frac{[I]}{K_i'}$$

Thus:

$$V_{\max}^{app} = \frac{V_{\max}}{\alpha'}$$

and:

$$K_m^{app} = \frac{K_m}{\alpha'}$$

Therefore:

$$\boxed{K_m \downarrow,\ V_{\max} \downarrow}$$

- **Mixed Inhibition**

The mixed inhibition takes place when the substrate binds to the enzyme-substrate complex with a higher affinity than free enzyme.

The general equation is:

$$v = \frac{V_{\max}[S]}{\alpha K_m + \alpha'[S]}$$

where:

$$\alpha = 1 + \frac{[I]}{K_i}$$

and:

$$\alpha' = 1 + \frac{[I]}{K_i'}$$

Thus:

$$V_{\max}^{app} = \frac{V_{\max}}{\alpha'}$$

and:

$$K_m^{app} = \frac{\alpha}{\alpha'} K_m$$

Hence, $V_{\max}$decreases, while apparent $K_m$may either increase or decrease.

- **Irreversible Inhibition**

Irreversible inhibition involves the formation of a bond between the enzyme and the inhibitor leading to loss of activity.

$$E + I \rightarrow E - I$$

The resulting enzyme–inhibitor complex is inactive.

Whereas reversible inhibition can be reversed once the inhibitor is removed, normal enzymatic reactions cannot.

1.7.2 Examples and Significance

Enzyme inhibition has major biochemical, therapeutic and toxicological importance.

- **Malonate and Succinate Dehydrogenase**

Malonate is an example of competitive inhibition. Malonate is structurally similar to succinate and acts as a competitor to succinate dehydrogenase.

The typical reaction is:

Succinate → Fumarate

Malonate binds to the active site of the enzyme but is not metabolized like succinate, thus reducing the metabolism of succinate.

❖ Cyanide and Cytochrome c Oxidase

Cyanide inhibits cytochrome c oxidase in the mitochondrial electron transport chain. This interferes with the synthesis of energy and oxidative metabolism.

❖ Fluoride and Enolase

Fluoride is capable of inhibiting the enzyme, enolase, which plays a role in glycolysis. This shows that inhibition of one enzyme affects the whole metabolic pathway.

❖ Therapeutic Significance

Many clinically useful drugs act through enzyme inhibition.

Table 6: Selected Therapeutic Enzyme Inhibitors

Drug/Class	**Principal Enzyme Target**	**General Significance**
Aspirin	Cyclooxygenase enzymes	Reduces prostaglandin formation
Statins	HMG-CoA reductase	Reduce cholesterol synthesis
ACE inhibitors	Angiotensin-converting enzyme	Reduce angiotensin II formation
Acetazolamide	Carbonic anhydrase	Used where carbonic-anhydrase inhibition is beneficial
Allopurinol	Xanthine oxidase pathway	Reduces uric-acid production

❖ Toxicological Significance

Some inhibitors produce harmful effects by interfering with essential enzyme systems.

Table 7: Examples of Toxic Enzyme Inhibition

Inhibitor	**Enzyme/System Affected**	**Major Consequence**
Cyanide	Cytochrome c oxidase	Blocks mitochondrial respiration
Organophosphate compounds	Acetylcholinesterase	Excess accumulation of acetylcholine

Certain heavy metals	Multiple enzyme systems	Disturb enzyme structure and function
Fluoride at inhibitory concentrations	Enolase	Reduces glycolytic activity

❖ **Significance in Metabolic Regulation**

Another way to regulate metabolism is by inhibition of enzymes. An enzyme earlier in the same pathway will be inhibited by the final result of a metabolic sequence during the feedback inhibition process.

For example:

$$A \overset{E_1}{\rightarrow} B \overset{E_2}{\rightarrow} C \overset{E_3}{\rightarrow} D$$

If Daccumulates, it may inhibit E_1, thereby slowing further production of D.

This system serves to avoid any wasteful build-up of end products and preserves energy in cells.

❖ **Significance in Drug Development**

Enzyme inhibition is an approach commonly used in drug discovery. In the case when excess activity of certain enzymes leads to disease, the discovery of selective inhibitors could serve as a treatment modality.

A successful treatment inhibitor is expected to have appropriate:

- selectivity,
- potency,
- durability of effect,
- accumulation,
- reversibility/persistence,
- toxicity.

1.8 REGULATION OF ENZYME ACTIVITY

Enzymatic activity should be well-controlled such that metabolic processes are carried out depending on the needs of individual cells and tissues. In case enzymes worked at full throttle all the time, then substrates would get exhausted fast, there will be an accumulation of unwanted products and energy will be wasted unnecessarily. Thus, controlling enzymes ensures that processes in the body take place in coordination and in harmony.

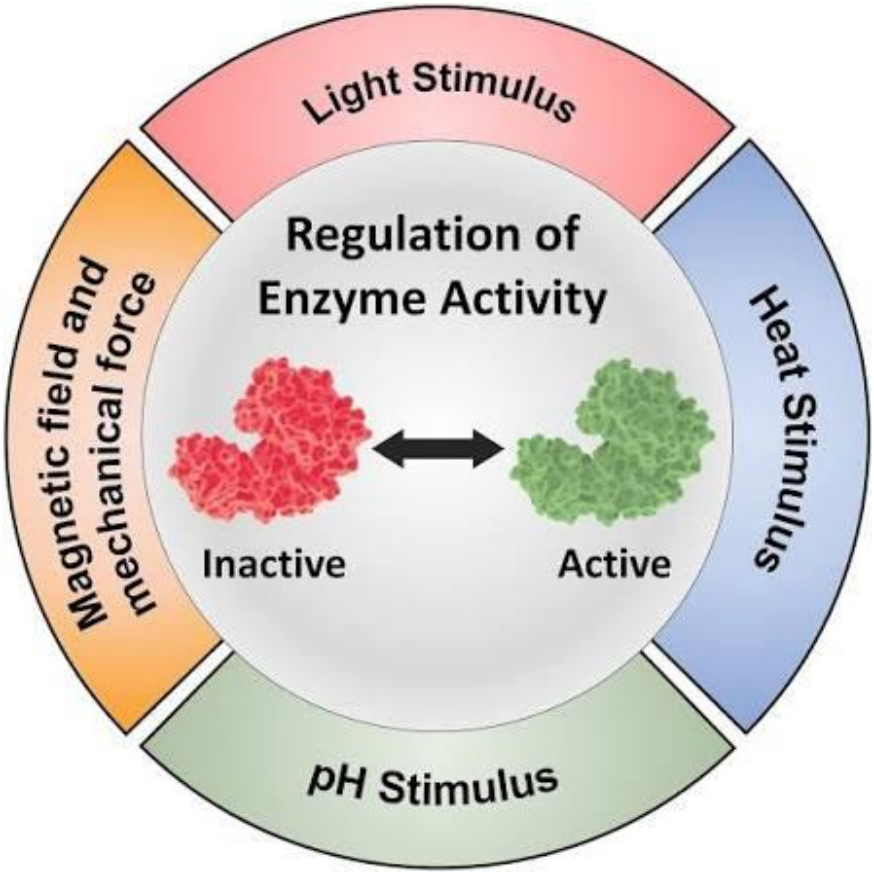


Figure 3: Regulation of Enzyme Activity by Activators and Inhibitors

(Image Source: https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.202200358)

Control of enzymes can be achieved by either varying the number of enzymes or by controlling the activities of enzymes already present in the body. The regulation of enzyme concentration is by means of enzyme induction, enzyme repression and enzyme degradation while rapid control of enzyme activity is done by allosteric enzyme control, covalent modifications, substrate availability and feedback control.

1.8.1 Enzyme Induction and Repression

Enzyme induction is defined as an increase in the production or intracellular level of a particular enzyme as a result of the right stimulus. This stimulus may include a substrate, a hormone, a drug, a nutrient, or another kind of regulatory factor. An increase in enzyme level increases the potential of the cell to execute that particular biochemical reaction.

Induction is usually slower than modification of an already existing enzyme but its effects can last longer.

Enzyme induction plays a very significant role in drug metabolism. Continuous exposure to certain substances leads to an increase in expression of particular enzymes responsible for the metabolism of that drug. Thus, the rate of metabolism of the inducing substance and other substrates of the enzyme system increases.

❖ **Enzyme Repression**

Enzyme repression is the reduction of the synthesis or quantity of an enzyme as a result of certain signals. Enzyme repression serves as a way by which metabolism can be slowed down since there is no need for an excessive amount of that enzyme.

Enzyme repression may be triggered if there is an adequate amount of the metabolic product.

Reduction of enzyme synthesis serves to save the amino acids and energy needed for the useless production of the enzymes.

The primary difference can then be stated as follows:

- Induction → enzyme synthesis or quantity increase
- Repression → enzyme synthesis or quantity decrease

Table 8: Comparison of Enzyme Induction and Repression

Characteristic	Enzyme Induction	Enzyme Repression
Basic effect	Increases enzyme synthesis/amount	Decreases enzyme synthesis/amount
Effect on metabolic capacity	Generally increases	Generally decreases

Major level of control	Gene expression and protein synthesis	Gene expression and protein synthesis
Response	Usually slower than direct enzyme regulation	Usually slower than direct enzyme regulation
Biological purpose	Increases capacity when enzyme activity is required	Prevents unnecessary enzyme production
Pharmaceutical importance	Can alter metabolism of drugs and other compounds	May reduce metabolic capacity for particular pathways

The processes of induction and repression must be clearly differentiated from enzyme activation and enzyme inhibition. While induction and repression affect the amount of enzymes, enzyme activation and inhibition affect the activity of pre-existing enzymes.

1.8.2 Allosteric Regulation

The term "allosteric regulation" describes a mechanism wherein an enzyme's activity can be modulated by interacting with another molecule that binds to an area of the enzyme apart from its active site. The allosteric site is the area where the molecule interacts, and the allosteric effector is the molecule itself.

Interaction of the allosteric effector causes a change in the structure of the enzyme, which may result in enhancement or reduction of enzyme activity.

Allosteric effectors include:

- Positive effectors or activators: enhance enzyme activity.
- Negative effectors or inhibitors: inhibit enzyme activity.

Allosteric enzymes are commonly multi-subunit and show cooperative action. Kinetic properties may also vary from those of Michaelis-Menten enzymes.

❖ **Feedback Regulation**

Feedback inhibition is an important process for metabolic control. Feedback inhibition occurs through the process whereby the end product in a certain metabolic pathway acts on an enzyme at the beginning of the pathway.

For instance, take the metabolic pathway of:

$$A \rightarrow B \rightarrow C \rightarrow D$$

If there is enough D, it can inhibit the early regulation stage. This would prevent the synthesis of more Dand the waste of substrates and energy.

Table 9: Major Mechanisms of Enzyme Regulation

Regulatory Mechanism	**Basic Principle**	**General Effect**
Enzyme induction	Increased synthesis of enzyme	Increases metabolic capacity
Enzyme repression	Decreased synthesis of enzyme	Decreases metabolic capacity
Allosteric activation	Activator binds regulatory site	Increases activity
Allosteric inhibition	Inhibitor binds regulatory site	Decreases activity
Feedback inhibition	End product inhibits an earlier regulatory step	Prevents excessive product formation
Covalent modification	Addition/removal of a chemical group modifies activity	May activate or inhibit enzyme
Substrate availability	Changes in substrate concentration alter reaction rate	Adjusts pathway activity to substrate supply

This allosteric control is highly effective because it ensures that pathways can be modulated rapidly without having to synthesize new enzyme molecules.

1.9 FACTORS AFFECTING ENZYME ACTIVITY

Several factors affect the speed of enzyme-mediated reactions. It is important to know about the factors that affect enzyme-mediated reactions in relation to biochemistry and in relation to enzymes as drugs.

The main factors include the concentrations of the enzyme, substrate and product; the effect of temperature, pH, activators, cofactors, inhibitors and the activation energy of the reaction. The factors affecting enzymes form an explicit part of the syllabus.

1.9.1 Enzyme Concentration

Where there is excess of substrate present, an increase in the rate of reaction will be noticed when enzyme concentration is increased.

Under appropriate conditions:

$$v \propto [E]$$

This equation is helpful in enzyme kinetics because the rate of reaction may be related to the quantity of the enzyme present.

1.9.2 Substrate Concentration

With low substrate concentrations, an increase in substrate concentration causes an increase in reaction velocity since more enzyme-substrate complexes are formed. With very high substrate concentrations, the enzyme is saturated and velocity approaches V_max.

This can be explained using the Michaelis-Menten equation as discussed in Section 1.6.

1.9.3 Temperature

The rise in temperature usually means higher molecular movements and higher chances of contact between the enzyme and substrate in an appropriate range. Outside the optimal range, excessive heat can alter the three-dimensional shape of the enzyme, leading to its denaturation.

Very cold temperatures do not usually affect the function of enzymes but may lead to denaturation. This is useful when storing biological materials.

1.9.4 pH

Enzymes work by ionising groups of amino acids and substrates. Enzyme conformation and interactions inside the active site are sensitive to changes in pH. There is a certain pH range in which an enzyme is most active.

Enzyme activity decreases or protein denaturation occurs in environments that are too acidic or too basic.

1.9.5 Product Concentration

Buildup of reaction products could lower the net reaction rates for some reversible reactions. Reaction products can regulate metabolic pathways by means of feedback systems.

1.9.6 Cofactors and Coenzymes

Many enzymes need metal ions, coenzymes, or other cofactors to be functional. If there is a deficiency or lack of the necessary component, the activity of the enzyme will be reduced.

An example is that some coenzymes come from vitamins. Thus, lack of nutrition may affect metabolism.

1.9.7 Activators

Some ions and molecules enhance the functioning of enzymes by increasing substrate attachment, structural stability, or involvement in catalysis. These are called enzyme activators.

1.9.8 Inhibitors

Enzyme inhibitors decrease catalysis efficiency. According to Section 1.7, enzyme inhibition can be either competitive, non-competitive, uncompetitive, mixed, or irreversible.

Table 10: Factors Affecting Enzyme Activity

Factor	General Effect on Enzyme Activity	Biochemical Basis
Enzyme concentration	Increased enzyme may increase velocity when substrate is sufficient	More catalytic sites become available
Substrate concentration	Increases velocity until saturation is approached	Increased formation of ES complex
Temperature	Increases activity to an optimum range; excessive heat decreases activity	Molecular motion followed by possible denaturation
pH	Activity is highest within an appropriate range	Alters ionization and protein conformation
Cofactors/coenzymes	Required factors support activity	Participate in catalytic reactions
Activators	Increase activity	Facilitate catalytic function
Inhibitors	Decrease activity	Interfere with enzyme binding or catalysis

Product concentration	May reduce net reaction rate or regulate pathway	Reversibility and feedback control

1.10 THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF ENZYMES AND ISOENZYMES

There is significant relevance of enzymes in medical science and pharmaceuticals. Some enzymes are given as such for medicinal use while some are analyzed in biological fluids to diagnose the disease or monitor its progress. Therapeutic and diagnostic uses of enzymes and isoenzymes are included in the syllabus.

Isoenzyme or isozyme is a molecule of an enzyme, which catalyzes the same chemical reaction as the other forms of the enzyme but differs from these in structure, physicochemical or kinetic properties or in distribution in various tissues. Since some isoenzymes are localized only in specific tissues, their identification helps to determine the origin of altered enzyme activity.

❖ Therapeutic Applications of Enzymes

Enzymes can be used therapeutically because of their ability to catalyse highly specific biochemical reactions. Their applications include thrombolysis, replacement of deficient digestive enzymes, degradation of particular biological substances and use in selected anticancer therapies.

Table 11: Selected Therapeutic Applications of Enzymes

Enzyme/Enzyme Preparation	Major Therapeutic Application	Basic Action
Alteplase and related plasminogen activators	Thrombolytic therapy in selected clinical situations	Promotes conversion of plasminogen to plasmin
Pancreatic enzyme preparations	Pancreatic enzyme replacement	Assist digestion of carbohydrates, proteins and fats
Lactase	Lactose intolerance	Hydrolyses lactose

L-Asparaginase	Selected haematological malignancies	Reduces availability of circulating asparagine
Dornase alfa	Cystic fibrosis	Degrades extracellular DNA in respiratory secretions
Hyaluronidase	Used to facilitate dispersion/absorption of certain injected substances in appropriate settings	Degrades hyaluronic acid in extracellular matrix

Enzyme therapy must be used carefully because proteins may have limited stability, specific routes of administration and potential immunological effects.

- **Diagnostic Applications of Enzymes**

Cell injury may change the number of certain enzymes in the blood and other bodily fluids. Enzyme determination may help in diagnosis and evaluation of tissue damage.

But the enzyme test should always be correlated with the clinical and other laboratory tests since one enzyme may be found in different tissues.

Table 12: Important Enzymes in Clinical Diagnosis

Enzyme	Major Clinical Association	Diagnostic Relevance
ALT	Hepatocellular injury	Commonly used in assessment of liver-cell injury
AST	Liver, skeletal muscle and other tissues	Used with other markers in evaluating tissue injury
ALP	Hepatobiliary system and bone	Useful in assessment of cholestatic/hepatobiliary and bone conditions
GGT	Hepatobiliary system	Useful alongside other liver-related biochemical tests
Amylase	Pancreatic and salivary sources	May assist evaluation of pancreatic disorders

Lipase	Pancreas	Common biochemical marker used in assessment of acute pancreatic injury
CK	Skeletal and cardiac muscle	Indicates muscle injury in appropriate clinical contexts

❖ **Diagnostic Importance of Isoenzymes**

Isoenzymes can be used to determine the site of origin of an enzyme since each tissue has a variable amount of each type of molecule. Lactate dehydrogenase (LDH) is one classic case of a molecule having isoenzymes. Creatine kinase is another molecule that is found in various molecular forms in various tissues. Traditionally, isoenzymes were a very important part of the diagnosis of tissue injury. In today's clinical practice, certain isoenzymes have been substituted by other specific molecules.

Table 13: Examples of Clinically Relevant Isoenzyme Systems

Enzyme System	Major Forms	General Significance
Lactate dehydrogenase (LDH)	LDH-1 to LDH-5	Different tissue distributions provide biochemical information about tissue origin
Creatine kinase (CK)	CK-MM, CK-MB, CK-BB	Different forms are associated predominantly with muscle, cardiac and brain/other tissues
Alkaline phosphatase (ALP)	Liver, bone and other tissue-associated forms	Helps in determining the possible source of increased ALP activity

Therefore, the functions of enzymes and isoenzymes in medicine include that of being able to use some as treatment and the other can be used for diagnosing purposes.

1.11 DIGESTION AND ABSORPTION OF NUTRIENTS

Nutrition supplies the body with compounds that are needed for the generation of energy, growth, repair, maintenance, and physiological regulation. The nutrients that are found in foods cannot be utilized by the body in their natural state. There is a need to digest these large molecules before they can be absorbed across the intestinal wall.

Digestion refers to the physical and chemical breakdown of food into absorbable forms.

Absorption refers to the uptake of digested foods across the intestinal wall into the bloodstream or lymphatic system.

The syllabus prescribed requires the study of the digestion and absorption of macronutrients and micronutrients, including vitamins and minerals.

1.11.1 Macronutrients

Macronutrients are nutrients that are needed in larger amounts. The main energy yielding macronutrients include carbohydrates, proteins, and lipids.

- **Digestion and Absorption of Carbohydrates**

Dietary carbohydrates comprise starch, disaccharides and other smaller quantities of carbohydrates. The digestive process breaks down digestible carbohydrates to monosaccharides that are absorbed into the intestines.

The salivary α-amylase enzyme breaks down carbs in the mouth. The salivary amylase enzyme cannot function in the stomach due to its low pH. Most of the digestion of carbohydrates occurs in the small intestine.

Starch is broken down by pancreatic α-amylase in the small intestine. Disaccharides and oligosaccharides are converted to monosaccharides by brush border enzymes in the small intestine. Among these enzymes are lactase, maltase, and sucrase.

For example:

$$\text{Lactose} + H_2O \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

The resulting monosaccharides—glucose, fructose, and galactose—are taken up by intestinal cells and transported to the liver through the portal system.

- **Digestion and Absorption of Proteins**

Absorption of dietary proteins depends on their hydrolysis into their component amino acids and smaller polypeptides.

It is in the stomach where gastric juice neutralises proteins, marking the beginning of the digesting process.

Protein digestion is carried out in the small intestines by means of a number of pancreatic proteolytic enzymes, such as trypsin, chymotrypsin, elastase, and carboxypeptidases.

It can be summarized in a general equation as:

Proteins → Polypeptides → Small peptides → Amino acids

Absorption of amino acids and peptides takes place primarily in the small intestine and enters the portal blood.

❖ **Digestion and Absorption of Lipids**

The diet lipids are mainly composed of triacylglycerides in combination with cholesterol, phospholipids and fat-soluble vitamins.

Due to their limited solubility in aqueous solution, fats need to undergo emulsification before being digested. The bile salts promote breaking down of dietary fat into smaller droplets, thus making more surface area for enzymes to act on them.

Triacylglycerides require pancreatic lipase and its cofactors for digestion. Lipid digestion end-products combine with bile salts in order to form mixed micelles which aid in the transport of lipid digestion products to the intestinal cell surface.

After absorption by intestinal cells, long chain fatty acids and monoacylglycerols are again converted back to triacylglycerides. Along with other lipids and proteins, the triacylglycerides are secreted from the intestinal cells in a form of chylomicrons.

Table 14: Digestion and Absorption of Major Macronutrients

Nutrient	**Major Digestive Components/Enzymes**	**Principal Absorbable Products**	**Main Route After Intestinal Absorption**
Carbohydrates	Salivary and pancreatic amylases; brush-border enzymes	Glucose, galactose, fructose	Portal blood

Proteins	Pepsin, pancreatic proteases and intestinal peptidases	Amino acids and small-peptide-derived products	Mainly portal blood
Lipids	Bile salts, pancreatic lipase and associated digestive processes	Fatty acids, monoacylglycerols and other lipid products	Long-chain lipid products mainly via chylomicrons and lymph

1.11.2 Micronutrients

Micronutrients are chemicals that are needed in smaller quantities, but are vital to healthy growth, metabolism, and physiology. The two types of micronutrients are vitamins and minerals.

Unlike carbohydrates, proteins, and fats, micronutrients are rarely considered primary sources of energy. Rather, they act as components of coenzymes, structures, antioxidants, electrolytes or physiologically active regulators.

Absorption of micronutrients is mainly done by the small intestines. However, there are variations regarding absorption location and mechanism from one micronutrient to another.

Some of the factors that affect micronutrient absorption are:

- the chemical nature of the micronutrient;
- dietary composition;
- intestinal acidity;
- state of gastrointestinal tract health;
- specialized transporters;
- micronutrient interactions with other food components;
- body's physiological needs.

The absence of micronutrients will affect enzymes, tissues, blood formation, bones and many other biochemical functions.

1.11.3 Vitamins and Minerals

❖ **Vitamins**

A person's metabolism and overall health depend on vitamins, which are compounds that are required in minute quantities. Coenzymes and active metabolites are two possible outcomes of vitamin metabolism.

Vitamins can be classified as follows:

- Fat soluble vitamins: These include vitamins A, D, E, and K.
- Water-soluble vitamins: These include vitamin C and the B complex vitamins.

The requirement for proper fat digestion and absorption stems from the fact that lipids are required for the absorption of fat-soluble vitamins. The portal circulation is a site of absorption for water-soluble vitamins.

Table 15: Major Vitamins, Principal Functions and Deficiency Associations

Vitamin	**Major Biochemical/Physiological Role**	**Important Deficiency Association**
Vitamin A	Vision, epithelial integrity and cellular differentiation	Night blindness and epithelial abnormalities
Vitamin D	Calcium and phosphate homeostasis; bone mineralization	Rickets/osteomalacia
Vitamin E	Antioxidant protection of cellular membranes	Neuromuscular/haematological manifestations in significant deficiency
Vitamin K	Required for normal activation of several coagulation-related proteins	Bleeding tendency
Vitamin B1 (Thiamine)	Coenzyme functions in carbohydrate metabolism	Beriberi and neurological manifestations
Vitamin B2 (Riboflavin)	Precursor of FAD and FMN	Characteristic mucocutaneous manifestations

Vitamin B3 (Niacin)	Precursor of NAD^+ and $NADP^+$	Pellagra
Vitamin B6	Amino-acid metabolism	Neurological and haematological manifestations
Folate (B9)	One-carbon metabolism and nucleotide synthesis	Megaloblastic anaemia
Vitamin B12	DNA-related metabolism and neurological function	Megaloblastic anaemia and neurological abnormalities
Vitamin C	Collagen-related reactions and antioxidant functions	Scurvy

Biochemical activities of vitamins and illnesses linked with vitamin deficiencies are particularly mentioned in this section of the syllabus.

- **Minerals**

The structural, catalytic, electric, and regulatory functions of the inorganic minerals are essential. As either significant minerals or trace elements, these minerals can be required at varying degrees of concentration.

Calcium is involved in bone and teeth formation, muscle contraction and cell signaling, among others. The mineral phosphorus is found in bones, nucleic acids, phospholipids and high-energy phosphate compounds. The magnesium element takes part in many enzymatic reactions and is especially critical in ATP-related reactions.

Iron is crucial in the production of hemoglobin and some electron transfer proteins. The mineral zinc is a structural or catalytic part of many proteins and enzymes. The element iodine is needed in the synthesis of thyroid hormones, while the mineral selenium works as an antioxidant protein.

Table 16: Important Minerals and Their Biological Significance

Mineral	Major Function	Important Deficiency Consequence
Calcium	Bone and teeth, muscle function, signalling	Impaired skeletal mineralization and neuromuscular disturbances
Phosphorus	Bone, ATP, nucleic acids, phospholipids	Disturbance of skeletal and metabolic functions in significant deficiency
Magnesium	Cofactor in numerous enzymatic reactions	Neuromuscular and metabolic disturbances
Iron	Haemoglobin and oxygen transport	Iron-deficiency anaemia
Zinc	Enzyme function, growth, immunity and tissue repair	Impaired growth, immunity and wound healing
Iodine	Thyroid hormone synthesis	Thyroid dysfunction and goitre in deficiency
Selenium	Component of antioxidant-related proteins	Disturbed antioxidant and other physiological functions

It is true that vitamins and minerals play a critical role in enzymology, where B-complex vitamins offer substrates for the formation of coenzymes and some minerals serve as cofactors of enzymes. As such, insufficient amounts of these nutrients affect the process of enzymology negatively.

In general, digestion breaks down complex food elements to assimilable units, whereas absorption of these nutrients into the inner environment takes place in the intestines. Thus, digestion and absorption of macronutrients, vitamins and minerals is important to ensure adequate metabolism and physiological processes within the body.

1.12 BIOCHEMICAL FUNCTIONS OF VITAMINS

Vitamins are organic micro-nutrients needed in fairly low amounts for the proper functioning of metabolic processes and maintenance of good health. While vitamins do not directly produce energy in the way that carbohydrates, fats and proteins do, they are very important for the chemical processes that enable the utilization of the latter nutrients. Vitamins may act as precursors to coenzymes in many

cases, while some other vitamins serve in protecting against oxidative damage, genetic regulations, epithelial functions, blood clotting, bone mineralization and cellular differentiation.

Liposoluble vitamins and water-soluble vitamins are the two main categories of vitamins according to their solubility. Most of the B-complex and C-vitamins are water-soluble, whereas vitamins A, D, E, and K are fat-soluble. Coursework covers biochemical vitamin functioning and related deficits.

1.12.1 Vitamin A

Important functions of the immune system, cell differentiation, development, eyesight, and epithelial tissue maintenance are performed by the retinoids, a class of vitamin A. In contrast to retinoic acid's effects on gene expression and cellular differentiation, the retina plays a role in vision.

Preserving the epithelium surfaces of the urinary, digestive, and respiratory tracts is one of vitamin A's most important functions.

1.12.2 Vitamin D

Vitamin D plays a significant role in the maintenance of calcium and phosphate balance and proper bone mineralization. The active hormone promotes the absorption of calcium and phosphate ions from the gastrointestinal tract.

The presence of sufficient amounts of vitamin D is necessary for proper skeletal formation and mineral metabolism.

1.12.3 Vitamin E

Vitamin E acts as a main antioxidant in the lipid phase. Vitamin E prevents oxidation of polyunsaturated fatty acids and membranes from free radicals.

Its role as an antioxidant is especially important in oxidatively stressed tissues and protection of lipid membranes from lipid peroxidation.

1.12.4 Vitamin K

Proteins involved in blood coagulation and bone metabolism require vitamin K for post-translational modifications. Vitamin K-dependent proteins are crucial for proper protein synthesis and have important biological roles.

Vitamin K is thus a crucial factor in haemostasis.

1.12.5 Vitamin B1 – Thiamine

Thiamine is transformed to the active form of TPP.

TPP is involved in some significant reactions such as oxidative decarboxylation of α-keto acids and transfer of carbon unit.

Thus, the importance of thiamine in carbohydrate and energy metabolism becomes obvious.

1.12.6 Vitamin B2 – Riboflavin

Riboflavin is an important precursor for the coenzymes FMN and FAD.

The coenzymes are involved in oxidoreductase reactions and transfer of electrons. Therefore, riboflavin plays an important role in cellular energy metabolism.

1.12.7 Vitamin B3 – Niacin

Niacin is required for the synthesis of NAD^+ and $NADP^+$ that play a major role in oxidation-reduction reactions.

NAD^+ plays a major role in catabolic reactions involved in energy production, while NADPH acts as a reducing agent for various biosynthetic reactions.

1.12.8 Vitamin B5 – Pantothenic Acid

Coenzyme A (CoA) contains pantothenic acid. Conjugated acyl groups are transferred by CoA, which is an essential component in the metabolism of carbohydrates, lipids, and amino acids.

Essential for energy generation and biosynthetic activities, acetyl-CoA is a byproduct of coenzyme A that connects numerous key metabolic pathways.

1.12.9 Vitamin B6 – Pyridoxine

Pyridoxal phosphate (PLP), which plays an essential role in amino acid metabolism, is formed from vitamin B6.

Pyridoxal phosphate plays a key role in transamination and decarboxylation reactions and hence plays an important role in the metabolism of amino acids.

1.12.10 Vitamin B7 – Biotin

Biotin is a coenzyme in carboxylation reactions. Biotin is an important compound used to transport active carbon dioxide in different metabolic activities like synthesis of fatty acids and intermediary metabolism.

1.12.11 Vitamin B9 – Folate

The transformation of folate into tetrahydrofolate derivatives results in the movement of single carbon units in metabolic processes. Metabolic processes are especially significant for the production of nucleotides and amino acids.

It follows that folate is crucial for DNA synthesis and cell division.

1.12.12 Vitamin B12 – Cobalamin

The biochemical activity of vitamin B12 occurs through particular metabolic reactions associated with methyl group transfers and carbon skeleton rearrangements. Vitamin B12 is required for normal blood cell production and proper nervous system functioning.

1.12.13 Vitamin C – Ascorbic Acid

Vitamin C is an antioxidant that is soluble in water and acts as a reducing agent in various biochemical reactions. Vitamin C is especially significant in the synthesis of collagen since it aids in carrying out hydroxylation reactions necessary in collagen formation.

Vitamin C enhances iron absorption by keeping iron in an easily absorbable reduced state.

Table 17: Major Biochemical Functions of Vitamins

Vitamin	**Important Active Form/Relationship**	**Principal Biochemical Functions**
Vitamin A	Retinal, retinoic acid	Vision, epithelial integrity, cellular differentiation
Vitamin D	Active vitamin D metabolite	Calcium/phosphate homeostasis and bone mineralization
Vitamin E	Tocopherols	Antioxidant protection of membranes
Vitamin K	Vitamin K-dependent cofactor function	Activation of coagulation-related and other proteins
Vitamin B1	TPP	Oxidative decarboxylation and carbon-unit transfer
Vitamin B2	FAD, FMN	Oxidation-reduction reactions

Vitamin B3	NAD^+, $NADP^+$	Electron/reducing-equivalent transfer
Vitamin B5	Coenzyme A	Acyl-group transfer
Vitamin B6	PLP	Amino-acid metabolism
Vitamin B7	Biotin	Carboxylation reactions
Vitamin B9	THF derivatives	One-carbon transfer and nucleotide synthesis
Vitamin B12	Cobalamin coenzyme forms	One-carbon-related metabolism and rearrangement reactions
Vitamin C	Ascorbate	Collagen-related hydroxylation, antioxidant action and iron absorption

1.13 VITAMIN DEFICIENCY AND ASSOCIATED DISEASES

Vitamin deficiency occurs due to insufficient intake or utilization of a specific vitamin within the body. Vitamin deficiency is usually caused by insufficient food intake, malabsorption, excessive bodily needs, disease, changes in metabolism, and interactions between specific drugs and nutrients. Since vitamins are part of various biochemical reactions, vitamin deficiencies have an impact on different organs of the body depending on the biochemical function of the vitamin that is deficient.

Table 18: Vitamin Deficiencies and Associated Disorders

Vitamin	**Important Deficiency Condition/Manifestation**	**Biochemical or Physiological Basis**
Vitamin A	Night blindness; xerophthalmic changes in severe deficiency	Impaired visual function and epithelial maintenance

Vitamin D	Rickets in children; osteomalacia in adults	Impaired calcium/phosphate homeostasis and mineralization
Vitamin E	Neurological and haemolytic manifestations in significant deficiency	Reduced antioxidant protection
Vitamin K	Increased bleeding tendency	Impaired activation of vitamin K-dependent coagulation proteins
Vitamin B1	Beriberi; neurological manifestations	Impaired carbohydrate and energy metabolism
Vitamin B2	Cheilosis, angular stomatitis and related mucocutaneous changes	Disturbance of flavin-dependent reactions
Vitamin B3	Pellagra	Impaired NAD/NADP-dependent metabolism
Vitamin B6	Anaemia, dermatitis and neurological manifestations	Disturbed amino-acid and related metabolism
Vitamin B9	Megaloblastic anaemia	Impaired nucleotide synthesis and cell division
Vitamin B12	Megaloblastic anaemia and neurological abnormalities	Disturbed DNA-related metabolism and neurological function
Vitamin C	Scurvy	Impaired collagen formation

- **Vitamin A Deficiency**

Deficiency of vitamin A impacts the vision and epithelial cells. The first noticeable symptom is the night blindness condition where adaptation to low light is affected. Severe deficiency can lead to ocular disorders associated with xerophthalmia and epithelial damage.

- **Vitamin D Deficiency**

A deficiency in vitamin D lowers the ability of calcium and phosphate metabolism in the body, making it difficult for the body to absorb minerals. Deficiency during childhood leads to rickets, while adult deficiency causes osteomalacia.

- **Vitamin E Deficiency**

Vitamin E deficiency is not common among healthy people but is possible in conditions that lead to malabsorption of fats. Deficiency of this vitamin causes nerve damage and increased oxidative stress to red blood cells.

- **Vitamin K Deficiency**

Deficiency of vitamin K causes disruption of proper functioning of certain clotting factors, and therefore, bleeding tendencies may arise.

- **Thiamine Deficiency**

The deficiency of thiamine will affect certain enzyme functions that relate to energy production. Deficiency of thiamine could lead to beriberi disease, but in extreme cases, it could also cause cardiovascular symptoms.

- **Riboflavin Deficiency**

A deficiency in riboflavin may cause problems for the skin and mucous membranes. Some examples include angular stomatitis and cheilosis.

- **Niacin Deficiency**

Niacin deficiency may lead to pellagra, where the clinical triad includes dermatitis, diarrhea, and neuropsychiatric symptoms. This condition arises due to dysfunction of reactions involving NAD and NADP.

- **Vitamin B6 Deficiency**

Deficiency of vitamin B6 might disrupt amino acid metabolism and could possibly result in dermatitis, anaemia, and neurological symptoms.

- **Folate Deficiency**

A lack of folate inhibits DNA synthesis especially for the fast-growing cells. One key consequence is megaloblastic anemia.

❖ **Vitamin B12 Deficiency**

Megaloblastic anaemia can also be caused by deficiency of vitamin B12. Unlike folate deficiency alone, vitamin B12 deficiency also results in neurologic symptoms.

❖ **Vitamin C Deficiency**

Vitamin C deficiency can cause scurvy. Deficiencies in the biochemical processes involving collagen cause symptoms like bleeding gums, poor wound healing, and fragile blood vessels.

It is crucial for pharmacists to understand this deficiency since treatment would only be effective by identifying the deficiency.

1.14 CLINICAL BIOCHEMISTRY

Clinical Biochemistry, sometimes referred to as Clinical Chemistry, involves the application of principles of biochemistry and laboratory analyses to the evaluation of health and illness. Various biological specimens like blood and urine are subjected to examination of enzymes, metabolites, proteins, electrolytes, and many more compounds. The information obtained through biochemical analyses can help with screening, diagnosing, evaluating the functions of various organs, disease monitoring, and treatment evaluation.

In this module, special attention will be given to Liver Function Tests, Renal Function Tests and ELISA as stated in the syllabus.

It is vital to understand that laboratory test results cannot be interpreted alone in most instances. Clinical significance of the result can only be achieved by interpreting it in relation to other test results, clinical findings, history, and reference values.

1.14.1 Liver Function Tests

The liver carries out many vital biochemical activities such as metabolism of carbohydrates, proteins and fats, production of plasma proteins, bilirubin metabolism, detoxification, bile secretion, and drug metabolism.

Liver Function Tests (LFTs) are tests carried out to assess various parameters of the hepatic/biliary system. It is worth noting that, contrary to popular belief, all LFTs do not test for liver function. In some instances, they mainly assess liver cell damage or cholestasis.

Important laboratory tests are bilirubin in the blood, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, albumin, and prothrombin time/INR (when indicated).

- **Alanine Aminotransferase**

Alanyl aminotransferase (ALT) is mainly found in liver cells. The levels of serum activity of ALT may rise due to liver cell damage leading to leakage of ALT from liver cells into the blood stream.

- **Aspartate Aminotransferase**

It is found in the liver as well as other sites such as skeletal muscles and heart muscles. AST cannot be regarded as an indicator of liver dysfunction because the elevation of AST can be due to other conditions.

- **Alkaline Phosphatase**

Alkaline phosphatase (ALP) is related to multiple tissues, especially the hepatobiliary tract and bone. Elevated levels of ALP can be seen in cholestasis or in bone-related diseases.

- **Gamma-Glutamyl Transferase**

The gamma glutamyl transpeptidase enzyme is usually correlated with other markers of hepatobiliary function. This enzyme may be of some value in assessing whether there is a greater likelihood that elevated ALP results from hepatobiliary pathology.

- **Bilirubin**

Bilirubin results from the breakdown of haem. Bilirubin is taken up by the liver and conjugated, thus facilitating its excretion through bile. Bilirubin can thus be measured in terms of its total and direct content.

- **Serum Albumin**

Albumin is formed mostly in the liver. The low concentration of albumin in serum could be due to poor functionality of the liver, but there are other causes that have nothing to do with the liver.

- **Prothrombin Time**

The liver produces various coagulation factors. Hence, prothrombin time and INR can offer relevant insight concerning the functioning of the liver in certain clinical scenarios even though other aspects affect both tests.

Table 19: Important Components of Liver Function Testing

Test	Principal Significance	Possible Interpretation of Abnormality
ALT	Marker of hepatocellular injury	Increased in several forms of hepatocellular injury
AST	Marker of cellular injury	May increase in liver or extrahepatic tissue injury
ALP	Hepatobiliary and bone-associated enzyme	May increase in cholestatic or bone-related conditions
GGT	Hepatobiliary-associated enzyme	Useful with other tests in evaluating hepatobiliary abnormalities
Total/direct bilirubin	Bilirubin metabolism and excretion	Increased in several causes of jaundice
Albumin	Hepatic protein synthesis among other influences	May decrease with impaired synthesis or other conditions
PT/INR	Coagulation-related synthetic function	May become prolonged when synthesis of coagulation factors is impaired

Abnormal patterns of findings tend to be more meaningful than single observations. Predominant elevation of aminotransferases implies a distinct biochemical pattern from that seen with predominant elevation of ALP.

1.14.2 Renal Function Tests

The kidneys help to control the internal chemical environment through the filtration of blood, removal of waste products, regulation of fluid and electrolyte balance, acid-base balance, and endocrine activity.

Renal Function Tests (RFTs) are biochemical tests performed for evaluating the filtration activities of the kidneys and the physiological functions. Some of the tests performed are serum creatinine, eGFR, urea, electrolytes, and urinary tests such as Albumin.

❖ **Serum Creatinine**

Creatinine is produced from the metabolic process involving creatine in the muscles. Creatinine is mostly excreted via the kidneys. Therefore, creatinine in the blood can be used as an endogenous indicator that is associated with renal filtration.

However, if the creatinine level increases, it means that the glomerular filtration rate has been reduced, but there are other variables like muscle mass that influence creatinine levels.

❖ **Estimated Glomerular Filtration Rate**

The estimated glomerular filtration rate (eGFR) gives an estimate of the filtering capability of the kidneys and is usually calculated using serum creatinine levels along with demographic factors using the chosen formula. The lower the value of eGFR, the lesser the filtering capability of the kidneys, though again this depends on the context.

❖ **Blood Urea**

Production of urea mainly occurs in the liver using nitrogen obtained from amino acids. Serum urea can increase in cases where there is poor elimination of urea through the kidney; however, the level of serum urea is not only influenced by the ability of the kidney to eliminate it but other factors like diet, hydration, and catabolism.

❖ **Urinary Albumin**

The regular function of the glomerulus allows only limited leakage of albumin in the urine. Any abnormal increase in urinary albumin excretion suggests kidney dysfunction, especially involving the glomerulus. Urinary albumin-creatinine ratio (ACR) is a test for detecting albuminuria.

❖ **Electrolytes**

Because of their crucial functions in regulating sodium, potassium, bicarbonate, and water balance, problems with renal function can cause disorders in electrolyte and acid-base balance.

Table 20: Common Renal Function Tests

Parameter	**Major Significance**	**General Interpretation**
Serum creatinine	Marker related to glomerular filtration	Often increases as filtration declines

eGFR	Estimate of filtration capacity	Lower values indicate reduced estimated filtration
Urea	Nitrogenous waste product	May increase when renal excretion is reduced; affected by non-renal factors
Urine albumin/ACR	Marker of renal damage	Increased values indicate abnormal albumin excretion
Sodium	Fluid and electrolyte balance	May change with renal and systemic disturbances
Potassium	Neuromuscular and cardiac function	May become abnormal in renal dysfunction
Bicarbonate	Acid–base balance	May decrease with impaired renal acid–base regulation

An assessment of the kidneys is best made when all of these biochemical factors are taken into account in combination, rather than using only one biochemical marker.

1.14.3 ELISA Test

The Enzyme-Linked Immunosorbent Assay (ELISA) refers to the immunoassay laboratory technique which is employed to identify or measure certain antigens or antibodies in biological samples. This test uses both the sensitivity of the antigen-antibody reaction as well as the enzyme-linked detection system.

It is commonly used in medical laboratories, pharmaceutical sciences, and other areas of biomedical science because of its sensitivity and specificity to biological substances.

- **Principle of ELISA**

ELISA is based on the reaction that occurs when a particular antigen binds to its specific antibody. In this case, one component of the reaction is attached to a solid surface. Once the right antigen-antibody reaction is completed, an enzyme-labeled substance is employed for the detection process.

After cleaning off all excess material, a suitable substrate is then added. The enzyme acts on the substrate to produce a colored product.

The basic enzyme reaction can be represented simply as:

Enzyme + Substrate → Detectable Product

In an appropriate setting, the intensity of the signal produced can be correlated to the level of the specific analyte in accordance with the ELISA design.

Major Types of ELISA

There are various ways to do ELISA, depending on the type of analyte.

- Direct ELISA: In direct ELISA, the antigen bound on the solid phase is measured by enzyme-labelled primary antibodies. The technique is simple as fewer antibodies need to bind.
- Indirect ELISA: In indirect ELISA, primary antibodies are recognized by the antigens and secondary antibodies labeled with enzyme recognize the primary antibodies. This technique is widely used in the detection of antibodies, and gives signal amplification.
- Sandwich ELISA: Two antibodies are used to encase the antigen in sandwich ELISA. One antibody binds to one spot on the antigen, while another binds to another site on the same antigen; these two antibodies work together to capture the antigen. High specificity can be achieved using the sandwich approach if the right antibodies are used.
- Competitive ELISA: In competitive ELISA, the analyte competes with another antigen or related reagent for limited binding capacity. Depending on the design of the assay, the signal measured is usually inversely proportional to the analyte concentration.

Table 21: Comparison of Major ELISA Formats

ELISA Type	Basic Principle	Common Purpose/Characteristic
Direct ELISA	Enzyme-labelled primary antibody directly detects immobilized antigen	Simple format with fewer binding steps
Indirect ELISA	Primary antibody is detected by enzyme-labelled secondary antibody	Frequently useful for antibody detection
Sandwich ELISA	Antigen is captured between capture and detection antibodies	High specificity for suitable antigen targets
Competitive ELISA	Target competes for a limited binding interaction	Useful for particular analytes and assay designs

General Steps of ELISA

While the specific process may differ depending on the specific kind of ELISA test performed, the overall steps performed in the lab include the coating or capture of the particular antigen/antibody, blocking of any unbound site if needed, the addition of the sample being tested, binding that occurs specifically, washing of any unbound elements, adding the enzyme detection reagent,

and finally the substrate measurement. Washing is very critical in the process because the lack of this step may cause high background readings.

Enzymes Commonly Used in ELISA

Labels for enzymes have been chosen as they have the ability to transform substrates into detectable products.

Table 22: Common Enzyme Labels Used in ELISA

Enzyme Label	General Role	Advantage
Horseradish peroxidase (HRP)	Converts suitable substrates to detectable products	Sensitive and widely used
Alkaline phosphatase (ALP)	Catalyses reactions producing measurable signals with appropriate substrates	Stable and commonly used in immunoassays

Applications of ELISA

ELISA has wide ranging uses in laboratory medicine and research. Depending upon the nature of the validated ELISA test kit being used, it can be used for the purposes of detecting and quantifying antibodies, antigens, hormones, proteins, and many other biological molecules.

Advantages and Limitations

The benefits associated with ELISA include analytical sensitivity, specificity due to antigen-antibody recognition, capability of handling multiple samples and adaptability for either quantitative or qualitative applications depending on the specific assay. However, accuracy relies on the availability of the right antibodies, assay validation, sample quality, washing techniques, reagent stability and appropriate controls. Technical problems and cross-reactivity might lead to erroneous findings. Consequently, ELISA findings need to be interpreted based on the specifics of the assay.

Table 23: Summary of Clinical Biochemistry Components Covered in Unit I

Area	Major Components	Principal Purpose
Liver function testing	ALT, AST, ALP, GGT, bilirubin, albumin, PT/INR	Assessment of hepatocellular injury, cholestasis, bilirubin handling and selected aspects of synthetic function
Renal function testing	Creatinine, eGFR, urea, urinary albumin, electrolytes	Assessment of renal filtration, renal damage and biochemical homeostasis
ELISA	Antigen–antibody reaction with enzyme-linked detection	Detection or measurement of selected biological analytes

REFERENCES

1. Abd El Rahman, R. A., & Sulieman, A. M. E. (2026). Classification of enzymes. In *Microbial Enzymes* (pp. 13-32). Academic Press.
2. Adeva-Andany, M. M., Pérez-Felpete, N., Fernández-Fernández, C., Donapetry-García, C., & Pazos-García, C. (2016). Liver glucose metabolism in humans. *Bioscience reports*, *36*(6), e00416.
3. Ahmad, I., Ahmed, I., Fatma, S., & Peres, H. (2021). Role of branched-chain amino acids on growth, physiology and metabolism of different fish species: A review. *Aquaculture Nutrition*, *27*(5), 1270-1289.
4. Ahmad, R. S., Hussain, M. B., Sultan, M. T., Arshad, M. S., Waheed, M., Shariati, M. A., ... & Hashempur, M. H. (2020). Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review. *Evidence-Based Complementary and Alternative Medicine*, *2020*(1), 7656919.
5. Akram, M., Ali Shah, S. M., Munir, N., Daniyal, M., Tahir, I. M., Mahmood, Z., ... & Zainab, R. (2019). Hexose monophosphate shunt, the role of its metabolites and associated disorders: A review. *Journal of Cellular Physiology*, *234*(9), 14473-14482.
6. Akram, M., Munir, N., Daniyal, M., Egbuna, C., Găman, M. A., Onyekere, P. F., & Olatunde, A. (2020). Vitamins and Minerals: Types, sources and their functions. In *Functional foods and nutraceuticals: bioactive components, formulations and innovations* (pp. 149-172). Cham: Springer International Publishing.
7. Alabduladhem, T. O., & Bordoni, B. (2022). Physiology, krebs cycle. In *StatPearls [Internet]*. StatPearls Publishing.
8. Al-Bayati, A., & Al-Khateeb, S. (2021). The effects of thyroid hormones and their abnormalities on intestinal and hepatic glucose metabolism. *Scholars International Journal of Biochemistry*, *4*, 26-36.
9. Anand, P. K. (2020). Lipids, inflammasomes, metabolism, and disease. *Immunological Reviews*, *297*(1), 108-122.
10. Anantrao, S. V., Mangala, K. J., Prashant, P., & Raut, M. R. R. (2023). *Principles Of Biomolecules And Metabolism*. Academic Guru Publishing House.

11. Ao, T., Kikuta, J., & Ishii, M. (2021). The effects of vitamin D on immune system and inflammatory diseases. *Biomolecules*, *11*(11), 1624.
12. Arany, Z., & Neinast, M. (2018). Branched chain amino acids in metabolic disease. *Current Diabetes Reports*, *18*(10), 76.
13. Argilés, J. M., Campos, N., Lopez-Pedrosa, J. M., Rueda, R., & Rodriguez-Mañas, L. (2016). Skeletal muscle regulates metabolism via interorgan crosstalk: roles in health and disease. *Journal of the American Medical Directors Association*, *17*(9), 789-796.
14. Aryal, B., Singh, A. K., Rotllan, N., Price, N., & Fernández-Hernando, C. (2017). MicroRNAs and lipid metabolism. *Current opinion in lipidology*, *28*(3), 273.
15. Aşkın, M. Ö., Uzunçakmak, M. T. K. Ü., Altunkalem, M. N., & Tüzün, M. Y. (2021). Vitamin deficiencies/hypervitaminosis and the skin. *Clinics in dermatology*, *39*(5), 847-857.
16. Athyros, V. G., Doumas, M., Imprialos, K. P., Stavropoulos, K., Georgianou, E., Katsimardou, A., & Karagiannis, A. (2018). Diabetes and lipid metabolism. *Hormones*, *17*(1), 61-67.
17. Auger, C., Alhasawi, A., Contavadoo, M., & Appanna, V. D. (2015). Dysfunctional mitochondrial bioenergetics and the pathogenesis of hepatic disorders. *Frontiers in cell and developmental biology*, *3*, 40.
18. Awuchi, C. G., Igwe, V. S., Amagwula, I. O., & Echeta, C. K. (2020). Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. *International Journal of Food Sciences*, *3*(1), 1-32.
19. Baig, S. A. (2026). *Microbial Enzyme Technology*. Authors Click Publishing.
20. Balaji, R., Duraisamy, R., & Kumar, M. P. (2019). Complications of diabetes mellitus: A review. *Drug Invention Today*, *12*(1), 98.
21. Banke, N. H., & Lewandowski, E. D. (2015). Impaired cytosolic NADH shuttling and elevated UCP3 contribute to inefficient citric acid cycle flux support of postischemic cardiac work in diabetic hearts. *Journal of molecular and cellular cardiology*, *79*, 13-20.
22. Batista-Gonzalez, A., Vidal, R., Criollo, A., & Carreño, L. J. (2020). New insights on the role of lipid metabolism in the metabolic reprogramming of macrophages. *Frontiers in immunology*, *10*, 2993.
23. Batista-Silva, W., Heinemann, B., Rugen, N., Nunes-Nesi, A., Araújo, W. L., Braun, H. P., & Hildebrandt, T. M. (2019). The role of amino acid metabolism during abiotic stress release. *Plant, cell & environment*, *42*(5), 1630-1644.

24. Bennett, A. (2018). *Enzyme Kinetics and Regulation*. Scientific e-Resources.
25. Bhatia, S., & Bhatia, S. (2018). Introduction to enzymes and their applications. *Introduction to pharmaceutical biotechnology*, *2*(4), 1-29.
26. Bishop, M. L. (2020). *Clinical chemistry: Principles, techniques, and correlations, enhanced edition: Principles, techniques, and correlations*. Jones & Bartlett Learning.
27. Blanco, A., & Blanco, G. (2022). *Medical biochemistry*. Academic Press.
28. Borkum, J. M. (2023). The tricarboxylic acid cycle as a central regulator of the rate of aging: implications for metabolic interventions. *Advanced biology*, *7*(7), 2300095.
29. Bröer, S., & Bröer, A. (2017). Amino acid homeostasis and signalling in mammalian cells and organisms. *Biochemical Journal*, *474*(12), 1935-1963.
30. Capuano, E., & Janssen, A. E. (2021). Food matrix and macronutrient digestion. *Annual Review of Food Science and Technology*, *12*(1), 193-212.

Unit 2…

BIOMOLECULES, BIOENERGETICS AND BIOLOGICAL OXIDATION

Biomolecules are compounds which serve as building blocks of all living beings. They play a role in cell organization, energy supply, metabolism, growth, regeneration, reproduction and genetic code transfer. Main types of biomolecules are carbohydrates, fats, amino acids, proteins and nucleic acids. Each type of molecules has unique structural properties and specific biological functions. Knowledge about biomolecules is important for pharmaceutical biochemistry as the changes in their synthesis, catabolism or use result in metabolic diseases.

2.1 INTRODUCTION TO BIOMOLECULES

Biomolecules refer to naturally occurring molecules that exist within the living cells and tissues. While some biomolecules are small in size, for instance, amino acids and monosaccharides, others are complex macromolecules, including proteins and nucleic acids. These molecules do not act independently but rather work together in a coordinated manner within biochemical processes that sustain cell life.

Elements like carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur make up the biomolecules. Carbon in particular is an element that is very important in making up the biomolecules due to its ability to form stable covalent bonds and make complex molecules.

Functions of Biomolecules

The role played by the biomolecules includes storage and source of metabolic energy in carbohydrates and lipids. Proteins act in a structural, catalytic, transport and regulatory way. Genetic information is stored and passed using nucleic acids. Amino acids act as building blocks of proteins.

Table 1: Major Classes of Biomolecules and Their Biological Roles

Biomolecule	Basic Components/Units	Major Biological Roles	Examples
Carbohydrates	Monosaccharides	Energy, energy storage, structural and recognition functions	Glucose, glycogen, ribose
Lipids	Fatty acids and other lipid components	Energy storage, membranes, signalling and insulation	Triacylglycerols, phospholipids, cholesterol
Amino acids	Individual amino-acid molecules	Protein synthesis and metabolic precursors	Glycine, alanine, lysine
Proteins	Amino acids linked by peptide bonds	Enzymatic, structural, transport, regulatory and defence functions	Haemoglobin, albumin, enzymes
Nucleic acids	Nucleotides	Storage, transmission and expression of genetic information	DNA and RNA

There must be a balance between these biological molecules for physiological functions to occur normally. Disruption of their metabolism may lead to various diseases like diabetes mellitus, dyslipidaemia, amino acid disorders, and nucleic acid metabolic disorders.

2.2 CARBOHYDRATES: INTRODUCTION AND BIOLOGICAL IMPORTANCE

Carbohydrates are organic molecules that are made up of carbon, hydrogen, and oxygen. The carbohydrates are very significant biological molecules as they are easily accessible sources of energy as well as help build cellular structures.

Monosaccharides are the basic carbohydrate molecules that can join together to form disaccharides, oligosaccharides, and polysaccharides.

Classification of Carbohydrates

Monosaccharides

- Monosaccharides represent simple sugars that cannot undergo hydrolysis to produce smaller carbohydrate molecules. The examples include glucose, fructose, galactose, and ribose.
- Of these, glucose stands out since it acts as a significant fuel for metabolism and has a key role in carbohydrate metabolism.

Disaccharides

Disaccharides contain two monosaccharide units linked by a glycosidic bond.

Important examples include:

- Sucrose – composed of glucose and fructose
- Lactose – composed of glucose and galactose
- Maltose – composed of two glucose units

For example, hydrolysis of lactose can be represented as:

$$\text{Lactose} + H_2O \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

❖ Oligosaccharides

Oligosaccharides are made up of only a few sugar units. They are common components of glycoproteins and glycolipids and play a role in recognition and cell-to-cell communication.

❖ Polysaccharides

Polysaccharides consist of numerous monosaccharides. The most prominent polysaccharide in humans is glycogen, which is primarily found in the liver and muscles. Cellulose is one of the other polysaccharides that are structurally significant for plants.

Table 2: Classification and Examples of Carbohydrates

Class	Basic Characteristic	Examples	Major Significance
Monosaccharides	Single sugar unit	Glucose, fructose, galactose, ribose	Energy metabolism and formation of larger carbohydrates
Disaccharides	Two monosaccharide units	Sucrose, lactose, maltose	Important dietary carbohydrates
Oligosaccharides	Small number of sugar residues	Components of glycoproteins and glycolipids	Cell recognition and communication
Polysaccharides	Large number of monosaccharide units	Glycogen, starch, cellulose	Energy storage or structural functions

Biological Importance of Carbohydrates

- Source of Energy: Carbohydrates are an important source of energy for metabolism. Glucose is metabolized to produce ATP that serves as the energy for various cellular functions. Some of the body tissues depend heavily on glucose under physiological conditions.
- Energy Reserves: Glucose in excess can be converted to glycogen which is stored in the liver and skeletal muscles. Glycogen of the liver serves as a reserve of blood glucose levels while glycogen in the muscles can act as fuel during muscular exertion.
- Components of Nucleotides: The pentoses ribose and deoxyribose are the constituents of RNA and DNA respectively. Thus carbohydrates serve as direct components of nucleic acid structures.
- Structural and Recognition Roles: Carbohydrates are found in glycoproteins and glycolipids and other constituents of cells. These compounds take part in the formation of cellular membranes, cell recognition, receptors and cellular communication.
- Metabolic Precursors: Carbohydrate metabolites serve as precursors for various molecules such as some amino acids and lipids. Thus, carbohydrate metabolism is interlinked with other metabolic systems.

2.3 LIPIDS: INTRODUCTION AND BIOLOGICAL IMPORTANCE

The lipids consist of a large number of organic substances that have low water solubility and increased non-polar solvents solubility. They differ from proteins and nucleic acids in that there is no common polymer structure for lipids. Some examples of lipids include fatty acids, triacylglycerols, phospholipids, glycolipids, cholesterol and steroids.

Major Types of Lipids

- They are essential parts of various complicated lipids as well as metabolic fuels.
- The triacylglycerol compounds, also referred to as triglycerides, comprise glycerol linked through ester linkages to three fatty acids. They are significant sources of metabolic energy stores.
- Phospholipids have unique polar and non-polar regions. They are crucial structural parts of biomembranes.
- Cholesterol is a vital part of animal cell membranes, and it also acts as a precursor to other important biological substances.

Table 3: Major Types of Lipids and Their Functions

Lipid Type	Examples	Major Biological Function
Fatty acids	Palmitic acid, oleic acid	Energy source and components of complex lipids
Triacylglycerols	Stored fats	Long-term energy storage
Phospholipids	Phosphatidylcholine and related phospholipids	Major components of cellular membranes
Glycolipids	Glycosphingolipids	Membrane structure and cellular recognition
Sterols	Cholesterol	Membrane function and precursor of steroid-related molecules

Biological Importance of Lipids

- Energy Storage: Lipids offer an effective way of storing energy over time. The triacylglycerols stored within the adipose tissue can be mobilized for use in metabolic processes whenever needed.
- Membrane Structural Elements: The phospholipids, cholesterol and glycolipids all have an important role in cell membranes by virtue of their structural characteristics.
- Temperature Control and Protective Function: The adipose tissue helps insulate the body and prevent loss of heat while offering mechanical protection to various organs.
- Producers of Metabolically Significant Substances: Cholesterol acts as a precursor of steroid hormones, bile salts, vitamin D derivatives, and some fatty acids act as precursors of signaling substances.
- Increased Absorption of Lipid-Soluble Vitamins: Normal metabolism of lipids promotes the absorption of vitamins that are fat soluble like vitamins A, D, E, and K.
- Pathological Role: Disturbances in lipid metabolism are involved in hyperlipidaemia, hypercholesterolemia, fatty liver, obesity and atherosclerotic cardiovascular disease. Thus lipid metabolism has considerable clinical significance.

2.4 AMINO ACIDS: INTRODUCTION AND BIOLOGICAL IMPORTANCE

Organic compounds known as amino acids include a carboxyl group, an amino group, and a unique side chain. Proteins are essentially elongated chains of amino acids.

A generalized α-amino acid can be represented as:

$$H_2N - CH(R) - COOH$$

where *R*represents the side chain that differs among individual amino acids.

The chemical properties of the side chain dictate the amino acid's features and the protein's activity.

Classification of Amino Acids

Several classifications of the amino acids exist based on different aspects, such as chemical composition of their side chains, nutritional and metabolic roles of the amino acids.

The distinction between essential and non-essential amino acids exists from the point of view of nutrition, with the former category of amino acids being unable to be synthesized by human organism in required quantities for normal functioning and thus requiring dietary intake, while the latter being capable of endogenous synthesis.

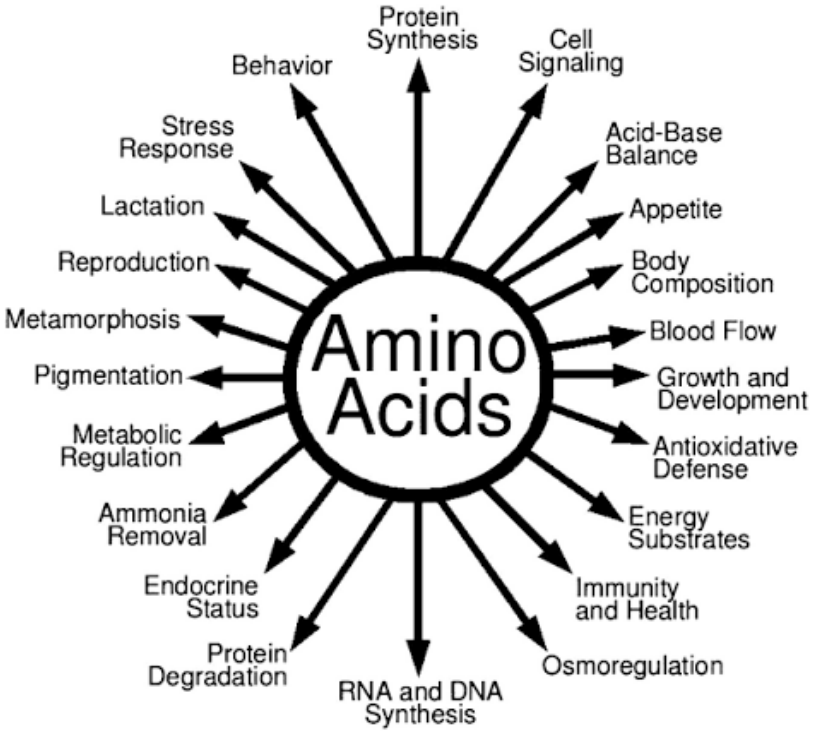


Figure 1: Major Biological and Physiological Functions of Amino Acids

(Image Source: https://advances.nutrition.org/article/S2161-8313(22)00554-3/fulltext)

Biological Importance of Amino Acids

Protein Building Blocks: The simplest function of amino acids is the synthesis of proteins. Peptide bonds bind amino acids together to make polypeptides that then form proteins.

Precursors of Bioactive Compounds: There are various amino acids that act as precursors to neurotransmitters, hormones and other nitrogenous compounds. Some examples are amino-acids contributing to the production of compounds like dopamine, noradrenaline, adrenaline, serotonin, and melatonin.

Participation in Energy Metabolism

In case of necessity, amino acid carbon skeleton may be involved into the process of energy production or synthesis of glucose and ketone bodies.

- Nitrogen Metabolism: The process of transfer and elimination of nitrogen takes place via the metabolism of amino acids. This process is strongly connected with processes of transamination, deamination, and urea synthesis.
- Precursors for Other Biochemical Molecules: Amino acids are involved into the synthesis of some specific substances necessary for the life of cells. Therefore, the biological significance of amino acids is much wider than protein synthesis.

2.5 PROTEINS: INTRODUCTION AND BIOLOGICAL IMPORTANCE

The most intricate nitrogen-containing macromolecules are proteins. Peptide bonds connect the amino acids that make up a protein.

If two amino acids' carboxyl groups react chemically with one another, a peptide bond is produced. For protein synthesis, a specific three-dimensional shape is required of the polypeptides formed by the polymerisation of many amino acids.

Biological functions of proteins depend on their three dimensional form and thus alterations in their form might affect the function of proteins.

Proteins may generally be classified as structural, catalytic, transport, regulatory, contractile and protective proteins.

Table 4: Major Biological Functions of Proteins

Function	Description	Examples
Catalytic	Accelerate biochemical reactions	Enzymes
Structural	Provide support and mechanical strength	Collagen, keratin
Transport	Carry molecules in blood or across membranes	Haemoglobin, albumin, transport proteins
Regulatory	Participate in cellular and physiological regulation	Peptide hormones and regulatory proteins
Protective	Participate in immune defence	Immunoglobulins

Contractile	Produce cellular or muscular movement	Actin and myosin
Storage	Store specific substances or amino-acid resources	Ferritin and selected storage proteins

- As Catalysts: Enzymes are generally proteins that act as catalysts in various biochemical processes related to digestion, metabolic activities, energy production, and synthesis of cell constituents.
- As Structure-Building Components: Structural proteins provide physical support to various tissues, where collagen is an important constituent of connective tissue and keratin is an important constituent of skin and other similar tissues.
- Transport Proteins: Various substances are transported in blood and across cell membrane with the help of proteins such as haemoglobin, which transports oxygen, and albumin, which transports several substances in blood.
- Regulatory Proteins: Several hormones, receptors, and other proteins control metabolism and growth processes.
- Defensive Proteins: Antibodies or immunoglobulins are proteins that recognize specific antigen molecules.

Maintenance of Fluid and Acid–Base Balance

Colloid osmotic pressure is created by proteins in the plasma, especially albumin. Protein molecules have ionizable groups, thus forming buffering solutions.

Clinical Importance

Protein biosynthesis, folding, breakdown, or deficiency could be linked with diseases. Testing of proteins such as albumin and antibodies is also relevant to diagnosis.

2.6 NUCLEIC ACIDS: INTRODUCTION AND BIOLOGICAL IMPORTANCE

The storage, transport, and expression of genetic information are greatly influenced by nucleic acids, which are big molecules. There are two main types of nucleic acids: DNA and RNA.

The basic building block of a nucleic acid is the nucleotide. Nucleotides are made up of the following three parts:

- A nitrogen base;
- Pentose sugar; and
- Phosphate group(s).

Purines and pyrimidines are the two further classes of nitrogen bases. Two examples of purines are adenine and guanine. Rhodamines are cytosine, thymine, and uracil. RNA has uracil, but DNA contains thymine.

DNA contains deoxyribose, while RNA contains ribose.

DNA

- Deoxyribonucleic acid (DNA) is the chief molecule carrying genetic information in human cells. The genetic information is stored in the arrangement of nucleotides of this molecule. DNA replication enables the transmission of genetic information from one generation of cells to another.
- The sequence of DNA contains information necessary for the formation of RNA and then proteins.

RNA

- RNA takes various functions in gene expression and protein synthesis processes. These include:
- mRNA (Messenger RNA) that is the medium for carrying genetic instructions that are used during protein synthesis.
- tRNA (Transfer RNA) that carries amino acids and is involved in decoding of genetic instructions during protein synthesis process.
- rRNA (Ribosomal RNA) that is an important component of ribosome structure**.**

Biological Importance of Nucleic Acids

Nucleic acids are required for the process of heredity, cell reproduction, and protein biosynthesis. DNA contains genes, while RNA is responsible for transforming this genetic information into practical molecules for cells. Nucleotides have significant importance in processes other than the

formation of nucleic acids. In particular, ATP and GTP serve as a medium of energy in the cell, while many nucleotide derivatives belong to signaling molecules and coenzymes. Mistakes in the process of DNA replication and injuries of the genetic substance can change the behavior of the cell. For this reason, there are specific mechanisms of DNA repair and genome stabilization. These topics create the background for further understanding of nucleotide metabolism, DNA replication, transcription, translation, genetic code, and DNA repair. Carbohydrates, lipids, amino acids, proteins, and nucleic acids create a complex biochemical network. Carbohydrates and lipids are important for energy and its storage; amino acids create proteins and serve as precursors; proteins have different functions; nucleic acids store and express genetic information. The knowledge of these basic properties is the prerequisite for understanding bioenergetics and biological oxidation in the next part of the unit.

2.7 FUNDAMENTALS OF BIOENERGETICS

Bioenergetics is the area of study within biochemistry that deals with energy conversion, transfer and consumption within living organisms. Each of the biological functions is characterized by the adequate amount of energy flow. The processes like muscular contraction, transport of substances, synthesis of protein and nucleic acids, regulation of ion concentration, signal transduction and biosynthesis are related to energy gained via metabolic processes.

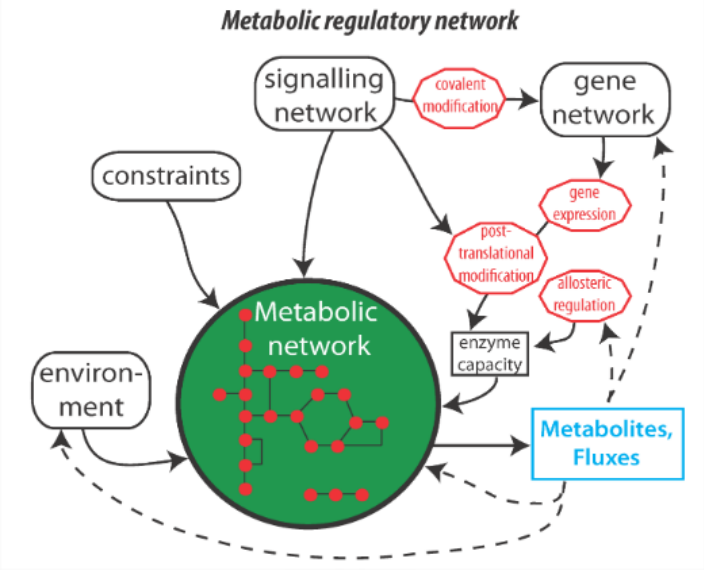


Figure 2: Integrated Regulatory Network of Cellular Metabolism

(Image Source: https://www.mdpi.com/2218-1989/2/3/529)

Living cells get their energy mostly by oxidizing organic substances like carbohydrates, fats, and, where needed, amino acids. A part of the energy received is saved as energy-rich compounds such as ATP and further utilized for energy-dependent functions.

The principles of thermodynamics, such as free energy, enthalpy, and entropy, control bioenergetics. Additionally, electron transfer is linked to oxidation-reduction reactions in bioenergetics. The electron transport chain and oxidative phosphorylation can be understood in this way.

2.7.1 Concept of Free Energy

The quantity of internal energy that can be utilised to perform work within a system at a given temperature and pressure is known as its free energy or Gibbs free energy (G). From a biochemical standpoint, the direction of an energetic process can be inferred from the change in free energy (ΔG).

The change in free energy for a reaction is given by:

$$\boxed{\Delta G = G_{\text{products}} - G_{\text{reactants}}}$$

The sign of ΔG indicates the thermodynamic favorability of a reaction under the given conditions.

Exergonic Reactions

When:

$$\Delta G < 0$$

The energy in the reaction is freed and described as an exergonic reaction. Under the above conditions, this reaction favors the forward direction thermodynamically.

Breaking down energy-filled compounds may entail exergonic reactions. This energy can be saved in ways that can aid future cellular work.

Endergonic Reactions

When:

$$\Delta G > 0$$

This type of reaction requires an input of energy and is known as an endergonic reaction. Biosynthesis reactions are usually endergonic and, thus, require energy releasing reactions.

Equilibrium

When:

$$\Delta G = 0$$

the system is at equilibrium, with no net thermodynamic driving force in either direction.

Table 5: Interpretation of Gibbs Free-Energy Change

Value of ΔG	Type of Process	Thermodynamic Meaning
$\Delta G < 0$	Exergonic	Thermodynamically favourable in the forward direction
$\Delta G > 0$	Endergonic	Requires energy input to proceed in the forward direction
$\Delta G = 0$	Equilibrium	No net free-energy change or net driving force

- **Standard Free-Energy Change**

Under well-defined standard conditions, ΔG^∘is utilised to symbolise the alteration in free energy. The standard free-energy change in biological reactions is denoted as ΔG^(∘^') because in the biochemical standard state there is a well defined concentration of hydrogen ions, which is approximately equal to pH 7.

Because the concentrations of the reactants and products are not standard, standard circumstances are not equivalent to real cellular conditions. So, the standard free-energy change and the concentrations of reactants and products will determine the value of ΔG for a biological reaction.

- **Coupled Reactions**

In cells, one can often couple an energetically unfavorable chemical reaction to a sufficiently favorable reaction to make it occur.

For instance, one can couple an endergonic biosynthesis reaction with ATP hydrolysis. The entire process is thermodynamically favorable when its Gibbs free energy is negative.

The above is the concept of energy coupling in cell metabolism.

2.7.2 Enthalpy and Entropy

Free energy properties in biochemical reactions are dependent on two major thermodynamic parameters which are enthalpy and entropy. Alongside temperature, they help in determining the thermodynamics of biochemical reactions.

Enthalpy

Enthalpy (H) denotes the energy related to heat in a system at constant pressure. The change in enthalpy during a chemical reaction can be shown using ΔH and is given as:

$$\boxed{\Delta H = H_{\text{products}} - H_{\text{reactants}}}$$

When:

$$\Delta H < 0$$

heat is released to the surroundings and the process is described as exothermic.

When:

$$\Delta H > 0$$

heat is absorbed from the surroundings and the process is described as endothermic.

It is necessary to differentiate between exothermic reactions and endothermic reactions and exergonic reactions and endergonic reactions. While exothermic and endothermic reactions relate to the enthalpy change, exergonic and endergonic reactions relate to the Gibbs energy change. Thus, the enthalpy of a reaction cannot decide whether the reaction is thermodynamically favourable**.**

Entropy

In thermodynamics, entropy (S) is a quantity that depends on both the number of microscopic states of a system and the dispersion of its energy. More scattered energy and molecular randomisation may be associated with high entropy, according to elementary biochemistry.

The change in entropy can be expressed as:

$$\boxed{\Delta S = S_{\text{products}} - S_{\text{reactants}}}$$

Whereas when ΔS is greater than zero, there is an increase in the entropy of the system, and when ΔS is less than zero, there is a decrease in entropy.

Living beings have cells with an ordered structure, yet this does not violate the laws of thermodynamics. Cells are open systems that constantly interact with their environment. Thus, an ordered state can be preserved without violating the second law of thermodynamics.

Table 6: Comparison of Free Energy, Enthalpy and Entropy

Thermodynamic Term	Symbol	Basic Meaning	Biochemical Importance
Gibbs free energy	G	Energy available to perform useful work under specified conditions	Indicates thermodynamic favourability of reactions
Enthalpy	H	Heat-related energy content at constant pressure	Indicates heat absorbed or released
Entropy	S	Measure related to energy dispersal and microscopic arrangements	Contributes to the direction and favourability of reactions
Temperature	T	Absolute temperature in kelvin	Determines the contribution of entropy to free-energy change

2.7.3 Relationship Between Free Energy, Enthalpy and Entropy

The Gibbs equation describes the connection between free energy, enthalpy, and entropy:

$$\Delta G = \Delta H - T\Delta S$$

Where:

- **ΔG** = Change in Gibbs free energy
- **ΔH** = Change in enthalpy
- **T** = Absolute temperature in Kelvin (K)
- **ΔS** = Change in entropy

In this equation, we see that the energy favorability of any reaction is not only related to the change in enthalpy and entropy but also to the temperature.

If $\Delta H$is negative, it means that ΔGwill be more likely to be negative. Similarly, if $\Delta S$is positive, it means that $-T\Delta S$will be negative.

Table 7: Influence of Enthalpy and Entropy on Reaction Favourability

ΔH	ΔS	General Thermodynamic Behaviour
Negative	Positive	Favourable at all temperatures
Positive	Negative	Unfavourable at all temperatures
Negative	Negative	Favourability depends on temperature
Positive	Positive	Favourability depends on temperature

In biology, the relationship can be used to explain the reasons for which certain reactions yield available energy whereas others need energy supply. The arrangement of reactions into pathways in cells ensures that favourable reactions can power unfavourable ones.

2.7.4 Redox Potential

Electron transfers between molecules occur in many biochemical reactions. They are called oxidation-reduction reactions or redox reactions. They play an important role in the generation of energy within cells.

In oxidation, the process involves a loss of electrons, while reduction involves gaining of electrons.

A useful mnemonic is:

OIL RIG: Oxidation Is Loss; Reduction Is Gain of electrons.

An electron donor becomes oxidized:

$$\text{Reduced donor} \rightarrow \text{Oxidized donor} + e^-$$

An electron acceptor becomes reduced:

$$\text{Oxidized acceptor} + e^- \rightarrow \text{Reduced acceptor}$$

Oxidation and reduction always occur together because electrons lost by one substance must be accepted by another.

<u>Oxidizing and Reducing Agents</u>

- An oxidising agent is a substance that can decrease itself by accepting electrons.

- A substance that undergoes oxidation and the loss of electrons is known as a reducing agent.
- Some important electron carriers in biological systems are NAD+/NADH, NADP+/NADPH and FAD/FADH2 and mitochondrial electron transport chain.

Redox Potential

The redox potential (E) refers to the ability of a redox system to either gain or lose electrons. In the case of specified biochemical standard conditions, it can be denoted by the standard reduction potential (E^(∘^')).

Electrons are more likely to be gained by a redox system with a high reduction potential than lost by one with a low one.

Table 8: Important Concepts in Biological Oxidation-Reduction

Concept	Meaning	Biological Significance
Oxidation	Loss of electrons	Occurs during oxidation of metabolic fuels
Reduction	Gain of electrons	Occurs when electron carriers accept reducing equivalents
Oxidizing agent	Accepts electrons and becomes reduced	Receives electrons in redox reactions
Reducing agent	Donates electrons and becomes oxidized	Supplies electrons to another molecule
Redox potential	Tendency of a redox couple to accept/donate electrons	Helps determine direction of electron transfer
NAD^+/NADH	Important biological redox couple	Transfers reducing equivalents in metabolism
$FAD/FADH_2$	Flavin redox couple	Participates in oxidation-reduction and electron-transfer reactions

Biological Importance of Redox Potential

In particular, redox potential becomes highly significant in the process of mitochondrial energy generation. The electrons, which are gained as a result of the oxidation of nutrients, are passed on to such carriers as NADH and FADH2 and eventually go to the respiratory chain to reach molecular oxygen.

The energy, which results from these electron transfer processes, helps to generate the electrochemical potential of protons in the inner mitochondrial membrane. This electrochemical potential helps to synthesize ATP via oxidative phosphorylation.

Hence, it can be stated that free energy, enthalpy, entropy and redox potential form the thermodynamic basis of energy transformation.

2.8 ENERGY-RICH COMPOUNDS

In living cells, energy is continuously required to undertake various activities such as biosynthesis, active transport, muscle contraction, cellular communication and the maintenance of ionic gradients. The energy that is liberated through oxidation of carbohydrates, fats and amino acids is not utilized directly in all biological processes within a cell. Rather, a considerable amount of energy from the oxidation of such substances is stored in an intermediate form in energy-rich molecules, from where it is available for transfer to chemical reactions requiring energy.

Molecules that undergo a very large negative change in free energy during hydrolysis or group transfer reactions are considered energy-rich. Protein synthesis, cellular communication, and some metabolic pathways all include energy-requiring processes, and GTP is an essential component of these pathways. ATP is the primary molecule that transports energy inside the cell. The bioenergetics section of the required curriculum includes ATP and GTP in particular.

2.8.1 ATP

In living systems, the nucleotide adenosine triphosphate (ATP) plays a crucial role as both a source and a carrier of chemical energy. Because it mediates between cellular reactions that provide energy and those that consume it, it is known as the cellular currency of energy.

ATP is made up of the following three major components:

- adenine which is a purine nitrogenous base;
- ribose which is a five-carbon sugar;

- three phosphate groups labeled α, β, and γ phosphates.

The union of adenine and ribose constitutes adenosine and the addition of three phosphates constitute ATP.

Hydrolysis of ATP

ATP can be hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate (P_i):

$$\boxed{ATP + H_2O \rightarrow ADP + P_i}$$

In regular biochemical conditions, ATP hydrolysis has a significantly negative standard free energy. Under normal physiological conditions, the exact value of free energy is dependent on various factors, such as the concentrations of ATP, ADP, phosphate, magnesium, and pH.

ATP may also be hydrolysed to adenosine monophosphate (AMP) and pyrophosphate (PP_i):

$$ATP + H_2O \rightarrow AMP + PP_i$$

Further hydrolysis of the pyrophosphate can greatly favor the net direction of some biosynthetic processes.

Why ATP Is an Effective Energy Carrier

ATP has a large phosphoryl group transfer potential. The hydrolysis of ATP is energetically favorable since the resulting products are more stable than ATP due to electrostatic stabilization, resonance effect, and solvation.

Rather than considering energy as stored "in the phosphate bond," it would be better to comprehend that energy is linked with the overall hydrolysis reaction of ATP.

Formation of ATP

- ATP is always being produced and used up. Important processes involved in producing ATP are as follows:
- Phosphorylation at the substrate level: The process of phosphate transfer from an energetic molecule into ADP results in the production of ATP.
- Oxidative phosphorylation: Mitochondrial electron transport generates a proton electrochemical gradient, which is utilised to produce ATP.

- Photophosphorylation of ATP can also occur in photosynthesizing organisms.

Functions of ATP

ATP acts as a source of energy or phosphoryl groups for many biological activities. This includes biochemical synthesis, active transport across membranes, muscle contraction, and various signaling mechanisms. In addition, ATP acts as a substrate in the synthesis of nucleic acids and protein phosphorylation by protein kinases.

Table 9: Major Cellular Functions of ATP

Function	Role of ATP	Example
Biosynthesis	Drives energy-requiring synthetic reactions	Protein and macromolecule synthesis
Active transport	Supplies energy to membrane pumps	Na^+/K^+-ATPase
Muscle contraction	Supports actin–myosin cycling	Skeletal and cardiac muscle activity
Phosphorylation	Donates phosphoryl groups	Protein kinase reactions
Cell signalling	Participates directly or indirectly in signalling pathways	Formation of cyclic AMP from ATP
Nucleic-acid metabolism	Serves as a nucleotide and energy source	RNA synthesis and related processes

ATP–ADP Cycle

The ATP-to-ADP conversion cycle is essential to the enzyme's activity. The process of converting ADP to ATP makes use of the energy that is produced during the oxidation of nutrients. Hydrolysis of ATP is the end outcome of these energy-intensive procedures.

In simplified form:

$$ADP + P_i + \text{energy} \rightarrow ATP$$

followed by:

$$ATP + H_2O \rightarrow ADP + P_i + \text{usable free energy}$$

This continuous regeneration is essential because cellular ATP is constantly being consumed.

2.8.2 GTP

Guanosine triphosphate (GTP) is yet another type of high-energy purine nucleotide. Chemically, GTP consists of guanine, ribose sugar and three phosphates. Just like ATP, GTP can release energy upon its hydrolysis.

GTP hydrolysis may be represented as:

$$\boxed{GTP + H_2O \rightarrow GDP + P_i}$$

Although ATP serves as the most general energy-transfer nucleotide, GTP has several specialized biological roles.

- **GTP in Protein Synthesis**

GTP plays an especially vital role in translation, where it functions through the different stages of protein synthesis, such as translation factor function during initiation, elongation and translocation.

- **GTP in Cell Signalling**

The importance of GTP comes from its involvement in the signaling pathways that include GTP-binding proteins or G proteins. G proteins have two possible forms – GTP-bound and GDP-bound forms. While the first is linked to an active signaling pathway, hydrolysis of GTP into GDP plays an important part in signal termination.

- **GTP in Metabolism**

In the citric acid cycle, succinate reacts with succinyl coenzyme A to produce GTP instantly. When nucleoside diphosphate kinase is involved in an energy-transferring activity, GTP can be transformed to ATP.

- **GTP in Gluconeogenesis**

GTP is needed for phosphoenolpyruvate carboxykinase (PEPCK) in gluconeogenesis because it facilitates the formation of phosphoen.

Table 10: Comparison of ATP and GTP

Characteristic	ATP	GTP
Nitrogenous base	Adenine	Guanine
Sugar	Ribose	Ribose
Number of phosphate groups	Three	Three
Hydrolysis product considered here	ADP + P_i	GDP + P_i
General role	Principal immediate energy-transfer nucleotide	Specialized energy and regulatory nucleotide
Major examples of use	Biosynthesis, transport, contraction, phosphorylation	Translation, G-protein signalling, gluconeogenesis
Metabolic relationship	Can support formation of GTP	Can transfer phosphate for ATP formation through nucleotide diphosphate kinase

ATP and GTP are not thus to be seen as competing systems of energy. They are linked nucleotides used differently in various cells.

2.8.3 Biological Significance of Energy-Rich Compounds

Compounds rich in energy serve as the important link between the energy yielding process and energy demanding processes. Without these compounds, the energy released from the oxidation of nutrients would not be utilized effectively.

- **Energy Coupling**

The primary role played by such compounds is that of energy coupling. Most biological synthesis reactions are thermodynamically unfavorable when done in isolation. However, coupling them to the hydrolysis of ATP or GTP renders the process favorable.

In essence, such high-energy compounds enable energy from catabolic reactions to drive anabolic and other energy-consuming reactions.

- **Metabolic Work**

ATP and its derivatives provide the necessary components for protein, lipid, carbohydrate, and nucleic acid synthesis, among others. Consequently, they are fundamental for growth, maintenance, and repair.

- **Mechanical Work**

ATP is used for mechanical work. For instance, muscle contraction is one of those processes where ATP is involved in the regulation of the contractile protein cycle.

- **Transport Work**

Active Transport Systems move materials across membranes against gradients of concentration or electrical charge. ATP-powered pumps are vital for ion distribution in cells.

- **Cell Signalling and Regulation**

Roles as regulators also apply to ATP and GTP. ATP acts as a phosphate donor in phosphorylation of proteins and precursor of signalling molecules. GTP acts as a regulator of various G-proteins and molecular switches.

Table 11: Biological Significance of Energy-Rich Compounds

Biological Process	Principal Energy-Rich Compound Involved	Significance
Energy coupling	ATP/GTP	Connects favourable and unfavourable biochemical reactions
Biosynthesis	Mainly ATP; GTP in selected processes	Supports synthesis of cellular components
Active transport	ATP	Maintains ion and solute gradients
Muscle contraction	ATP	Provides energy for contractile cycling

Protein synthesis	ATP and GTP	ATP supports amino-acid activation; GTP supports translation steps
Cell signalling	ATP and GTP	Supports phosphorylation and G-protein signalling
Gluconeogenesis	ATP and GTP	Supplies energy for energy-requiring steps
Metabolic regulation	ATP, ADP, AMP and GTP-related systems	Helps coordinate cellular energy status and metabolic activity

❖ Other Energy-Rich Compounds

While ATP and GTP are the primary focus of discussion in this chapter, there are many other biological molecules that have high group-transfer potential. The examples of such are phosphoenol pyruvate, 1,3 bisphosphoglycerate, creatine phosphate, and acetyl Co-A.

Table 12: Selected Energy-Rich Compounds and Their Roles

Compound	Type of Energy-Rich/Activated Group	Major Biochemical Role
ATP	Phosphoryl groups	General cellular energy transfer
GTP	Phosphoryl groups	Translation, signalling and selected metabolic reactions
Phosphoenolpyruvate	High-transfer-potential phosphate	Participates in substrate-level phosphorylation in glycolysis
1,3-Bisphosphoglycerate	High-transfer-potential phosphate	Supports ATP formation during glycolysis
Creatine phosphate	Phosphoryl group	Rapid buffering/regeneration of ATP in muscle and other tissues
Acetyl-CoA	Activated thioester-linked acetyl group	Transfers acetyl groups and connects major metabolic pathways

To conclude, ATP is the chief molecule used by cells to transfer energy immediately, while GTP has specific functions to play in protein synthesis and signaling. Along with other molecules of high energy transfer potential, ATP and GTP make it possible for cells to efficiently capture, transfer, and use energy.

2.9 BIOLOGICAL OXIDATION

In biological oxidation, the energy in food is transformed into a form that cells can store through a series of oxidation-reduction events. As part of the process of carbohydrate, lipid, and protein metabolism, reducing agents such as NAD^+ and FAD are produced. These reducing agents subsequently yield NADH and $FADH_2$, respectively. The mitochondria's electron transport chain receives electron donations from NADH and $FADH_2$.

Coordination between ATP production and electron reduction to molecular oxygen is essential in aerobic respiring cells. This example involves a number of important processes, including the ETC, oxidative phosphorylation, and ATP generation. The majority of the reactions occur in mitochondria, as the name implies.

2.9.1 Electron Transport Chain

The inner mitochondrial membrane is home to an array of electron carriers known as the electron transport chain. The process by which electrons are transferred from NADH and $FADH_2$ to oxygen molecules is the primary focus. Proton (H^+) pumping from the mitochondrial matrix to the intermembrane space is powered by the process's energy.

Coenzyme Q (ubiquinone) and cytochrome c are two mobile electron carriers that make up the Electron Transport Chain, which also includes the four primary respiratory complexes.

- **Complex I: NADH–Ubiquinone Oxidoreductase**

The electrons that come from NADH go to Complex I. They then move through flavin and iron-sulfur centers until reaching coenzyme Q. The energy released in this process results in proton transfer over the mitochondrial inner membrane.

In doing so, NADH is reduced back to NAD^+ and can be used again for metabolism.

- **Complex II: Succinate Dehydrogenase**

Complex II is another enzyme involved in the citric acid cycle. The electrons produced by the oxidation of succinate are transferred through FAD/$FADH_2$ and iron-sulfur clusters to coenzyme Q.

In contrast to Complexes I, III, and IV, Complex II does not transport protons across the mitochondrial inner membrane.

❖ **Coenzyme Q**

Ubiquinone, also known as coenzyme Q, is an electron carrier that can move across membranes and is water-insoluble. Ubiquinone obtains electrons from complexes I, II, and some other mitochondrial dehydrogenases and gives them to complex III.

❖ **Complex III: Ubiquinol–Cytochrome c Oxidoreductase**

Electrons transferred by complex III from reduced coenzyme Q to cytochrome c. The transfer of electrons is linked to the proton transport across the inner mitochondrial membrane.

❖ **Cytochrome c**

On the inner mitochondrial membrane's surface lies cytochrome c, a protein that transfers electrons. The electrons are donated from Complex III to Complex IV.

❖ **Complex IV: Cytochrome c Oxidase**

Molecular oxygen is the final destination of the electrons transported from complex IV by cytochrome c. Reducing molecular oxygen to water, it serves as an electron acceptor. The movement of proton is likewise carried out via complex IV.

A simplified terminal reaction is:

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

Table 13: Major Components of the Electron Transport Chain

Component	Major Function	Electron Transfer	Proton Pumping
Complex I	Oxidizes NADH	NADH → CoQ	Yes
Complex II	Transfers electrons from succinate-linked $FADH_2$	Complex II → CoQ	No
Coenzyme Q	Mobile lipid-soluble carrier	Complex I/II and other sources → Complex III	No
Complex III	Transfers electrons from reduced CoQ	CoQ → cytochrome c	Yes
Cytochrome c	Mobile protein electron carrier	Complex III → Complex IV	No
Complex IV	Transfers electrons to oxygen	Cytochrome c → O_2	Yes

The overall direction of electron transfer can be summarized as:

$$\text{NADH} \rightarrow \text{Complex I} \rightarrow \text{CoQ} \rightarrow \text{Complex III} \rightarrow \text{Cytochrome c} \rightarrow \text{Complex IV} \rightarrow O_2$$ Electrons entering through Complex II reach coenzyme Q without passing through Complex I.

2.9.2 Oxidative Phosphorylation

The metabolic process known as oxidative phosphorylation converts ADP to ATP by using the energy released when reduced coenzymes are oxidised, mainly NADH and $FADH_k$. The electron transport chain (ETC) is involved in this process, which is the main way that aerobic cells produce ATP.

The inner mitochondrial membrane is the site of oxidative phosphorylation, which calls for a number of conditions to be met, including a functional electron transport chain, molecular oxygen, ATP, and inorganic phosphate. The energy released at Complexes I, III, and IV is used to pump protons (H^+) out of the mitochondrial matrix into the intermembrane space as electrons are moved through the ETC. In doing so, the proton motive force—an electrochemical gradient across the inner mitochondrial membrane—is established.

The proton motive force consists of two components:

- A **proton concentration gradient (ΔpH)** across the inner mitochondrial membrane.
- An **electrical potential (membrane potential)** generated by the unequal distribution of protons across the membrane.

The ATP synthase complex is the principal pathway for H^+ ions to re-enter the mitochondrial matrix, due to the inner mitochondrial membrane's high proton permeability. In order to catalyse the production of ATP from ADP and inorganic phosphate, ATP synthase makes use of the energy generated during this proton flow.

Thus, oxidative phosphorylation represents a functional coupling between electron transport and ATP synthesis. Any disruption of electron transport, proton-gradient formation, or membrane integrity can impair oxidative phosphorylation and consequently reduce cellular ATP production.

Table 14: Requirements for Oxidative Phosphorylation

Requirement	Role
NADH and $FADH_2$	Supply reducing equivalents/electrons
Electron transport chain	Transfers electrons and supports proton pumping

Oxygen	Final electron acceptor
Intact inner mitochondrial membrane	Maintains proton electrochemical gradient
ADP	Substrate for ATP synthesis
Inorganic phosphate (P_i)	Combines with ADP during ATP formation
ATP synthase	Catalyses ATP formation using proton-motive force

2.9.3 Mechanism of ATP Synthesis

Chemiosmotic theory explains how oxidative phosphorylation forms ATP. This theory states that an electrochemical gradient of proton concentration across the inner mitochondrial membrane is produced by electron transport. Accumulated kinetic energy (ATP) is produced during the gradient creation process.

The enzyme responsible for producing ATP is known as ATP synthase or Complex V. There are two main parts to this enzyme, and they are the F_0 and F_1 components.

The inner mitochondrial membrane can allow proton flow due to the F_0 component. Conformational changes in ATP synthase are caused by the passage of protons. Afterward, the F_1 component receives the changes and uses them to create ATP by combining ADP and inorganic phosphate.

The central reaction is:

$$\boxed{ADP + P_i \rightarrow ATP}$$

Thus, ATP synthesis involves the following sequence:

Nutrient oxidation → NADH/$FADH_2$ formation → electron transport → proton gradient → ATP synthase activity → ATP formation

The formation of ATP does not result from the transfer of phosphate via electrons to ADP. Instead, the proton motive force created due to electron transport drives the synthesis of ATP.

From a modern biochemical perspective, the oxidation of mitochondrial NADH produces about 2.5 ATP, whereas the entry of electrons into the chain from FAD-linked reactions via coenzyme Q results in about 1.5 ATP.

2.9.4 Regulation of Oxidative Phosphorylation

Regulation of oxidative phosphorylation ensures that ATP production is proportional to energy requirements within the cell. Availability of ADP is especially crucial for regulation of the process of respiration in mitochondria.

With increase in ATP utilization by the cell, ATP is metabolized to form ADP. ADP activates ATP synthesis as well as increases electron transport and oxygen consumption. Reduced levels of ADP lead to decreased oxidative phosphorylation.

Other significant regulators include oxygen availability, availability of reducing agents, inorganic phosphate, respiratory substrates, and function of the mitochondrial membrane.

Table 15: Major Factors Regulating Oxidative Phosphorylation

Regulatory Factor	Effect
ADP availability	Increased ADP generally stimulates ATP synthesis and respiration
ATP demand	Increased cellular work promotes oxidative phosphorylation indirectly through ADP production
Oxygen availability	Required as the terminal electron acceptor
NADH/$FADH_2$ supply	Provides electrons to the respiratory chain
Inorganic phosphate	Required for ATP formation
Substrate availability	Influences production of reducing equivalents
Inner mitochondrial membrane integrity	Required to maintain proton-motive force

❖ **Inhibitors and Uncouplers**

- Some chemicals are capable of blocking oxidative phosphorylation, either by interfering with electron transport or ATP synthase.
- Cyanide for instance acts on cytochrome c oxidase (complex IV), thus interfering with the proper electron transfer to oxygen. On the other hand, oligomycin blocks ATP synthase from allowing protons to pass through the F0 component of this enzyme.

- Uncouplers, on the other hand, are chemicals that allow the disintegration of proton gradient without blocking the process of electron transfer.
- In physiological uncoupling in special tissues, uncoupling protein 1 (UCP1) in brown fat cells allows the controlled proton leakage.

2.9.5 Mitochondrial Dysfunction

Mitochondrial dysfunction means a dysfunction of mitochondrial structure or activity which leads to decreased capabilities of mitochondria to carry out their functions, especially oxidative metabolism.

Metabolism relies heavily on proteins encoded by nuclear genes, while mitochondrial DNA is physically distinct. Therefore, mitochondrial malfunction can result from a variety of internal and external sources, including mutations in nuclear or mitochondrial genes.

❖ **Consequences of Mitochondrial Dysfunction**

The other major effect is decreased ATP formation. Organs having high energy demands like the brain, muscles, and heart may be highly affected by the malfunctioning of mitochondria in energy production.

Other effects of mitochondria on the cell include alteration of calcium metabolism, increased production of reactive oxygen species, and the regulation of cell death and survival.

Table 16: Major Consequences of Mitochondrial Dysfunction

Abnormality	Biochemical Consequence
Impaired electron transport	Reduced efficiency of electron transfer
Reduced proton gradient	Decreased driving force for ATP synthesis
Impaired ATP synthase function	Reduced ATP production
Increased electron leakage	Increased formation of reactive oxygen species
Disturbed calcium handling	Altered cellular signalling and homeostasis
Severe mitochondrial damage	May contribute to activation of cell-death pathways

The mitochondrial diseases are diverse and may occur in many different tissues. The manifestations vary depending upon the defect itself and the energy demands of the tissues.

2.9.6 Oxidative Stress and Its Clinical Significance

Normal aerobic metabolism leads to the production of ROS by cells. While at regulated levels, certain forms of reactive species take part in cell signaling, excessive production compared to the antioxidant mechanisms can be damaging to the cell. Oxidative stress is characterized by a disruption of oxidant and antioxidant systems enough to disrupt redox signaling and/or lead to molecular injury.

Reactive oxygen species of importance include:

- superoxide radical (O_2^(·-));
- hydrogen peroxide (H_2 O_2);
- hydroxyl radical (OH^·).

An important source of ROS in the cell is the mitochondria because a small fraction of the electrons may reduce oxygen prematurely during electron transport.

❖ **Cellular Effects of Oxidative Stress**

- ROS at high levels may affect different categories of biological molecules.
- Lipid peroxidation may impact membrane lipids, changing the properties of membranes.
- Protein oxidation may change the properties of the amino-acid side chains, thus causing protein denaturation.
- DNA damage may cause the formation of base modifications and DNA strand breaks, and its inefficient repair may lead to genomic instability.
- The very mitochondria may be damaged, leading to a vicious circle in which mitochondria cause oxidative stress.

❖ **Antioxidant Defence Systems**

Cells possess enzymatic and non-enzymatic antioxidant mechanisms that limit oxidative damage.

Superoxide dismutase (SOD) facilitates conversion of superoxide to hydrogen peroxide:

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

Catalase converts hydrogen peroxide into water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione-mediated processes play a key role in peroxide breakdown and cellular redox homeostasis.

The non-enzyme based antioxidants include substances like glutathione and dietary antioxidant compounds such as vitamin C and E.

❖ **Clinical Significance**

It has been proposed that oxidative stress is part of the pathogenesis of several illnesses, such as cardiovascular disease, metabolic disorders, neurodegenerative diseases, inflammation and tissue injury, but the involvement of oxidative stress is variable according to the type of illness, and hence, oxidative stress cannot be considered as the cause alone for these multi-factorial diseases.

The interaction between mitochondrial dysfunction and oxidative stress is noteworthy. Mitochondrial dysfunction with regard to the electron transport chain can lead to increased ROS, while oxidative stress can cause dysfunction of mitochondrial components.

As a conclusion, biological oxidation enables the conversion of the energy gained by the consumption of nutrients into energy usable for ATP generation. Electrons of NADH and FAD-dependent pathways are transferred through the mitochondria respiratory chain to oxygen. The resulting transmembrane movement of protons is responsible for the generation of the electrochemical gradient for the ATP synthase. Regulating the process ensures the adequacy of ATP production according to the cellular need, while disruption of the mitochondrial energy production is linked to the occurrence of oxidative stress.

REFERENCES

1. Carrà, S. (2018). Biomolecules, Networks and Bioenergetics: System Approach to Biology. In *Stepping Stones to Synthetic Biology* (pp. 55-82). Cham: Springer International Publishing.
2. Censani, M., Hammad, H. T., Christos, P. J., & Schumaker, T. (2018). Vitamin D deficiency associated with markers of cardiovascular disease in children with obesity. *Global pediatric health*, *5*, 2333794X17751773.
3. Chandel, N. S. (2021). Carbohydrate metabolism. *Cold Spring Harbor perspectives in biology*, *13*(1), a040568.
4. Chandel, N. S. (2021). Glycolysis. *Cold Spring Harbor perspectives in biology*, *13*(5), a040535.
5. Chandel, N. S. (2021). Lipid metabolism. *Cold Spring Harbor Perspectives in Biology*, *13*(9), a040576.
6. Chatterjee, S. (2023). Bioenergetics and primitive metabolism. In *From Stardust to First Cells: The Origin and Evolution of Early Life* (pp. 67-74). Cham: Springer International Publishing.
7. Chen, L., Chen, X. W., Huang, X., Song, B. L., Wang, Y., & Wang, Y. (2019). Regulation of glucose and lipid metabolism in health and disease. *Science China life sciences*, *62*(11), 1420-1458.
8. Cheng, C. (2022). *Enzyme-based organic synthesis*. John Wiley & Sons.
9. Choi, I., Son, H., & Baek, J. H. (2021). Tricarboxylic acid (TCA) cycle intermediates: regulators of immune responses. *Life*, *11*(1), 69.
10. Chouchani, E. T., & Kajimura, S. (2019). Metabolic adaptation and maladaptation in adipose tissue. *Nature metabolism*, *1*(2), 189-200.
11. Choudhary, M. (2024). Applications of Biochemistry in Modern Healthcare. *57*(1), 1-5.
12. Collin, C. B., Gebhardt, T., Golebiewski, M., Karaderi, T., Hillemanns, M., Khan, F. M., ... & Kuepfer, L. (2022). Computational models for clinical applications in personalized medicine—guidelines and recommendations for data integration and model validation. *Journal of personalized medicine*, *12*(2), 166.
13. Combs Jr, G. F., & McClung, J. P. (2022). *The vitamins: fundamental aspects in nutrition and health*. Academic press.

14. Crabbé, A., Jensen, P. Ø., Bjarnsholt, T., & Coenye, T. (2019). Antimicrobial tolerance and metabolic adaptations in microbial biofilms. *Trends in microbiology*, *27*(10), 850-863.
15. Curtius, H. C., & Roth, M. (Eds.). (2018). *Clinical biochemistry. Principles and methods. Vol. 1*. Walter de Gruyter GmbH & Co KG.
16. d'Alessandro, A., Nemkov, T., Sun, K., Liu, H., Song, A., Monte, A. A., ... & Roach, R. C. (2016). AltitudeOmics: red blood cell metabolic adaptation to high altitude hypoxia. *Journal of proteome research*, *15*(10), 3883-3895.
17. Da Poian, A. T., & Castanho, M. A. (2021). The families of biological molecules. In *Integrative Human Biochemistry: A Textbook for Medical Biochemistry* (pp. 89-185). Cham: Springer International Publishing.
18. Darnton-Hill, I. (2019). Public health aspects in the prevention and control of vitamin deficiencies. *Current Developments in Nutrition*, *3*(9), nzz075.
19. Delaunay, S., Helm, M., & Frye, M. (2024). RNA modifications in physiology and disease: towards clinical applications. *Nature Reviews Genetics*, *25*(2), 104-122.
20. Devindra, S., Bhanuprakash Reddy, G., Sivakumar, B., Reddy, S., Kalyanasundaram, S., Ibrahim, A., ... & Madhavan Nair, K. Chemical Nature, Physiology and Metabolism of Nutrition.
21. Di Giulio, R. T., Benson, W. H., Sanders, B. M., & Van Veld, P. A. (2020). Biochemical mechanisms: metabolism, adaptation, and toxicity. In *Fundamentals of aquatic toxicology* (pp. 523-561). CRC Press.
22. Dimastrogiovanni, G., Córdoba, M., Navarro, I., Jáuregui, O., & Porte, C. (2015). Alteration of cellular lipids and lipid metabolism markers in RTL-W1 cells exposed to model endocrine disrupters. *Aquatic Toxicology*, *165*, 277-285.
23. Djordjevic, I. B. (2016). Fundamentals of biological thermodynamics, biomolecules, cellular genetics, and bioenergetics. In *Quantum Biological Information Theory* (pp. 75-142). Cham: Springer International Publishing.
24. Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., ... & Yin, Y. (2016). The role of leucine and its metabolites in protein and energy metabolism. *Amino acids*, *48*(1), 41-51.
25. Eid, S., Sas, K. M., Abcouwer, S. F., Feldman, E. L., Gardner, T. W., Pennathur, S., & Fort, P. E. (2019). New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. *Diabetologia*, *62*(9), 1539-1549.

26. Falkowska, A., Gutowska, I., Goschorska, M., Nowacki, P., Chlubek, D., & Baranowska-Bosiacka, I. (2015). Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. *International journal of molecular sciences*, *16*(11), 25959-25981.
27. Feingold, K. R. (2022). Lipid and lipoprotein metabolism. *Endocrinology and Metabolism Clinics*, *51*(3), 437-458.
28. Feingold, K. R. (2024). Introduction to lipids and lipoproteins. *Endotext [internet]*.
29. Fothergill, E., Guo, J., Howard, L., Kerns, J. C., Knuth, N. D., Brychta, R., ... & Hall, K. D. (2016). Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. *Obesity*, *24*(8), 1612-1619.
30. Franca Gois, P. H., Wolley, M., Ranganathan, D., & Seguro, A. C. (2018). Vitamin D deficiency in chronic kidney disease: recent evidence and controversies. *International journal of environmental research and public health*, *15*(8), 1773.

Unit 3…

CARBOHYDRATE METABOLISM AND DIABETES MELLITUS

Carbohydrates are significant metabolic fuel in human beings where glucose is considered the central component of energy metabolism. Glucose once absorbed in the human body can be oxidized for ATP formation, converted to glycogen, other compounds, or even formed from non-carbohydrate sources. Carbohydrate metabolism thus involves different interlinked pathways which play a vital role in maintaining the energy and blood glucose level balance. This topic will discuss the various metabolic pathways of carbohydrates and their regulation, and then their clinical significance regarding diabetes mellitus.

3.1 INTRODUCTION TO CARBOHYDRATE METABOLISM

Carbohydrate metabolism entails the biochemical reactions that occur during digestion, assimilation, utilization, storage, and synthesis of carbohydrates. Glucose is the most important carbohydrate involved in metabolism and is essential in tissues that have high demands for glucose.

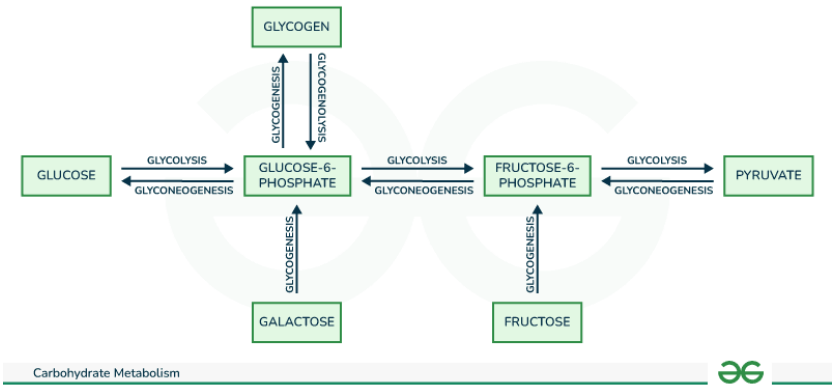


Figure 1: Overview of Major Pathways in Carbohydrate Metabolism

(Image Source: https://www.geeksforgeeks.org/biology/carbohydrate-metabolism/)

The many tissues in the body use glucose, which is taken into the bloodstream after a carbohydrate-rich meal. Glucose has multiple functions in the body, including converting it to energy through glycolysis, storing it as glycogen, using it in the pentose pathway, and providing carbon for other molecules. Gluconeogenesis and glycogen breakdown work together to keep blood glucose levels stable during fasting.

The citric acid cycle, glycogenesis, glycogenolysis, gluconeogenesis, and pentose pathway are the main metabolic routes for carbohydrates. Cellular energy requirements and hormones like adrenaline, glucagon, and insulin control these metabolic processes.

Table 1: Major Pathways of Carbohydrate Metabolism

Pathway	Major Site	Principal Function
Glycolysis	Cytosol	Conversion of glucose to pyruvate with formation of ATP and NADH
TCA cycle	Mitochondrial matrix*	Oxidation of acetyl-CoA and production of NADH, $FADH_2$ and GTP
Glycogenesis	Cytosol, mainly liver and muscle	Storage of glucose as glycogen
Glycogenolysis	Cytosol, mainly liver and muscle	Breakdown of glycogen
Gluconeogenesis	Mainly liver; also kidney	Formation of glucose from non-carbohydrate precursors
Pentose phosphate pathway	Cytosol	Formation of NADPH and ribose-5-phosphate

*While succinate dehydrogenase is located on the inner mitochondrial membrane, most TCA cycle events occur in the mitochondrial matrix. Because diabetes mellitus can emerge from issues with carbohydrate utilisation or hormone control, understanding carbohydrates metabolism is important from a medical perspective.

3.2 GLYCOLYSIS

Glycolysis refers to the pathway wherein a single molecule of glucose gets converted to two pyruvate molecules via a series of enzymatic reactions.

Glycolysis is especially significant in cells lacking mitochondria, for example, the matured red blood cells and provides an efficient source of ATP in conditions where there is an oxygen shortage.

The glycolytic pathway may be split into two stages:

1. The energy-investment stage, where ATP gets utilized.
2. The energy-repayment stage, where ATP and NADH get produced.

3.2.1 Steps of Glycolysis

In the metabolic route known as glycolysis, one molecule of glucose is transformed into two molecules of pyruvate by means of 10 consecutive enzyme-catalyzed reactions. There is a general division into an energy-investment phase and an energy-generation phase that happens in the cytoplasm of cells along the pathway. Glycolysis begins with the consumption of ATP and continues with the generation of both ATP and NADH.

Step 1: Phosphorylation of Glucose

Phosphorylation of glucose to produce glucose-6-phosphate (G6P) is the initial step in glycolysis. Hexokinase is responsible for catalysing this reaction in the majority of tissues, while glucokinase is mainly responsible for this in the liver and pancreatic β-cells.

$$\textbf{Glucose} + \textbf{ATP} \rightarrow \textbf{Glucose-6-phosphate} + \textbf{ADP}$$

During this reaction, one molecule of ATP is used up. Phosphorylation aids glucose uptake and storage within cells, setting the stage for metabolic events to come.

Step 2: Isomerization of Glucose-6-Phosphate

Phosphoglucose isomerase is an enzyme that can reverse the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P).

$$\textbf{Glucose-6-phosphate} \rightleftharpoons \textbf{Fructose-6-phosphate}$$

In order to set the stage for the following phosphorylation event, this process reorganises the carbonyl group inside the sugar molecule.

Step 3: Formation of Fructose-1,6-Bisphosphate

Fructose-1,6-bisphosphate (F-1,6-BP) is formed by phosphorylating fructose-6-phosphate. One molecule of ATP is required for the reaction, which is catalysed by phosphofructokinase-1 (PFK-1).

$$\textbf{Fructose-6-phosphate} + \textbf{ATP} \rightarrow \textbf{Fructose-1,6-bisphosphate} + \textbf{ADP}$$

Glycolysis relies on this crucial, irreversible, and committed process. Glycolytic flux rate is strongly influenced by PFK-1 activity.

Step 4: Cleavage of Fructose-1,6-Bisphosphate

The enzyme aldolase breaks down fructose-1,6-bisphosphate into two molecules that each contain three carbon phosphate atoms.

$$\textbf{Fructose-1,6-bisphosphate} \rightleftharpoons \textbf{Glyceraldehyde-3-phosphate} + \textbf{Dihydroxyacetone phosphate}$$

Group 3 phosphate and dihydroxyacetone phosphate are the end results. The molecules then go on to the glycolysis energy-generating step.

Step 5: Isomerization of Dihydroxyacetone Phosphate

The enzyme triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde-3-phosphate quickly and irreversibly.

$$\textbf{Dihydroxyacetone phosphate} \rightleftharpoons \textbf{Glyceraldehyde-3-phosphate}$$

As a result of this reaction, both three-carbon molecules generated from one glucose molecule are converted into G3P. Therefore, the subsequent reactions of glycolysis occur **twice for every molecule of glucose**.

Step 6: Oxidation of Glyceraldehyde-3-Phosphate

1,3-bisphosphoglycerate (1,3-BPG) is formed by oxidising and phosphorylating glyceraldehyde-3-phosphate. In this process, glyceraldehyde-3-phosphate dehydrogenase acts as a catalyst.

$$\textbf{Glyceraldehyde-3-phosphate} + \textbf{NAD}^+ + \textbf{Pi} \rightarrow \textbf{1,3-Bisphosphoglycerate} + \textbf{NADH} + \textbf{H}^+$$

This process results in the reduction of NAD^+ to NADH. Under these conditions, glycolysis via this pathway produces two molecules of NADH per molecule of glucose, as two molecules of G3P are formed from one molecule of glucose.

Step 7: Formation of ATP

1-Phosphoglycerate undergoes a transfer reaction with ADP to form 3-phosphoglycerate and ATP. Phosphoglycerate kinase is the enzyme that facilitates the process.

1,3-Bisphosphoglycerate + ADP ⇌ 3-Phosphoglycerate + ATP

In this process, known as substrate-level phosphorylation, a phosphate group is transferred from a high-energy metabolic intermediate to ADP, directly resulting in the generation of ATP. This phase results in the generation of two ATP molecules since each glucose molecule yields two 1,3-BPG molecules.

Step 8: Formation of 2-Phosphoglycerate

Reversible conversion of 3-phosphoglycerate into 2-phosphoglycerate is catalysed by the enzyme phosphoglycerate mutase.

3-Phosphoglycerate ⇌ 2-Phosphoglycerate

The phosphate group is moved from the third carbon atom of the glycerate molecule to the second carbon atom in this process. After the reaction, the chemical is in the right place to undergo dehydration.

Step 9: Formation of Phosphoenolpyruvate

Phosphoenolpyruvate (PEP) is produced when the enzyme enolase removes a water molecule from 2-phosphoglycerate.

2-Phosphoglycerate ⇌ Phosphoenolpyruvate + H_2O

PEP's high phosphoryl-transfer potential is due to its high-energy phosphate bond. Because of this, it is a crucial step in glycolysis's last reaction, which generates ATP.

Step 10: Formation of Pyruvate

The enzyme pyruvate kinase converts phosphoenolpyruvate to pyruvate in the last phase of glycolysis. The process of ATP synthesis involves the transfer of the phosphate group from PEP to ADP.

Phosphoenolpyruvate + ADP → Pyruvate + ATP

This process, like many others involving phosphorylation at the substrate level, is practically irreversible within living cells. This process yields two molecules of ATP for every molecule of glucose because it forms two molecules of PEP from a single molecule of glucose.

Overall Outcome of Glycolysis

One molecule of glucose can be transformed into two molecules of pyruvate across the entire glycolytic pathway. The energy-generation phase produces four ATP molecules, while the energy-investment phase consumes two, therefore a net gain of two ATP molecules occurs. Furthermore, two NADH molecules are generated.

The overall reaction can be summarized as:

$$\textbf{Glucose} + 2\ \textbf{NAD}^+ + 2\ \textbf{ADP} + 2\ \textbf{Pi} \rightarrow 2\ \textbf{Pyruvate} + 2\ \textbf{NADH} + 2\ \textbf{H}^+ + 2\ \textbf{ATP} + 2\ \textbf{H}_2\textbf{O}$$

Thus, glycolysis provides a rapid source of ATP and metabolic intermediates and serves as a central pathway linking carbohydrate metabolism with other major metabolic processes.

Energy Yield of Glycolysis

For each glucose molecule:

Total ATP used = 2

Total ATP generated = 4

ATP gain = 2

NADH formed = 2

Pyruvate formed = 2

The general equation can be written as:

$$\text{"Glucose"} + 2\text{ADP} + 2\text{P_i} + 2\text{NAD}\hat{}+ \rightarrow 2\text{"Pyruvate"} + 2\text{ATP} + 2\text{NADH} + 2\text{H}\hat{}+ + 2\text{H_2O}$$

In aerobic condition, the cytosolic NADH may act as a reducing agent for oxidative phosphory.

Table 2: Energy Balance of Glycolysis per Glucose Molecule

Component	Number
ATP utilized	2
ATP produced directly	4
Net ATP produced	2
NADH produced	2
Pyruvate produced	2

3.2.2 Regulation and Significance

Glycolysis regulation happens mainly during the irreversible steps, where there are three key enzymes namely, hexokinase, PFK-1 and pyruvate kinase. This happens in order to control the rate of glucose breakdown with the body's metabolic needs in mind.

Regulation by Hexokinase and Glucokinase

Hexokinase is widespread among tissues and acts via inhibition of its own end product, glucose-6-phosphate. Glucokinase is abundant within the liver cells and pancreatic beta-cells and exhibits distinct kinetics and regulation from that of hexokinase. It participates in the phosphorylation of glucose in the liver especially during its excess.

Regulation of PFK-1

PFK-1 is a key enzyme in glycolysis regulation. The cellular energy level controls the enzyme. A adequate amount of energy is indicated by high levels of citrate and ATP, which inhibit PFK-1, and by the fact that AMP induces glycolysis. One powerful PFK-1 stimulator is fructose-2,6 bisphosphate.

Regulation of Pyruvate Kinase

Pyruvate kinase is responsible for the last ATP-forming step in glycolysis. The enzyme's functioning depends on cellular metabolites and hormones in the liver.

❖ Biological Significance of Glycolysis

Glycolysis is the major source of ATP production in organisms that do not have mitochondria. This is in addition to providing pyruvate for oxidation in the mitochondria when there is oxygen available, and also metabolic precursors for many biosynthetic pathways.

Clinically significant because there is abnormal use of glucose in diseases such as diabetes mellitus, tissue hypoxia, and certain enzyme deficiencies.

3.3 CITRIC ACID CYCLE (TCA CYCLE)

One of the most important metabolic pathways in the body, the citric acid cycle (or Krebs cycle) converts acetyl-CoA to carbon dioxide (CO_2). Most of the energy that is released during acetyl-CoA oxidation is stored in the reduced coenzymes NADH and $FADH_k$, along with GTP (or ATP, depending on the tissue) during this process. By participating in oxidative phosphorylation, these molecules contribute to the generation of ATP.

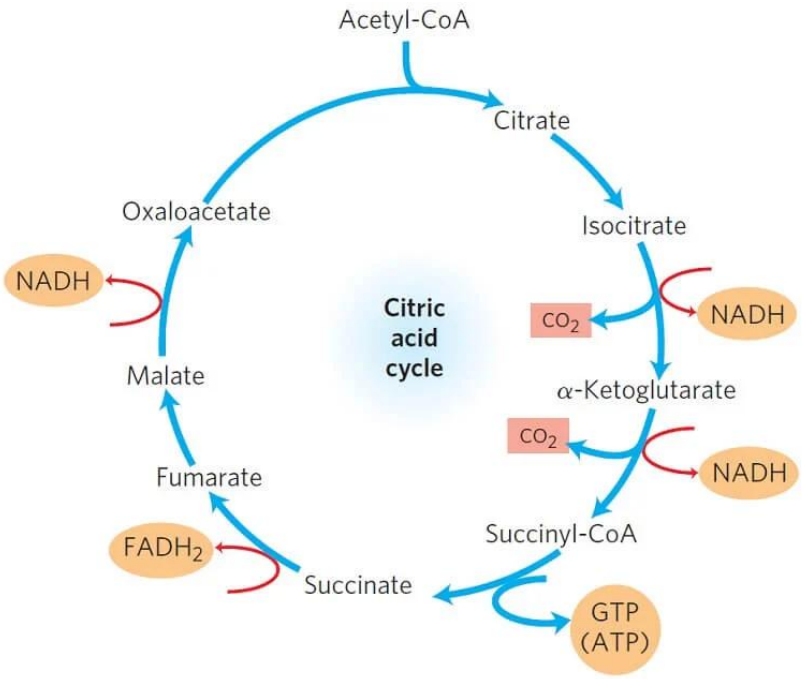


Figure 2: Citric Acid Cycle (TCA Cycle) and Its Major Metabolic Products

(Image Source: https://microbenotes.com/krebs-cycle/)

The mitochondrial matrix is the site of the majority of TCA cycle processes in eukaryotic cells. Succinate dehydrogenase, an essential enzyme found within mitochondria, is an outlier. Enabling

oxidative phosphorylation and the TCA cycle to be directly linked, this enzyme catalyses the conversion of succinate to fumarate while also serving as Complex II of the electron transport chain.

Carbohydrate, fatty acid, and amino acid catabolism can all produce acetyl-CoA, which can then enter the TCA cycle. Therefore, all three main nutrition classes are metabolically integrated via the TCA cycle, making it a key metabolic hub. A number of intermediates in the TCA cycle are not only involved in energy production, but also in the manufacture of glucose, heme, amino acids, and other crucial cellular components.

Glucose undergoes glycolysis to produce pyruvate, which is a necessary intermediate for the carbon atoms that come from glucose to join the TCA cycle. In addition to reducing NAD^+ to NADH, this process also releases CO_2 and creates acetyl-CoA.

$$\textbf{Pyruvate + CoA-SH + NAD}^+ \rightarrow \textbf{Acetyl-CoA + CO}_2 \textbf{ + NADH + H}^+$$

The acetyl group of acetyl-CoA then enters the TCA cycle by condensing with **oxaloacetate**, a four-carbon molecule, to form the six-carbon compound **citrate**. This reaction is catalysed by **citrate synthase** and represents the first step of the citric acid cycle. Through a series of subsequent enzymatic reactions, citrate is progressively oxidized, ultimately regenerating oxaloacetate and completing the cycle.

3.3.1 Reactions of TCA Cycle

The citric acid cycle consists of **eight sequential enzyme-catalysed reactions** through which the acetyl group of acetyl-CoA is oxidized to carbon dioxide, while energy is conserved in the form of NADH, $FADH_2$, and GTP. The reactions occur in a cyclic sequence, with oxaloacetate being regenerated at the end of each cycle to combine with another molecule of acetyl-CoA.

Reaction 1: Formation of Citrate

The TCA cycle begins with the condensation of **acetyl-CoA** with **oxaloacetate** to form the six-carbon compound citrate. The reaction is catalysed by **citrate synthase**.

$$\textbf{Acetyl-CoA + Oxaloacetate + H}_2\textbf{O} \rightarrow \textbf{Citrate + CoA-SH}$$

This reaction represents the first step of the cycle and is highly favourable under physiological conditions. The release of CoA-SH and the hydrolysis of the high-energy thioester bond of acetyl-CoA contribute to the thermodynamic favourability of the reaction.

Reaction 2: Isomerization of Citrate to Isocitrate

Citrate is reversibly converted into **isocitrate** through an isomerization reaction catalysed by the enzyme **aconitase**. The reaction occurs through the intermediate **cis-aconitate** and involves the removal and subsequent addition of water.

Citrate ⇌ Isocitrate

This rearrangement positions the hydroxyl group appropriately for the subsequent oxidative decarboxylation reaction.

Reaction 3: Oxidative Decarboxylation of Isocitrate

Isocitrate dehydrogenase catalyses the oxidative decarboxylation of isocitrate to form **α-**ketoglutarate (2-oxoglutarate). During this reaction, one molecule of carbon dioxide is released and NAD^+ is reduced to NADH.

Isocitrate + NAD^+ → α-Ketoglutarate + CO_2 + NADH + H^+

This is an important regulatory step of the TCA cycle and represents the first oxidative decarboxylation reaction in the cycle.

Reaction 4: Formation of Succinyl-CoA

α-Ketoglutarate undergoes oxidative decarboxylation to form succinyl-CoA. The reaction is catalysed by the α-ketoglutarate dehydrogenase complex and is accompanied by the release of carbon dioxide and reduction of NAD^+ to NADH.

α-Ketoglutarate + CoA-SH + NAD^+ → Succinyl-CoA + CO_2 + NADH + H^+

This reaction is essentially irreversible under physiological conditions and represents another major regulatory step of the TCA cycle.

Reaction 5: Formation of Succinate and GTP

Succinyl-CoA is converted into **succinate** by the enzyme **succinyl-CoA synthetase**, also known as succinate thiokinase. The energy released from the hydrolysis of the high-energy thioester bond is coupled to the formation of GTP from GDP and inorganic phosphate.

Succinyl-CoA + GDP + Pi ⇌ Succinate + CoA-SH + GTP

In some tissues, ATP rather than GTP is generated by the corresponding isoenzyme. This reaction represents substrate-level phosphorylation, as a nucleoside triphosphate is produced directly without involvement of the electron transport chain.

Reaction 6: Oxidation of Succinate to Fumarate

The oxidation of succinate to **fumarate** is catalysed by **succinate dehydrogenase**. During this reaction, FAD is reduced to $FADH_2$.

Succinate + FAD → Fumarate + $FADH_2$

Succinate dehydrogenase is unique because it is located in the inner mitochondrial membrane and also functions as **Complex II of the electron transport chain**. The electrons generated during succinate oxidation are subsequently transferred through the electron transport system.

Reaction 7: Formation of Malate

Fumarate is hydrated to form **L-malate** in a reversible reaction catalysed by the enzyme **fumarase**, also known as fumarate hydratase.

Fumarate + H_2O ⇌ Malate

The addition of water across the double bond of fumarate results in the formation of malate, which serves as the substrate for the final reaction of the cycle.

Reaction 8: Regeneration of Oxaloacetate

In the final reaction of the TCA cycle, **malate dehydrogenase** catalyses the oxidation of malate to regenerate **oxaloacetate**. NAD^+ is reduced to NADH during this reaction.

Malate + NAD^+ ⇌ Oxaloacetate + NADH + H^+

The oxaloacetate regenerated in this reaction is available to combine with another molecule of acetyl-CoA, allowing the cycle to continue.

Overall Outcome of One TCA Cycle

For each molecule of acetyl-CoA entering the TCA cycle, the pathway produces **three molecules of NADH, one molecule of $FADH_2$, one molecule of GTP (or ATP), and two molecules of CO_2**. The NADH and $FADH_2$ generated during the cycle transfer their high-energy electrons to the electron transport chain, where they contribute to ATP production through oxidative phosphorylation.

Thus, the TCA cycle serves not only as a major pathway for the oxidation of acetyl-CoA but also as a central metabolic hub that connects carbohydrate, lipid, and amino acid metabolism

- **Energy Yield of the TCA Cycle**

For every acetyl-CoA, one cycle of the citric acid cycle yields:

- 3 NADH
- 1 FADH2
- 1 GTP
- 2 CO2

These NADH and FADH2 then pass their electrons to the electron transport chain and help synthesize ATP via oxidative phosphorylation.

Based on approximate contemporary P/O ratios, the resulting reducing equivalents and GTP are equal to around 10 ATP equivalents.

Table 3: Products of One Turn of the TCA Cycle

Product	Amount per Acetyl-CoA	Major Significance
NADH	3	Supplies electrons to the ETC
$FADH_2$	1	Supplies electrons through Complex II
GTP	1	Direct energy-rich nucleotide product
CO_2	2	Released during oxidative decarboxylation
Approximate ATP equivalents	10	Includes oxidative phosphorylation of NADH/$FADH_2$ and GTP

3.3.2 Regulation and Significance

The cell's energy requirements and the availability of substrates determine how the TCA cycle is regulated. The reactions that citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase catalyse are the most important regulators. The control of the pyruvate dehydrogenase complex is crucial for the entrance of acetyl-CoA from carbohydrates.

Oxidation reactions are typically slowed by energy-rich molecules like ATP and NADH, whereas enzymes are activated by molecules that signal for additional energy, such as ADP and calcium.

Table 4: Major Regulatory Points of the TCA Cycle

Enzyme	Important Inhibitory Signals	Important Activating/Promoting Signals
Citrate synthase	ATP, NADH, citrate, succinyl-CoA in appropriate contexts	Substrate availability, especially oxaloacetate and acetyl-CoA
Isocitrate dehydrogenase	ATP, NADH	ADP; Ca^{2+} in certain tissues
α-Ketoglutarate dehydrogenase	NADH, succinyl-CoA	Ca^{2+} in certain tissues

❖ **Biological Significance of the TCA Cycle**

The TCA cycle plays an essential role in cellular metabolism, since it carries out both anabolic and catabolic roles and hence it is referred to as amphibolic pathway.

The main significance of TCA cycle lies in the fact that it oxidizes acetyl CoA, reduces the required substrates for oxidative phosphorylation, and provides the intermediate compounds for biosynthesis. Citrate provides the carbon for fatty acid and cholesterol biosynthesis, α-ketoglutarate and oxaloacetate are involved in amino acid metabolism, while succinyl CoA is involved in heme biosynthesis.

The removal of the TCA cycle intermediate compounds for biosynthetic purposes has to be counteracted by anaplerotic processes which provide the missing intermediates. One such process involves the conversion of pyruvate to oxaloacetate by pyruvate carboxylase.

As a result, the TCA cycle is pivotal for the regulation of amino acid, carbohydrate, and lipid metabolism. The glucose cycle begins with glycolysis, which yields pyruvate. From pyruvate, acetyl-CoA can be produced, and then it can be incorporated into the TCA cycle. Consequently, the electrons are transferred to the electron transport chain via the generated NADH and FADH2.

3.4 GLUCONEOGENESIS

Gluconeogenesis refers to the pathway used for the formation of glucose from non-carbohydrate sources. This process is especially significant when there is no food intake in the body or when there

is no more availability of dietary glucose or glycogen stores. It ensures that there is enough glucose present in the blood for tissues requiring glucose. The tissues requiring glucose are the brain (generally) and the red blood cells, as these require glucose due to the absence of mitochondria.

This process occurs mostly in the liver and the contribution from kidney cortex increases with extended periods of fasting. Most reactions happen in the cytoplasm although some reactions take place in the mitochondria and endoplasmic reticulum.

Gluconeogenic precursors include lactate, glycerol, and glucogenic amino acids. The lactate is formed by anaerobic glycolysis and the glycerol from the breakdown of triacylglycerol.

- **Major Steps of Gluconeogenesis**

Gluconeogenesis is more than just the reversal of glycolysis. Reversal of most of the reactions that take place during glycolysis is possible, but for the three very irreversible steps of glycolysis, it is not possible without any bypass mechanism.

1. Conversion of Pyruvate to Phosphoenolpyruvate

There are two stages to it. In the first, pyruvate carboxylase converts pyruvate to oxaloacetate. At this stage, ATP and biotin are utilised.

$$\text{Pyruvate} + CO_2 + ATP \rightarrow \text{Oxaloacetate} + ADP + P_i$$

Phosphoenolpyruvate carboxykinase (PEPCK) uses GTP to convert oxaloacetate to phosphoenolpyruvate (PEP). Because oxaloacetate cannot diffuse freely across the mitochondrial inner membrane, carbon transfer from the mitochondria to the cytosol must take place.

2. Conversion of Fructose-1,6-Bisphosphate to Fructose-6-Phosphate

The conversion of fructose-1,6-bisphosphate to fructose-6-phosphate is carried out by fructose-1,6-bisphosphatase. Bypassing the PFK-1 response is possible with the aforementioned method.

3. Conversion of Glucose-6-Phosphate to Glucose

The penultimate phase of bypass is the action of glucose-6-phosphatase, which converts glucose-6-phosphate to free glucose. Muscles do not contain this endoplasmic reticulum–related enzyme, which is abundant in the kidneys and liver. Therefore, the circulation cannot receive glucose directly from muscle glycogen.

Table 5: Major Bypass Reactions of Gluconeogenesis

Glycolytic Reaction Bypassed	Gluconeogenic Enzyme(s)	Major Conversion
Pyruvate kinase	Pyruvate carboxylase and PEPCK	Pyruvate → Oxaloacetate → PEP
PFK-1	Fructose-1,6-bisphosphatase	Fructose-1,6-bisphosphate → Fructose-6-phosphate
Hexokinase/Glucokinase	Glucose-6-phosphatase	Glucose-6-phosphate → Glucose

- **Regulation of Gluconeogenesis**

Glycolysis regulates gluconeogenesis antagonistically. The activation of glucogenic factors triggers glucose production in the liver in response to dangerously low blood sugar levels. Glucagon stimulates gluconeogenesis during fasting, in contrast to insulin's inhibition of it during fed circumstances.

The ratio of glycolysis to gluconeogenesis in the liver is regulated by fructose-2,6-bisphosphate. Fructose-2,6-bisphosphate activates PFK-1 and inhibits fructose-1,6-bisphosphatase. Reducing fructose-2,6-bisphosphate levels in the liver promotes gluconeogenesis.

- **Biological Significance**

Gluconeogenesis contributes to maintaining glucose levels in blood while fasting and generating glucose when there is no dietary carbohydrates. Additionally, gluconeogenesis plays a role in the cycling of lactate formed in the periphery. Lactate formed in the working muscle and red blood cells is transferred to the liver and recycled back to glucose through Cori's cycle.

3.5 HEXOSE MONOPHOSPHATE (HMP) SHUNT

Hexose Monophosphate Shunt

The hexose monophosphate (HMP) shunt or pentose phosphate pathway (PPP) is the alternative pathway of glucose catabolism. The pathway takes place in the cytosol and starts with the utilization of glucose-6-phosphate.

The HMP shunt is not an energy-yielding process like glycolysis; rather, it is involved in generating NADPH and ribose-5-phosphate.

It plays an important role in tissues where NADPH is needed for reductive biosynthesis or protection against free radicals, such as the liver, fat tissues, adrenal cortex, lactating mammary gland, and RBCs.

Hexose monophosphate shunt comprises of two main stages:

Oxidative stage Non-oxidative stage.

- **Oxidative Phase**

Oxidative step is irreversible in nature and yields NADPH. In the oxidative step, glucose-6-phosphate undergoes oxidation in various steps to yield ribulose-5-phosphate, carbon dioxide and NADPH.

Glucose-6-phosphate dehydrogenase is the first and main regulated enzyme.

Two molecules of NADPH are produced for every glucose-6-phosphate which undergoes oxidative steps.

- **Non-Oxidative Phase**

The second stage involves reversible reactions with sugar phosphate conversions. The ribulose-5-phosphate can thus be transformed into ribose-5-phosphate, which is necessary for nucleotide production.

The pentoses can be transformed into glycolysis precursors like fructose-6-phosphate and glyceraldehyde-3-phosphate if ribose is not required. Transketolase and transaldolase are two key enzymes involved in this process. Thiamin pyrophosphate is an essential cofactor for the second enzyme.

Table 6: Major Features of the HMP Shunt

Feature	Oxidative Phase	Non-Oxidative Phase
Nature	Irreversible	Reversible

Major starting substrate	Glucose-6-phosphate	Pentose phosphates
Major products/functions	NADPH, ribulose-5-phosphate and CO_2	Ribose-5-phosphate and glycolytic intermediates
Important enzymes	G6PD, 6-phosphogluconate dehydrogenase	Transketolase, transaldolase
Main importance	NADPH generation	Sugar-phosphate interconversion

❖ **Biological Importance of NADPH**

NADPH is involved in supplying reducing potential to various metabolic pathways. It is needed for:

- Synthesis of fatty acids and cholesterol;
- Steroid hormone synthesis;
- Glutathione reductase activity;
- Antioxidant system function;
- Cytochrome P450 system activation;
- Respiratory burst in phagocytes.

In erythrocytes, NADPH is especially crucial in ensuring the reduction of glutathione. This process protects haemoglobin and cell membrane from oxidation.

❖ **Ribose-5-Phosphate Formation**

Ribose-5-phosphate is needed in the formation of nucleotides and nucleic acids. This metabolic pathway may thus supply nutrients to fast-growing cells and tissues where nucleotide biosynthesis occurs.

❖ **Clinical Significance: G6PD Deficiency**

G6PD deficiency means that there is impairment in the capability of red blood cells to produce NADPH by using the HMP pathway.

When such people are exposed to certain oxidizing medications, infections, or food items such as fava beans, there is a triggering of haemolysis.

The clinical significance of G6PD deficiency underscores the importance of the HMP pathway for antioxidant defense.

Table 7: Major Functions of the HMP Shunt

Product/Function	Biological Importance
NADPH	Provides reducing power for biosynthetic reactions
Reduced glutathione maintenance	Protects cells, particularly erythrocytes, against oxidative damage
Ribose-5-phosphate	Required for nucleotide and nucleic-acid synthesis
Sugar interconversion	Connects pentose metabolism with glycolytic intermediates
NADPH oxidase support	Contributes to antimicrobial respiratory burst in phagocytes

3.6 GLYCOGEN METABOLISM

Animals store carbs as glycogen, a complex polysaccharide composed of glucose molecules with many branches. The liver and muscles are the primary tissue locations for glycogen, but it is present in other tissues to a lesser extent. While the liver is responsible for maintaining steady blood glucose levels—particularly in times of starvation—muscle glycogen is responsible for supplying energy molecules such as glucose to working muscles.

Glycogen metabolism includes the following two opposing reactions: the synthesis of glycogen, known as glycogenesis, and the catabolism of glycogen, called glycogenolysis.

Table 8: Comparison of Liver and Muscle Glycogen

Feature	Liver Glycogen	Muscle Glycogen
Principal role	Helps maintain blood glucose	Provides fuel for muscle contraction
Response to glucagon	Important	Little/no direct glucagon response
Response to adrenaline	Yes	Yes

Glucose-6-phosphatase	Present	Absent
Can directly release free glucose into blood	Yes	No
Main use during increased demand	Blood-glucose support	Local ATP production

3.6.1 Glycogenesis

Glycogenesis is the metabolic process by which glucose is transformed into glycogen, which can be stored for energy. When energy is present, the cytoplasmic reaction occurs most frequently in the muscles and liver.

Steps of Glycogenesis

Step 1: Glucose-6-phosphate Formation

Glucose is transformed into glucose-6-phosphate through the action of hexokinase or glucokinase.

Step 2: Glucose-1-phosphate Formation

Glucose-6-phosphate undergoes transformation to glucose-1-phosphate using the enzyme phosphoglucomutase.

Step 3: Formation of UDP-Glucose

The reaction between glucose-1-phosphate and UTP leads to UDP-glucose formation.

Step 4: Glycogen Synthesis Initiation

Glycogen synthesis starts with the formation of the primer by protein glycogenin.

Step 5: Glycogen Chain Elongation

The formation of α-1,4 glycosidic linkages is facilitated by glycogen synthase, an enzyme that plays a crucial role in glycogenesis by attaching glucose from UDP-glucose to the expanding glycogen chain.

Step 6: Branching

Formation of α-1,6 glycosidic bonds through the action of the branching enzyme results in branch formation.

Regulation of Glycogenesis

Glycogenesis is most stimulated during the fed state. Glycogen synthesis is promoted by insulin because it promotes activation of glycogen synthase.

The process of phosphorylation and dephosphorylation can regulate glycogen synthase. Phosphorylated forms are often less active than their unphosphorylated counterparts.

The meal's high carbohydrate content makes glucose readily available, which in turn stimulates the liver to produce glycogen. Skeletal muscle uses glycogen synthesis to restock its glycogen stores when they are depleted.

Biological Significance of Glycogenesis

Glycogenesis prevents the buildup of free glucose inside the cells and also provides a source of fast-acting carbohydrates. Glycogen present in the liver is responsible for maintaining blood glucose levels in the short run, while that in muscles acts as a local source of energy.

3.6.2 Glycogenolysis

Glycogenolysis is the degradation of glycogen to provide glucose units if energy or glucose is needed. Glycogenolysis takes place mainly in the liver and skeletal muscles.

Glycogenolysis is not merely the reverse of glycogenesis since it utilizes different enzymes.

Steps of Glycogenolysis

Step 1: Phosphorylative Breakdown of Glycogen

Alpha 1-4 glycosidic bonds present at the non-reducing ends of glycogen molecules are broken down using glycogen phosphorylase, yielding glucose-1-phosphate.

However, glycogen phosphorylase does not have the ability to cleave branches.

Step 2: Debranching and Degradation

The enzyme debranching removes branches from the glycogen molecules and allows its further degradation. A little amount of glucose is formed as a result of the breakdown of α-1,6-linked branch residues.

Step 3: Formation of Glucose-6-Phosphate

The phosphoglucomutase enzyme is responsible for converting glucose-1-phosphate to glucose-6-phosphate.

The location of the subsequent glucose-6-phosphate processing determines whether it occurs in the skeletal muscle or the liver.

The enzyme glucose-6-phosphatase delivers glucose into the bloodstream after converting glucose-6-phosphate if the activity is occurring in the liver.

But glucose-6-phosphatase does not exist.

Regulation of Glycogenolysis

- Glycogen degradation depends on hormonal inputs and energy needs of the cells.
- Glucagon induces glycogenolysis of the liver in case of a decrease in blood glucose level. Its predominant effect takes place in the liver as opposed to skeletal muscles.
- Epinephrine (adrenaline) causes glycogenolysis in the liver and skeletal muscles, especially in stressful situations and physical exercises.
- Glycogenolysis in skeletal muscles may be induced by high Ca^{2+} concentration during muscle contraction and energy status of the cell.

Insulin inhibits glycogenolysis and promotes glycogen synthesis.

Table 9: Comparison of Glycogenesis and Glycogenolysis

Feature	Glycogenesis	Glycogenolysis
Definition	Synthesis of glycogen	Breakdown of glycogen
Metabolic role	Glucose storage	Mobilization of stored carbohydrate
Major regulatory enzyme	Glycogen synthase	Glycogen phosphorylase
Activated predominantly when	Glucose/energy availability is high	Glucose or energy demand increases
Effect of insulin	Stimulates	Inhibits
Effect of glucagon in liver	Inhibits synthesis/favours opposite pathway	Stimulates
Important product	Glycogen	Glucose-1-phosphate
Major tissues	Liver and skeletal muscle	Liver and skeletal muscle

Glycogenesis and Glycogenolysis are a system that acts as a response to carbohydrate metabolism. They act in a complementary way to regulate the metabolism of glucose in the body. The regulation of these processes is vital for maintaining glucose levels in the blood and for ensuring enough energy in the skeletal muscles.

3.7 REGULATION OF BLOOD GLUCOSE LEVELS

Glucose concentration in the blood is regulated to be kept at a certain range via the concerted efforts of metabolic pathways and hormones. This process is called blood glucose homeostasis. It is crucial to keep glucose concentration in an appropriate range because glucose is a vital metabolic fuel in regular circumstances for the brain, whereas red blood cells require glucose for ATP production exclusively.

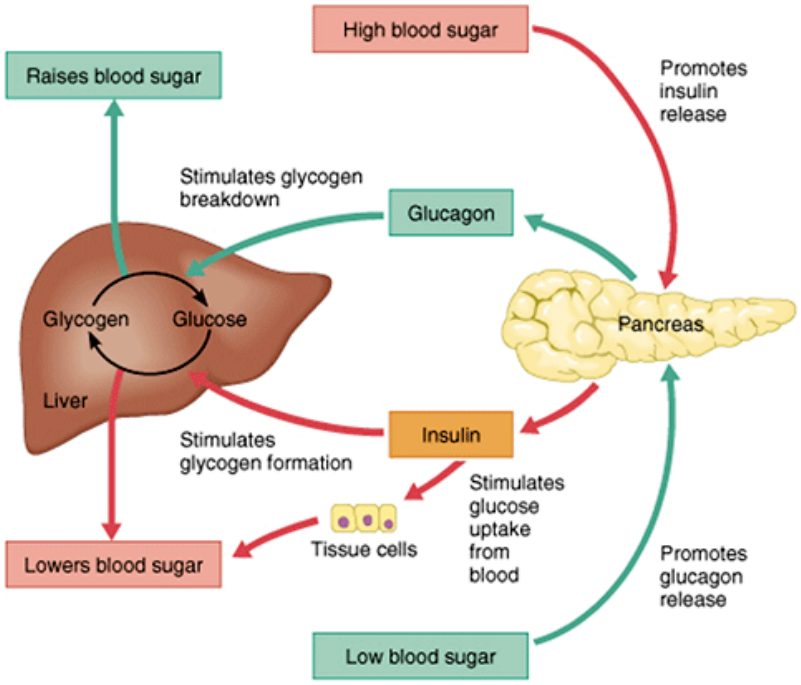


Figure 3: Hormonal Regulation of Blood Glucose by Insulin and Glucagon

(Image Source: https://www.pharmaguideline.com/2022/01/hormonal-regulation-of-blood-glucose-level.html)

Blood glucose concentration depends on the balance between the input of glucose into the blood and its elimination and use by cells. Glucose can enter the blood due to intestinal absorption following

meal consumption, as well as hepatic breakdown of glycogen and its synthesis from non-carbohydrate precursors. Its elimination occurs during cellular absorption, oxidation for energy production, glycogen formation and conversion into other metabolites.

The key importance of the liver in blood glucose control is conditioned by the fact that it is capable of storing glucose as glycogen and releasing glucose into the blood flow. Endocrine pancreas is also crucial in the regulation of blood glucose concentration due to insulin and glucagon secretion.

- **Role of Insulin**

Pancreatic islet β cells secrete insulin, a polypeptide hormone, when blood glucose and other nutrient levels rise. When the body is fed, it produces this hormone, which encourages nutrient storage and utilisation.

There are multiple ways in which insulin lowers blood glucose levels. By facilitating the passage of GLUT 4 glucose transporters to cell membranes, insulin enhances glucose absorption by insulin-sensitive organs such adipose tissues and muscle fibres. Insulin promotes glucose oxidation and glycolysis, in addition to enhancing hepatic and muscular glycogen production.

In addition, insulin inhibits glucose production in the liver by inhibiting glycogenolysis and gluconeogenesis. If energy input exceeds immediate needs, then insulin increases fatty-acid and triglyceride synthesis while decreasing lipolysis.

- **Role of Glucagon**

Glucagon is produced in the islet cells of the pancreas especially when the blood glucose level is low. The primary target of action of glucagon is the liver.

Glucagon facilitates glycogenolysis and gluconeogenesis leading to more glucose output in the blood. When in fasting state, glucagon is involved in maintaining glucose levels in the blood which is dependent on glucose.

Glucagon also facilitates the metabolic shift towards fat acid metabolism in fasting. The skeletal muscle does not react to glucagon for glycogenolysis

- **Other Hormones Affecting Blood Glucose**

There are some hormones that have opposing actions to some of the functions of insulin, which will help prevent hypoglycemia.

Adrenaline is a hormone that stimulates fuel release in cases of stress and exercise and glycogenolysis.

Cortisol is an example of a hormone that provides glucose during periods of stress, mainly through the stimulation of gluconeogenesis and substrate mobilization.

The growth hormone helps to conserve glucose by decreasing glucose uptake and increasing fat use.

Table 10: Major Hormonal Influences on Blood Glucose

Hormone	Major Source	Major Metabolic Actions Relevant to Glucose	General Effect on Blood Glucose
Insulin	Pancreatic β-cells	Increases glucose uptake in muscle/adipose tissue, glycogenesis and utilization; suppresses hepatic glucose production	Decreases
Glucagon	Pancreatic α-cells	Stimulates hepatic glycogenolysis and gluconeogenesis	Increases
Adrenaline	Adrenal medulla	Promotes rapid glycogen breakdown and fuel mobilization	Increases
Cortisol	Adrenal cortex	Supports gluconeogenesis and substrate mobilization	Tends to increase
Growth hormone	Anterior pituitary	Reduces glucose utilization in some tissues and promotes lipid utilization	Tends to increase

- **Role of the Liver in Blood Glucose Homeostasis**

Following a carbohydrate-rich diet, the liver absorbs glucose and produces some of it as glycogen. Once the level of blood glucose starts to fall between meals, glycogen in the liver is metabolized and glucose is secreted into the bloodstream.

In times of more extended fasting, hepatic gluconeogenesis becomes more prominent. Glucose production from the precursors such as lactate, glycerol, and glucogenic amino acids occurs in the liver. In fasting, the kidney also contributes to gluconeogenesis to some extent.

Therefore, the liver serves as a metabolic buffer between the states of eating and fasting.

Table 11: Major Processes Affecting Blood Glucose

Process	Major Effect	Predominant Physiological Situation
Intestinal glucose absorption	Adds glucose to blood	After a carbohydrate-containing meal
Glycogenesis	Stores glucose as glycogen	Fed state
Glycogenolysis	Mobilizes stored glycogen	Between meals and early fasting
Glycolysis	Utilizes glucose for energy/metabolism	Fed and energy-requiring conditions
Gluconeogenesis	Produces new glucose	Fasting and starvation
Peripheral glucose uptake	Removes glucose from circulation	Enhanced by insulin in muscle and adipose tissue

Disruption of these regulatory systems can cause either hyperglycemia or hypoglycemia. Hyperglycemia that persists is a distinguishing feature of diabetes mellitus which is covered later in this unit.

3.8 METABOLIC ADAPTATIONS

Metabolism of the body constantly varies depending on the presence of nutrients and fasting periods. The basic stages of metabolism can be generally classified into fed state, fasting state and starvation state.

The main aim of this process is provision of necessary energy supply to tissues along with maintenance of glucose level in the blood and saving the vital substances in the body. The use of carbohydrates, fats and proteins largely varies during these states.

3.8.1 Fed State

Fed or absorptive stage is that following food consumption and digestion. The concentration of blood glucose, amino acids, and other nutrients is increased due to their release from the GI system. Blood glucose stimulates the synthesis of insulin, while the ratio of insulin and glucagon increases.

The process of anabolism prevails in the fed stage because nutrients are utilized for energy supply or storage.

- **Carbohydrate Metabolism**

Glucose can be easily used by many tissues. Glucose utilization results in increased glycogenesis and glycolysis in the liver. Skeletal muscles increase glucose utilization through the effect of insulin to restore their glycogen reserves.

- **Lipid Metabolism**

Fatty acid and triacylglycerol synthesis are promoted when there is enough carbohydrate and energy available. Adipose tissue uses the fatty acids from lipoproteins in circulation, and even glucose can supply glycerol-3-phosphate for triacylglycerol synthesis. Insulin inhibits lipolysis in adipose tissue.

- **Protein Metabolism**

In order to make proteins and other compounds that contain nitrogen, the body uses the amino acids that are found in food. Insulin promotes the production of new proteins while simultaneously reducing their breakdown.

Table 12: Major Metabolic Features of the Fed State

Tissue	Major Metabolic Activities
Liver	Glycolysis, glycogenesis, fatty-acid synthesis and other biosynthetic processes
Skeletal muscle	Glucose uptake, glycogen synthesis and protein synthesis
Adipose tissue	Glucose uptake and triacylglycerol storage
Brain	Primarily utilizes glucose as metabolic fuel
Red blood cells	Utilize glucose through glycolysis and produce lactate

Thus, the fed state is characterized by nutrient utilization, biosynthesis and energy storage.

3.8.2 Fasting State

The fasting or post-absorptive condition starts when the absorption of nutrients from the previous meal has decreased significantly. The insulin level drops, whereas that of glucagon becomes more significant.

In metabolic terms, the priority moves from the accumulation of nutrients to the use of internal energy stores.

- **Maintenance of Blood Glucose**

Hepatic glycogenolysis contributes significantly to blood glucose during early fasting. However, as fasting progresses and hepatic glycogen stores get depleted, gluconeogenesis becomes more significant.

Major gluconeogenic substrates are lactate, glycerol, and glucogenic amino acids.

- **Lipid Mobilization**

Adipose tissue lipolysis is induced by low insulin and high counter regulatory hormones. Hydrolysis breaks down lipids into glycolipids and fatty acids.

The liver and muscles both benefit from fatty acids as an additional energy source. The process of gluconeogenesis can transport glycerol to the liver, where it is converted to glucose.

- **Protein Metabolism**

A few amino acids from protein breakdown could participate in the process of gluconeogenesis. But excessive use of protein will be very dangerous, therefore, adaptive changes in the case of extended fasting will lead towards protein conservation mechanisms.

Table 13: Major Changes During the Fasting State

Metabolic Process	General Change
Insulin secretion	Decreases
Relative glucagon influence	Increases
Hepatic glycogenolysis	Increases initially
Gluconeogenesis	Increases
Adipose lipolysis	Increases
Fatty-acid oxidation	Increases
Glycogen synthesis	Decreases
Fat synthesis	Decreases

Thus, fasting is characterized by an integrated process of switching from glucose storage to glucose formation and lipid breakdown.

3.8.3 Prolonged Starvation

Chronic starvation is characterized by continued food starvation beyond the time when glycogen stores in the liver can sustain the body. The glycogen stores become highly depleted, and metabolism changes to conserve glucose and reduce loss of body proteins.

- **Increased Gluconeogenesis**

Gluconeogenesis is still vital because there are some cells that still need glucose. The liver and kidney synthesize glucose from non-carbohydrate precursors.

The red blood cells still need glucose because they have no mitochondria. Glucose is metabolized to lactate, which can later on be used in gluconeogenesis by the liver.

- **Increased Fatty-Acid Oxidation**

Lipolysis continues in adipose tissue by secreting fatty acids. The majority of tissues become more reliant on fatty acids as an energy source, thus decreasing their dependency on glucose.

Fatty acid oxidation in the liver produces a large quantity of acetyl-CoA. Whenever there is excess acetyl-CoA that cannot be oxidized through the citric acid cycle, it is transformed into ketone bodies.

- **Ketone-Body Utilization**

Ketone bodies serve as significant sources of energy during starvation over an extended period. Adaptation allows the brain to produce a significant amount of energy by using ketone bodies, which helps in lowering its dependence on glucose.

This is essential because lowered glucose need reduces the need for gluconeogenesis from amino acids.

- **Protein Conservation**

In the early stages of fasting, amino acids play a major role in the process of gluconeogenesis. In the case of extended starvation and use of ketones, the demand for glucose from amino acids declines. Thus, the rate of degradation of protein becomes reduced in comparison with early fasting stages.

Protein conservation is physiologically significant since body proteins serve many different functions.

Table 14: Comparison of Fed, Fasting and Prolonged Starvation States

Feature	Fed State	Fasting State	Prolonged Starvation
Insulin	High	Low	Very low
Glucagon influence	Relatively low	Increased	Increased
Major glucose source	Dietary carbohydrate	Liver glycogen + gluconeogenesis	Gluconeogenesis
Glycogenesis	Increased	Decreased	Minimal
Glycogenolysis	Low	Increased initially	Limited after glycogen depletion
Gluconeogenesis	Low	Increased	Important
Lipolysis	Suppressed	Increased	Markedly increased
Fatty-acid oxidation	Relatively lower	Increased	High
Ketone-body production	Low	Begins to increase with fasting	Markedly increased
Brain fuel	Mainly glucose	Mainly glucose initially	Increasing use of ketone bodies plus glucose
Protein breakdown	Low/net synthesis favoured	Increased contribution to substrate supply	Relatively reduced after adaptation to conserve protein

The fed state, fasting state, and starvation illustrate the integration of human metabolism. When fed, insulin causes the use of glucose in the body. In the fasting state, glycogen degradation, gluconeogenesis, and fatty acid oxidation ensure there is energy in the body. When starving for an extended period, there is increased fatty acid oxidation and ketone body utilization, thus decreasing

glucose needs and protecting the proteins in the body. The above process is also useful in the study of abnormalities in fuel metabolism in diabetes mellitus

3.9 DIABETES MELLITUS

Metabolic disorders known together as diabetes mellitus (DM) include insulin secretion or action abnormalities, or both, leading to persistently high blood sugar levels. In order for the body's metabolism of carbs, lipids, and proteins to work in tandem, the hormone insulin is crucial. Metabolic abnormalities beyond glucose metabolism will occur as a result of any deficiencies in insulin synthesis and activity.

Both type 1 and type 2 diabetes mellitus are common, however there are two distinct forms of the disease. The β-cells of the pancreas are destroyed in an autoimmune process, resulting in type 1 diabetes. The symptoms of type 2 diabetes include insulin resistance and the slow decline in β-cell activity. Other forms of diabetes include gestational diabetes and those that have a hereditary or other causal component.

Table 15: Major Types of Diabetes Mellitus

Type	Principal Abnormality	Major Biochemical Feature
Type 1 DM	Autoimmune β-cell destruction	Marked/absolute insulin deficiency
Type 2 DM	Insulin resistance with progressive β-cell dysfunction	Reduced effectiveness of insulin with relative insulin deficiency
Gestational diabetes	Hyperglycaemia first recognized during pregnancy	Pregnancy-associated insulin resistance with inadequate compensatory insulin secretion
Other specific types	Genetic, pancreatic, endocrine or drug-related causes	Depends on the underlying defect

Diabetes mellitus plays a significant role in biochemistry due to the role played by hormonal imbalance in the metabolic processes of carbohydrates, fats and proteins.

3.9.1 Biochemical Basis of Diabetes Mellitus

Diabetes Mellitus biochemical mechanism is strongly linked to insulin deficiency or resistance. Normally, the role of insulin is to assist in glucose uptake and metabolism in insulin-sensitive tissues, stimulate glycogenesis, synthesis of lipids and proteins while inhibiting gluconeogenesis and lipolysis in the liver and fat tissue.

If insulin resistance occurs, the above process gets disrupted leading to an increase in blood glucose level.

- **Impaired Glucose Uptake**

In skeletal muscles and adipose tissues, insulin is responsible for stimulating glucose uptake by translocating GLUT4 transporters to the cell membrane. Insulin resistance or absence of insulin leads to the inability of insulin stimulation of glucose uptake, which causes hyperglycemia.

Some tissues do not need insulin to stimulate glucose uptake. The brain and erythrocytes are two such tissues.

- **Increased Hepatic Glucose Production**

Insulin is responsible for inhibiting hepatic glucose production via gluconeogenesis and glycogenolysis. In the presence of inadequate activity of insulin, accompanied by dominance of counteracting hormones, hepatic glucose production is stimulated.

Increased glucose production and decreased glucose utilization together significantly contribute to hyperglycemia.

- **Altered Glycogen Metabolism**

Decreased effective action of insulin reduces glycogen formation especially in the liver and muscles. Glucose production by the liver can be increased even if the individual suffers from hyperglycemia.

- **Altered Lipid Metabolism**

In normal circumstances, insulin acts to reduce lipolysis in the fat cells. Insulin deficiency results in the breakdown of the triacylglycerols into free fatty acids and glycerol.

The free fatty acids move to the liver where they are oxidized by β-oxidation. In cases of extreme insulin deficiency, there is excessive synthesis of ketone bodies.

❖ **Altered Protein Metabolism**

Insulin increases protein synthesis and decreases protein degradation. Inadequate effects of insulin will increase proteolysis, leading to the liberation of amino acids that can be used for gluconeogenesis by the liver.

Table 16: Major Biochemical Changes in Diabetes Mellitus

Metabolic Process	Normal Effect of Insulin	Change with Inadequate Insulin Action
Peripheral glucose uptake	Increased	Decreased
Glycogenesis	Increased	Decreased
Hepatic gluconeogenesis	Suppressed	Increased
Hepatic glycogenolysis	Suppressed	Increased
Lipolysis	Suppressed	Increased, especially with severe insulin deficiency
Ketogenesis	Suppressed	May increase markedly
Protein synthesis	Promoted	Reduced
Proteolysis	Suppressed	Increased in poorly controlled states

Therefore, hyperglycemia seen in diabetes is not due to an abnormality in one pathway alone but occurs due to a combination of defective glucose disposal and overproduction of glucose, along with metabolic abnormalities in proteins and lipids.

3.9.2 Metabolic Derangements in Diabetes Mellitus

Continuous deficiency of insulin or its resistance causes specific disorders in metabolism of carbohydrates, fats and proteins. This varies depending on the type and level of metabolic control.

❖ Carbohydrate Metabolism

Carbohydrate malfunction that occurs is hyperglycaemia. This is because there is insufficient uptake of glucose via insulin in tissues such as skeletal muscles, together with high rates of glucose synthesis in the liver resulting in increased blood glucose. As blood glucose rises above what the kidney can absorb, there is presence of glucose in urine; glycosuria. The presence of glucose in the tubular fluid causes osmotic action with resultant excessive loss of water; osmotic diuresis, polyuria. Water loss causes thirst, polydipsia. There is loss of glucose in urine which serves as energy source.

❖ Lipid Metabolism

Insulin dysfunction causes changes in lipid metabolism and storage. When there is a serious deficiency of insulin, adipose tissue lipolysis leads to an increase in free fatty acids in circulation. The liver converts some of the generated acetyl-CoA into ketones acetoacetate, β-hydroxybutyrate, and acetone.

Overproduction of ketones can cause the condition of ketonaemia and ketonuria. As acetoacetate and β-hydroxybutyrate are acidic, overproduction of them can cause metabolic acidosis.

Changes in lipid metabolism also occur in type 2 diabetes, which might be manifested in elevated triglycerides, decreased HDL and quantitative changes in LDL particles.

❖ Protein Metabolism

Uncontrolled diabetes leads to enhanced protein degradation and reduced synthesis of proteins. The released amino acids may be used in gluconeogenesis.

With serious cases of insufficient insulin, the loss of protein, fat, and carbohydrate leads to weight loss, especially in type 1 diabetes.

Table 17: Metabolic Derangements and Their Consequences in Diabetes

Derangement	Biochemical Change	Possible Consequence
Reduced glucose utilization	Decreased insulin-dependent glucose uptake	Hyperglycaemia
Increased hepatic glucose output	Increased gluconeogenesis/glycogenolysis	Fasting hyperglycaemia

Glycosuria	Urinary loss of glucose	Osmotic diuresis
Osmotic diuresis	Increased urinary water loss	Polyuria and dehydration
Increased lipolysis	Increased free fatty acids	Increased hepatic fatty-acid oxidation
Excess ketogenesis	Increased ketone bodies	Ketoacidosis in severe cases
Increased proteolysis	Amino-acid mobilization	Muscle wasting and gluconeogenic substrate supply

- **Diabetic Ketoacidosis**

The condition of diabetic ketoacidosis (DKA) is one that is commonly observed in patients suffering from Type 1 diabetes, but can occur in other cases as well.

Insulin deficiency and heightened levels of counter-regulatory hormones contribute to this condition. Lipolysis due to the diminished effects of insulin increases the levels of free fatty acids entering the liver, resulting in ketone body formation.

Metabolic acidosis occurs because of acidic ketone bodies building up inside the body. DKA is always accompanied by the following factors: hyperglycemia, electrolyte imbalance and dehydration.

- **Hyperosmolar Hyperglycaemic State**

The HHS is more prevalent among patients with type 2 diabetes than among those with type 1 diabetes. The HHS is associated with high levels of glucose and osmolality but low amounts of ketoacidosis.

- **Advanced Glycation End Products**

Hyperglycemia causes non-enzymatic interaction between glucose and proteins leading to the production of AGEs.

AGE production may cause structural and functional changes on proteins leading to vascular and tissue anomalies. Hyperglycemia may cause oxidative and cellular stress through biochemical mechanisms.

3.9.3 Related Metabolic Disorders

There are various metabolic disorders that have been linked to diabetes mellitus. Some of them arise as a result of significant insulin deficiency, while other metabolic disorders usually occur with insulin resistance.

- **Insulin Resistance**

Insulin resistance refers to a state whereby normal or high levels of insulin cause a subnormal physiological response. Insulin resistance often occurs in the liver, muscles and fat tissues.

To begin with, the body responds to the presence of insulin resistance by secreting increased amounts of insulin from the β-cells. When the β-cells are unable to secrete sufficient insulin, the body develops type 2 diabetes.

- **Metabolic Syndrome**

Cardiometabolic disease risk factors are often associated with central obesity, hypertension, diabetes, elevated triglycerides, and low HDL cholesterol; these conditions are together referred to as metabolic syndrome.

In this group, there is a high degree of association with insulin resistance.

- **Dyslipidaemia**

There exists a significant link between diabetes and dyslipidemia, especially type 2 diabetes. This usually involves high levels of triglycerides with low levels of HDL cholesterol, and this may involve alterations in the qualities of LDL particles.

These disturbances in lipids account for the heightened incidence of atherosclerosis in diabetic individuals.

- **Ketosis and Ketoacidosis**

Definition of ketosis is excess production and accumulation of ketone bodies. Moderate and physiological ketosis may result from prolonged fasting, but diabetic conditions can lead to excess accumulation of ketone bodies.

Excessive accumulation of ketones leading to decreased levels of blood pH leads to ketoacidosis. Ketoacidosis resulting from diabetic conditions is known as diabetic ketoacidosis.

❖ Lactic Acidosis

Lactic acidosis is due to an excess build-up of lactate with an acid base disorder. Lactic acidosis happens whenever there is more lactate being produced than the body can process. This can happen because of hypoxic state or certain metabolic problems. Lactic acidosis does not commonly happen with diabetes patients.

Table 18: Important Metabolic Disorders Related to Diabetes

Disorder	Principal Biochemical Abnormality	Major Significance
Insulin resistance	Reduced tissue response to insulin	Central feature of type 2 diabetes
Metabolic syndrome	Cluster of glucose, lipid, blood-pressure and adiposity abnormalities	Increased diabetes and cardiovascular risk
Dyslipidaemia	Abnormal plasma lipid/lipoprotein profile	Increases cardiovascular risk
Ketosis	Increased ketone-body production	May be physiological or pathological depending on degree/context
Diabetic ketoacidosis	Severe ketonaemia with metabolic acidosis	Acute medical emergency
Hyperosmolar hyperglycaemic state	Severe hyperglycaemia and hyperosmolality	Acute metabolic emergency
Lactic acidosis	Excessive lactate accumulation with acidosis	Occurs in specific pathological conditions

❖ Long-Term Metabolic Consequences

Chronic hyperglycemia may lead to progressive damage of blood vessels and organs. Complications of diabetes are usually divided into microvascular and macrovascular complications.

Diabetic nephropathy, neuropathy, and diabetic retinopathy are microvascular consequences. Cardiovascular disease, brain disease, and peripheral arterial disease are examples of macrovascular consequences.

In conclusion, diabetes mellitus can be defined as a disease that results from faulty metabolism of fuel and not an abnormality of glucose. Diabetes mellitus results from poor insulin secretion and its action, resulting in disturbances in carbohydrate, protein, and lipid metabolism and leading to hyperglycemia. In extreme cases, diabetes leads to the production of ketones, dehydration and imbalance in body electrolytes.

REFERENCES

1. Fu, Y., Zou, T., Shen, X., Nelson, P. J., Li, J., Wu, C., ... & Dong, Q. (2021). Lipid metabolism in cancer progression and therapeutic strategies. *MedComm*, *2*(1), 27-59.
2. Garst, A. D., Bassalo, M. C., Pines, G., Lynch, S. A., Halweg-Edwards, A. L., Liu, R., ... & Gill, R. T. (2017). Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. *Nature biotechnology*, *35*(1), 48-55.
3. Gasmi, A., Peana, M., Arshad, M., Butnariu, M., Menzel, A., & Bjørklund, G. (2021). Krebs cycle: activators, inhibitors and their roles in the modulation of carcinogenesis. *Archives of toxicology*, *95*(4), 1161-1178.
4. Girigoswami, K., Harini, K., & Girigoswami, A. (2025). Bioenergetic of enzymatic reaction. In *Introduction to Metabolic Engineering and Application* (pp. 281-294). Cham: Springer Nature Switzerland.
5. Griffiths, J. K. (2020). Vitamin deficiencies. In *Hunter's tropical medicine and emerging infectious diseases* (pp. 1042-1047). Elsevier.
6. Guasch-Ferré, M., Santos, J. L., Martínez-González, M. A., Clish, C. B., Razquin, C., Wang, D., ... & Ruiz-Canela, M. (2020). Glycolysis/gluconeogenesis-and tricarboxylic acid cycle–related metabolites, Mediterranean diet, and type 2 diabetes. *The American journal of clinical nutrition*, *111*(4), 835-844.
7. Guo, H., & Mi, P. (2024). Polymer–drug and polymer–protein conjugated nanocarriers: design, drug delivery, imaging, therapy, and clinical applications. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *16*(4), e1988.
8. Gupta, A. (2018). Metabolism of Carbohydrates. In *Comprehensive Biochemistry for Dentistry: Textbook for Dental Students* (pp. 403-440). Singapore: Springer Singapore.
9. Gyamfi, D., Awuah, E. O., & Owusu, S. (2019). Lipid metabolism: an overview. *The molecular nutrition of fats*, 17-32.
10. Haddad, A., & Mohiuddin, S. S. (2023). Biochemistry, citric acid cycle. In *StatPearls [internet]*. StatPearls Publishing.
11. Hallan, S., Afkarian, M., Zelnick, L. R., Kestenbaum, B., Sharma, S., Saito, R., ... & de Boer, I. H. (2017). Metabolomics and gene expression analysis reveal down-regulation of the citric acid (TCA) cycle in non-diabetic CKD patients. *EBioMedicine*, *26*, 68-77.

12. Han, X. (2016). Lipidomics for studying metabolism. *Nature Reviews Endocrinology*, *12*(11), 668-679.
13. Hanna, M., Jaqua, E., Nguyen, V., & Clay, J. B. (2022). Vitamins: functions and uses in medicine. *Perm J*, *26*(2), 89-97.
14. He, Z., Pan, L., Xu, Q., Zhou, Y., Li, P., Li, Z., & Wang, Q. (2024). Studies on the characteristics of polycyclic aromatic hydrocarbons accumulation in lipids and the disturbance of lipid metabolism of Ruditapes philippinarum. *Chemosphere*, *364*, 143304.
15. Hildebrandt, T. M. (2018). Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. *Plant molecular biology*, *98*(1), 121-135.
16. Hildebrandt, T. M., Nesi, A. N., Araújo, W. L., & Braun, H. P. (2015). Amino acid catabolism in plants. *Molecular plant*, *8*(11), 1563-1579.
17. Holeček, M. (2018). Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. *Nutrition & metabolism*, *15*(1), 33.
18. Holeček, M. (2023). Role of impaired glycolysis in perturbations of amino acid metabolism in diabetes mellitus. *International journal of molecular sciences*, *24*(2), 1724.
19. Hong, J., Tong, H., Wang, X., Lv, X., He, L., Yang, X., ... & Lu, Y. (2023). Embryonic diapause due to high glucose is related to changes in glycolysis and oxidative phosphorylation, as well as abnormalities in the TCA cycle and amino acid metabolism. *Frontiers in Endocrinology*, *14*, 1135837.
20. Huang, C., & Freter, C. (2015). Lipid metabolism, apoptosis and cancer therapy. *International journal of molecular sciences*, *16*(1), 924-949.
21. Hubler, M. J., & Kennedy, A. J. (2016). Role of lipids in the metabolism and activation of immune cells. *The Journal of nutritional biochemistry*, *34*, 1-7.
22. Hui, S., Ghergurovich, J. M., Morscher, R. J., Jang, C., Teng, X., Lu, W., ... & Rabinowitz, J. D. (2017). Glucose feeds the TCA cycle via circulating lactate. *Nature*, *551*(7678), 115-118.
23. Jenzer, H., & Sadeghi-Reeves, L. (2020). Nutrigenomics-associated impacts of nutrients on genes and enzymes with special consideration of aromatase. *Frontiers in nutrition*, *7*, 37.
24. Ji, Y., Wu, Z., Dai, Z., Sun, K., Wang, J., & Wu, G. (2016). Nutritional epigenetics with a focus on amino acids: implications for the development and treatment of metabolic syndrome. *The Journal of nutritional biochemistry*, *27*, 1-8.

25. Jun, S., Datta, S., Wang, L., Pegany, R., Cano, M., & Handa, J. T. (2019). The impact of lipids, lipid oxidation, and inflammation on AMD, and the potential role of miRNAs on lipid metabolism in the RPE. *Experimental eye research*, *181*, 346-355.
26. Kelly, B., & Pearce, E. L. (2020). Amino assets: how amino acids support immunity. *Cell metabolism*, *32*(2), 154-175.
27. Khandbahale, S. V., Pagar, K. R., & Khankari, R. V. (2019). Introduction to enzymes. *Asian Journal of Research in Pharmaceutical Science*, *9*(2), 123-130.
28. Kiani, A. K., Dhuli, K., Donato, K., Aquilanti, B., Velluti, V., Matera, G., ... & Bertelli, M. (2022). Main nutritional deficiencies. *Journal of preventive medicine and hygiene*, *63*(2 Suppl 3), E93.
29. Ko, C. W., Qu, J., Black, D. D., & Tso, P. (2020). Regulation of intestinal lipid metabolism: current concepts and relevance to disease. *Nature Reviews Gastroenterology & Hepatology*, *17*(3), 169-183.
30. Kontoghiorghes, G. J., & Kontoghiorghe, C. N. (2020). Iron and chelation in biochemistry and medicine: new approaches to controlling iron metabolism and treating related diseases. *Cells*, *9*(6), 1456.

Unit 4…

LIPIDS AND LIPID METABOLISM

Lipids are a class of varied organic molecules that have several roles to play in the body structure and functioning of the human being. They offer an excellent reserve of energy; are an integral part of the biological membrane, help in fat-soluble vitamin absorption and also act as precursors for various metabolically active compounds. Most lipids are very sparingly soluble in water and thus require special biochemical processes for their digestion, transportation, storage and utilization. Understanding lipid metabolism will enable us to understand energy metabolism as well as diseases like hyperlipidaemia, fatty liver and atherosclerosis.

4.1 INTRODUCTION, CLASSIFICATION AND FUNCTIONS OF LIPIDS

Lipids represent a diverse set of organic molecules which are not very soluble in water but readily soluble in non-polar organic solvents. In general, most biological lipids have either fatty acids or molecules similar in structure to the metabolites of lipids.

Lipids are acquired through food as well as produced internally. Lipids are usually stored in adipose tissue in the form of triacylglycerols (triglycerides) and released when energy is needed. The other types of lipids, especially phospholipids and cholesterol, play a role as structural components of cell membranes.

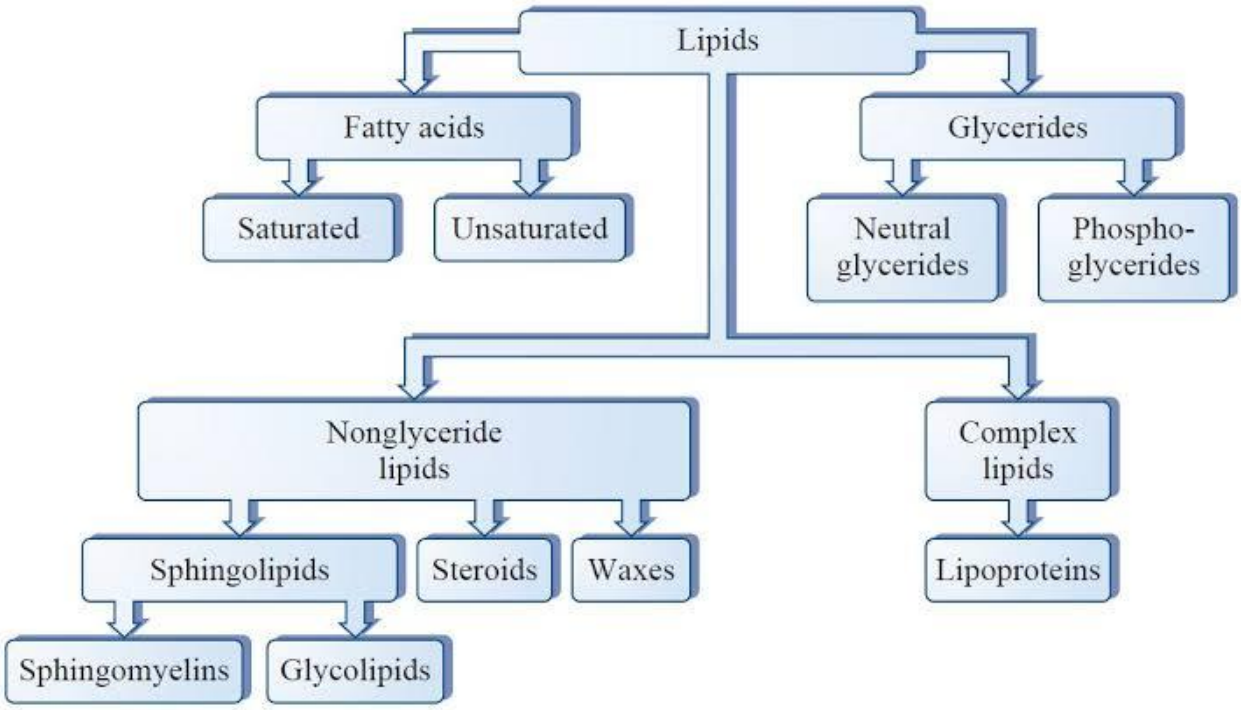


Figure 1: Classification of Lipids and Their Major Types

(Image Source: https://alevelbiology.co.uk/notes/lipids-introduction-and-classification/)

❖ **Classification of Lipids**

The lipids are basically of three types: Simple Lipids, Compound Lipids and Derived Lipids.

Simple Lipids are the ester derivatives of fatty acids with some alcohol. The best examples of simple lipids are fats and oils, which are made up mostly of triglycerides. The other example is that of waxes.

Compound lipids have some extra chemical groups besides fatty acids and alcohol. Phospholipids and Glycolipids are the best examples of compound lipids.

The derived lipids are those lipids which can be found when the simple and complex lipids are hydrolyzed or modified. Some of the examples of such lipids are the fatty acids, sterols like cholesterol and steroid derivatives.

Table 1: Major Classification of Lipids

Class	Major Examples	Principal Importance
Simple lipids	Triacylglycerols, waxes	Energy storage and protective functions
Complex lipids	Phospholipids, glycolipids	Cell membranes and specialized cellular functions
Derived lipids	Fatty acids, cholesterol, steroid derivatives	Energy metabolism, membrane structure and precursor functions

❖ Fatty Acids

The primary building blocks of many different types of lipids are fatty acids. The hydrocarbon chains of these fatty acids are characterised by the presence of carboxyl groups at the very end, allowing for their categorisation according to the carbon-carbon double bonds that they contain.

Saturated fatty acids are a type of fatty acid that does not contain any carbon chains. Unsaturated fatty acids, in contrast, are those that contain both carbon and carbon double bonds.

Since the human body is unable to manufacture enough of certain unsaturated fatty acids, they are considered nutritionally essential. The two primary necessary fatty acids in humans are linoleic acid and α-linolenic acid.

❖ Functions of Lipids

Lipids play multiple roles within the body.

Energy storage: Triacylglycerides serve as the main energy storage form. The oxidation of fatty acids produces a significant amount of energy.

Structure: Phospholipids, glycolipids, and cholesterol play an important role in the structure of biological membranes.

Thermal insulation: Lipids serve as a thermal insulator and protect the body from losing its warmth and provide cushioning for some internal organs.

Vitamin absorption: Dietary lipids enable the absorption of the fat-soluble vitamins A, D, E, and K.

Biosynthetic roles: Cholesterol acts as a precursor for steroid hormones, bile acids, and vitamin D. Certain polyunsaturated fatty acids act as biosynthetic precursors for lipid signaling molecules.

Table 2: Major Biological Functions of Lipids

Function	Major Lipid Involved	Biological Importance
Energy storage	Triacylglycerols	Long-term energy reserve
Membrane structure	Phospholipids, glycolipids, cholesterol	Maintain membrane organization and properties
Thermal insulation	Stored triacylglycerol in adipose tissue	Reduces heat loss
Organ protection	Adipose tissue	Provides mechanical cushioning
Vitamin absorption	Dietary lipids	Supports absorption of vitamins A, D, E and K
Precursor function	Cholesterol, polyunsaturated fatty acids	Formation of hormones, bile acids and signalling molecules

Thus, lipids are not merely energy-storage molecules; they are essential for cellular structure, metabolic regulation, signalling and normal physiological function.

4.2 LIPOPROTEINS AND LIPID TRANSPORT

The lipids like triacylglycerols and cholesterol have poor solubility in water in which blood exists. Hence, the transport of these substances needs special molecules called lipoproteins.

The composition of lipoproteins includes lipids and some special proteins named apolipoproteins. The hydrophobic interior is made up mostly of triacylglycerols and cholesteryl esters while phospholipids, free cholesterol, and apolipoproteins are present on the surface, making the molecules move in plasma.

The apolipoproteins have both structural and metabolic roles. They help to maintain the structure of lipoproteins, act as ligands for receptors on cells, and activate some metabolic enzymes.

There are four major plasma lipoproteins – chylomicrons, (VLDL), (LDL) and (HDL).

Table 3: Major Classes of Plasma Lipoproteins

Lipoprotein	Major Lipid Carried	Principal Origin	Major Function
Chylomicrons	Dietary triacylglycerols	Intestine	Transport dietary lipids to peripheral tissues
VLDL	Endogenous triacylglycerols	Liver	Transport liver-derived triacylglycerols
LDL	Cholesterol/cholesteryl esters	Derived mainly from VLDL metabolism	Delivers cholesterol to peripheral tissues
HDL	Cholesterol	Liver and intestine; remodeled in plasma	Participates in reverse cholesterol transport and lipoprotein metabolism

4.2.1 Chylomicrons and VLDL

- **Chylomicrons**

Chylomicrons are large triacylglycerol-laden lipoproteins that are synthesized in the intestinal epithelium following ingestion of lipids. They are major carriers of dietary triacylglycerols from the intestine to peripheral tissues.

Fatty acid carriers like chylomicrons usually contain the apolipoprotein B-48 (ApoB-48) as a significant component. Once released into the circulation, more apolipoproteins are added by HDL.

In capillaries of tissues such as fat tissue and muscles, lipoprotein lipase (LPL) will hydrolyze triacylglycerols in chylomicrons. The free fatty acids will now be utilized by the tissues for energy production or storage.

Upon the removal of triacylglycerols, chylomicrons will become smaller and eventually called chylomicron remnants. Chylomicron remnants will then be taken up by the liver.

- **Very-Low-Density Lipoproteins**

Very low density lipoproteins (VLDL) are formed mainly in the liver. The main role of VLDL is to carry triacylglycerols that have been synthesized within the liver to peripheral tissues.

VLDL possesses ApoB-100 as its principal apolipoprotein. VLDL, just like chylomicrons, undergoes lipolysis by lipoprotein lipase, resulting in release of free fatty acids.

Due to loss of triacylglycerols, VLDL will become increasingly smaller and denser, thereby forming remnants and eventually LDL.

Table 4: Comparison of Chylomicrons and VLDL

Feature	Chylomicrons	VLDL
Site of formation	Intestinal epithelial cells	Liver
Main lipid transported	Dietary triacylglycerol	Endogenous triacylglycerol
Major structural apolipoprotein	ApoB-48	ApoB-100
Major function	Transport dietary fat	Transport liver-derived triacylglycerol
Action of LPL	Releases fatty acids from triacylglycerol	Releases fatty acids from triacylglycerol
Major metabolic fate	Chylomicron remnants taken up by liver	Converted through remnant stages, contributing to LDL formation

4.2.2 LDL and HDL

- **Low-Density Lipoproteins**

Low density lipoprotein (LDL) refers to cholesterol containing lipoproteins that result from the catabolism of VLDL and its remnants.

The primary role of LDL is to transport cholesterol to peripheral tissues. LDL can be endocytosed via LDL receptors that identify ApoB-100. The cholesterol is utilized by cells for synthesis of cell membranes and other cholesterol-based molecules.

High levels of LDL cholesterol in circulation are significant since LDL is capable of entering the intima of blood vessels and causing atherosclerosis. Due to this, LDL cholesterol is often considered an atherogenic lipoprotein marker.

❖ High-Density Lipoproteins

High-density lipoproteins (HDL) are smaller-sized lipoproteins that are high in proteins and play roles in various lipid transport activities. An important apolipoprotein in HDL is ApoA-I.

One of the important roles played by HDL is that of reverse cholesterol transport, where the surplus of cholesterol is taken up by HDL and then delivered to the liver.

HDL provides storage and transfer of some of the apolipoproteins necessary in the metabolism of other lipoproteins.

The higher levels of HDL cholesterol have been shown to correlate with the lower levels of cardiovascular risks epidemiologically; but HDL and cardiovascular risk is more complicated than HDL being labeled as "good cholesterol."

Table 5: Comparison of LDL and HDL

Feature	LDL	HDL
Major lipid association	Cholesterol/cholesteryl esters	Cholesterol transport and exchange
Important apolipoprotein	ApoB-100	ApoA-I
Major role	Delivers cholesterol to peripheral tissues	Participates in reverse cholesterol transport
Receptor/metabolic importance	ApoB-100 interacts with LDL receptor	Accepts and transports cholesterol from peripheral cells
Clinical association	Elevated LDL-C is strongly associated with atherosclerotic cardiovascular risk	HDL-C is inversely associated with risk epidemiologically, although HDL function is complex

This means that the various lipoprotein fractions constitute a complete transport system. Chylomicrons are involved in the transport of dietary triacylglycerols, VLDL in the transport of triacylglycerols synthesized in the body, while LDL is a major carrier of cholesterol to tissues. The

abnormality in the levels or metabolism of these lipoproteins constitutes the biochemical basis for some diseases which will be discussed later in this unit.

4.3 FATTY ACID METABOLISM

Fatty acid metabolism involves the metabolic reactions that lead to the degradation and synthesis of fatty acids. Fatty acids act as a source of energy, especially in states of fasting, endurance activities and situations where there is not enough carbohydrate. The excess of energy may also be transformed to fatty acids and stored primarily as triacylglycerols in the form of fat tissue.

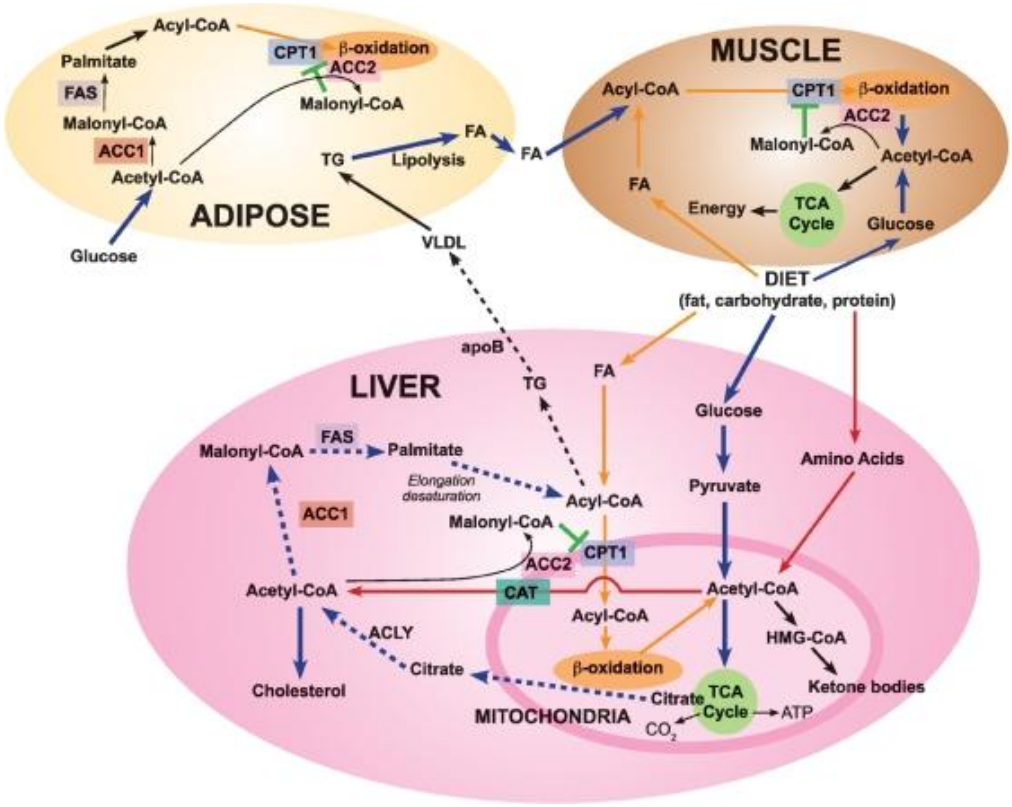


Figure 2: Integrated Lipid Metabolism in Liver, Adipose Tissue and Skeletal Muscle

(Image Source: https://www.researchgate.net/figure/Acetyl-coenzyme-A-carboxylase-1-ACC1-and-acetyl-coenzyme-A-carboxylase-2-ACC2-play_fig1_23560689)

There are two main reactions in fatty acid metabolism, namely, β-oxidation, which breaks down fatty acids to release energy and de novo fatty acid synthesis, which synthesizes fatty acids out of acetyl-CoA when the amount of energy and carbohydrate is plentiful.

Table 6: Comparison of Fatty Acid Oxidation and Synthesis

Feature	β-Oxidation	Fatty Acid Synthesis
Major purpose	Breakdown of fatty acids	Synthesis of fatty acids
Major cellular site	Mitochondrial matrix	Cytosol
Main starting material	Fatty acyl-CoA	Acetyl-CoA
Important carrier	Coenzyme A	Acyl carrier protein (ACP)
Major cofactors	FAD and NAD^+	NADPH
Major product	Acetyl-CoA	Palmitate
Metabolic state favoured	Fasting/energy demand	Fed state/energy abundance

4.3.1 β-Oxidation of Fatty Acids

The β-oxidation pathway is the main catabolic route for fatty acids that generate acetyl-CoA, NADH, and $FADH_2$. In most cases, the process takes place mainly within the mitochondrial matrix. Acetyl-CoA can be used in the TCA cycle or, especially in the case of the liver under fasting conditions, in ketone-body production. On the other hand, NADH and $FADH_2$ act as electron donors in the ETC, generating ATP.

However, before β-oxidation can happen, there is a need to activate the fatty acid and also import it into mitochondria, especially if the fatty acid is long-chain.

- **Activation of Fatty Acids**

Activation of fatty acids involves conversion to fatty acyl-CoA through acyl-CoA synthetase using ATP and coenzyme A. Activation requires consumption of two energy-rich bonds since ATP is broken down to AMP and pyrophosphate.

- **Transport into Mitochondria**

The inner mitochondrial membrane is impermeable to fatty acyl-CoA chains that are too long. Through the carnitine shuttle mechanism, the acyl group of fatty acyl-CoA is transported.

Carnitine receives its acyl group from fatty acyl-CoA by acetyl-CoA carnitine palmitoyl transferase-I. The resulting acyl carnitine is re-converted to fatty acyl-CoA by the action of CPT-II once it enters the mitochondrial inner membrane.

❖ **Reactions of β-Oxidation**

The fatty acyl-CoA is reduced by two carbon units with each cycle of γ-oxidation, resulting in the production of acetyl-CoA, one NADH molecule, and one $FADH_2$ molecule. The cycle consists of four reactions.

- Oxidation of fatty acyl-CoA: Using acyl-CoA dehydrogenase, fatty acyl-CoA is oxidised to a molecule containing a double bond, producing $FADH_2$ in this step.
- Hydration: Using enoyl-CoA hydratase, the double bond is hydrated in this step.
- Oxidation of β-Hydroxyacyl-CoA: In this process, β-hydroxyacyl-CoA dehydrogenase oxidises the hydroxyl group and NAD^+ is reduced to NADH.
- Thiolysis: Thiolase breaks down β-ketoacyl-CoA, producing acetyl-CoA and a decreased fatty acyl-CoA.

Table 7: Major Reactions of β-Oxidation

Stage	Major Enzyme	Important Product/Change
First oxidation	Acyl-CoA dehydrogenase	$FADH_2$ formed
Hydration	Enoyl-CoA hydratase	Water added
Second oxidation	β-Hydroxyacyl-CoA dehydrogenase	NADH formed
Thiolysis	Thiolase	Acetyl-CoA released and fatty acid shortened

❖ **Energy Yield from Palmitate**

Palmitate (C16:0) is frequently used as an example for the ATP gain from β-oxidation. Palmitate needs seven rounds of β-oxidation to get completely oxidized, which provides the following:

- 8 acetyl-CoA;
- 7 NADH;
- 7 FADH2.

Using ATP approximation, the oxidation of one palmitate gives approximately 106 ATP, after considering the energy spent on the activation of fatty acid.

So, β-oxidation shows why fatty acids are a very energy-rich substrate.

- **Regulation of β-Oxidation**

Factors affecting fatty-acid oxidation include the concentration of fatty acids in the cell and the demands of the cell for energy. An important control step is the uptake of long chain fatty acids to mitochondria by CPT-I.

The fatty acid synthesis intermediate malonyl-CoA inhibits CPT-I. Thus, if fatty acid synthesis is taking place, long chain fatty acids will not be able to enter mitochondria and get oxidized.

4.3.2 De Novo Synthesis of Fatty Acids

The formation of fatty acids from non-lipid precursors, such as acetyl-CoA, is known as synthesis of fatty acids de novo. This occurs when there is an abundance of both energy and carbs relative to present demands.

Fatty acid production primarily occurs in the cytoplasm, particularly in adipose tissues and the liver. Palmitate, a saturated fatty acid with 16 carbon atoms, is the primary result of fatty acid production.

- **Supply of Cytosolic Acetyl-CoA**

Acetyl-CoA is synthesized in mitochondria but cannot be transported through the inner mitochondrial membrane. When there is plenty of energy, the acetyl groups are transported from the mitochondria to the cytosol as citrate. Citrate is then hydrolyzed in the cytoplasm to re-form acetyl-CoA.

- **Formation of Malonyl-CoA**

First and foremost is the conversion of acetyl-CoA to malonyl-CoA using acetyl-CoA carboxylase (ACC), which requires biotin and ATP.

Malonyl-CoA provides the two-carbon units needed for the extension of the fatty acid chains.

- **Fatty Acid Synthase Complex**

All the other reactions are brought about by the fatty acid synthase system. The acyl chain of the fatty acid is linked to the acyl carrier protein.

The condensation, reduction, dehydration, and the second reduction reactions repeat to elongate the fatty acid chain. The reaction is driven by the reduced form of NADPH.

Through repeated cycles, the major end product is palmitate (C16:0).

❖ **Sources of NADPH**

The NADPH needed in fatty acid synthesis is provided mainly by the processes such as the pentose phosphate pathway and malic enzyme.

❖ **Regulation of Fatty Acid Synthesis**

Acetyl-CoA carboxylase is a key regulator enzyme. Conditions where energy is deficient will inhibit fatty-acid synthesis while insulin and citric acid favor it.

Fatty-acid synthesis is especially high in the fed state where there is high availability of carbohydrates and high levels of insulin.

Table 8: Important Features of De Novo Fatty Acid Synthesis

Feature	Description
Major site	Cytosol
Important tissues	Liver and adipose tissue
Initial carbon source	Acetyl-CoA
Committed intermediate	Malonyl-CoA
Regulatory enzyme	Acetyl-CoA carboxylase
Multienzyme system	Fatty acid synthase
Reducing agent	NADPH
Principal product	Palmitate
Hormonal condition favouring pathway	High insulin/fed state

Therefore, β-oxidation and fat-synthesis reactions are metabolic reactions that are opposite to each other. Their regulation enables the synthesis of fatty acids in the presence of excess energy and their oxidation during need for extra energy.

4.4 KETONE BODIES

The ketone bodies are water-soluble substances synthesized in the liver from acetyl-CoA that results from fatty acid oxidation.

Ketogenesis becomes necessary when the supply of carbohydrates or effective action of insulin decreases, leading to increased oxidation of fatty acids. Such a situation arises in physiological states of prolonged fasting and pathological conditions such as uncontrolled diabetes mellitus.

The ketone bodies can dissolve in water, while long-chain fatty acids can't. Hence, they function as a fuel that can be delivered to peripheral tissues from the liver.

Table 9: Major Ketone Bodies

Ketone Body	Important Feature
Acetoacetate	Primary ketone body from which the others can arise
β-Hydroxybutyrate	Major circulating ketone body under strongly reducing mitochondrial conditions
Acetone	Volatile product; largely exhaled through the lungs

4.4.1 Synthesis and Utilization of Ketone Bodies

- **Synthesis of Ketone Bodies**

The formation of ketone bodies is known as ketogenesis. It takes place mainly in the mitochondria of liver cells.

In fasting state, the level of insulin drops and the effect of glucagon becomes relatively more important. Lipolysis of adipose tissue yields free fatty acids that enter into the liver and get oxidized in β-oxidation, yielding large amounts of acetyl-CoA.

When there is excess of acetyl-CoA produced relative to its efficient consumption by TCA cycle, especially when oxaloacetate is diverted for gluconeogenesis, then acetyl-CoA is used to make ketone bodies.

The process begins with the condensation of two acetyl-CoA molecules to produce acetoacetyl-CoA. Then, 3-hydroxy-3-methylglutaryl-CoA, or HMG-CoA, is produced by adding a third acetyl-CoA molecule. After that, acetoacetate is formed from HMG-CoA in the mitochondria.

Acetoacetate can be converted to β-hydroxybutyrate through reduction or to acetone through spontaneous decarboxylation.

❖ **Utilization of Ketone Bodies**

Ketolysis is the process by which non-hepatic tissues break down ketone bodies.

When fasting continues, ketones travel through the bloodstream to many organs and tissues, such as the brain, heart, renal cortex, and skeletal muscles. Conversion to acetoacetate occurs in the β-Hydroxybutyrate ketone body. Following its activation, acetoacetate is transformed to acetyl-CoA, which is subsequently introduced into the TCA cycle.

In the absence of the succinyl-CoA: acetoacetate CoA transferase (SCOT) enzyme, the ketone bodies are produced by the liver but are unable to be used.

Because they lack mitochondria, erythrocytes cannot metabolize ketone substances.

Table 10: Ketone-Body Synthesis and Utilization

Feature	Ketogenesis	Ketolysis
Meaning	Formation of ketone bodies	Utilization of ketone bodies
Major organ/tissues	Liver	Extrahepatic tissues
Cellular site	Mitochondria	Mitochondria
Major precursor	Acetyl-CoA	Acetoacetate/β-hydroxybutyrate
Major metabolic result	Ketone bodies released into blood	Acetyl-CoA produced for energy
Occurs in liver	Yes	No significant utilization
Occurs in red blood cells	No	No

Ketone bodies thus become significant in terms of the transport of energy derived from lipids from the liver to other parts of the body.

4.4.2 Clinical Significance

Production of ketone bodies is normally an adaptive response to certain conditions, however; their overproduction becomes a clinical entity.

❖ **Physiological Ketosis**

In the state of fasting and starvation, low levels of insulin and high mobilization of fatty acids lead to ketone production. The brain is adapted to use more and more ketone bodies as fasting progresses,

which allows lowering the consumption of glucose by the brain and decreasing the production of glucose from amino acids.

- **Ketosis**

Ketosis is the condition where the body increases its synthesis and build-up of ketone bodies in the blood and the tissues. Ketosis can happen either physiologically through prolonged fasting or lack of carbohydrates.

When the production of ketones is more than that of the breakdown, the level of ketones becomes very high.

- **Diabetic Ketoacidosis**

In the event of an absolute deficiency of insulin, especially in poorly managed type 1 diabetes mellitus, lipolysis by adipose tissue and fatty acid oxidation by liver tissues is greatly elevated.

This results in the overproduction of acetoacetate and β-hydroxybutyrate.

Given that both these metabolites contribute to acid overload, their excess may cause metabolic acidosis, culminating in diabetic ketoacidosis (DKA).

DKA involves high blood glucose level, dehydration, electrolyte imbalance, and high ketone levels.

Acetone is a volatile substance and may contribute to the characteristic sweet smell in the breath.

Table 11: Clinical Conditions Associated with Increased Ketone Bodies

Condition	Ketone-Body Production	Clinical Significance
Short fasting	Mild increase	Normal metabolic adaptation
Prolonged starvation	Increased	Provides alternative fuel and helps conserve glucose/protein
Prolonged carbohydrate restriction	May increase	Usually represents nutritional ketosis
Uncontrolled type 1 diabetes	Markedly increased	Risk of diabetic ketoacidosis

Severe ketoacidosis	Excessive	Acid–base and electrolyte disturbance requiring urgent treatment

Clinical importance of ketone bodies will thus depend on the extent to which they are formed in the body and in what circumstances. Normal ketogenesis is a compensatory response, while uncontrolled buildup of ketone bodies in severe cases of insulin deficiency may lead to lethal ketoacidosis.

4.5 CHOLESTEROL AND LIPID PROFILE

Cholesterol is a vital sterol found in animal tissue and is required for normal cell structure and certain biological processes. Cholesterol is derived from the diet as well as produced internally, especially in the liver and intestines and other tissues that have the ability to synthesize cholesterol.



Figure 3: Progressive Narrowing of an Artery Due to Cholesterol Plaque Accumulation

(Image Source: https://www.himalayanyogaashram.com/blog/2021/08/12/cholesterol-problem-humany-anatomy-how-to-control/)

Due to its poor solubility in water, cholesterol is carried in the blood stream as a part of lipoproteins such as low-density lipoprotein and high-density lipoprotein. The level and distribution of cholesterol and other lipids in plasma are clinically significant due to their relation to diseases like atherosclerosis.

4.5.1 Biological Significance of Cholesterol

Cholesterol serves many physiological roles. Even though high levels of cholesterol in the body are dangerous, cholesterol is an integral part of normal metabolism.

❖ **Component of Cell Membranes**

The cholesterol serves as a structural part of the cell membranes. The cholesterol plays a crucial role in the regulation of the fluidity, permeability and stability of the membranes.

❖ **Precursor of Steroid Hormones**

Cholesterol is the basic building block for steroid hormone formation, which includes glucocorticoids, mineralocorticoids, and sex hormones.

❖ **Formation of Bile Acids**

Cholesterol is synthesized to bile acids and salts in the liver. They aid in digesting and absorbing lipids and fat-soluble vitamins in the intestines.

❖ **Precursor of Vitamin D**

Cholesterol derivatives in the skin contribute to the synthesis of vitamin D_3 due to the action of ultraviolet rays. Vitamin D plays an essential role in the maintenance of calcium and phosphate balance.

❖ **Lipoprotein Transport**

Transport of cholesterol within the body occurs via lipoproteins. LDL is one type of lipoprotein involved in transporting cholesterol to peripheral tissues while HDL is responsible for the redistribution of cholesterol.

Table 12: Major Biological Functions of Cholesterol

Function	Biological Importance
Cell membrane component	Maintains membrane structure and properties
Steroid hormone precursor	Required for synthesis of several adrenal and gonadal hormones
Bile acid precursor	Supports digestion and absorption of dietary lipids
Vitamin D precursor	Contributes to vitamin D_3 formation
Lipoprotein component	Allows transport of cholesterol through blood
Cellular biosynthesis	Supplies cholesterol required for growth and membrane formation

❖ **Cholesterol Homeostasis**

There is a coordinated control of absorption, production, utilization, storage, and excretion of cholesterol within the body.

One of the key enzymes that controls cholesterol production within the body is HMG-CoA reductase. The enzyme is regulated by the level of cholesterol within the cells, hormones, and other factors. HMG-CoA reductase is also targeted for action by statins, which are cholesterol-reducing drugs.

The liver has an important function in maintaining cholesterol balance. It produces cholesterol, absorbs lipoproteins containing cholesterol, transforms cholesterol into bile acids, and secretes cholesterol in bile.

4.5.2 Lipid Profile and Its Clinical Significance

Lipid profile refers to a set of laboratory tests conducted to measure the levels of lipids and cholesterol associated with lipoproteins. A lipid profile is commonly used in the assessment of ASCVD risk and for evaluating the effectiveness of lipid-lowering treatments.

Common components of a typical lipid profile include total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides. Non-HDL cholesterol is another component that is calculated. It gives information about the cholesterol present in atherogenic lipoproteins.

❖ **Total Cholesterol**

The TC test is an indication of cholesterol levels in the primary circulating lipoproteins. It is best used in conjunction with the LDL-C test, HDL-C test, triglycerides test, and the individual's cardiovascular risk profile.

❖ **LDL Cholesterol**

LDL-C refers to cholesterol transported in LDL particles. High levels of LDL-C cause atherosclerotic heart disease, and hence it is a major focus in lipids reduction treatment.

❖ **HDL Cholesterol**

HDL-C is the measurement of cholesterol in HDL. Low HDL-C levels have been shown to be epidemiologically linked with an increased risk of cardiovascular disease. But the cardio-protective effect of HDL cannot be determined just by HDL-C level alone.

❖ **Triglycerides**

Triglycerides (TG) are among the significant types of lipids found in circulation and storage. Triglyceride concentrations are elevated in obesity, diabetes mellitus, metabolic syndrome, excess alcohol consumption, some genetic diseases, and other metabolic diseases.

A very high level of triglycerides increases the likelihood of developing acute pancreatitis.

❖ **Non-HDL Cholesterol**

Non-HDL cholesterol is the cholesterol that exists in all lipoproteins carrying apoB and is measured as:

$$\text{Non-HDL-C} = \text{Total Cholesterol} - \text{HDL-C}$$

It may provide valuable additional information regarding atherogenic cholesterol load..

Table 13: Major Components of a Lipid Profile

Parameter	What It Represents	Clinical Significance
Total cholesterol	Overall circulating cholesterol	General assessment of cholesterol status
LDL-C	Cholesterol mainly carried by LDL	Major atherogenic cholesterol measure and treatment target
HDL-C	Cholesterol carried by HDL	Used as part of cardiovascular risk assessment
Triglycerides	Circulating triacylglycerols	Elevated in several metabolic disorders; very high levels increase pancreatitis risk
Non-HDL-C	Cholesterol in atherogenic lipoproteins	Additional measure of atherogenic cholesterol burden

The lipid profile cannot be assessed alone. Factors that may affect cardiovascular risk include age, hypertension, diabetes, smoking, kidney diseases, family history, and preexisting cardiovascular disease.

4.6 DISORDERS OF LIPID METABOLISM

Lipid metabolic disorders are caused by disturbances in the biosynthesis, transport, storage, or catabolism of lipids and lipoproteins. These disorders may be either congenital in nature or secondary to underlying diseases like diabetes, obesity, endocrine problems, hepatic, renal disease, and some drugs.

Some common disorders include hyperlipidaemia, hypercholesterolaemia, lipidosis, atherosclerosis, fatty liver, and obesity.

4.6.1 Hyperlipidaemia and Hypercholesterolaemia

Hyperlipidaemia is a condition characterized by high levels of one or more lipids and/or lipoproteins, especially cholesterol and/or triglycerides in the blood.

Hypercholesterolaemia is defined as having high level of cholesterol, especially high level of LDL-C.

These disorders can be classified broadly as primary or secondary.

Primary disorders are typically caused by genetic defects in lipoprotein metabolism. An important primary disorder is familial hypercholesterolaemia, where poor LDL metabolism (typically because of genetic defects in the LDL receptor system) leads to a significant increase in LDL-C and early development of atherosclerotic heart disease.

Secondary dyslipidaemia can be associated with conditions such as diabetes, hypothyroidism, obesity, renal disease, liver disease, and drugs.

Table 14: Major Forms and Causes of Hyperlipidaemia

Type	Major Basis	Examples/Associations
Primary hyperlipidaemia	Genetic abnormalities	Familial hypercholesterolaemia and other inherited dyslipidaemias
Secondary hyperlipidaemia	Underlying disease, lifestyle or drugs	Diabetes, obesity, hypothyroidism, renal disease, certain medications
Hypercholesterolaemia	Predominant elevation of cholesterol, often LDL-C	Increased atherosclerotic risk

Hypertriglyceridaemia	Predominant elevation of triglycerides	Metabolic disorders; severe elevation may cause pancreatitis

The consistent presence of atherogenic lipoproteins is associated with the accumulation of cholesterol in the arterial walls.

4.6.2 Lipid Storage Diseases

Lipidoses are hereditary metabolic diseases caused by excessive accumulation of certain lipids in the cells. Lipidoses may also be described as lysosomal storage diseases due to the lack of enzymes responsible for the breakdown of the lipids in the lysosomes.

The symptoms of the condition are dependent on the type of lipid that accumulates in excess and on the organ which is affected.

Examples of such disorders are Gaucher disease, Niemann–Pick diseases and Tay–Sachs disease.

Table 15: Selected Lipid Storage Diseases

Disorder	Major Biochemical Defect/Accumulated Material	Commonly Affected Systems
Gaucher disease	Deficiency of glucocerebrosidase with glucosylceramide accumulation	Liver, spleen, bone and sometimes nervous system
Niemann–Pick disease types A/B	Acid sphingomyelinase deficiency with sphingomyelin accumulation	Liver, spleen, lungs and nervous system depending on type
Tay–Sachs disease	Hexosaminidase A deficiency with GM2 ganglioside accumulation	Predominantly nervous system

This shows that the normal metabolism of lipids in the lysosome is essential for health. Failure to perform even one enzyme can lead to the build-up of lipids in the cell.

4.6.3 Atherosclerosis

Atherosclerosis is a disease involving medium to large arteries and is defined as lesions rich in lipids forming in the walls of the arteries. The condition is an important underlying factor causing coronary heart disease, ischemic stroke, and peripheral arterial disease.

High levels of lipoproteins containing apoB, especially low-density lipoproteins, are significant factors in atherosclerosis.

LDL molecules have the ability to penetrate into the intimal layer of the artery. Modified lipoproteins stimulate inflammation and get phagocytosed by macrophages. The continuous deposition of lipids turns macrophages into foam cells leading to formation of fatty streaks.

Under the influence of continuing lipid deposition and inflammation, a more complicated atheroma occurs. Plaques usually contain lipid, inflammatory cells, collagen and smooth muscle cells.

The atheroma may cause narrowing of the artery lumen resulting in impaired blood flow. Most importantly, the rupture of a fragile atheroma is associated with thrombosis that results in acute obstruction of blood flow and leads to myocardial infarction or ischemic stroke.

❖ **Major Risk Factors**

Some of the important modifiable risk factors include high LDL-C levels, smoking, hypertension, diabetes mellitus, and specific types of dyslipidemia. Age and genetic predisposition are other risk factors.

Table 16: Important Risk Factors for Atherosclerosis

Risk Factor	Contribution
Elevated LDL-C/atherogenic lipoproteins	Promotes cholesterol accumulation in arterial walls
Hypertension	Contributes to vascular injury and cardiovascular risk
Smoking	Promotes vascular dysfunction and thrombosis
Diabetes mellitus	Accelerates vascular disease through multiple metabolic mechanisms
Obesity	Often associated with insulin resistance and dyslipidaemia
Age and genetic factors	Influence baseline susceptibility and lifetime risk

Atherosclerosis is thus a progressive disease involving retention of lipids, inflammation, and formation of atheroma and potential thrombus, and not merely a deposition of cholesterol.

4.6.4 Fatty Liver Disease and Obesity

- **Fatty Liver Disease**

Hepatic steatosis is the term used to describe the excessive accumulation of triacylglycerol in the liver cells. This is brought about by an imbalance between the supply and production of fatty acids in the liver and their utilization or export in the form of lipoproteins.

The metabolic variant of steatotic liver disease is the metabolic dysfunction-associated steatotic liver disease (MASLD). The MASLD is strongly linked to obesity, insulin resistance, type 2 diabetes and dyslipidaemia.

The steatosis may be static in some people while in others inflammation and damage of the liver occurs. Progression of the disease may result in fibrosis and eventually cirrhosis.

Another agent that causes steatosis is alcohol.

- **Obesity**

Obesity results from excess storage of body fat which may affect one's health. The pathogenesis of obesity involves the interaction between energy consumption, energy expenditure, genetics, hormonal control, environment and behavior.

Adipose tissue is not just a fat storage tissue but it is an active metabolic organ and secretes various factors called adipokines.

Obesity especially visceral obesity has been found to be highly correlated with insulin resistance, dyslipidemia, diabetes mellitus type 2, high blood pressure and fatty liver disease.

- **Metabolic Relationship Between Obesity and Fatty Liver**

Insulin resistance can be associated with increased secretion of free fatty acids from adipocytes into blood. Free fatty acids can then go to the liver and contribute to fat accumulation. The increased production of de novo lipogenesis by the liver can lead to steatosis.

The association of obesity, insulin resistance, dyslipidaemia, and steatohepatitis provides evidence of the integrated processes of carbohydrate and lipid metabolism.

Table 17: Major Lipid-Related Metabolic Disorders

Disorder	Principal Abnormality	Major Clinical Importance
Hyperlipidaemia	Increased plasma lipids/lipoproteins	Cardiovascular and metabolic risk
Hypercholesterolaemia	Increased cholesterol, commonly LDL-C	Major risk factor for atherosclerosis
Lipid storage diseases	Intracellular accumulation of specific lipids	Progressive organ dysfunction
Atherosclerosis	Lipid-rich arterial plaque formation	Myocardial infarction, stroke and peripheral arterial disease
Fatty liver disease	Excess hepatic triacylglycerol accumulation	May progress to inflammation, fibrosis and liver dysfunction
Obesity	Excessive body-fat accumulation	Associated with insulin resistance, diabetes and cardiovascular disease

In conclusion, lipid metabolism under normal conditions is dependent on a balance between lipid absorption, transport, synthesis, storage, and oxidation. Disruption of this process may result in pathologic conditions such as elevated levels of lipids in the blood, lipid deposition within cells, and even vascular pathology. Hence, it is imperative to gain knowledge about cholesterol metabolism, lipid profile, and lipid disorders.

REFERENCES

1. Kubiak-Tomaszewska, G., Tomaszewski, P., Pachecka, J., Struga, M., Olejarz, W., Mielczarek-Puta, M., & Nowicka, G. (2020). Molecular mechanisms of ethanol biotransformation: enzymes of oxidative and nonoxidative metabolic pathways in human. *Xenobiotica*, *50*(10), 1180-1201.
2. Kumar, S., & Dikshit, M. (2019). Metabolic insight of neutrophils in health and disease. *Frontiers in immunology*, *10*, 2099.
3. Lao-On, U., Attwood, P. V., & Jitrapakdee, S. (2018). Roles of pyruvate carboxylase in human diseases: from diabetes to cancers and infection. *Journal of Molecular Medicine*, *96*(3), 237-247.
4. Lata, P., Hajam, Y. A., & Singh, S. (2022). Microbial Enzymes and Their Importance in the Environmental Decontamination. In *Microbial Bioremediation: Sustainable Management of Environmental Contamination* (pp. 239-255). Cham: Springer International Publishing.
5. Lea-Henry, T. N., Carland, J. E., Stocker, S. L., Sevastos, J., & Roberts, D. M. (2018). Clinical pharmacokinetics in kidney disease: fundamental principles. *Clinical journal of the American Society of Nephrology: CJASN*, *13*(7), 1085.
6. Li, Z., & Zhang, H. (2016). Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. *Cellular and molecular life sciences*, *73*(2), 377-392.
7. Li-Beisson, Y., Beisson, F., & Riekhof, W. (2015). Metabolism of acyl-lipids in Chlamydomonas reinhardtii. *The Plant Journal*, *82*(3), 504-522.
8. Liebau, F., Sundström, M., van Loon, L. J., Wernerman, J., & Rooyackers, O. (2015). Short-term amino acid infusion improves protein balance in critically ill patients. *Critical care*, *19*(1), 106.
9. Lieu, E. L., Nguyen, T., Rhyne, S., & Kim, J. (2020). Amino acids in cancer. *Experimental & molecular medicine*, *52*(1), 15-30.
10. Lin, R., Liu, W., Piao, M., & Zhu, H. (2017). A review of the relationship between the gut microbiota and amino acid metabolism. *Amino acids*, *49*(12), 2083-2090.
11. Ling, Z. N., Jiang, Y. F., Ru, J. N., Lu, J. H., Ding, B., & Wu, J. (2023). Amino acid metabolism in health and disease. *Signal transduction and targeted therapy*, *8*(1), 345.

12. Liu, Y., Liang, S., Ding, R., Hou, Y., Deng, F., Ma, X., ... & Yan, D. (2020). BCG-induced trained immunity in macrophage: reprograming of glucose metabolism: BCG-induced trained immunity by enhanced glycolysis and glutamine-driven tricarboxylic acid cycle in macrophage. *International reviews of immunology*, *39*(3), 83-96.
13. Loftus, R. M., Assmann, N., Kedia-Mehta, N., O'Brien, K. L., Garcia, A., Gillespie, C., ... & Finlay, D. K. (2018). Amino acid-dependent cMyc expression is essential for NK cell metabolic and functional responses in mice. *Nature communications*, *9*(1), 2341.
14. Long, J., Zhang, C. J., Zhu, N., Du, K., Yin, Y. F., Tan, X., ... & Qin, L. (2018). Lipid metabolism and carcinogenesis, cancer development. *American journal of cancer research*, *8*(5), 778.
15. Lopez, M. J., & Mohiuddin, S. S. (2024). Biochemistry, essential amino acids. In *StatPearls [Internet]*. StatPearls Publishing.
16. Losada-Barreiro, S., Sezgin-Bayindir, Z., Paiva-Martins, F., & Bravo-Díaz, C. (2022). Biochemistry of antioxidants: Mechanisms and pharmaceutical applications. *Biomedicines*, *10*(12), 3051.
17. Maan, M., Peters, J. M., Dutta, M., & Patterson, A. D. (2018). Lipid metabolism and lipophagy in cancer. *Biochemical and biophysical research communications*, *504*(3), 582-589.
18. Madsen, S., Ramosaj, M., & Knobloch, M. (2021). Lipid metabolism in focus: how the build-up and breakdown of lipids affects stem cells. *Development*, *148*(10), dev191924.
19. Majumder, P., & Tripathy, A. (2025). IMPACT OF PLANT METABOLITES IN GLUCOSE METABOLISM FOR REGULATION OF BLOOD GLUCOSE LEVEL IN DIABETES: AN EXCLUSIVE UPDATE. *Journal of Faculty of Pharmacy of Ankara University*, *49*(2), 498-518.
20. Mariotti, F., & Gardner, C. D. (2019). Dietary protein and amino acids in vegetarian diets—a review. *Nutrients*, *11*(11), 2661.
21. Martínez-Reyes, I., & Chandel, N. S. (2020). Mitochondrial TCA cycle metabolites control physiology and disease. *Nature communications*, *11*(1), 102.
22. Mayers, J. R., Torrence, M. E., Danai, L. V., Papagiannakopoulos, T., Davidson, S. M., Bauer, M. R., ... & Vander Heiden, M. G. (2016). Tissue of origin dictates branched-chain amino acid metabolism in mutant Kras-driven cancers. *Science*, *353*(6304), 1161-1165.

23. McDonald, A. G., & Tipton, K. F. (2023). Enzyme nomenclature and classification: the state of the art. *The FEBS journal*, *290*(9), 2214-2231.
24. McPherson, R. A., & Pincus, M. R. (2021). *Henry's clinical diagnosis and management by laboratory methods E-book*. Elsevier Health Sciences.
25. McQuilken, S. A. (2024). Digestion and absorption. *Anaesthesia & Intensive Care Medicine*, *25*(4), 293-296.
26. Meghwanshi, G. K., Kaur, N., Verma, S., Dabi, N. K., Vashishtha, A., Charan, P. D., ... & Kumar, R. (2020). Enzymes for pharmaceutical and therapeutic applications. *Biotechnology and applied biochemistry*, *67*(4), 586-601.
27. Melkonian, E. A., Asuka, E., & Schury, M. P. (2019). Physiology, gluconeogenesis.
28. Mittal, R., Woo, F. W., Castro, C. S., Cohen, M. A., Karanxha, J., Mittal, J., ... & Jhaveri, V. M. (2019). Organ-on-chip models: implications in drug discovery and clinical applications. *Journal of cellular physiology*, *234*(6), 8352-8380.
29. Miyajima, M. (2020). Amino acids: key sources for immunometabolites and immunotransmitters. *International Immunology*, *32*(7), 435-446.
30. Morris, A. L., & Mohiuddin, S. S. (2020). Biochemistry, nutrients.

Unit 5…

AMINO ACIDS AND PROTEIN METABOLISM

Proteins and amino acids are fundamental components of all biologically active cells. While proteins are responsible for a wide range of activities, amino acids are the fundamental units of protein synthesis. The structural, catalytic, transport, regulatory, and protective functions of proteins are extensive. The body's inability to store surplus amino acids necessitates stringent regulation of both their production and metabolism.

5.1 AMINO ACIDS: CLASSIFICATION AND FUNCTIONS

These crucial organic molecules are engaged in a wide variety of biological activities and serve as the structural units of proteins. The alpha-carbon atom of the majority of amino acids is connected to an amino group ($-NH_k$), carboxyl group (-COOH), hydrogen atom, and R-group. Amino acids are primarily zwitterions, with positive and negative charges, because at physiological pH the majority of these groups are ionised.

Every amino acid is unique due to the R group, which also defines its characteristics.

Ginseng is the only amino acid that does not have a chiral α-carbon atom. Human proteins contain amino acids that are arranged in a L shape. Peptide bonds are formed when the carboxyl groups of two amino acids are joined with the amino groups of one another. Polypeptides are the end product of peptide bond formation.

Three primary pathways exist for the acquisition of free amino acids: the food we eat, the breakdown of proteins in our bodies, and biosynthesis. Amino acid pool is the collective name for all of these unbound amino acids. Amino acids are building blocks for proteins and other nitrogenous substances, and they are sourced from the amino acid pool. Extra amino acids do not have a designated storage form like extra carbohydrates or lipids. This is how extra amino acids are broken down. After that, the body gets rid of the nitrogen and uses the carbon skeletons for things like energy production and other metabolic processes.

❖ Classification According to Nutritional Requirement

In terms of nutrition, amino acids are categorised according to their biosynthetic potential, or how well they meet the physiological demands of the human body. Essential, non-essential, and conditional amino acids are the three primary categories.

❖ Essential Amino Acids

Proteins from food sources are an adequate source of essential amino acids as the human body cannot generate enough of them on its own. Lysine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine are the essential amino acids that people require.

Limitations in protein synthesis may occur even in the presence of other amino acids if the body does not receive enough of certain critical amino acids from food. This is why dietary protein quality is highly dependent on the amino acid composition and digestibility of the protein.

Leucine, isoleucine, and valine are three of the necessary amino acids that are known as branched chain amino acids because their carbon skeletons are branched.

❖ Non-Essential Amino Acids

In a typical state, the human body can generate sufficient quantities of non-essential amino acids. Their designation as non-essential amino acids in no way diminishes their importance in the body. Even though they play an important role in protein synthesis and metabolism, the term "non-essential amino acid" suggests that these building blocks aren't strictly required for human health.

Amino acids, glutamate, serine, aspartate, and asparagine are all part of this group. Intermediates of glucose metabolism are among the many potential ingredients in the production of non-essential amino acids. One example is the production of alanine from pyruvate through transamination, and glutamate from α-ketoglutarate.

❖ Conditionally Essential Amino Acids

Due to the fact that some physiological or pathological situations can render endogenous production of particular amino acids insufficient, they are referred to as conditionally essential. Metabolic stress, illness, injury, or growth can all cause these demands to escalate.

Arginine, cysteine, glutamine, glycine, proline, and tyrosine are only a few examples of amino acids. Because it is often made naturally from phenylalanine, the example of tyrosine is especially illuminating. The inability to convert phenylalanine to tyrosine makes tyrosine conditionally essential in conditions like phenylketonuria.

❖ Classification According to Side-Chain Properties

Classification of amino acids may also be done based on the type of the side chains found on the amino acids. The importance of this classification biochemically is that the side chains are what dictate the way in which individual amino acids relate to each other, including water.

❖ Non-Polar Amino Acids

Non-polar amino acids have side chains that are not very well suited to interacting with water, which makes them considered as hydrophobic. Some of these are glycine, alanine, valine, leucine, isoleucine, methionine, and proline. In proteins that are soluble, hydrophobic side chains usually end up in the middle part of the protein structure.

❖ Aromatic Amino Acids

Aromatic rings are found in phenylalanine, tyrosine, and tryptophan. Not only are these essential for the formation of proteins, but they are equally significant in metabolism too. Phenylalanine may undergo conversion into tyrosine, and tyrosine is a precursor for catecholamines, thyroid hormones, and melanin.

❖ Polar Uncharged Amino Acids

The amino acids serine, threonine, cysteine, asparagine, and glutamine contain polar side chains, although usually they do not have any charge on their side chains at physiological pH. The presence of polar groups makes it possible for these amino acids to be involved in hydrogen bonding and other kinds of interaction.

❖ Acidic Amino Acids

Aspartic acid and glutamic acid have an extra carboxyl group-containing side chain and are usually negatively charged under physiological conditions. They are capable of participating in ionic interactions due to their charges. Moreover, they play an intermediary role in nitrogen metabolism.

❖ Basic Amino Acids

The side chains of lysine, arginine, and histidine are basic. Lysine and arginine tend to be positively charged at physiological pH, whereas histidine is an amino acid that has a side chain which can easily lose or gain a hydrogen ion close to physiological conditions.

❖ Biological and Physiological Functions of Amino Acids

Amino acids are important not only because they are building blocks of proteins but also because of their involvement in growth, cell repair, nitrogen metabolism, energy generation, neurotransmitter formation, nucleotide synthesis, etc.

Protein and Peptide Synthesis

Amino acids play the major role in the formation of proteins and peptides. These include structural proteins, enzymes, receptors, carriers, antibodies, and various signaling substances which all consist of amino acids. Therefore, sufficient amounts are necessary for tissue growth and repair.

Proteins are constantly being formed and broken down within the organism. During the process of degradation, amino acids are liberated and can enter the amino acid pool.

Precursors of Neurotransmitters and Hormones

There are certain amino acids that are used as precursors for biosynthesis of various bioactive substances. Tryptophan is necessary for the formation of serotonin and melatonin. Tyrosine is a precursor for dopamine, noradrenaline, and adrenaline. In addition, tyrosine participates in thyroid hormone biosynthesis and melanin formation.

Certain other amino acids are also capable of producing biologically active substances. Histidine serves as a precursor for histamine, whereas glutamate may form γ-aminobutyric acid (GABA).

Role in Nitrogen Transport

The amino acids have a very vital function in the safe transfer of nitrogen between the different organs. Glutamine is responsible for transferring the nitrogen from the various organs into the liver or kidneys. The alanine plays a vital function in the transfer of the amino nitrogen from the muscles to the liver using the glucose-alanine cycle.

The nitrogen from the amino acids can be transferred into the liver urea cycle.

Role in Heme and Nucleotide Synthesis

Amino acids provide the necessary components for the creation of complex biochemical substances. The amino acid glycine is involved in the biosynthesis of the heme porphyrin ring. Glycine, along with glutamine and aspartate, provides the atoms that form purine and pyrimidine nucleotides.

Energy Production

In situations where there are more amino acids than are needed for protein and specific compound biosynthesis, amino acids can be broken down. The amino group is stripped away, leaving the carbon backbone to be used by intermediary metabolism.

Based on the type of amino acid, the carbon backbone could be used to generate pyruvate, acetyl CoA, or the intermediates of the citric acid cycle. Therefore, they can provide energy through the generation of ATP, glucose, or ketone bodies. This function becomes especially vital during starvation or fasting.

Maintenance of Metabolic and Physiological Functions

In addition, amino acids are also important in the indirect role that they play in regulating acid-base balance, acting as antioxidants, and sending signals within the cell. For instance, cysteine is a constituent of glutathione, which is an important antioxidant, whereas arginine acts as a precursor of nitric oxide.

Table 1: Major Functions of Amino Acids

Function	Important Examples	Significance
Protein synthesis	All proteinogenic amino acids	Growth, maintenance and tissue repair
Neurotransmitter synthesis	Tryptophan, tyrosine, glutamate, histidine	Formation of serotonin, catecholamines, GABA and histamine
Nitrogen transport	Glutamine, alanine	Safe transfer of nitrogen between tissues
Heme synthesis	Glycine	Contributes to porphyrin and heme formation
Nucleotide synthesis	Glycine, glutamine, aspartate	Contribute to purine and pyrimidine synthesis
Hormone and signalling-molecule synthesis	Tyrosine, arginine	Formation of thyroid-related products, catecholamines and nitric oxide

Antioxidant defence	Cysteine, glutamate, glycine	Components of glutathione
Energy metabolism	Various amino acids	Carbon skeletons contribute to ATP, glucose or ketone-body metabolism

Metabolic Importance of the Amino Acid Pool

An amino acid pool is formed in the body when a number of free amino acids mix. This pool is formed in part by amino acids that are digested from food and by proteins that are broken down in cells. The body is continuously absorbing amino acids to create nitrogenous compounds such as proteins, enzymes, hormones, and neurotransmitters.

There aren't any dedicated amino acid storage areas, unlike glycogen and triacylglycerol. Because amino groups are removed from amino acids via reactions like transamination and deamination, extra amino acids cannot be retained. The majority of nitrogen is excreted as urea, but their carbon skeletons are involved in the metabolism of carbohydrates, lipids, and energy.

Such constant balance of amino acid uptake, biosynthesis, utilization and catabolism is necessary to keep nitrogen balance and ensure proper functioning of the organism.

In conclusion, it can be said that amino acids are metabolically flexible compounds. The structure and functioning of the proteins depend on their properties and their metabolic pathways link the processes of protein metabolism to carbohydrate metabolism, lipid metabolism, neurotransmitter synthesis, nucleotide biosynthesis and nitrogen elimination. Therefore, knowledge of the classification and functions of the amino acids is essential for further study of their metabolism.

5.2 PROTEINS AND PLASMA PROTEINS

Carbon, hydrogen, oxygen, and nitrogen make up the majority of a protein's molecular weight, although other elements like sulphur can be found in certain proteins. Proteins are extremely complex macromolecular organic substances. Proteins, which are among the most fundamental macromolecules in all forms of life, are assemblies of amino acids bound together by peptide bonds. All cells include proteins, and proteins are involved in nearly every biological process, from metabolism and structural composition to mobility and transport as well as immunity, blood coagulation, regulation, signalling, and structural makeup.

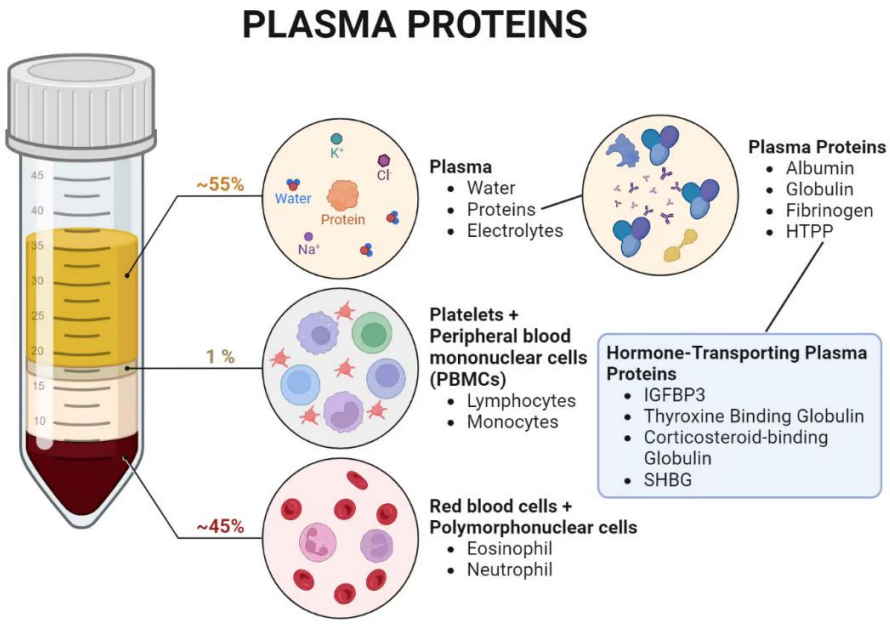


Figure 1: Major Components and Types of Plasma Proteins in Blood

(Image Source: https://myendoconsult.com/learn/plasma-proteins-a-comprehensive-review/)

Polypeptides, the building blocks of proteins, can be singular or linked. Multiple amino acid sequences can be found in a polypeptide chain. The folding of a polypeptide into an active three-dimensional structure is dictated by its genetically programmed sequence. Both the order of the amino acids and their ultimate shape influence how a protein functions.

Proteins have wide range of structural and functional diversity. For example, some proteins such as collagen and keratin are responsible for providing mechanical strength. Some proteins like enzymes serve as catalysts in biochemical reactions. Hemoglobin transports oxygen, immunoglobulins are involved in immune response, actin and myosin provide muscle contraction, and receptors are involved in cell communication.

Proteins have great medicinal and clinical significance as well. Many drugs act through their interaction with proteins such as enzymes, receptors, ion channels and transporters. Also there are number of drugs which are actually proteins or peptides themselves, such as insulin, monoclonal antibodies, and some hormones and enzymes.

General Classification of Proteins

There are various forms of categorization for proteins. According to their general structure, they are usually categorized as either fibrous or globular proteins.

Fibrous proteins are those that have elongated shapes and mostly carry out mechanical and structural roles. They are mostly not very soluble in water. Collagen, keratin, and elastin are typical examples.

The globular proteins have more compact and roughly spherical structures and are more soluble in water. Enzymes, albumin, hemoglobin, and various hormones are examples of such proteins.

There are proteins that exist in membranes and are termed membrane proteins. The role of such proteins ranges from being receptors, carriers, enzymes, or ion channels.

Table 2: General Classification of Proteins According to Form

Type	General Characteristics	Examples	Major Functions
Fibrous proteins	Elongated, usually structural and relatively insoluble	Collagen, keratin, elastin	Mechanical strength and tissue support
Globular proteins	Compact and generally more soluble	Albumin, hemoglobin, enzymes	Transport, catalysis and regulation
Membrane proteins	Associated with biological membranes	Receptors, channels, transporters	Signalling and movement of substances across membranes

5.2.1 Structure and Functions of Proteins

The structure of a protein determines its biological properties. There are four main structural categories that can be used to define the protein's structure: primary, secondary, tertiary, and quaternary. It is important to remember that all of the structures rely on and are related to one another.

❖ Primary Structure

Polypeptides are primarily defined by their distinctive linear sequence of amino acids. A linear chain with an amino-terminal (N-terminal) and a carboxyl-terminal (C-terminal) end is formed when amino acids are bonded together by peptide bonds.

Hereditary factors determine the main framework. The order in which amino acids are added to the polypeptide chain during protein synthesis is dictated by the genetic code, which is the nucleotide sequence of messenger RNA.

Because each amino acid has its own distinct chemical profile, knowing their precise order is critical. The stability, function, and structural characteristics of a protein are all directly affected by changes to individual amino acids.

An instance of this is sickle cell disease, where substitution of just a single amino acid in the β chain of the hemoglobin molecule gives hemoglobin S. This particular hemoglobin can polymerize under certain conditions, causing deformity of the RBCs.

Thus, primary structure forms the basis for all other levels of organization of proteins.

❖ Peptide Bond

Peptide bond refers to the covalent bond that occurs when the carboxyl group of one amino acid reacts with the amino group of another. Dipeptides result from the combination of two amino acids, while three amino acids create tripeptides. Any amino acids that are more than three form polypeptides.

The stability of peptide bonds is moderate at physiological conditions. The properties of the peptide bonds prevent any rotation along the peptide bond.

❖ Secondary Structure

A polypeptide chain's secondary structure consists of its regular local folding. The primary cause is the establishment of hydrogen bonds along the polypeptide chain's backbone.

The α-helix and the β-sheet are the two primary secondary structures.

The polypeptide chain's backbone takes on a helical shape in the α-helix. The polypeptide chain's carbonyl and amide groups establish hydrogen bonds, which stabilise this. It is from the helix structure that the side chains emerge.

The β-sheet houses the adjacent expanded portions of the polypeptide chain. Hydrogen bonds connect them. The arrangement of these β-sheets might be either parallel or antiparallel.

Assembling the secondary structures, the twists and turns aid in protein three-dimensional structure determination.

Protein stability and local polypeptide chain folding are both provided by the secondary structure.

❖ Tertiary Structure

The three-dimensional form of a single polypeptide chain is known as tertiary structure. The tertiary structure of a protein is formed when the secondary structures connect with each other through bonds.

❖ Several forces stabilize tertiary structure.

Hydrophobic forces are especially significant in many soluble proteins. The non-polar groups usually reside inside the molecule, hidden from the solvent.

- Hydrogen bonding occurs between appropriate polar groups.
- Ionic forces result from interactions between opposite charged side chains.
- Van der Waals forces further stabilize the folding as long as atoms are properly positioned.

Disulfide bonds are covalent bonds which can be formed between two cysteines, providing an extra level of structural stability in proper proteins, especially in many extracellular proteins.

The proper folding is crucial for biological activity. For example, in the case of an enzyme, different amino acids located far apart in the primary sequence come into close proximity due to proper folding and form the active site.

Many proteins have certain regions responsible for the specific biological activity and called domains. A domain has its own proper folding and performs a particular biological function, for example, ligand binding or catalytic activity.

❖ Quaternary Structure

Quaternary structure refers to the structure of proteins that contain more than one polypeptide chain or subunit.

Subunits may be identical or different. They interact to make up the protein molecule.

Hemoglobin is one of the examples of a protein whose structure is quaternary. Hemoglobin A, which is found in adults, comprises four polypeptide subunits, two alpha and two beta.

The interaction between these subunits leads to cooperativity of oxygen binding and release.

Quaternary structure, therefore, gives proteins functions that cannot be obtained by isolated subunits.

- **Protein Folding**

The newly formed polypeptides should fold into the right three-dimensional conformation in order to carry out their usual role. Even though most of the information needed for such a process is inherent in the primary structure of the protein, there are proteins called molecular chaperones which help in the folding process of other proteins.

If the wrong process occurs, it leads to non-functional proteins and sometimes formation of protein aggregates. Therefore, there are mechanisms within the cell to recognize the wrong folding process and try to correct the mistakes made in the folding process or degrade the proteins.

So protein folding is not just an issue of structure but of functionality as well.

- **Functions of Proteins**

Proteins perform an exceptionally broad range of physiological and biochemical functions.

Catalytic Function

The majority of biological catalysts are enzymes, and enzymes are proteins mostly. They enhance the reaction rate but do not get consumed themselves. Examples of enzymes are the digestive enzymes, such as pepsin, glycolysis enzymes, and DNA synthesis enzymes.

Through the activity of enzymes, complicated biochemical reactions can take place effectively.

Structural Function

Structural proteins help give cells and tissues strength and organization. Collagen is one example of a structural protein that helps give connective tissue its form. Keratin is another type of structural protein that helps give skin, hair, and nails their physical characteristics. Elastin helps make tissues more elastic.

Transport Function

Transport proteins move molecules through the bloodstream or across biological membranes. Hemoglobin transports oxygen, transferrin transports iron, while albumin transports several substances including fatty acids, bilirubin, hormones and many drugs.

Membrane transport proteins aid in the passage of ions, glucose, amino acids and other substances across cellular membranes.

Storage Function

Some proteins function as reservoirs for biologically essential compounds. For instance, ferritin serves as a reservoir for iron in the body.

Protective and Immune Function

The immunoglobulin proteins or antibodies recognize certain antigens and function in adaptive immunity. Certain complement proteins along with some other proteins found in the blood plasma are also used in immunity.

Contractile and Motor Functions

The contractile proteins are actin and myosin. Some other motor proteins are involved in cell locomotion, such as vesicle and organelle movements.

Regulatory Function

Proteins act as regulators of cellular physiology. Receptors and signaling molecules are proteins. A number of hormones such as insulin and glucagon belong to this category.

Proteins also take part in regulation of gene expression, metabolism and cell growth and differentiation.

Blood Coagulation

Several parts of the coagulation system are proteins. For instance, fibrinogen is changed to fibrin during the process of blood clotting. Some clotting factors are involved in the series of reactions that regulate hemostasis.

Maintenance of Fluid and Acid–Base Balance

The proteins present in blood plasma, particularly albumin, play an important role in forming colloid osmotic pressure. They also have ionizable groups that are able to accept or donate hydrogen ions, thus acting as buffers.

Table 3: Major Functions of Proteins

Function	Examples	Biological Importance
Catalytic	Metabolic and digestive enzymes	Accelerate biochemical reactions
Structural	Collagen, keratin, elastin	Provide strength, support and elasticity

Transport	Hemoglobin, albumin, transferrin	Transport oxygen, drugs, iron and other substances
Storage	Ferritin	Stores biologically important substances
Protective	Immunoglobulins, complement proteins	Participate in immune defence
Contractile	Actin, myosin	Produce muscle contraction and movement
Regulatory	Insulin, receptors, regulatory proteins	Control metabolism and cellular signalling
Coagulation	Fibrinogen, clotting factors	Maintain normal hemostasis
Homeostatic	Albumin and other plasma proteins	Contribute to fluid and acid–base balance

❖ **Denaturation of Proteins**

Denaturation is the process whereby there is modification of the natural three dimensional arrangement of proteins without necessarily affecting the peptide bonds in their primary structure.

Denaturation can arise from a variety of physical and chemical agents such as:

- High temperatures;
- Extremely low/high pH values;
- Organic solvents;
- Detergents;
- Certain metallic ions;

And other protein-denaturing substances.

Heat interferes with hydrogen bonding within protein molecules while changes in pH interferes with the ionization of side chains in amino acids.

In an enzyme system, the modification of the shape of its active site leads to inhibition of its catalytic activity. Denaturation thus causes a reduction in biological activity.

An example of denaturation is the clotting of egg white protein when heated. It is just like the case in drugs which have therapeutic proteins and where proper conditions of storage are critical.

❖ **Denaturation and Renaturation**

Sometimes, by simply taking away the denaturant, the protein can return back to its original state and activity. This phenomenon is called renaturation. Sometimes, however, denaturation is so strong that it results in aggregation or even structural modification that will prevent renaturation.

5.2.2 Plasma Proteins and Their Functions

Plasma proteins are proteins found in the fluid part of the blood. They constitute a significant portion of the extracellular protein system, and their functions include maintaining blood volume, transporting substances, defense, clotting and buffering.

The principal classes of plasma proteins are albumin, globulin, and fibrinogen. These proteins are synthesized mainly by the liver except for immunoglobulin, which is synthesized by plasma cells.

Abnormal plasma protein levels can be used clinically to indicate alterations in hepatic production, protein loss, inflammation, immune function and metabolism.

❖ **Plasma and Serum**

The distinction between plasma and serum must be understood properly.

Plasma is the fluid portion of blood collected without allowing clotting with the help of an anticoagulant. Hence, it includes fibrinogen and all other clotting factors.

Serum is the fluid extracted from clotted blood. Fibrinogen is largely excluded from serum but many other proteins are present.

Table 4: Difference Between Plasma and Serum

Feature	Plasma	Serum
Obtained from	Anticoagulated blood	Blood allowed to clot
Fibrinogen	Present	Essentially absent after clot formation
Clotting factors	Generally retained	Some consumed or altered during clotting
Common laboratory use	Coagulation and various biochemical tests	Many routine biochemical and immunological tests

❖ Albumin

Albumin is the major protein of plasma which is produced by liver cells. It carries out two extremely significant roles including maintaining plasma oncotic pressure and the transport of many substances.

❖ Maintenance of Oncotic Pressure

Fluid constantly shifts between the vascular and interstitial spaces.

Plasma proteins, especially albumin, assist in keeping the fluid within the blood vessels through colloid oncotic pressure.

If there is a considerable drop in plasma albumin levels, then there would be less retaining ability, resulting in fluid shift into interstitial space. This could lead to edema formation.

There are various conditions where hypoalbuminemia could develop, such as reduced production in the liver, increased loss through the kidneys and GI tract, and inflammation.

❖ Transport Function of Albumin

Albumin has many binding sites and can bind many endogenous and exogenous substances. Albumin carries:

- unconjugated bilirubin;
- free fatty acids;
- some hormones;
- calcium;
- various metabolites; and
- many drugs.

The binding of albumin plays a significant role in pharmacology. When a drug reaches the bloodstream, some part of the drug is protein-bound, whereas another part is free. The free part of the drug usually becomes the part that is readily able to cross the membranes, act on receptors, and be eliminated.

A change in the level of albumin or competition for binding sites will change the free part of the drug, but it depends on pharmacokinetics of the drug in question.

❖ Globulins

Globulins constitute a diverse class of plasma proteins. By means of electrophoretic analysis, they can be subdivided into four classes, i.e., α_1, α_2, β, and γ fractions.

α_1-Globulins

The α_1 fraction consists of various transport proteins and regulatory proteins. An example of such protein is α_1-antitrypsin which inhibits proteolytic enzymes in tissues.

α_2-Globulins

Various α_2 proteins include haptoglobin, ceruloplasmin and α_2-macroglobulin. The first protein binds hemoglobin released into blood, whereas ceruloplasmin takes part in copper transportation.

β-Globulins

This fraction includes numerous transport proteins and others. Transferrin is one of the proteins that carry out iron through the blood flow. Components of the complement system are able to move in this region during electrophoretic analysis.

γ-Globulins

The γ fraction has many immunoglobulins including the main antibody classes. The proteins in this fraction are synthesized in plasma cells but not in hepatocytes.

Immunoglobulins bind specific antigens and take part in their neutralization or destruction.

Table 5: Important Globulin Fractions

Fraction	Important Examples	Major Function
α_1	α_1-Antitrypsin	Protease inhibition and protection of tissues
α_2	Haptoglobin, ceruloplasmin, α_2-macroglobulin	Binding, transport and enzyme inhibition
β	Transferrin, some complement proteins	Iron transport and other plasma functions
γ	Immunoglobulins	Antibody-mediated immune defence

❖ Fibrinogen

Fibrinogen is a large soluble glycoprotein of plasma and is manufactured by the liver. It is essential in the process of coagulation of blood.

The process of clotting of blood is set in motion when there is an injury to the vessel and fibrinogen is converted to fibrin by the action of thrombin which forms a network of insoluble fibrin which aids in the formation of the blood clot.

Since fibrinogen is used up in the process of blood clotting, it is found in plasma but not in serum.

❖ Other Important Plasma Proteins

Despite the fact that albumin, globulins, and fibrinogen make up the majority, there are many other kinds of proteins present in plasma that serve specific roles.

Transferrin transports iron ions and regulates the concentration of free iron.

Haptoglobin binds free hemoglobin molecules that come from the lysis of erythrocytes.

Ceruloplasmin binds a significant portion of free copper and is a ferroxidase enzyme.

The complement system proteins take part in nonspecific immunity and antigen-antibody immunity.

Lipoproteins are protein-lipid complexes that transport cholesterol and triacylglycerols in blood.

Table 6: Major Plasma Proteins and Their Functions

Plasma Protein	Major Source	Principal Function
Albumin	Liver	Oncotic pressure and transport of many substances
Transferrin	Liver	Iron transport
Haptoglobin	Liver	Binds free hemoglobin
Ceruloplasmin	Liver	Copper transport and ferroxidase activity
Immunoglobulins	Plasma cells	Antibody-mediated immune defence
Fibrinogen	Liver	Precursor of fibrin in coagulation
Complement proteins	Mainly liver and other cells	Immune defence

Clotting proteins	Mainly liver	Hemostasis

Functions of Plasma Proteins

- Maintenance of Blood Volume and Fluid Distribution: The albumin proteins and other plasma proteins affect the plasma oncotic pressure. This enables the movement of water across the capillaries to maintain blood volume and fluid distribution in tissues.
- Biological Substance Transport: Plasma proteins act as a carrier of substances that are insoluble in water or need to be transported in a regulated manner. This includes fatty acids, bilirubin, hormones, metallic ions, vitamins and drugs.
- Immunity: Immunoglobulins react specifically with antigens whereas the complement proteins play a role in the destruction or elimination of the microorganisms or damaged cells.
- Thus, plasma proteins play an important part in immune defense mechanisms of the body.
- Coagulation: Fibrinogen and many other coagulation proteins help in the formation of clots in the blood vessels in response to vascular injury.
- Buffers: Plasma proteins have ionizable amino and carboxyl group and ionizable side chain. Thus, they can release or accept H^+ and act as buffers.
- Enzyme inhibitors: Some plasma proteins control the activity of certain enzymes which may cause harm to the body. The examples of such proteins include alpha1-antitrypsin and alpha2-macroglobulin.

Table 7: Physiological Functions of Plasma Proteins

Function	Important Protein(s)	Significance
Oncotic pressure	Albumin	Helps maintain vascular fluid volume
Transport	Albumin, transferrin, ceruloplasmin	Carries drugs, iron, copper, hormones and metabolites
Immune defence	Immunoglobulins, complement proteins	Protects against foreign agents
Coagulation	Fibrinogen and clotting factors	Promotes hemostasis
Buffering	Albumin and other proteins	Contributes to blood pH regulation

Protease regulation	α_1-Antitrypsin, α_2-macroglobulin	Limits excessive proteolytic activity

❖ Clinical Significance of Plasma Proteins

Estimation of protein levels in the blood plasma or serum is an essential aspect of clinical biochemistry. The changes in the protein levels can give a lot of information about synthetic functions of the liver, protein loss from kidneys, inflammatory reactions, malnutrition, and immune disorders.

Hypoproteinemia describes reduced levels of plasma proteins and could happen due to decreased production, increased loss, increased degradation, or redistribution of proteins.

Hypoalbuminemia might result from serious liver diseases, as albumin is produced by the liver. It can also happen because of the loss of albumin by kidneys in proteinuria caused by renal disease, or by gastrointestinal system in some circumstances. Also, inflammation could affect the level of albumin in the serum in several ways.

The elevation of specific fractions of globulins might happen in case of inflammatory or immune reactions. The production of a single immunoglobulin could form a specific pattern on the serum protein electrophoresis and might be helpful in diagnosis of plasma cell disorders.

❖ Serum Protein Electrophoresis

The principle behind Serum Protein Electrophoresis (SPEP) involves the separation of the proteins of the blood based on their electrical characteristics. The common profile consists of albumin, then the α_1, α_2, β and γ regions.

Abnormalities in one or more of these components can help in the diagnosis of the disease. Inflammatory conditions might affect the different globulin components, whereas the production of a single antibody will result in an abnormal narrow peak.

This test is evaluated in conjunction with other tests.

❖ Albumin and Drug Binding

Plasma protein-drug interactions are especially relevant in pharmaceutical sciences. The fact is that many medications are distributed in the blood in association with protein. Drug-protein complex serves as a reversible depot in circulation, while unbound fraction of the medication is used in tissue distribution and for interaction with pharmacological receptors.

Several conditions may affect the level of protein binding of medication, such as:

- plasma concentration of albumin;
- association of the drug with albumin;
- drug concentration;
- endogenous substance or competition from other drugs;
- renal or liver diseases; and
- physiological state.

Alterations of protein binding are not clinically meaningful for all medications because distribution and elimination of medications may also be affected. However, it is necessary to know about plasma protein binding in the case of highly protein-bound medications.

❖ Acute-Phase Proteins

There exist certain proteins in blood plasma which become elevated or diminished in quantity upon inflammation, infection, or tissue damage and are known as acute-phase proteins.

The positive acute-phase proteins become elevated in an inflammatory state, and include C-reactive protein, fibrinogen, and haptoglobin. Certain other proteins such as albumin may get diminished and are thus termed negative acute-phase proteins.

Acute-phase protein determination, particularly C-reactive protein, can help in evaluating the inflammation state.

❖ Importance in Pharmacy and Clinical Practice

The study of proteins and plasma proteins is directly relevant to pharmacy practice. Drugs target proteins and use proteins as a means of treatment and diagnosis. Enzymes and receptors are examples of protein targets for many drugs, whereas recombinant proteins and monoclonal antibodies are becoming increasingly important drug therapies.

Plasma proteins have particular relevance to understanding how drugs are distributed. Changes in albumin and other binding proteins may change the ratio of bound and free drug. Additionally, measurement of levels of albumin, immunoglobulins, acute-phase proteins and other plasma proteins offers valuable laboratory information in the assessment of diseases.

Hence, proteins play a crucial role in the organization of human structure and function. Biological activity of proteins is highly dependent on their exact structural organization. Plasma proteins

comprise a very important class of proteins, which participate in oncotic pressure formation, transport processes, immunity, blood clotting, buffering action, enzyme control, and drug binding. The relevance of these processes and functions for biochemical and clinical practice make the study of proteins a fundamental subject in biochemistry and pharmacology.

5.3 GENERAL METABOLISM OF AMINO ACIDS

The sources of amino acids for the human body include the proteins from the diet, breakdown of body proteins, and endogenous synthesis. These amino acids are constantly utilized in the synthesis of various proteins and other organic molecules containing nitrogen. In contrast to carbohydrates and fats, there is no special storage form of amino acids in the body. Thus, extra amino acids are catabolized but not stored.

Two main stages can be defined in amino acid catabolism: elimination/transfer of the nitrogen atom from the molecule and catabolism of the remaining carbon skeleton. The main product of nitrogen disposal is urea, whereas the carbon skeletons are catabolized in the same ways as carbohydrates.

Main reactions of amino acid catabolism are transamination, deamination and decarboxylation.

Table 7: Major Reactions in Amino Acid Metabolism

Reaction	Major Change	Main Significance
Transamination	Transfer of an amino group to an α-keto acid	Collects and redistributes amino nitrogen
Oxidative deamination	Removal of amino group as ammonia with oxidation	Releases ammonia for nitrogen disposal
Non-oxidative deamination	Removal of amino group without an oxidation step	Catabolism of selected amino acids
Decarboxylation	Removal of carboxyl group as CO_2	Produces biologically active amines

5.3.1 Transamination and Deamination

- **Transamination**

Transamination refers to the reversible process of transferring the amino group of an amino acid to an alpha-keto acid, resulting in the formation of a new amino acid and a new keto acid but not any free ammonia.

This process is facilitated by enzymes called aminotransferases or transaminases, which usually require the vitamin B6 derivative called pyridoxal phosphate (PLP).

An example of the amino acid acceptor is α-ketoglutarate, which is converted to glutamate. By this process, amino groups from other amino acids can thus be combined to form glutamate.

The two clinically significant aminotransferases are:

Alanine aminotransferase (ALT) transfers the amino group of alanine to α-ketoglutarate, forming pyruvate and glutamate.

Aspartate aminotransferase (AST) transfers the amino group of aspartate to α-ketoglutarate, forming oxaloacetate and glutamate.

Table 8: Important Transamination Reactions

Amino Acid	Amino-Group Acceptor	Products	Enzyme
Alanine	α-Ketoglutarate	Pyruvate + glutamate	ALT
Aspartate	α-Ketoglutarate	Oxaloacetate + glutamate	AST

The transamination process is vital because it facilitates the transfer of nitrogen from one amino acid to another and leads to its concentration primarily in glutamate. After this, the carbon skeletons become available for entry into intermediary metabolic processes.

Increased ALT and AST enzyme activity levels in the bloodstream may be seen after cell damage, especially involving the liver cells. However, this observation depends upon the complete clinical scenario.

❖ Deamination

Deamination refers to the process of removing an amino group from an amino acid that produces ammonia along with a carbon skeleton. Ammonia needs to be managed carefully since excess amounts can be harmful to the body, especially to the nervous system.

The deamination process can take place by oxidation or non-oxidation methods.

❖ Oxidative Deamination

Deamination by oxidation involves the loss of the amino group along with the oxidation of the compound. One of the key examples of deamination by oxidation in amino acid metabolism is oxidative deamination of glutamate.

It is catalyzed by glutamate dehydrogenase and is mainly confined to mitochondria. The amino acid glutamate is converted to α-ketoglutarate along with the liberation of ammonium, where NAD^+ or $NADP^+$ act as electron acceptors.

The significance of this reaction is that transamination frequently adds the amino group to α-ketoglutarate resulting in the formation of glutamate, which is further subject to oxidative deamination. Thus, the process of transamination followed by oxidative deamination is known as transdeamination.

❖ Non-Oxidative Deamination

During non-oxidative deamination, there is no oxidation involved in removing the amino group. Some amino acids may be subjected to this reaction via particular enzymes.

Some examples of such reactions involve serine and threonine being subjected to specific dehydratase/deaminase enzymes that produce their respective α-keto acids and ammonium.

Table 9: Comparison of Transamination and Deamination

Feature	Transamination	Deamination
Basic process	Transfer of amino group	Removal of amino group
Free ammonia directly produced	No	Yes in relevant deamination reactions
Important enzymes	Aminotransferases	Glutamate dehydrogenase and specific deaminating enzymes

Important cofactor	PLP for most aminotransferases	Depends on enzyme
Major significance	Transfers and collects amino nitrogen	Releases nitrogen for disposal
Important product	Often glutamate	Ammonium and carbon skeleton

Transamination and deamination combined create a very effective process to detach amino nitrogen from carbon skeletons in the amino acids. After that, the nitrogen may enter into the process of urea synthesis, whereas the carbon skeletons are available for metabolism in other processes.

5.3.2 Decarboxylation

The decarboxylation of amino acids is a process where the carboxyl group is removed as carbon dioxide. Decarboxylation reactions in amino acids are catalysed by amino acid decarboxylases enzymes. Pyridoxal phosphate is usually used as a coenzyme for these enzymes.

It is worth noting that decarboxylation of amino acids is very significant because some of the products formed are amines. These amines act as neurotransmitters and other biological agents such as regulators.

For instance, histidine is decarboxylated to give rise to histamine. Histamine acts in allergic and inflammatory responses as well as regulation of acid secretion in the stomach.

Glutamate is decarboxylated to give γ-aminobutyric acid (GABA), a neurotransmitter in the brain.

5-hydroxytryptophan from tryptophan is decarboxylated to give 5-hydroxytryptamine (serotonin)

L-DOPA from tyrosine is decarboxylated to give dopamine.

Table 10: Important Products Formed by Decarboxylation

Precursor	Product	Major Biological Role
Histidine	Histamine	Inflammatory/allergic responses and gastric secretion
Glutamate	GABA	Inhibitory neurotransmission
5-Hydroxytryptophan	Serotonin	Neurotransmission and physiological regulation
L-DOPA	Dopamine	Neurotransmission and precursor of catecholamines

Decarboxylation is different from transamination and deamination in terms of the metabolic function it performs. The function of decarboxylation is not to get rid of nitrogen but to transform amino acid precursors to biologically active substances.

Transamination, deamination and decarboxylation are some of the main reactions in amino acid metabolism. Transamination is a reaction where the amino group is transferred, deamination is a reaction where the nitrogen is released for excretion, and decarboxylation produces biological amines.

5.4 UREA CYCLE AND NITROGEN DISPOSAL

Metabolism of amino acids constantly produces nitrogen in the form of ammonia/ammonium ions. While nitrogen is required for the formation of amino acids, nucleotides and other substances, large amounts of ammonia can be very poisonous, especially for the brain. That is why organisms transform extra nitrogen mostly into urea, which is non-toxic and soluble in water, and which is then excreted through urine via bloodstream.

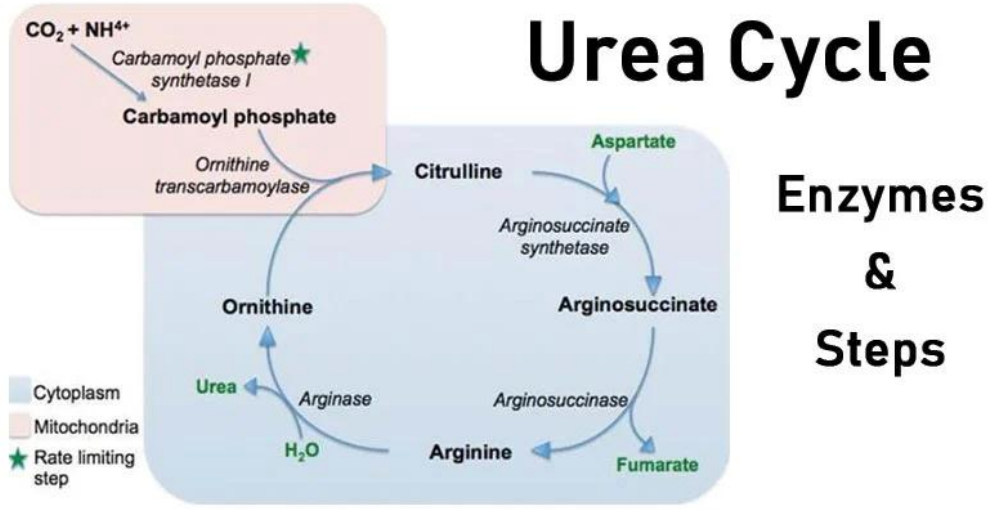


Figure 2: Urea Cycle Showing Major Enzymatic Steps and Cellular Compartments

(Image Source: https://microbenotes.com/urea-cycle-enzymes-and-steps/)

Urea cycle, or ornithine cycle, takes place predominantly in the liver. This cycle has its reactions located in the mitochondrial matrix and cytosol of hepatocytes. Nitrogen atoms of urea come from ammonia and aspartate, while carbon atom comes from bicarbonate.

❖ **Formation of Urea**

The reactions in the urea cycle occur in five major stages.

1. Carbamoyl phosphate formation: Ammonia reacts with bicarbonate in the mitochondrial matrix and produces carbamoyl phosphate. The process involves carbamoyl phosphate synthetase I (CPS I) enzyme and two ATP molecules. CPS I enzyme is activated by N-acetylglutamate.
2. Citrulline formation: In this stage, carbamoyl phosphate donates its carbamoyl group to ornithine, producing citrulline. The process involves the ornithine transcarbamylase enzyme.
3. Argininosuccinate formation: Citrulline reacts with aspartate in the cytosolic compartment to give rise to argininosuccinate. The process involves argininosuccinate synthetase and ATP molecule.
4. Arginine formation: In this reaction, argininosuccinate lyase enzyme hydrolyses argininosuccinate to produce arginine and fumarate. The fumarate produced can be used in pathways linked to the TCA cycle.
5. Urea formation: Hydrolysis of arginine leads to the production of urea and ornithine. The ornithine is transported back to the mitochondrial matrix.

Table 11: Major Reactions of the Urea Cycle

Step	Major Reaction	Enzyme	Location
1	Ammonia → carbamoyl phosphate	Carbamoyl phosphate synthetase I	Mitochondria
2	Carbamoyl phosphate + ornithine → citrulline	Ornithine transcarbamylase	Mitochondria
3	Citrulline + aspartate → argininosuccinate	Argininosuccinate synthetase	Cytosol
4	Argininosuccinate → arginine + fumarate	Argininosuccinate lyase	Cytosol
5	Arginine → urea + ornithine	Arginase	Cytosol

❖ Energy Requirement

For the urea cycle to take place, three ATPs are needed; however, four high-energy phosphate bonds are utilized. The production of carbamoyl phosphate utilizes two ATPs, whereas the process of citrulline becoming argininosuccinate causes the ATP to become AMP and pyrophosphate.

Though urea production needs energy, this energy use is necessary for the body's ability to dispose of toxic nitrogen.

❖ Nitrogen Transport and Disposal

The ammonia produced in the peripheral tissues is not usually transported in significant amounts as ammonia. Rather, the nitrogen compounds that reach the liver are mostly those which have relatively low toxicity such as glutamine and alanine.

Glutamine is synthesized in the body by adding ammonia to glutamate using glutamine synthetase. Glutamine is involved in the transportation of nitrogen from various parts of the body to the liver or kidneys.

Nitrogen from amino groups in the skeletal muscles may also be moved to pyruvate to create alanine. The alanine reaches the liver and helps in the production of urea, while its carbon skeleton helps in the synthesis of glucose.

This forms part of the glucose-alanine cycle. The other organ that is responsible for nitrogen elimination includes the kidneys which are involved in the urinary elimination of ammonium ions (NH_4^+).

Table 12: Major Forms of Nitrogen Transport and Disposal

Form/Process	Major Role	Principal Site
Glutamine	Safe transport of ammonia nitrogen	Peripheral tissues → liver/kidney
Alanine	Transfers amino nitrogen from muscle to liver	Muscle → liver
Urea	Major nitrogenous end product	Formed in liver, excreted by kidneys
Ammonium	Nitrogen excretion and acid–base regulation	Kidney

❖ Hyperammonaemia

Hyperammonaemia is defined as having too much ammonia in the bloodstream. It can be caused by serious malfunctioning of the liver or by genetic disorders that affect the enzymes in the urea cycle.

Due to its harmful effect on the nervous system, serious cases of hyperammonaemia lead to neurological problems that can be fatal. This makes the urea cycle very important for balancing nitrogen.

5.5 FATE OF AMINO ACID CARBON SKELETONS

After the amino group is removed or transferred, the rest of the structure of an amino acid is called its carbon skeleton, and is generally seen metabolically as an α-keto acid. Unlike the amino nitrogen which has to be eventually excreted, the carbon skeleton is a reusable structure in a number of different metabolic pathways.

Based on the type of amino acid, the carbon skeletons are formed from pyruvate, acetyl-CoA, acetoacetate, α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate. These compounds link amino acid metabolism to glycolysis, TCA cycle, gluconeogenesis and ketone body metabolism.

The carbon skeleton, therefore, can be used in the following ways:

- Generation of ATP by oxidative metabolism;
- Glucose formation by gluconeogenesis;
- Ketone body formation;
- Lipid formation if there is enough energy;
- TCA cycle intermediate replenishment.

Table 13: Major Metabolic Fates of Amino Acid Carbon Skeletons

Metabolic Product	Possible Metabolic Fate
Pyruvate	Glucose formation or oxidation for energy
α-Ketoglutarate	Enters TCA cycle; may support gluconeogenesis
Succinyl-CoA	Enters TCA cycle; may support gluconeogenesis
Fumarate	Enters TCA cycle; may support gluconeogenesis
Oxaloacetate	Gluconeogenesis or TCA cycle

Acetyl-CoA	Energy production, lipid synthesis or ketogenesis
Acetoacetate/acetoacetyl-CoA	Ketone-body or lipid-related metabolism

5.5.1 Glucogenic and Ketogenic Amino Acids

Amino acids are classified into glucogenic amino acids, ketogenic amino acids, or both depending on the fate of their carbon skeleton.

Glucogenic Amino Acids

Glucogenic amino acids are amino acids that can form intermediates from their carbon chain that will help in the formation of glucose by undergoing gluconeogenesis.

The examples of glucogenic amino acids are alanine, glycine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, histidine, methionine, and valine. Alanine, for example, forms pyruvate, while aspartate contributes to oxaloacetate formation.

Ketogenic Amino Acids

The ketogenic amino acids will generate carbon skeletons that may be used to form acetyl-CoA and/or acetoacetate/acetoacetyl-CoA; therefore, the generation of ketones or lipids may occur via the utilization of these amino acids.

Leucine and lysine are pure ketogenic amino acids; they will not generate any net glucose from their degradation process by gluconeogenesis.

Glucogenic and Ketogenic Amino Acids

There are certain amino acids that will generate products that will generate glucose as well as ketones. These include phenylalanine, tyrosine, tryptophan, threonine and isoleucine**.**

- **Physiological Significance**

Glucogenic and Ketogenic Amino Acids

The classification of glucogenic and ketogenic amino acids is especially important in fasting and extended starvation. Glucogenic amino acids are able to supply carbon to the process of gluconeogenesis in the liver and thus maintain glucose levels in blood for glucose-dependent cells.

Ketogenic amino acids are involved in the supply of acetyl-CoA or ketone body precursors. In extended fasting, ketone bodies become increasingly important source of energy for various cells, including brain after metabolic adaptation.

Therefore, catabolism of amino acids connects metabolism of proteins with carbohydrates and fats. The nitrogen moiety is excreted via the urea cycle, and the carbon skeleton is used again either for the synthesis of glucose, ketone bodies, biosynthesis, or energy production.

5.6 METABOLISM OF PHENYLALANINE AND TYROSINE

Phenylalanine is a critical aromatic amino acid that is acquired through dietary protein sources. The main metabolic route starts with its conversion to tyrosine, a process that is mediated by phenylalanine hydroxylase and requires the presence of tetrahydrobiopterin (BH_4).

This indicates that tyrosine is acquired from both diet and phenylalanine. The biological functions include participation in the formation of proteins as well as acting as a building block for synthesis of catecholamines, thyroid hormones, and melanin.

Degradation of tyrosine involves a series of chemical processes leading to the production of fumarate and acetoacetate, where fumarate participates in glycolysis while acetoacetate acts as a ketone body. Therefore, tyrosine and phenylalanine are both glucogenic and ketogenic amino acids.

Table 14: Major Metabolic Roles of Phenylalanine and Tyrosine

Amino Acid	Major Metabolic Role	Important Products
Phenylalanine	Converted to tyrosine	Tyrosine
Tyrosine	Protein synthesis and formation of specialized compounds	Catecholamines, melanin, thyroid hormones
Tyrosine catabolism	Produces glucogenic and ketogenic products	Fumarate and acetoacetate

5.7 DISORDERS OF AMINO ACID METABOLISM

Amino acid metabolism disorders are usually due to the inheritance of an enzyme that is needed for the breakdown or modification of the amino acid. The disorder leads to an accumulation of the amino

acid and a deficiency of other necessary products. These conditions fall into the category of inborn metabolic errors.

5.7.1 Phenylketonuria, Albinism and Alkaptonuria

- **Phenylketonuria**

The most common cause of phenylketonuria (PKU) is the lack of the enzyme phenylalanine hydroxylase, which causes improper metabolism of phenylalanine to tyrosine. Phenylalanine and other metabolic products build up in the body.

If left untreated, phenylketonuria can negatively impact neural development. This makes diagnosis during infancy and limiting phenylalanine consumption crucial. In classical phenylketonuria, tyrosine is considered conditionally essential.

- **Albinism**

Albinism consists of hereditary diseases involving the reduced ability or absence of ability to produce melanin. In certain types of oculocutaneous albinism, mutations in the tyrosinase protein, or other enzymes required for melanin formation, cause the condition.

Low levels of melanin lead to the low pigmentation of the skin, hair, and eyes and may result in vision problems and photosensitivity.

- **Alkaptonuria**

Alkaptonuria is due to a lack of homogentisate 1,2-dioxygenase, which participates in the breakdown of phenylalanine and tyrosine. This results in buildup of homogentisic acid.

A typical manifestation includes dark-colored urine after standing for some time. Prolonged buildup of the pigment in connective tissue, called ochronosis, may be another feature and can cause joint problems.

5.7.2 Tyrosinemia and Other Inborn Errors of Amino Acid Metabolism

Tyrosinemias are genetic conditions that occur because of an impairment of enzymes that participate in the catabolism of tyrosine. Each one is caused by a lack of a particular enzyme. The most common form is Type I tyrosinemia, which occurs due to the absence of fumarylacetoacetate hydrolase.

Some other inborn metabolic disorders include deficiencies in branched-chain and aromatic amino acid catabolism. One such disorder is MSUD, which occurs due to a lack of the branched-chain α-ketoacid dehydrogenase complex.

Table 15: Selected Disorders of Amino Acid Metabolism

Disorder	Major Biochemical Defect	Important Feature
Phenylketonuria	Phenylalanine hydroxylase deficiency in classical PKU	Phenylalanine accumulation and neurological risk
Albinism	Defect in melanin synthesis	Reduced pigmentation
Alkaptonuria	Homogentisate 1,2-dioxygenase deficiency	Homogentisic acid accumulation
Tyrosinemia type I	Fumarylacetoacetate hydrolase deficiency	Severe hepatic and renal involvement
Maple syrup urine disease	Branched-chain α-ketoacid dehydrogenase complex deficiency	Accumulation of branched-chain amino acids and their keto acids

5.8 AMINO ACID-DERIVED NEUROTRANSMITTERS AND HORMONES

Many amino acids serve as precursors to neurotransmitters, hormones, and other signalling molecules. The two that are especially significant are tryptophan and tyrosine, since the metabolic breakdown of these amino acids leads to the formation of physiologically relevant molecules.

5.8.1 Serotonin and Melatonin

Serotonin (5-hydroxytryptamine or 5-HT) is formed from the amino acid tryptophan. The process starts by transforming tryptophan into 5-hydroxytryptophan which is then decarboxylated into serotonin.

Serotonin acts as a vital neurotransmitter involved in the regulation of mood, appetite, sleep and gastrointestinal functioning.

Melatonin is produced mainly from serotonin in the pineal gland. Secretion of melatonin is significantly influenced by the light/dark cycle and is very important for regulating circadian rhythms and sleep/wake cycles.

Table 16: Tryptophan-Derived Compounds

Precursor	Product	Major Function
Tryptophan	Serotonin	Neurotransmission, mood, appetite and gastrointestinal regulation
Serotonin	Melatonin	Regulation of circadian rhythm and sleep

5.8.2 Dopamine, Noradrenaline and Adrenaline

Dopamine, noradrenaline, and adrenaline belong to the class of catecholamines derived from tyrosine.

First, tyrosine undergoes transformation into L-DOPA through tyrosine hydroxylase and then into dopamine. Further on, dopamine can be transformed into noradrenaline and further on into adrenaline.

Dopamine is a vital neurotransmitter taking part in such processes as movement, motivation, and rewards.

Noradrenaline can serve both as a neurotransmitter and hormone and plays its role in sympathetic nervous system responses.

Adrenaline is one of the hormones playing a major role in the reaction of the body to acute stress and promoting metabolic fuel mobilization.

Table 17: Major Amino Acid-Derived Neurotransmitters and Hormones

Compound	Major Precursor	Principal Role
Serotonin	Tryptophan	Neurotransmission and behavioural regulation
Melatonin	Tryptophan via serotonin	Circadian rhythm
Dopamine	Tyrosine	Neurotransmission
Noradrenaline	Tyrosine via dopamine	Sympathetic nervous-system activity
Adrenaline	Tyrosine via catecholamine pathway	Acute stress response

5.9 HEME CATABOLISM AND JAUNDICE

Heme plays an essential part in the structure of hemoglobin, myoglobin and some enzymes. Old red blood cells are scavenged by reticuloendothelial macrophages, especially spleen, liver and bone marrow macrophages. Hemoglobin is broken down into its globin and heme parts.

Heme is degraded, but the former is converted to amino acids. Heme oxygenase releases carbon monoxide and iron by converting heme to biliverdin. Afterward, bilirubin is formed from biliverdin.

Blood carries unconjugated bilirubin linked to albumin, which is nearly insoluble in water. Bilirubin is mainly conjugated with glucuronic acid in the liver; this process makes it more water-soluble. Conjugated bilirubin is secreted to bile and enters the intestines, where microbial oxidation generates products such as urobilinogen. Metabolites are eventually excreted primarily with feces and partially in urine.

Jaundice

Jaundice is described as the yellow staining of the skin, sclera, and mucosa due to elevated bilirubin level in the blood. Jaundice can be categorized based on its mechanism into three types including prehepatic, hepatic or post-hepatic.

The first category is prehepatic where excess bilirubin production takes place. The second type is hepatic jaundice which is caused by problems in the liver involving the uptake, conjugation and elimination of bilirubin. The last category is post-hepatic or obstructive.

Table 18: Major Types of Jaundice

Type	Major Cause	Principal Biochemical Problem
Pre-hepatic	Increased red-cell destruction	Excess bilirubin production
Hepatic	Hepatocellular dysfunction	Impaired bilirubin processing/excretion
Post-hepatic	Biliary obstruction	Impaired excretion of conjugated bilirubin

Red cell turnover and heme breakdown are, thus, related to bilirubin production and metabolism. Alterations occurring at various levels of this process result in hyperbilirubinemia and jaundice, which renders bilirubin analysis clinically valuable for investigating liver, blood, and bile diseases.

REFERENCES

1. Mrowicka, M., Mrowicki, J., Dragan, G., & Majsterek, I. (2023). The importance of thiamine (vitamin B1) in humans. *Bioscience Reports*, *43*(10), BSR20230374.
2. Munir, R., Lisec, J., Swinnen, J. V., & Zaidi, N. (2019). Lipid metabolism in cancer cells under metabolic stress. *British journal of cancer*, *120*(12), 1090-1098.
3. Mutlu, A. S., Duffy, J., & Wang, M. C. (2021). Lipid metabolism and lipid signals in aging and longevity. *Developmental cell*, *56*(10), 1394-1407.
4. Nakazawa, M. S., Keith, B., & Simon, M. C. (2016). Oxygen availability and metabolic adaptations. *Nature Reviews Cancer*, *16*(10), 663-673.
5. Neinast, M. D., Jang, C., Hui, S., Murashige, D. S., Chu, Q., Morscher, R. J., ... & Arany, Z. (2019). Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. *Cell metabolism*, *29*(2), 417-429.
6. Neinast, M., Murashige, D., & Arany, Z. (2019). Branched chain amino acids. *Annual review of physiology*, *81*(1), 139-164.
7. Neis, E. P., Dejong, C. H., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients*, *7*(4), 2930-2946.
8. Nie, C., He, T., Zhang, W., Zhang, G., & Ma, X. (2018). Branched chain amino acids: beyond nutrition metabolism. *International journal of molecular sciences*, *19*(4), 954.
9. Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A., & Alshaer, W. (2022). Liposomes: structure, composition, types, and clinical applications. *Heliyon*, *8*(5).
10. OGIDI, O., & IYONSI, O. F. (2025). Biochemistry of drug metabolism: a review. *Quantum Journal of Medical and Health Sciences*, *4*(4), 16-34.
11. Ooijevaar, R. E., Terveer, E. M., Verspaget, H. W., Kuijper, E. J., & Keller, J. J. (2019). Clinical application and potential of fecal microbiota transplantation. *Annual review of medicine*, *70*(1), 335-351.
12. Papsdorf, K., & Brunet, A. (2019). Linking lipid metabolism to chromatin regulation in aging. *Trends in cell biology*, *29*(2), 97-116.
13. Parhofer, K. G. (2015). Interaction between glucose and lipid metabolism: more than diabetic dyslipidemia. *Diabetes & metabolism journal*, *39*(5), 353.

14. Park, J., Hsueh, P. C., Li, Z., & Ho, P. C. (2023). Microenvironment-driven metabolic adaptations guiding CD8+ T cell anti-tumor immunity. *Immunity*, *56*(1), 32-42.
15. Parthasarathy, A., Cross, P. J., Dobson, R. C., Adams, L. E., Savka, M. A., & Hudson, A. O. (2018). A three-ring circus: metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. *Frontiers in molecular biosciences*, *5*, 29.
16. Patel, T., Rahimi, N., & Cassagnol, M. (2026). Biochemistry, cytochrome P450. In *StatPearls [Internet]*. StatPearls Publishing.
17. Peters, S. A. (2021). *Physiologically based pharmacokinetic (PBPK) modeling and simulations: principles, methods, and applications in the pharmaceutical industry*. John Wiley & Sons.
18. Phillips, J. (2019). *Fundamentals of Enzymology*. Scientific e-Resources.
19. Pino, R. M., & Singh, J. (2020). Appropriate clinical use of lactate measurements. *Anesthesiology*, *134*(4), 637-644.
20. Pontzer, H., Durazo-Arvizu, R., Dugas, L. R., Plange-Rhule, J., Bovet, P., Forrester, T. E., ... & Luke, A. (2016). Constrained total energy expenditure and metabolic adaptation to physical activity in adult humans. *Current biology*, *26*(3), 410-417.
21. Popa, D. S., Bigman, G., & Rusu, M. E. (2021). The role of vitamin K in humans: implication in aging and age-associated diseases. *Antioxidants*, *10*(4), 566.
22. Pope, L. E., & Dixon, S. J. (2023). Regulation of ferroptosis by lipid metabolism. *Trends in cell biology*, *33*(12), 1077-1087.
23. Popovic, M. (2023). Introducing the Physical Driving Forces into Biology.
24. Portune, K. J., Beaumont, M., Davila, A. M., Tomé, D., Blachier, F., & Sanz, Y. (2016). Gut microbiota role in dietary protein metabolism and health-related outcomes: The two sides of the coin. *Trends in Food Science & Technology*, *57*, 213-232.
25. Rai, S., Kumar, S., Kumar, S., & Banerjee, C. (2025). Cellular Metabolism and Bioenergetics. In *Bioprocess Engineering and Technology* (pp. 79-114). CRC Press.
26. Rao, S., Li, T., Li, X., Hou, L., & Sun, W. (2025). Vitamin A and its related diseases. *Food Science & Nutrition*, *13*(7), e70630.
27. Razak, M. A., Begum, P. S., Viswanath, B., & Rajagopal, S. (2017). Multifarious beneficial effect of nonessential amino acid, glycine: a review. *Oxidative medicine and cellular longevity*, *2017*(1), 1716701.

28. Refugjati, E., Li, Z., Umano, G. R., Galuppo, B., Van Name, M., Samuels, S., ... & Santoro, N. (2025). Biomarkers of Glycolysis and the Tricarboxylic Acid Cycle in Youth with and without Obesity. *Hormone research in paediatrics*.
29. Roosterman, D., & Cottrell, G. S. (2021). Rethinking the citric acid cycle: connecting pyruvate carboxylase and citrate synthase to the flow of energy and material.
30. Sabater, B. (2022). Entropy perspectives of molecular and evolutionary biology. *International journal of molecular sciences*, *23*(8), 4098.
31. Salih, K. J., Sabir, D. K., & Abdoul, H. J. (2022). Glycolysis regulation to maintain blood glucose homeostasis. *Kurdistan Journal of Applied Research*, *7*(1), 114-124.
32. Salvador, M. M., de Cedrón, M. G., Rubio, J. M., Martínez, S. F., Martínez, R. S., Casado, E., ... & Sereno, M. (2017). Lipid metabolism and lung cancer. *Critical reviews in oncology/hematology*, *112*, 31-40.
33. Schmidt-Rohr, K. (2020). Oxygen is the high-energy molecule powering complex multicellular life: Fundamental corrections to traditional bioenergetics. *ACS omega*, *5*(5),
34. Schurr, A. (2017). Lactate, not pyruvate, is the end product of glucose metabolism via glycolysis. *Carbohydrate*, *2*, 22-35.
35. Sharma, M., Tuaine, J., McLaren, B., Waters, D. L., Black, K., Jones, L. M., & McCormick, S. P. (2016). Chemotherapy agents alter plasma lipids in breast cancer patients and show differential effects on lipid metabolism genes in liver cells. *PloS one*, *11*(1), e0148049.
36. Shi, H., Fang, R., Li, Y., Li, L., Zhang, W., Wang, H., ... & Ye, L. (2016). The oncoprotein HBXIP suppresses gluconeogenesis through modulating PCK1 to enhance the growth of hepatoma cells. *Cancer letters*, *382*(2), 147-156.
37. Siltari, A., Syvälä, H., Lou, Y. R., Gao, Y., & Murtola, T. J. (2022). Role of lipids and lipid metabolism in prostate cancer progression and the tumor's immune environment. *Cancers*, *14*(17), 4293.
38. Sivanand, S., & Vander Heiden, M. G. (2020). Emerging roles for branched-chain amino acid metabolism in cancer. *Cancer cell*, *37*(2), 147-156.
39. Smith, J. A., Murach, K. A., Dyar, K. A., & Zierath, J. R. (2023). Exercise metabolism and adaptation in skeletal muscle. *Nature Reviews Molecular Cell Biology*, *24*(9), 607-632.
40. Snaebjornsson, M. T., Janaki-Raman, S., & Schulze, A. (2020). Greasing the wheels of the cancer machine: the role of lipid metabolism in cancer. *Cell metabolism*, *31*(1), 62-76.

Unit 6…

NUCLEIC ACID METABOLISM AND GENETIC INFORMATION TRANSFER

Nucleic acids are key biomolecules whose function is the storage and transmission of genetic information. Nucleotides, which are basic building blocks of nucleic acids, are involved in energy transfers, cell communication and metabolic processes as well. Regulation of nucleotides' synthesis and breakdown is vital. Knowledge about nucleic acids and their metabolism serves as a basis for understanding DNA replication and other genetic processes.

6.1 NUCLEIC ACIDS, NUCLEOTIDES AND GENOME ORGANIZATION

Biological polymers with a high molecular weight are known as nucleic acids. Their function in the inheritance, processing, and storage of genetic information is crucial. Nucleotides are the building blocks of nucleic acids. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are fundamental types of nucleic acids. The primary function of DNA is to store genetic information, but RNA is crucial for gene expression, gene transfer, and gene regulation.

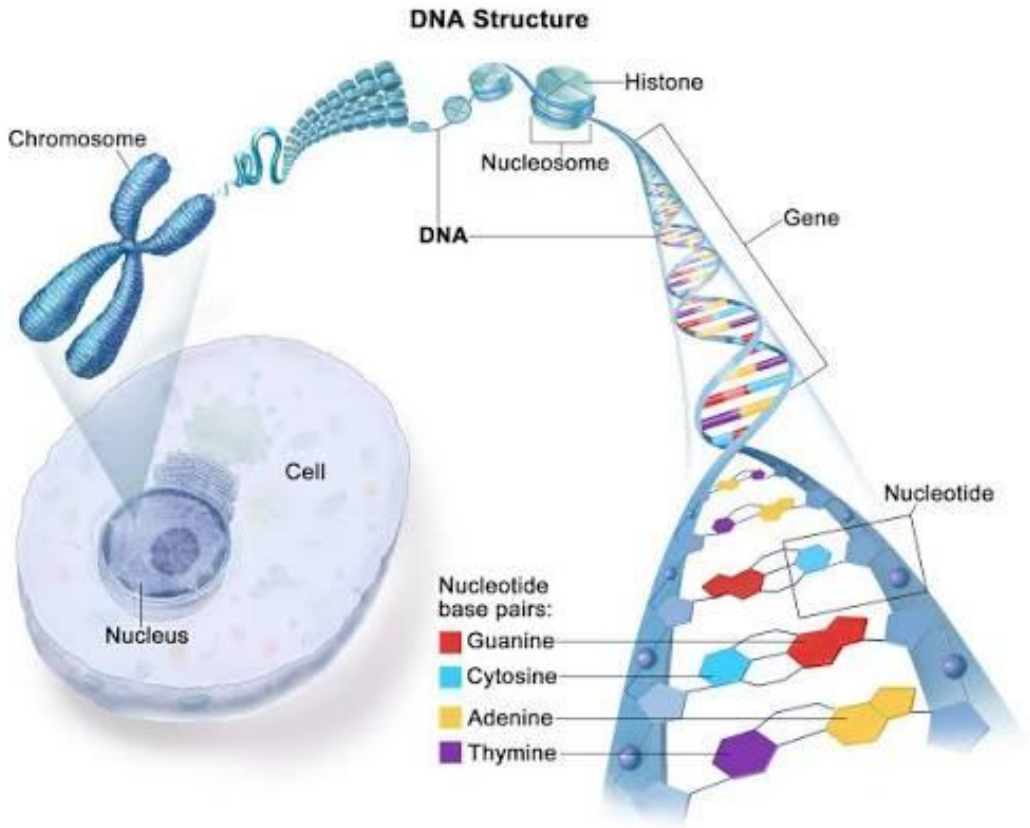


Figure 1: Structural Organisation of DNA from Nucleotide to Chromosome

(Image Source: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/nucleotide)

The genetic code of a cell is defined by the sequence of its nucleotides. Growth, metabolism, differentiation, reproduction, and inheritance are just a few of the many processes impacted by genetic material, which also regulates protein synthesis. When the sequence of nucleotides changes, it is called a mutation. Mutations can change the way proteins fold or even change the way genes work.

❖ Nucleosides and Nucleotides

Nucleic acids consist of three primary components: a nitrogenous base, a pentose sugar, and a phosphate group. The presence of a nitrogenous base linked to a pentose sugar results in the formation of a nucleoside. Nucleotides are formed when nucleosides are attached to one or more phosphate groups.

Nitrogenous Base + Pentose Sugar → Nucleoside

Nucleoside + Phosphate Group → Nucleotide

The nitrogenous bases can be classified as follows:

- Purines: These consist of Adenine (A) and Guanine (G). They have a double-ring structure.
- Pyrimidines: These include Cytosine (C), Thymine (T), and Uracil (U). They have a single-ring structure

DNA includes deoxyribose sugar and nitrogenous bases of adenine, guanine, cytosine and thymine. RNA includes ribose sugar along with the nitrogenous bases of adenine, guanine, cytosine and uracil. Thus, adenine, guanine and cytosine are found in both DNA and RNA, while thymine is present in DNA and uracil in RNA.

Table 1: Basic Components of Nucleic Acids

Component	**Types/Examples**	**Significance**
Pentose sugar	Ribose, deoxyribose	Forms an important structural component of nucleotides
Purine bases	Adenine, guanine	Double-ring bases present in DNA and RNA
Pyrimidine bases	Cytosine, thymine, uracil	Participate in storage and expression of genetic information
Phosphate group	One or more phosphate groups	Forms part of the sugar-phosphate backbone
Nucleoside	Base + sugar	Precursor of nucleotide formation
Nucleotide	Base + sugar + phosphate	Basic structural unit of DNA and RNA

The phosphodiester linkages between the nucleotides of nucleic acids occur at 3′-5′ positions. The phosphate group links the sugar of one nucleotide to the sugar of the other nucleotide and thus forms a backbone of sugar and phosphate. The bases are attached to the backbone and hold the genetic information within their sequence.

Each polynucleotide has two distinct ends referred to as 5′ end and 3′ end. The direction is vital during DNA replication and RNA synthesis since polynucleotide chains usually are synthesized from 5′ to 3′ ends.

❖ **DNA**

Deoxyribonucleic acid (DNA) is the main genetic material in man and other living beings. It carries genetic information required for the development, growth, reproduction and functioning of the cells.

DNA typically exists in a double helical form that includes two polynucleotide chains. These two chains have opposite polarity and are called antiparallel. The chain going from 5' end to 3' end is called one chain and vice versa is called the other chain.

Both the DNA chains are joined by hydrogen bonds between their complementary nitrogen bases:

Adenine (A) ≡ Thymine (T) – Two hydrogen bonds

Guanine (G) ≡ Cytosine (C) – Three hydrogen bonds

Genes are the functional regions of DNA. There are many genes whose function is to code for proteins and there are other genes which synthesize functional RNA molecules. Expression of genes is controlled as per need of individual cells.

DNA performs several important roles which include:

- Information storage.
- Genetic information transfer from one generation to the next.
- Genetic information transfer from the parent cell to the daughter cells.
- As a template for RNA synthesis.
- Protein synthesis control via gene expression.
- Cellular growth, metabolism and development regulation.

Replication of DNA takes place before cell division. The process involves production of an exact copy of the DNA molecule so that the daughter cells receive all the necessary genetic information.

❖ **RNA**

Ribonucleic acid (RNA) is another form of nucleic acid that plays an important role in the creation of proteins and the storage of genetic information. Although most RNA molecules are unidirectional, folding can cause some segments to take on complementary structures, setting them apart from DNA.

While DNA contains the sugar ribose, RNA molecules typically include the nucleotide uracil (U) instead of thymine. The many forms of RNA each have specific functions inside cells. There are three main categories of RNA, according to conventional wisdom: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

The genetic code is transferred from the DNA to the ribosomes by messenger RNA, or mRNA. Codons are groups of three bases that are used to write genetic information in messenger RNA molecules. A codon is a nucleotide or a signal that specifies an amino acid during protein synthesis.

Certain amino acids are transported to the ribosome by transfer RNA (tRNA). Anticodons are unique to each tRNA and are able to recognise complementing codons on messenger RNA.

Proteins and ribosomal RNA (rRNA) come together to produce ribosomes. It is the job of ribosomes to synthesise proteins.

- In addition to these three primary kinds of RNA, there are several others, including miRNA, snRNA and others. All these RNA molecules are involved in processes of RNA processing and gene expression regulation.
- General pathway of genetic information transfer looks like:
- DNA → Transcription → RNA → Translation → Protein
- Transcription involves copying of genetic information from DNA to RNA. During translation information stored in mRNA is used by ribosomes to code the order of amino acids in a protein.

Biological Role of Nucleotides

- In addition to their structural role in formation of DNA and RNA, nucleotides serve as biochemical compounds participating in several other important processes.
- For example, adenosine triphosphate (ATP) is one of the key nucleotides involved in energy exchange processes in living cells. The energy stored in ATP molecule is consumed for muscle contraction, active transport, biosynthesis and many other cellular processes.
- Similarly, guanosine triphosphate (GTP) is another nucleotide acting as a source of energy and playing an important role in protein synthesis and some other intracellular signal transduction pathways.
- Cyclic nucleotides, including cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) function as intracellular second messengers transmitting signals from hormones and other extracellular signal molecules to targets inside the cell.
- Some nucleotides are also important components of coenzymes playing significant role in metabolism of living organisms. NAD^+, FAD and Coenzyme A (CoA) are some examples of

such compounds involved in oxidation-reduction processes, energy metabolism and transfer of groups.

Thus, nucleotides have the following functions:

- Formation of DNA and RNA;
- Storing and transporting of energy in cells;
- Intracellular signal transduction;
- Metabolic regulation;
- Synthesis of coenzymes;
- Biosynthetic processes.

❖ **Organization of the Mammalian Genome**

Genome refers to the entire genetic composition of an organism. In mammalian cells, most of the DNA is found inside the nucleus in the form of chromosomes. The rest of the DNA occurs in mitochondria.

Somatic cells in humans have 46 chromosomes, which are formed of 23 pairs. Out of the 23 pairs, 22 are autosomes and one pair is made up of sex chromosomes. Each pair of chromosomes is derived from the mother and the father.

The haploid nuclear genome of a human being is estimated to have 3.2 billion base pairs. This is only a small part that codes for proteins. There are other parts that have other functions.

❖ **Packaging of DNA into Chromatin**

The DNA molecule is very long and needs to be effectively organized in order to fit into the nucleus. Therefore, nuclear DNA is linked to proteins called histones. The linking of DNA and proteins results in chromatin.

The basic unit of organization of chromatin is the nucleosome. In the nucleosome, the DNA molecule wraps around the histone proteins. Such organization is a very effective way of organizing DNA and making it accessible.

The scheme may look like this:

DNA Double Helix → Nucleosome → Chromatin → Condensed Chromatin → Chromosome

There are different stages of compaction of chromatin. In the process of cell division, there occurs the formation of chromosomes from chromatin.

❖ Euchromatin and Heterochromatin

The two main categories of chromatin are euchromatin and heterochromatin.

Euchromatin is less condensed and hence accessible to various enzymes and proteins that mediate transcription. This leads to increased transcriptional activity of genes residing within euchromatin regions.

Heterochromatin is denser and hence less transcriptionally active. This helps in chromosome structure, protection and genome stability.

It can therefore be concluded that chromatin condensation determines its accessibility and hence its gene expression.

Table 2: Levels of Mammalian Genome Organization

Level	Description	Significance
DNA	Double-stranded molecule containing nucleotide sequences	Stores genetic information
Nucleosome	DNA wrapped around histone proteins	Basic unit of DNA packaging
Chromatin	DNA associated with histones and other proteins	Packages DNA and regulates its accessibility
Chromosome	Highly condensed and organized chromatin	Ensures proper distribution of DNA during cell division
Genome	Complete genetic material of an organism	Contains the total genetic information of the organism

❖ Genes and Genome Function

A gene is defined as a functional segment of DNA that encodes for RNA or protein products via its sequence. In most cases, protein genes have introns and exons. Exons code for mature RNA, while introns are edited out of the mature mRNA transcripts.

There are also regulatory sequences within the genome which are responsible for gene expression. Consequently, not all DNA segments code for proteins as many of these serve as regulators and help organize the genome.

In addition to being needed for the packaging of DNA within the cell nucleus, genome organization is required to regulate many biological processes such as gene transcription, DNA replication and DNA repair. Changes in the chemical properties of both DNA and histone proteins may affect the activity of a gene without any changes in its nucleotide sequence.

- **Mitochondrial DNA**

Aside from the nuclear DNA in mammals, there is an insignificant quantity of genetic material present within mitochondria, which is referred to as mtDNA or mitochondrial DNA.

MtDNA is comprised of genes responsible for certain parts involved in energy generation within mitochondria, including rRNA and tRNA of mitochondria. However, most of the proteins responsible for structure and function of mitochondria are coded in nuclear DNA and transferred to mitochondria.

On the whole, the well-organized structure of nucleic acids is necessary for efficient storage, protection, replication and utilization of genetic information. DNA represents a source of information, RNA is responsible for its expression and regulation, while nucleotides play their own role in metabolism and signaling processes.

6.2 NUCLEOTIDE METABOLISM

The need for nucleotides is continuous due to the requirements in DNA and RNA biosynthesis as well as in other physiological functions. This occurs by a balance in the production, salvage, and degradation of the nucleotides.

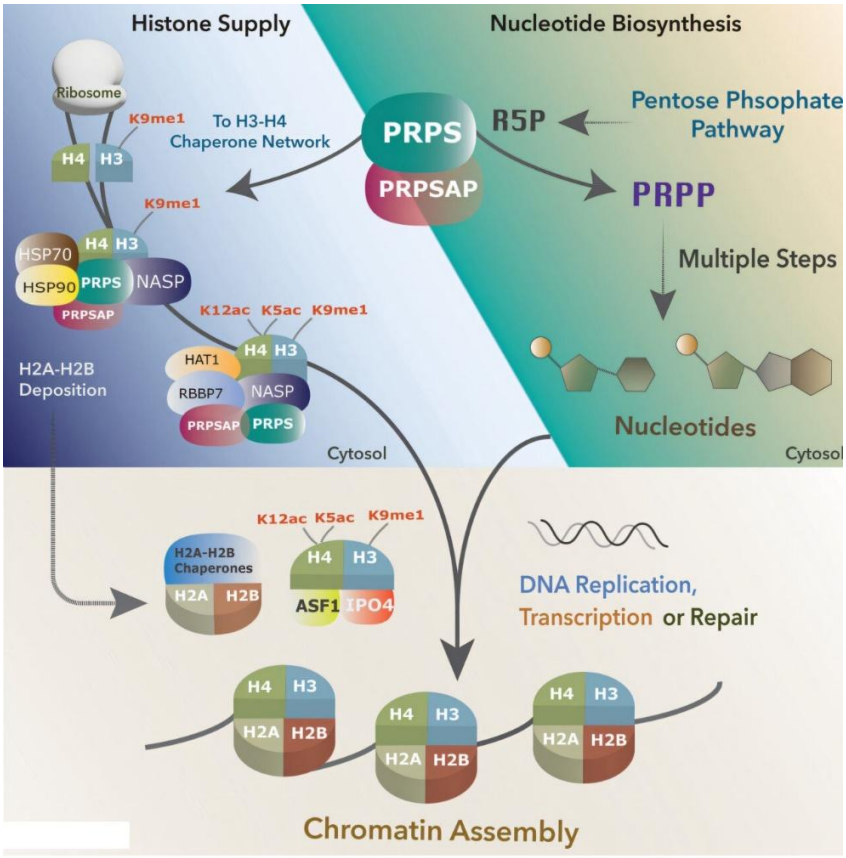


Figure 2: Integration of Histone Supply, Nucleotide Biosynthesis and Chromatin Assembly

(Image Source: https://www.sciencedirect.com/science/article/pii/S1097276525009050)

Nucleotides may be produced either via de novo synthesis where the nucleotides are built from simple precursors or salvage synthesis where the bases and nucleosides are used.

The synthesis of purine and pyrimidine nucleotides is quite different in the formation of their nitrogenous rings.

Table 3: General Comparison of Purine and Pyrimidine Metabolism

Feature	**Purines**	**Pyrimidines**
Major bases	Adenine, guanine	Cytosine, thymine, uracil
Ring synthesis	Ring is assembled on ribose phosphate	Ring is formed first and then attached to ribose phosphate

Important common precursor	IMP	UMP
Major degradation product in humans	Uric acid	Soluble smaller metabolites

6.2.1 Purine and Pyrimidine Biosynthesis

- **Purine Nucleotide Biosynthesis**

For de novo purine biosynthesis, the purine skeleton is built up on a ribose phosphate base. The ribose phosphate is provided by ribose-5-phosphate, which is synthesized primarily through the pentose phosphate pathway and gets converted into 5-phosphoribosyl-1-pyrophosphate (PRPP).

The different atoms in the purine ring come from various compounds such as glycine, glutamine, aspartic acid, carbon dioxide and the C1 donors from tetrahydrofolate derivatives.

The first purine nucleotide synthesized is inosine monophosphate (IMP). Then, IMP acts as a branching point from where adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are synthesized.

Synthesis of purines takes much energy.

Pyrimidine Nucleotide Biosynthesis

Pyrimidine biosynthesis is different from purine biosynthesis in that the pyrimidine ring is synthesized before the attachment of the ring to the ribose-phosphate portion.

The synthesis starts with the production of carbamoyl phosphate in the cytoplasm through an enzyme called carbamoyl phosphate synthetase II (CPS II). The carbamoyl phosphate gets added with aspartate and goes through further steps resulting in the formation of the pyrimidine ring.

The ring-containing compound finally gets added to PRPP, and this results in the formation of uridine monophosphate (UMP). UMP is the precursor for other pyrimidine nucleotides such as UDP, UTP, and CTP.

Table 4: Comparison of Purine and Pyrimidine Biosynthesis

Feature	**Purine Biosynthesis**	**Pyrimidine Biosynthesis**
Initial framework	PRPP/ribose phosphate	Pyrimidine ring

Ring formation	Built directly on ribose	Ring synthesized first
Important amino-acid contributors	Glycine, glutamine, aspartate	Mainly glutamine and aspartate
First major nucleotide product	IMP	UMP
Major subsequent products	AMP and GMP	UTP, CTP and related nucleotides

- **Salvage Pathways**

Purines and pyrimidines can be salvaged by recycling free bases. This is an economical process because it utilizes previously formed bases and does not require starting from scratch. Purine salvage plays a crucial role in specific organ.

6.2.2 Purine Catabolism and Uric Acid Formation

Nucleotides of purines are constantly being broken down as part of the regular process of degradation. The final products of degradation in humans are uric acid.

Adenosine monophosphate and guanosine monophosphate undergo degradation via different pathways but end up forming uric acid. Purine derivatives of adenine form hypoxanthine as an intermediate product, while purine derivatives of guanine form xanthine.

Conversion of hypoxanthine to xanthine and then of xanthine to uric acid involves oxidation, and both oxidation steps occur due to xanthine oxidoreductase, which includes such forms as xanthine oxidase.

Table 5: Important Intermediates in Purine Catabolism

Starting Purine	**Important Intermediate(s)**	**Final Product in Humans**
Adenine nucleotides	Hypoxanthine → xanthine	Uric acid
Guanine nucleotides	Guanine → xanthine	Uric acid

❖ Uric Acid Formation and Excretion

Uric acid is mainly synthesized from purine degradation and is mostly found as urate in blood at physiological pH. Uric acid is mostly excreted via the kidney but the gastrointestinal system also plays an important role in its elimination.

The serum level of uric acid is determined by the rate of its synthesis and secretion. Excessive production and decreased secretion of uric acid or both together lead to an elevated level of serum urate.

An elevated level of urate continuously for a long period of time may lead to the formation and deposition of crystals of monosodium urate in the body. This biochemical imbalance becomes the cause of hyperuricemia and gout.

Table 6: Summary of Nucleotide Metabolism

Process	**Main Function/Product**	**Significance**
De novo synthesis	Formation of new nucleotides	Supplies nucleotides for DNA, RNA and cellular functions
Salvage pathway	Recycling of bases/nucleosides	Conserves cellular resources and energy
Purine degradation	Formation of uric acid	Disposal of purine nitrogenous bases
Uric acid excretion	Removal mainly through kidneys	Maintains urate balance

6.3 HYPERURICEMIA AND GOUT

Hyperuricemia is defined as a biochemical disorder that involves an increased level of urate in the blood. Uric acid is the end product of purine breakdown in humans. The level of uric acid is influenced by its synthesis and elimination, which mainly occurs through the renal system but also partially in the gastrointestinal system.

Hyperuricemia may be caused by increased synthesis of uric acid, reduced renal elimination of uric acid, or a combination of both factors. An increased rate of purine breakdown and cell turnover as well as metabolic disorders may cause an increase in uric acid synthesis, while reduced renal elimination may be caused either by kidney failure or medication.

Hyperuricemia may not necessarily cause any symptoms. When the levels of urate stay high enough, monosodium urate crystals may precipitate and get deposited in the joints and other tissues.

- **Gout**

Gout is an inflammatory condition where there is an accumulation of monosodium urate crystals, especially in the joints. These crystals induce a strong inflammatory reaction that results in an attack of pain and inflammation in the joint.

Gout usually attacks one joint only, and the most common site is the first metatarsophalangeal joint of the big toe. Crystal accumulation may result in the development of chronic gout with the formation of nodules known as tophi. Urate may cause urinary tract stones in some people.

Table 7: Hyperuricemia and Gout

Feature	**Hyperuricemia**	**Gout**
Basic abnormality	Increased blood urate concentration	Inflammatory disease due to urate crystal deposition
Major mechanisms	Increased production and/or decreased excretion	Persistent urate supersaturation with crystal formation
Symptoms	Often asymptomatic	Acute joint pain, swelling and inflammation
Long-term significance	Increases risk of urate crystallization	Recurrent arthritis, tophi and joint damage
Renal association	Reduced excretion may contribute	Urate stones may occur

Evaluation for the presence of gout based on biochemical analysis of urate in the serum is relevant for disorders of purine metabolism despite hyperuricemia.

6.4 GENETIC INFORMATION TRANSFER

The genetic code present in the DNA should be correctly replicated and expressed for proper functioning of the cells. The main processes that take place include DNA replication, transcription, and translation. These processes together help in maintaining the genetic code and production of functional proteins.

6.4.1 Central Dogma and DNA Replication

❖ **Central Dogma of Molecular Biology**

It gives a general view of how genetic information flows in living beings from DNA to RNA to Proteins. The DNA contains genetic information in its structure, and RNA carries it or decodes it into a useful language, and proteins do various tasks in the body.

Three main mechanisms linked with information transfer are:

- Replication: DNA to DNA
- Transcription: DNA to RNA
- Translation: RNA to Protein

DNA Replication

DNA replication is the copying of DNA prior to cell division. DNA replication is semiconservative, meaning that the resultant DNA molecule will have one original strand and one new strand.

Replication starts at certain sites called origins of replication. Here, the DNA double helix is unzipped so that both of the parental strands serve as templates to form a complementary strand.

The process involves different enzymes and proteins. Helicases unwind the DNA strands, whereas topoisomerases reduce torsional stress. Primase forms RNA primers, and the DNA polymerases synthesize new DNA from 5′ to 3′. DNA ligase links discontinuous DNA fragments.

Replication strategies vary from one parent template to another due to the antiparallel nature of DNA strands. At first, the leading strand replicates continuously, but as the Okazaki fragments are later joined, the trailing strand replicates discontinuously. DNA replication is very important for genome stability and passing on information from one cell generation to another.

6.4.2 Transcription

RNA synthesis is known as transcription where an RNA molecule is synthesized from DNA. Transcription mainly takes place in the nucleus of eukaryotes and it is accomplished by RNA polymerases.

During transcription, RNA polymerase utilizes DNA strand as the template for forming a complementary RNA molecule in 5′→3′ direction. Transcription has three major phases including initiation, elongation and termination.

The initiation phase involves the recognition of a promoter region that is related to a particular gene. In the elongation phase, RNA polymerase synthesizes a complementary RNA molecule moving on the DNA template. The termination phase terminates RNA synthesis.

In eukaryotes, primary precursor of mRNA is processed to form a mature mRNA. Some of the key processes involved are 5′ capping, splicing and addition of 3′poly(A) tail. Splicing process removes introns and connects exons together.

Table 8: Major Stages of Transcription

Stage	**Major Event**
Initiation	Transcription machinery assembles at the promoter
Elongation	RNA chain is synthesized using DNA as template
Termination	RNA synthesis ends
RNA processing	Capping, splicing and polyadenylation produce mature eukaryotic mRNA

Transcription serves as one of the key stages of regulation of gene expression that helps express various genes in accordance with cell needs.

6.4.3 Translation and Genetic Code

- **Translation**

Translation is the procedure through which the base sequence of mRNA is decoded for protein formation. It takes place in ribosomes and involves components such as mRNA, tRNA, rRNA, amino acids, and other protein factors.

In addition to carrying a single amino acid, the tRNA molecule also has an anticodon that works in tandem with the mRNA codon. In order for translation to begin, aminoacyl-tRNA synthetase must covalently attach the amino acid to the correct tRNA.

There are essentially three phases to translation: beginning, middle, and end. The ribosome binds to the messenger RNA and the initiator transfer RNA during the initiation process. Peptide bonds are formed during elongation by adding amino acids progressively. Once a stop codon reaches the ribosome, translation comes to a halt.

Table 9: Major Components of Translation

Component	**Function**
mRNA	Provides the codon sequence
tRNA	Carries amino acids and recognizes codons
Ribosome/rRNA	Provides the site and catalytic machinery for protein synthesis
Amino acids	Building blocks of the polypeptide
Aminoacyl-tRNA synthetases	Attach amino acids to corresponding tRNAs

❖ Genetic Code

The genetic code refers to the set of instructions that interpret the nucleotide sequences of an mRNA molecule into amino acids in the process of protein synthesis. Three consecutive nucleotides form a codon.

There exist sixty-four possible combinations in the genetic code. Out of these combinations, six are used to terminate the sequence while sixty-one specify amino acids. Initiation codons include the codon AUG, which codes for methionine, while UAA, UAG, and UGA are used as termination codons.

Some major features of the genetic code are listed below. First, it is considered a triplet code because it consists of three-nucleotide sequences; second, it is degenerate, since each amino acid can be specified by several codons; third, it is unambiguous since the same codon represents one specific amino acid.

Replication ensures the preservation of genetic information, transcription involves the conversion of DNA into RNA, while translation is the use of the mRNA code in order to produce proteins. This is how the molecular mechanism of genetic information transfer and expression works.

6.5 REGULATION AND INHIBITION OF PROTEIN SYNTHESIS

The synthesis of proteins is a highly regulated cellular process where proteins are synthesized in the right quantity, time, and place. This regulation can happen at various levels of the gene expression process such as transcription, RNA processing, mRNA stability, translation and post-translational modification.

Transcriptional regulation is one of the key methods of control of protein synthesis. The regulatory proteins and transcription factors affect the efficiency of RNA polymerase in the transcription of certain genes. Moreover, in eukaryotic cells, the alteration in the structure of chromatin affects the access of DNA to the transcription process.

The translation process of protein synthesis includes the regulation of the initiation of the translation, stability and availability of mRNA, translation factors, and amino acids.

- **Inhibition of Protein Synthesis**

A number of pharmaceuticals, such as antibiotics and toxins, inhibit either transcription or translation.

Actinomycin D (Dactinomycin) attaches to DNA and interferes with RNA synthesis. Rifampicin prevents transcription in bacteria by inhibiting the activity of bacterial DNA-dependent RNA polymerase.

A number of antibiotics have a direct effect on ribosomes of bacteria. Tetracycline interferes with binding of aminoacyl-tRNA to ribosome of bacteria. Chloramphenicol inhibits peptidyl transferase of bacteria. Macrolides, such as Erythromycin, block the process of bacterial protein synthesis by attaching to 50S ribosomal subunit.

Toxins can also disrupt the process of protein synthesis. Diphtheria toxin blocks eukaryotic protein synthesis by blocking elongation factor 2 (EF-2).

Table 10: Selected Inhibitors of Transcription and Translation

Agent	Main Target	**Major Effect**
Actinomycin D	DNA-dependent RNA synthesis	Inhibits transcription
Rifampicin	Bacterial RNA polymerase	Inhibits bacterial transcription
Tetracyclines	Bacterial 30S ribosomal subunit	Prevent aminoacyl-tRNA binding
Chloramphenicol	Bacterial 50S ribosomal subunit	Inhibits peptidyl transferase activity
Erythromycin	Bacterial 50S ribosomal subunit	Inhibits protein synthesis
Diphtheria toxin	EF-2	Inhibits eukaryotic translation

Knowing about these inhibitors is essential in pharmacology and medicine because they help in explaining the modes of action of antibacterial agents and some poisons.

6.6 DNA DAMAGE AND REPAIR

There are always factors in our environment that have the ability to modify the DNA structure chemically. Damage can occur due to physiological processes or through the actions of environmental factors. Incorrect repair of DNA damage can lead to mutation, malfunction of cells, genomic instability, or even cell death.

This is why there are multiple repair mechanisms for DNA in cells.

6.6.1 DNA Damage and Repair Mechanisms

DNA holds the genetic instructions necessary for healthy growth, cell division, and proper functioning of cells. Though DNA is chemically stable, it is constantly subjected to agents that cause damage to it. It has been suggested that many cases of DNA damage are seen in cells as a result of normal metabolism and interaction with the environment. Most of these damages are corrected by DNA repair process before becoming mutations.

DNA damage and mutation are two different terms. DNA damage involves abnormal chemical or physical changes in the DNA while mutation involves the change in nucleotide sequence. In case the DNA damage is not fixed prior to DNA replication, then mutations take place.

❖ Types and Causes of DNA Damage

Damage to DNA can result either due to endogenous or exogenous causes.

Endogenous DNA damage results from events that take place inside the cell due to normal body functions and metabolism. Oxygen radicals formed during body functions can oxidize the DNA bases and also damage the sugar-phosphate backbone. DNA bases can also be chemically altered in the form of deamination and depurination. There can also be errors during DNA replication.

Exogenous DNA damage results from external influences. Some of the significant sources include ultraviolet radiation, ionizing radiation and certain chemicals. Ultraviolet radiation forms abnormal crosslinks between adjacent pyrimidine bases while ionizing radiation causes single strand and double strand breaks in the DNA molecule.

❖ Oxidative Base Damage

Oxidative damage to DNA molecules includes chemical alteration of DNA bases by oxidative agents like hydroxyl radicals. Examples of oxidative damage include 8-oxoguanine, which can mispair with adenine during replication. In addition to antioxidant defense mechanisms to minimize oxidative damage, there are DNA repair mechanisms.

Despite the antioxidant defenses in cells, DNA repair is necessary for any oxidative damage that may arise.

❖ Pyrimidine Dimers

The formation of covalent bonds between adjacent pyrimidines as a result of exposure to ultraviolet radiation includes cyclobutane pyrimidine dimers, which may disrupt the structure of the DNA helix.

These lesions can affect the processes of DNA replication and transcription. The process of nucleotide excision repair plays an important role in eliminating such lesions.

❖ Base Mismatches

The incorporation of a wrong base in the newly formed strand of DNA during replication process may occur. The enzymes responsible for replication have proofreading properties which enable them to rectify most of these errors. Nonetheless, those mismatches not fixed by proofreading will be repaired by mismatch repair system.

If left unchecked, mismatches can cause mutations during the next round of replication.

❖ **Single-Strand and Double-Strand Breaks**

When there is an interruption of the sugar-phosphate backbone of a single DNA strand, there will be a break known as a single-strand break. This can occur due to oxidation, spontaneously or by chemical agents. These breaks can be easily corrected because the other strand of DNA can act as a template.

The second type of DNA break is a double-strand break whereby there is a break on both strands. This is considered more harmful since there will be no integrity of the DNA molecule. This kind of break can be caused by ionization, during replication or by chemicals.

❖ **DNA Cross-Links and Bulky Lesions**

Chemical substances can cause abnormal covalent bonding in the same DNA strand or in both strands of DNA. The interstrand cross-linking is especially destructive since it inhibits the separation of DNA strands, making replication and transcription impossible.

A bulky modification of DNA can cause structural distortion of the DNA helix and inhibit the interaction between DNA and enzymes in cells.

Table 11: Major Types of DNA Damage

Type of DNA Damage	Common Cause	Major Effect	Possible Consequence
Oxidative base damage	Reactive oxygen species	Chemical modification of bases	Incorrect base pairing and mutation
Pyrimidine dimers	Ultraviolet radiation	Distortion of DNA helix	Interference with replication and transcription
Base mismatch	DNA replication errors	Incorrect base pairing	Permanent mutation if not corrected
Single-strand break	ROS and chemical damage	Break in one DNA strand	Disturbed DNA integrity

Double-strand break	Ionizing radiation and replication-associated damage	Break in both strands	Chromosomal instability
DNA cross-link	Certain chemical agents	Prevents normal strand separation	Impaired replication and transcription
Bulky DNA lesion	Chemical or environmental agents	Distorts DNA helix	Blocks normal DNA processing

❖ **DNA Repair Mechanisms**

Cells have several repair pathways since one pathway is unable to correct all DNA damages effectively. The different pathways recognize distinct lesions and restore the DNA structure by the action of specific enzymes.

There are mainly five types of DNA repair pathways namely; base excision repair, nucleotide excision repair, mismatch repair, homologous recombination and non-homologous end joining.

❖ **Base Excision Repair**

BER acts to correct small lesions which are not bulky in nature and affect single DNA bases. These lesions may be caused by oxidation or deamination among others.

The process starts with DNA glycosylase recognizing the abnormal DNA base and removing it thereby creating an AP site. AP endonuclease comes in to cut the DNA backbone at the site of damage. The missing base is replaced via DNA synthesis followed by ligation of the DNA strand.

BER is especially critical in the correction of chemical lesions that occur during normal cellular function.

❖ **Nucleotide Excision Repair**

Nucleotide Excision Repair (NER) deals with the repair of bulky adducts in DNA which distort the DNA structure considerably.

During NER, the damaged segment is identified and a portion of the affected DNA strand including the lesion is removed. DNA polymerase synthesizes the new DNA using the normal DNA strand as a template and DNA ligase repairs the break in the DNA strand.

Malfunctioning of nucleotide excision repair leads to conditions like xeroderma pigmentosa, where patients are highly sensitive to UV radiation.

- **Mismatch Repair**

The function of mismatch repair is the fixing of errors that are not corrected by proofreading activity of DNA polymerase during replication.

This includes the presence of improperly paired nucleotides and small insertions or deletions.

Recognition of the erroneous stretch on the newly formed DNA followed by removal of this part and resynthesis of the correct sequence based on the template parent DNA is involved in mismatch repair.

Thus, mismatch repair increases the fidelity of DNA replication. Mutations in the genes involved in MMR can lead to Lynch syndrome.

- **Homologous Recombination**

DNA double strand break repair is done through homologous recombination. This process involves the use of a homologous sequence of DNA, which in this case is mostly the sister chromatid, to make the necessary repairs.

Being that homologous recombination uses a full sequence of homologous DNA, it can replace genetic material with relative ease. It becomes very significant in cell cycles where a sister chromatid is present.

- **Non-Homologous End Joining**

The non-homologous end joining (NHEJ) pathway is a mechanism used to mend double-stranded breaks without requiring a homologous DNA template. In this mechanism, the broken DNA ends are recognized and then rejoined.

The NHEJ process enables immediate repair of the hazardous double-stranded breaks. The drawback of the NHEJ process is that the ends of DNA can be processed which may cause either loss or gain of nucleotides.

Table 12: Major DNA Repair Mechanisms

Repair Mechanism	Principal Damage Repaired	Basic Mechanism	Significance
Base excision repair (BER)	Small or chemically modified bases	Damaged base is removed and replaced	Repairs common endogenous base damage
Nucleotide excision repair (NER)	Bulky, helix-distorting lesions	Short damaged DNA segment is excised and resynthesized	Important in repair of UV-induced damage
Mismatch repair (MMR)	Replication-associated mismatches	Incorrect newly synthesized region is removed and replaced	Maintains replication accuracy
Homologous recombination (HR)	Double-strand breaks	Uses homologous DNA as a repair template	Generally high-fidelity repair
Non-homologous end joining (NHEJ)	Double-strand breaks	Broken DNA ends are directly rejoined	Rapid repair but potentially error-prone

❖ **Cellular Response to Severe DNA Damage**

DNA repair is very closely related to the regulation of the cell cycle. If there is a large amount of DNA damage that has occurred, the cell signaling systems have the capability of holding back the progression of the cell cycle until it can be fixed.

Should the DNA damage be too great for repair to take place successfully, then the cell will go through apoptosis. This is programmed cell death that prevents any further replication and propagation of faulty DNA information.

❖ **Biological and Clinical Importance**

DNA repair is a critical process in order to keep genome stability during life span. The inability of these processes results in accumulation of the DNA lesions and might be linked to aging, malfunctioning of cells and development of cancer.

Some major problems which appear due to insufficient DNA repair are:

- mutation increase;
- genomic and chromosomal instability;
- replication and transcription malfunction;
- cell malfunctions or cell death;
- hypersensitivity to radiation and chemical exposure; and
- greater risk of cancer and hereditary DNA repair deficiencies.

Therefore, DNA lesions and their repair are an ongoing balance in the cell. Whereas, BER and NER mainly eliminate different forms of DNA damage, MMR corrects replication mistakes and HR and NHEJ repair dangerous DNA breaks.

6.6.2 Disorders of Defective DNA Repair

The DNA molecules are constantly exposed to harmful agents that result from both natural cellular activities and from external sources like UV light, ionizing radiation, and some chemicals. Usually, the DNA molecules are repaired through special repair processes. However, when genes encoding the repair processes have pathogenic mutations, the repair processes might become inefficient. This means that the DNA molecule becomes vulnerable to DNA damages and mutations that might eventually lead to genomic instability and abnormal cellular function.

Hereditary DNA repair syndromes are important since they help understand how maintenance of genome integrity affects human health. The specific manifestation of DNA repair syndromes is associated with the type of repair process that is affected. For example, some hereditary syndromes result in an increased susceptibility to radiation, while others affect the central nervous system and cause a predisposition to specific cancers.

❖ **Xeroderma Pigmentosum**

Xeroderma pigmentosum is a very rare condition that results from genetic defects in DNA repair of damage caused by UV radiation. The majority of XPD syndromes feature malfunctions in certain parts of the NER repair system.

UV light can cause various lesions of DNA, especially such as cyclobutane pyrimidine dimers, as well as other UV photoproducts that distort DNA helix. NER system recognizes distorted DNA helix, excises the short section of the damaged DNA and substitutes it based on the complementary DNA chain. In XP cases, NER fails, leading to accumulation of DNA lesions and mutations caused by UV rays.

People with xeroderma pigmentosum develop severe photosensitivity, pigment abnormalities and precancerous changes in sun-exposed skin. Moreover, the most serious manifestation of xeroderma pigmentosum is extremely high risk of cancer formation in very early age. Some forms of xeroderma pigmentosum might also result in progressive neurological problems.

Protection from the UV radiation and dermatological control are the key to management of xeroderma pigmentosum patients.

- **Ataxia-Telangiectasia**

Ataxia-telangiectasia (A-T) is an inherited, multisystemic disease resulting from mutations in the ATM gene. This gene codes for an important protein in the process of signaling in cells responding to DNA double strand breaks.

In the event of such DNA breakage, ATM becomes involved in the activation of the proteins responsible for controlling the cell cycle, repairing damaged DNA, or causing apoptosis, if the DNA damage is critical. Malfunction of the ATM protein results in the disruption of the process of responding to the DNA damage.

A-T is manifested through the presence of progressive cerebellar ataxia that causes problems with coordination and mobility. Telangiectasias - small dilated blood vessels - are often seen on the patient's face and skin near the eyes. In some cases, there might be an immune dysfunction and frequent infections.

Cells of such patients demonstrate higher sensitivity to ionizing radiation, and the patients become more susceptible to developing malignancies, especially lymphoid tumors. Therefore, A-T shows how crucial DNA damage responses are not only in DNA repair but also in cell-cycle regulation and tumor suppression.

- **Lynch Syndrome**

Lynch Syndrome is a genetic condition which leads to an increased tendency for cancers due to pathogenic mutations of DNA mismatch repair (MMR) pathway.

Mismatch repair is a process that involves correction of occasional mistakes during DNA replication, including incorrect base pairings and insertions/deletions. In case there is some defect in mismatch repair, replication errors increase and accumulate mostly in repeated regions of the DNA sequence called microsatellites, leading to microsatellite instability (MSI).

People who suffer from Lynch Syndrome have high tendency for developing such cancer types as colon cancer and may be prone to endometrial and other types of cancers. Being a genetic disorder, the diagnosis of Lynch Syndrome has implications for relatives of the patient.

❖ **Other Disorders Associated with DNA Repair Defects**

There are a number of other hereditary diseases that emphasize the clinical significance of maintaining DNA integrity. For instance, Fanconi anemia entails deficiencies in the DNA repair mechanisms for interstrand cross-links and it is characterized by bone marrow failure and developmental problems among others. Bloom syndrome results from malfunctioning of the BLM DNA helicase which leads to instability of chromosomes as well as cancer susceptibility.

Cockayne syndrome entails defects in DNA repair of specific types of transcription-blocking DNA lesions. Cockayne syndrome manifests itself with growth deficiencies, neurological impairments and photosensitivity. This contrasts xeroderma pigmentosum to some extent.

Table 13: Selected Disorders Associated with Defective DNA Repair

Disorder	Major Molecular/Repair Defect	Important Clinical Features
Xeroderma pigmentosum	Defective nucleotide excision repair of UV-induced DNA damage in most forms	Severe UV sensitivity, pigmentation abnormalities and markedly increased skin-cancer risk
Ataxia-telangiectasia	Defective ATM-mediated response to DNA double-strand breaks	Progressive ataxia, telangiectasia, immune dysfunction, radiation sensitivity and increased cancer risk
Lynch syndrome	Defective DNA mismatch repair	Microsatellite instability and increased risk of colorectal, endometrial and other cancers

Fanconi anemia	Defective repair of DNA interstrand cross-links	Bone-marrow failure, developmental abnormalities and cancer predisposition
Bloom syndrome	Defective BLM helicase and increased chromosomal instability	Growth abnormalities and increased cancer susceptibility
Cockayne syndrome	Defective repair of transcription-blocking DNA lesions	Photosensitivity, growth failure and neurological abnormalities

❖ **Clinical Significance of Defective DNA Repair**

It is clinically important to know about DNA repair disorders since they clearly demonstrate the necessity not only of proper DNA replication, but also of constant DNA lesions' recognition and repairing in order to ensure genomic stability. The defects in various repair pathways give rise to various disease syndromes since each of these pathways deals with certain type of DNA damage.

The clinical outcomes of improper DNA repair are:

- Mutations accumulation: the lesions which are not repaired will become mutations upon next DNA replication.
- Genomic instability: chromosome breaks, reorganizations and other abnormalities can occur.
- Cancer predisposition: the mutations in genes responsible for cell growth and division regulation can induce cancer.
- Radiation sensitivity: inability to repair DNA lesions produced by radiation causes excessive cellular damage.
- Nervous system abnormalities: some repair disorders especially affect those neurons that live a long time and have high metabolism.
- Abnormalities in development and immunity: the defects in genome maintenance can lead to the development and immune disorders.

Thus, DNA repair systems are vital for cells. Xeroderma pigmentosum, ataxia-telangiectasia and Lynch syndrome are very good examples of the diseases caused by malfunctioning of nucleotide excision repair, DNA damage signal transduction and mismatch repair, respectively. It is also clinically important to understand these disorders for pharmacy and biomedical scientists since the DNA repair pathways regulate carcinogenesis, reactions to radiation and effects of certain anticancer agents.

REFERENCES

1. Sreemathy, V., & Dandekar, S. P. (2023). *Applied Nutrition including Dietetics and Applied Biochemistry for Nurses-E-Book*. Elsevier Health Sciences.
2. Stach, K., Stach, W., & Augoff, K. (2021). Vitamin B6 in health and disease. *Nutrients*, *13*(9), 3229.
3. Stanford, K. I., Middelbeek, R. J., & Goodyear, L. J. (2015). Exercise effects on white adipose tissue: beiging and metabolic adaptations. *Diabetes*, *64*(7), 2361-2368.
4. Stowe, D. F. (2025). *Evolution of Bioenergetics from Elements to Life: Emergence of High Energy Mitochondria*. Springer Nature.
5. Sulieman, A. M. E. (Ed.). (2025). *Microbial Enzymes: Classification, Biochemistry, Production and Applications*. Academic Press.
6. Sultana, S., & McClure, J. (2023). Intermediary metabolism. *Anaesthesia & Intensive Care Medicine*, *24*(2), 139-147.
7. Tardy, A. L., Pouteau, E., Marquez, D., Yilmaz, C., & Scholey, A. (2020). Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. *Nutrients*, *12*(1), 228.
8. Tayeb, H. H., & Sainsbury, F. (2018). Nanoemulsions in drug delivery: formulation to medical application. *Nanomedicine*, *13*(19), 2507-2525.
9. Thelen, A. M., & Zoncu, R. (2017). Emerging roles for the lysosome in lipid metabolism. *Trends in cell biology*, *27*(11), 833-850.
10. Tomé, D. (2021). Amino acid metabolism and signalling pathways: potential targets in the control of infection and immunity. *Nutrition & diabetes*, *11*(1), 20.
11. Torres, N., Tobón-Cornejo, S., Velazquez-Villegas, L. A., Noriega, L. G., Alemán-Escondrillas, G., & Tovar, A. R. (2023). Amino acid catabolism: an overlooked area of metabolism. *Nutrients*, *15*(15), 3378.
12. Tyszka-Czochara, M., Bukowska-Strakova, K., Kocemba-Pilarczyk, K. A., & Majka, M. (2018). Caffeic acid targets AMPK signaling and regulates tricarboxylic acid cycle anaplerosis while metformin downregulates HIF-1α-induced glycolytic enzymes in human cervical squamous cell carcinoma lines. *Nutrients*, *10*(7), 841.

13. Vettore, L., Westbrook, R. L., & Tennant, D. A. (2020). New aspects of amino acid metabolism in cancer: Cancer Metabolism. *British journal of cancer*, *122*(2), 150-156.
14. Wang, H., Franco, F., Tsui, Y. C., Xie, X., Trefny, M. P., Zappasodi, R., ... & Ho, P. C. (2020). CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nature immunology*, *21*(3), 298-308.
15. Wang, J., Yang, P., Yu, T., Gao, M., Liu, D., Zhang, J., ... & Liu, Y. (2022). Lactylation of PKM2 suppresses inflammatory metabolic adaptation in pro-inflammatory macrophages. *International journal of biological sciences*, *18*(16), 6210.
16. Wang, R., & Lou, L. (2024). The central role of the citric acid cycle in energy metabolism: from metabolic intermediates to regulatory mechanisms. *Biological Evidence*, *14*.
17. Wei, Z., Liu, X., Cheng, C., Yu, W., & Yi, P. (2021). Metabolism of amino acids in cancer. *Frontiers in cell and developmental biology*, *8*, 603837.
18. Weinstein, D. A., Steuerwald, U., De Souza, C. F., & Derks, T. G. (2018). Inborn errors of metabolism with hypoglycemia: glycogen storage diseases and inherited disorders of gluconeogenesis. *Pediatric Clinics*, *65*(2), 247-265.
19. Weis, S., Carlos, A. R., Moita, M. R., Singh, S., Blankenhaus, B., Cardoso, S., ... & Soares, M. P. (2017). Metabolic adaptation establishes disease tolerance to sepsis. *Cell*, *169*(7), 1263-1275.
20. Werner, C., Doenst, T., & Schwarzer, M. (2016). Metabolic pathways and cycles. In *The scientist's guide to cardiac metabolism* (pp. 39-55). Academic Press.
21. Wu, G. (2021). *Amino acids: biochemistry and nutrition*. CRC press.
22. Yadav, N. N., Yadav, S., & Pareek, A. (2021). Structure and function of biological biomolecules: Carbohydrates, amino acids, proteins, nucleic acids, lipids and biomembranes. In *Biochemistry: Fundamentals and Bioenergetics* (pp. 118-213). Bentham Science Publishers.
23. Yadav, S., Yadav, K. S., & Yadav, P. (2021). Bioenergetics, Metabolism of Biomolecules, Photosynthesis and Respiration, Transcription and Translation, Recombinant DNA Technology. In *Biochemistry: Fundamentals and Bioenergetics* (pp. 361-410). Bentham Science Publishers.

24. Yamashita, S., Masuda, D., & Matsuzawa, Y. (2020). Pemafibrate, a new selective PPARα modulator: drug concept and its clinical applications for dyslipidemia and metabolic diseases. *Current atherosclerosis reports*, *22*(1), 5.
25. Yanamadala, V. (2024). Carbohydrate metabolism. In *Essential Medical Biochemistry and Metabolic Disease: A Pocket Guide for Medical Students and Residents* (pp. 1-34). Cham: Springer Nature Switzerland.
26. Yanamadala, V. (2024). *Essential Medical Biochemistry and Metabolic Disease: A Pocket Guide for Medical Students and Residents*. Springer Nature.
27. Yang, Z., Yan, C., Ma, J., Peng, P., Ren, X., Cai, S., ... & Gao, Q. (2023). Lactylome analysis suggests lactylation-dependent mechanisms of metabolic adaptation in hepatocellular carcinoma. *Nature metabolism*, *5*(1), 61-79.
28. Yap, Y. W., Rusu, P. M., Chan, A. Y., Fam, B. C., Jungmann, A., Solon-Biet, S. M., ... & Rose, A. J. (2020). Restriction of essential amino acids dictates the systemic metabolic response to dietary protein dilution. *Nature communications*, *11*(1), 2894.
29. Yin, F. (2023). Lipid metabolism and Alzheimer's disease: clinical evidence, mechanistic link and therapeutic promise. *The FEBS journal*, *290*(6), 1420-1453.
30. Yoon, M. S. (2016). The emerging role of branched-chain amino acids in insulin resistance and metabolism. *Nutrients*, *8*(7), 405.
31. Yu, D., Richardson, N. E., Green, C. L., Spicer, A. B., Murphy, M. E., Flores, V., ... & Lamming, D. W. (2021). The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. *Cell metabolism*, *33*(5), 905-922.
32. Zeng, Q., Gong, Y., Zhu, N., Shi, Y., Zhang, C., & Qin, L. (2024). Lipids and lipid metabolism in cellular senescence: Emerging targets for age-related diseases. *Ageing Research Reviews*, *97*, 102294.
33. Zeng, S. L., Li, S. Z., Xiao, P. T., Cai, Y. Y., Chu, C., Chen, B. Z., ... & Liu, E. H. (2020). Citrus polymethoxyflavones attenuate metabolic syndrome by regulating gut microbiome and amino acid metabolism. *Science advances*, *6*(1), eaax6208.
34. Zeng, Z., Liu, F., & Li, S. (2017). Metabolic adaptations in pregnancy: a review. *Annals of Nutrition and Metabolism*, *70*(1), 59-65.

35. Zhang, G. F., Jensen, M. V., Gray, S. M., El, K., Wang, Y., Lu, D., ... & Newgard, C. B. (2021). Reductive TCA cycle metabolism fuels glutamine-and glucose-stimulated insulin secretion. *Cell metabolism*, *33*(4), 804-817.
36. Zhang, J., Pavlova, N. N., & Thompson, C. B. (2017). Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. *The EMBO journal*, *36*(10), 1302-1315.
37. Zhang, S., Zeng, X., Ren, M., Mao, X., & Qiao, S. (2017). Novel metabolic and physiological functions of branched chain amino acids: a review. *Journal of animal science and biotechnology*, *8*(1), 10.
38. Zhao, J., Zhang, X., Liu, H., Brown, M. A., & Qiao, S. (2019). Dietary protein and gut microbiota composition and function. *Current Protein and Peptide Science*, *20*(2), 145-154.
39. Zheng, M., Zhang, W., Chen, X., Guo, H., Wu, H., Xu, Y., ... & Yang, B. (2023). The impact of lipids on the cancer–immunity cycle and strategies for modulating lipid metabolism to improve cancer immunotherapy. *Acta Pharmaceutica Sinica B*, *13*(4), 1488-1497.
40. Zou, K., Hinkley, J. M., Park, S., Zheng, D., Jones, T. E., Pories, W. J., ... & Houmard, J. A. (2018). Altered tricarboxylic acid cycle flux in primary myotubes from severely obese humans. *International journal of obesity (2005)*, *43*(4), 895.

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