METABOLIC PATHWAY IN ANIMALS AND PLANTS

Sunita Ojha
Dr. Prithpal Singh Matreja

ALEXIS PRESS
JERSEY CITY, USA

METABOLIC PATHWAY IN ANIMALS AND PLANTS

METABOLIC PATHWAY IN ANIMALS AND PLANTS

Sunita Ojha

Dr. Prithpal Singh Matreja

ALEXIS PRESS

ALEXIS PRESS

Published by: Alexis Press, LLC, Jersey City, USA
www.alexispress.us

First Published 2022

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication Data

Includes bibliographical references and index.

Metabolic Pathway in Animals and Plants by *Sunita Ojha, Dr. Prithpal Singh Matreja*

ISBN 978-1-64532-438-6

CONTENTS

CHAPTER 1

AN INTRODUCTION OF METABOLIC PATHWAY AND THEIR BIOENERGETICS

Sunita ojha, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id- ojhasunita@jnujaipur.ac.in

ABSTRACT:

The biochemical alterations in an organism's or the chemistry of the cell. These modifications generate the resources and energy that cells and creatures need to develop, procreate, and maintain health.A cellular process called metabolism uses various sets of enzymes to create and break down compounds in a series of stages. The metabolism process is important for the growth, development and the maintained the structural integrity of the cell. The explanation of the metabolic process and the function of the bioenergetics study from the metabolic processes were covered in this paper.

KEYWORDS:

Adenosine Triphosphate, Anabolic Pathway, Catabolic Pathway, Electron Transport Chain, Free Energy.

INTRODUCTION

A metabolic route is a connected chain of molecular processes taking place inside a cell in biology. Metabolites are the reactants, products, and intermediates of a biochemical reaction that are altered by a series of chemical processes that are catalyzed by enzymes. Typically, the output of one enzyme serves as the fuel for the following one in a biochemical process. Side products, on the other hand, are discarded as garbage and taken out of the cell. These enzymes frequently need nutritional vitamins, minerals, and other cofactors to work properly. Different metabolic pathways have different roles depending on where they are located in eukaryotic cells and how important they are to that particular cell section.

For instance, the mitochondrial membrane is the site of the electron transport chain and oxidative phosphorylation. In comparison, a cell's cytoplasm is where glycolysis, the pentose phosphate pathway, and fatty acid production take place. The capacity to either create molecules while utilizing energy (anabolic pathway) or break down complicated molecules while releasing energy during the process distinguishes two different kinds of metabolic pathways (catabolic pathway). The energy produced from one of the two routes is used up by the other, making them complementary to one another. The energy needed to carry out the biosynthesis of an anabolic pathway is produced by the catabolic pathway's degradative process[1]–[3].

The amphibolic pathway, which can be either catabolic or anabolic depending on the need for or supply of energy, is an additional metabolic route in addition to the two separate metabolic pathways. An organism requires pathways to maintain equilibrium, and the flow of molecules through a route is controlled by the substrate's supply and the requirements of the cell. A metabolic pathway's output can either be used right away, used to start another metabolic pathway, or saved for later use. An intricate web of linked paths makes up a cell's metabolism and allows for the creation and destruction of substances (anabolism and catabolism).Plants are known for their incredible molecular variety, which is sustained by extremely

sophisticated metabolic apparatus. Plant metabolic enzymes descended from eukaryotic and bacterial progenitors, were moved there, and underwent unparalleled rates of gene replication and functionalization in terrestrial plants, which led to further diversification. In contrast to bacteria, which frequently undergo horizontal gene transfers and receive numerous sources of energy and organic carbon, terrestrial plants primarily depend on the organic carbon produced by CO_2 and have undergone very few, if any, gene transfers in recent times. As a result, under a variety of genetic conditions, plant biochemical networks have developed gradually and on top of pre-existing networks. This study seeks to broaden our understanding of plant metabolic evolution and establish a paradigm for further investigation of the complicated metabolic network's evolutionary processes. For the logical building and remodeling of plant metabolic networks, it is also empirically necessary to understand the fundamental metabolic and genetic limitations.

An energy-releasing pathway known as a catabolic pathway is a chain of reactions that results in the production of adenosine triphosphate (ATP) and guanosine triphosphate (GTP), respectively, from the energy carriers adenosine diphosphate (ADP) and guanosine diphosphate. The overall process is therefore thermodynamically advantageous because the end products have reduced free energy.□ A catabolic route is an exergonic mechanism that converts energy-containing substances like carbs, lipids, and proteins into molecular energy in the shape of ATP, GTP, NADH, NADPH, $FADH_2$, etc. Ammonia, water, and carbon dioxide are frequently the final byproducts. The organism can make new proteins by combining an endergonic anabolic process with the initial anabolic pathway components. The phosphorylation of fructose-6-phosphate by the enzyme phosphofructokinase to produce the intermediary fructose-1,6-bisphosphate along with the breakdown of ATP in the glycolysis route is an illustration of a linked process. Due to the metabolic pathway's extremely advantageous thermodynamics, the ensuing chemical process is irrevocable in the cell.

All organic things possess a basic collection of biochemical processes that produce energy. These routes convert the energy produced during food degradation into ATP and other energy-producing tiny compounds (e.g. GTP, NADPH, $FADH_2$). Anaerobic metabolism via glycolysis is a function of all organisms. Additionally, the citric acid cycle and oxidative phosphorylation enable most creatures to carry out oxygen breathing more effectively. Additionally, photosynthesis is a process by which plants, algae, and cyanobacteria anabolically create substances from non-living materials. Energy movement through biological systems is the focus of the biochemistry and cell biology subfield of bioenergetics. Thousands of different cellular processes, including cellular respiration and the numerous other metabolic and enzymatic processes that result in the production and utilization of energy in forms like adenosine triphosphate (ATP) molecules, are being studied in this active area of biological research. In other words, bioenergetics aims to explain how living things obtain and change energy to carry out biological tasks. Thus, bioenergetics depends on the research of biochemical processes.

Anabolic pathways need an energy intake in opposition to catabolic pathways to build polymers like polypeptides, nucleic acids, proteins, carbohydrates, and lipids. A cell's solitary anabolic response is negative because of a positive Gibbs Free Energy (+G). As a result, a connection with an exergonic process is required to provide a molecular energy intake. By reducing the total activation energy of an anabolic pathway and enabling the reaction to occur, the linked reaction of the catabolic pathway has an impact on the kinetics of the reaction.□ A non-spontaneous reply is what an endergonic response is. In a biosynthesis route, an anabolic pathway joins smaller molecules to create bigger, more complicated compounds.□ One illustration is the reversible process of glycolysis, also known as

gluconeogenesis, which takes place in the liver and occasionally the kidney to keep a healthy blood glucose level and provide sufficient glucose to the brain and muscle cells. Although gluconeogenesis and the opposite route of glycolysis are comparable, the natural occurrence of the pathway is made possible by the presence of four different enzymes from glycolysis: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase.

According to the supply or demand for energy, an amphibolic route can be either catabolic or anabolic. Adenosine triphosphate (ATP), which saves its energy in phosphoanhydride molecules, is the unit of energy in a living organism. The energy is used for the cell's production, to promote mobility, and to control active transport. The glyoxylate cycle and citric acid cycle are two examples of amphibolic processes. Both energy-producing and energy-using routes are present in these groups of molecular processes. An example of the TCA cycle's amphibolic characteristics can be seen to the right. The tricarboxylic acid (TCA) cycle is an option, and the glyoxylate shunt pathway reroutes the TCA cycle to conserve high-energy carbon sources as potential future energy sources and avoid the complete decomposition of carbon molecules. Only plants and microbes have this route, which functions without the presence of glucose molecules.

The area of biochemistry known as bioenergetics is concerned with the energy needed to form and dissolve molecular interactions in the compounds found in living things. Another way to describe it is the study of energy interactions, changes, and transductions in biological things. All organic things can use energy from a range of biochemical processes. Because energy plays a crucial part in these biological processes, some of the key processes in the study of biological entities include growth, evolution, anabolism, and catabolism. Energy changes are essential to life; living things live thanks to the energy transfer between their organs and surroundings. Some creatures, like autotrophs, can obtain energy from sunshine (via photosynthesis) without consuming or breaking down nutrition. Other animals, such as heterotrophs, depend on ingesting nutrients from food to obtain energy by rupturing molecular bonds in nutrients during biochemical processes like glycolysis and the citric acid cycle.

Importantly, autotrophs and heterotrophs are members of a global biochemical network as a direct result of the First Law of Thermodynamics. By consuming autotrophs (plants), heterotrophs use energy that was originally converted by the plants during photosynthesis. Chemical connections are formed and dissolved in biological things as a result of energy transmission and transmutation. When weak bonds are dissolved and stronger bonds are formed, energy is made available for work (such as mechanical labor) or other processes (such as chemical production and hormonal processes in development). Stronger connections can be created, which permits the discharge of useful energy. The primary "energy currency" for organisms is adenosine triphosphate (ATP), which is produced from the environment's available starting materials by metabolic and catabolic processes[4], [5].

When ATP is used in biological processes, it is broken down into adenosine diphosphate (ADP) and inorganic phosphate. The proportion of ATP to ADP amounts in a cell is referred to as the cell's "energy charge." If there is more ATP than ADP accessible, the cell can use ATP to perform work; however, if there is more ADP than ATP, the cell must make ATP via oxidative phosphorylation. A cell can use this energy charge to communicate information about biological requirements. Oxidative phosphorylation is the process by which living things convert energy sources into ATP. In comparison to the stronger bonds created when ATP is digested (broken down by water) into adenosine diphosphate and inorganic phosphate, the terminal phosphate bonds of ATP are comparatively feeble. Here, energy is

released as a consequence of the thermodynamically advantageous free energy of hydrolysis; this energy is not present in the phosphoanhydride link between the end phosphate group and the remainder of the ATP molecule. ATP serves as a storage to hold energy in the cells of a body.

Every biological organism uses chemical energy from such molecular bond rearrangements to power its biological processes. Energy is obtained by living things from both organic and artificial sources; for example, ATP can be created from several molecular components. To generate ATP, lithotrophs can, for instance, decompose rocks like nitrates or types of sulfur like basic sulfur, sulfites, and hydrogen sulfide. In photosynthesis, autotrophs use light energy to create ATP, while heterotrophs must ingest organic substances, primarily proteins, lipids, and carbs. Due to losses in digestion, respiration, and thermogenesis, the body truly obtains less energy than what is contained in the meal. Although some nutrients can also be metabolized anaerobically by different organisms, most environmental materials that an organism consumes are mixed with oxygen to release energy. Because the nutrients are reacting with air, using these compounds is a type of delayed burning. The materials are oxidized slowly enough that the organisms do not produce fire. The oxidation produces energy that can either be used by the creature for other processes, like dissolving molecular bonds, or it can develop as heat.

The reaction of the bioenergetics: Energy is naturally released during exergonic processes. Indicating thermodynamic favorability is negative G. (Gibbs free energy). Reactants are transferred from a stable state through an energetically unstable transition state to a more stable, lower-energy state by activation energy. Simpler products are formed from more complex reactants. Most often catabolic. Gibbs's free energy is negative because reactants possess greater energy than products (G 0). Endergonic refers to a molecular reaction that uses energy. Alternative to exergonic. Because H > 0, which denotes that reactant bonds are more difficult to break than product bonds and that products have weaker bonds than reactants, it has a positive G. Endergonic processes won't take place at steady temperature because they are thermodynamically advantageous (Figure. 1). There are anabolic endergonic responses. G is the change in Gibbs free energy, H is the change in enthalpy, T is the change in temperature (in kelvins), and S is the change in entropy.

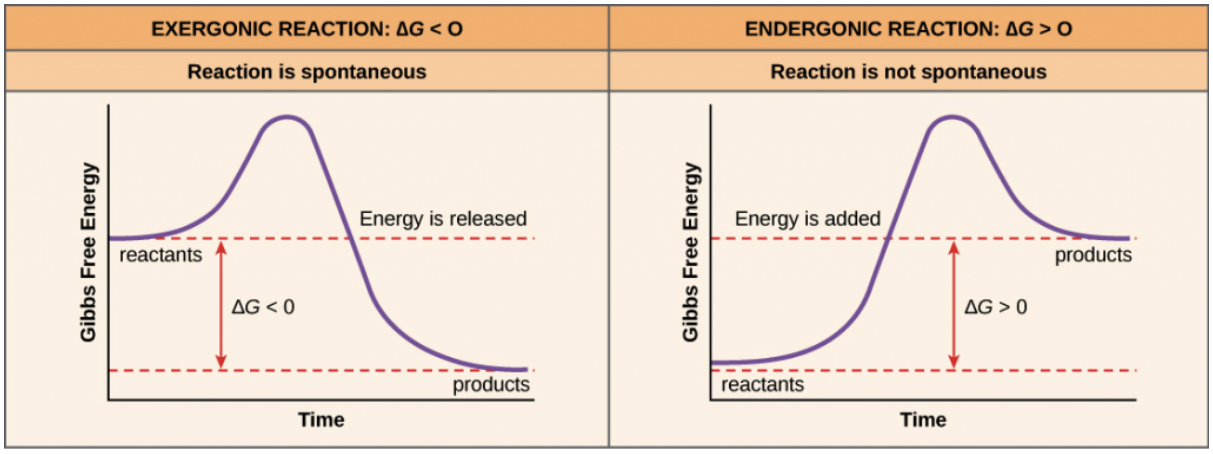

Figure 1: bioenergetics reaction: Diagram showing the bioenergetics (exothermic and the endothermic reaction) (Bioscience notes).

Bioenergetic examples: Pyruvate is produced when glucose is broken down by glycolysis, producing two ATP per molecule. A cell cannot carry out glycolysis to liberate energy from glucose when it has a large energy charge, or when there is more ATP than ADP. A

glycolysis byproduct called pyruvate can enter other biochemical pathways (gluconeogenesis, etc.). NADH, a product of glycolysis, contributes electrons to the electron transport pathway. The production of glucose occurs when the energy charge of the cell is low (the quantity of ADP is higher than that of ATP), and these substances include proteins, amino acids, lipids, pyruvate, etc. A possible breakdown of proteins into amino acids, which are required to produce glucose. Acetyl coenzyme A first reacts with oxaloacetate to produce citrate in the citric acid cycle. Metabolites containing carbon are produced by eight mechanisms.

These molecules are successively oxidized, and $FADH_2$ and NADH are the products that retain the oxidation energy. These reduced electron carriers are re-oxidized when they transfer electrons to the electron transport chain. Ketone molecules are used as cell energy during ketosis instead of using glucose. Cells turn to ketosis for energy when blood glucose levels are low, as they are when you're hungry. The electron transport chain's feeble double bonds in O_2 are converted into energy during oxidative phosphorylation. In electron transport chain compounds, NADPH, $FADH_2$, and NADH can all add electrons to redox reactions. Complexes of enzymes in the mitochondria undergo redox reactions. These redox reactions move electrons "down" the path of electron transport that is powered by protons. Through ATP synthase, this disparity in proton content causes the creation of ATP. In plants, photosynthesis produces carbohydrates from water and carbon dioxide using solar energy. The chloroplasts react. Plant cells can produce ATP by photophosphorylation after producing glucose. ATP is produced by humans when they consume vitamin D.

DISCUSSION

The comparatively recent background of metabolic pathway research is reviewed in this paper. We describe computer-aided methods for creating biochemical networks. Null space and convex cone, two significant mathematical ideas used in route analysis, are described. It is shown how these ideas can be transformed into useful biochemical ideas. For instance, it is demonstrated that the simplest vectors, for which the term "elementary flux modes" was created, covering the region of all permissible fluxes in stable states, correlate to essential system paths. A reaction system that represents the glyoxylate cycle and the nearby processes of aspartate and glutamate production is used to explain the ideas. The relationships between route analysis and the idea of metabolic regulation are described. There are several promising industrial and gene labeling uses addressed. Biochemists and biotechnologists will be able to sketch the complete metabolic map of a cell and remake it through logical and targeted metabolic engineering once they have a better grasp of the layout of cellular metabolism and the vast quantity of genetic data currently accessible[6]–[8].

A key biological theory is the concept of metabolic pathways. Their gradual finding has historically served as the foundation for their definition. To analyze their powers and functions, such as metabolic flexibility and resilience and optimum growth rates, new network-based meanings of pathways are needed to accommodate the genome-scale metabolic networks that are currently being rebuilt from the annotation of genome sequences. This need has resulted in the creation of a novel analytically based study of intricate metabolic networks that lists all of their various paths and takes into consideration all of the coenzyme and metabolite needs. Applications include the creation of provable theories about network structure and function, the design of tailored biological systems, and the clarification of characteristics that cannot be explained by straightforward explanations of individual components such as product yield, network robustness, correlated reactions, and predictions of minimal media. These characteristics have recently been investigated in genome-scale networks. As a result, network-based paths are becoming a key model for the study of biological processes.

Prokaryotic metabolic pathways are more biochemically complex than is typically thought. Phylogenetic groups are characterized by distinctive molecular characteristics. The evolutionary events that unintentionally became set in the DNA of a shared progenitor are what determine the higher tiers of characteristics grouping. Phylogenetic trees can now be used to rank prokaryotes. The evolutionary development of prokaryote groups that contain these pathways, makes it possible to track the evolutionary stages that underpin the creation and control of suitably complicated metabolic pathways. Essentially, the method involves using maximal parsimony reasoning principles to infer primordial character traits at ever-deeper evolutionary levels. A case-in-point model for studies that ought to be possible with many important metabolic systems is created using the current viewpoint on the development of the biochemical route for the production of aromatic amino acids. Before the development of branches leading to tyrosine and tryptophan, phenylalanine biosynthesis most likely first evolved. The development of an enzyme system that predates the separation of the main groups of contemporary eubacteria follows the evolution of non-enzymatic processes that may have occurred in early systems (Gram-positive bacteria, Gram-negative purple bacteria, and cyanobacteria).

The information, techniques, uses, and constraints of fish bioenergetics or dietary energetics are reviewed in this paper. In an aquaculture environment, fish bioenergetics is primarily the subject. To support their life functions, all organic creatures depend on the world for sources of renewable energy. Energy is not a nutrient in and of itself; rather, it is the culmination of several processes that lead to the absorption of particular nutrients that produce energy. Animals' use of nutritional components can be examined in a fairly straightforward manner through the study of the equilibrium between food energy supply, consumption, and growth. Any research on dietary energetics must take into account the absorption of meals' minerals and energy. As a single food source, some ingredients in fish feed might not be very agreeable (palatable) to fish. It is also difficult to make feed granules with the right physical properties (water stability) using a large number of individual ingredients. Fish development models based on nutrients have been developed in some cases. These simulations might offer a potential replacement for the existing bioenergetics modeling. These study models, though, are very speculative and contain a lot of biochemical and physiological specifics. They can't be used as forecast models or as parts of feeding or control systems because they are too complicated and costly to do so. As ubiquitous as the genetic code is the ability to harness energy as ion gradients across membranes. We use fresh knowledge of anaerobe metabolism to suggest geological roots for the widespread use of chemiosmotic coupling and in particular Na+/H+ transporters. Alkaline hydrothermal vents with thin FeS walls may experience natural proton gradients that promote carbon assimilation and the emergence of protocells. Protocell membranes that were originally porous would ultimately become less permeable, causing cells reliant on natural H+ gradients to transport Na+ ions. Our theory explains both the profound difference between bacteria and archaea as well as the Na+/H+ flexibility of bioenergetic proteins[9], [10].

Exergonic electron transport between reducing and oxidizing substrates is coupled to the production of chemiosmotic potential in living cells to enable energy harvesting. Prokaryotes use a broad range of redox substrates, leading to a wide diversity of electron transfer chain arrangements, but the assemblage of molecular structures of the enzymes and redox cofactors used to build these systems is astonishingly tiny and consistent. The main categories of electron transport networks are described, along with the distinctive electrochemical characteristics that characterize them. We suggest that the global molecule structure and operation of these chemiosmotic systems can be explained by fundamental thermodynamic principles. The evolutionary path going from hypothesized energy metabolisms in early life

to the chemiosmotic variety of living species is discussed using evidence from palaeogeochemistry and genetic genealogy. Following Occam's razor concept, we only took into account the beginning of life possibilities that are continuous with existing life for this purpose. The evolving features of bioenergetic systems are the title of a Special Issue that this paper is a component of.

CONCLUSION

All living things require energy to sustain their systems, develop and propagate, and adapt to their surroundings. The collection of chemical reactions known as metabolism allows creatures to convert the molecular energy contained in biomolecules into energy that could be used for biological functions. Complex series of regulated biological processes, or metabolic pathways, are involved in cellular metabolism. These mechanisms enable creatures to develop and propagate, preserve their structural integrity, and adapt to changes in their surroundings. Groups of molecular interactions occurring inside cells enable the biochemical processes vital to existence. These processes are arranged through metabolic pathways, which together make up cellular metabolism. The general mechanism by which biological systems obtain and use renewable energy to perform their different tasks is known as bioenergetic metabolism. The most practical thermodynamic principle in biology is free energy. Only when G, the shift in free energy, is negative can a response happen on its own.

REFERENCES:

[1] D. C. Wallace, W. Fan, and V. Procaccio, “Mitochondrial energetics and therapeutics,” *Annual Review of Pathology: Mechanisms of Disease*. 2010. doi: 10.1146/annurev.pathol.4.110807.092314.

[2] S. Demaré, A. Kothari, N. A. Calcutt, and P. Fernyhough, “Metformin as a potential therapeutic for neurological disease: mobilizing AMPK to repair the nervous system,” *Expert Review of Neurotherapeutics*. 2021. doi: 10.1080/14737175.2021.1847645.

[3] A. Garcia-Ropero, C. G. Santos-Gallego, M. U. Zafar, and J. J. Badimon, “Metabolism of the failing heart and the impact of SGLT2 inhibitors,” *Expert Opin. Drug Metab. Toxicol.*, 2019, doi: 10.1080/17425255.2019.1588886.

[4] D. L. Kirchman, “Introduction to geomicrobiology,” in *Processes in Microbial Ecology*, 2013. doi: 10.1093/acprof:oso/9780199586936.003.0013.

[5] M. Chaker *et al.*, “Rho GTPase effectors and NAD metabolism in cancer immune suppression,” *Expert Opinion on Therapeutic Targets*. 2018. doi: 10.1080/14728222.2018.1413091.

[6] N. Lehninger, “Lehninger□: principios de bioquímica,” *J. Shoulder Elb. Surg.*, 2014.

[7] R. A. Harvey and D. R. Ferrier, “Biochemistry (Lippincott Illustrated Reviews Series),” *Biochem. (Lippincott Illus. Rev. Ser.*, 2010.

[8] D. N. Silachev *et al.*, “Magnetic resonance spectroscopy of the ischemic brain under lithium treatment. Link to mitochondrial disorders under stroke,” *Chem. Biol. Interact.*, 2015, doi: 10.1016/j.cbi.2015.06.012.

[9] L. G. Closs, “Principles of Geochemistry (Fourth Edition),” *Geochim. Cosmochim. Acta*, 1983, doi: 10.1016/0016-7037(83)90289-2.

[10] M. Kumari *et al.*, “Activation of type i interferon signaling breaks mitochondrial bioenergetics and drives lethal cardiomyopathy,” *Circ. Res.*, 2019.

CHAPTER 2

GLYCOLYSIS PATHWAY; HISTORY, REACTION, AND REGULATION

Ms. Anu Singh, Assistant Professor
Department of Food Technology, Jaipur National University, Jaipur, India
Email Id-anu.singh@jnujaipur.ac.in

ABSTRACT:
The breakdown of the glucose molecule occurs during glycolysis, a ten-step process that is a component of the digestion of carbohydrates. The anaerobic reaction causes a glycolysis process that occurs in place in the cell's cytosol. One glucose molecule is broken down into two pyruvate molecules in the glycolysis route, which are then added to the electron transport chain. The Hexokinase, phosphofructokinase, and pyruvate kinase enzyme biochemical system heavily controls glycolysis. The background of the glycolysis pathway's genesis was covered in this paper. We go over the various stages of metabolism and how they are controlled.

KEYWORDS:

Aerobic Glycolysis, Cancer Cells, Fructose Phosphate, Glucose Molecule, Pyruvate Kinase.

INTRODUCTION

Suppose that we gave one molecule of glucose to you and one molecule of glucose to Lactobacillus acidophilus the friendly bacteria that transform milk into yogurt. What would the bacteria and you both do with your glucose molecules. Overall, the breakdown of glucose in one of your cells would vary significantly from that of Lactobacillus; for more information, see the fermentation page. But in both situations, the initial stages would be the same: you and the bacterium would both need to divide the glucose molecule in half by sending it through glycolysis. Through a sequence of processes known as glycolysis, glucose is divided into two pyruvate molecules, each of which has three carbons. Glycolysis is a metabolic process that developed long ago and is present in the vast majority of creatures that are living today. Glycolysis is the first step in the process of cellular metabolism in animals. However, many anaerobic organisms organisms that do not use oxygen also have this route because glycolysis does not require oxygen[1], [2].

History: It took nearly 100 years to completely explain the glycolysis pathway as it is understood today. To comprehend the route as a whole, the findings of numerous smaller trials had to be merged. The first stages in comprehending glycolysis started in the nineteenth century with the wine business. For economic reasons, the French wine industry attempted to study why wine sometimes turned distasteful, instead of fermenting into alcohol. The lengthy journey to elucidating the glycolysis pathway started with the 1850s study on this topic by the French scientist Louis Pasteur. His research demonstrated that yeasts, living microbes, are responsible for fermentation. He also demonstrated that yeasts consumed less glucose under aerobic circumstances of fermentation than they did under anaerobic conditions. Eduard Buchner's non-cellular fermentation studies from the 1890s gave researchers insight into the individual stages of glycolysis.

Buchner showed that the use of a non-living yeast extract made it feasible to convert glucose to ethanol due to the activity of the extract's enzymes. In addition to revolutionizing biochemistry, this experiment made it possible for future researchers to study this route in a

more regulated laboratory environment. More components of glycolysis were uncovered by researchers Arthur Harden and William Young throughout several trials (1905–1911). They found how ATP regulated the amount of glucose consumed during the fermentation of booze. They also clarified the function of fructose 1,6-bisphosphate as a glycolysis intermediary Production of CO2 spiked quickly before slowing. Harden and Young observed that if an inorganic phosphate (Pi) was introduced to the mixture, the process would resume. By conducting additional tests, Harden and Young were able to isolate fructose diphosphate after deducing that this process generated organic phosphate esters. (F-1,6-DP). In a subsequent experiment, Arthur Harden, William Young, Nick Sheppard, and others discovered that fermentation requires both a heat-sensitive high-molecular-weight subcellular fraction (the enzymes) and a heat-insensitive low-molecular-weight cytoplasm fraction (ADP, ATP, NAD+, and other cofactors).

Dialyzed (purified) yeast liquid was used in this experiment after it was discovered that it could not germinate or even produce a sugar phosphate. With the inclusion of boiled, undialyzed yeast extract, this combination was saved. All proteins in the yeast extract become inert when it is boiled. The fact that the fermentation could be finished with dialyzed juice and cooked extract indicates that the cofactors weren't proteins. Otto Meyerhof was able to connect a few of the various parts of the glycolysis puzzle found by Buchner, Harden, and Young in the 1920s. To intentionally generate the route from glycogen to lactic acid, Meyerhof and his colleagues were able to separate various glycolytic enzymes from muscle tissue. Meyerhof and researcher Renate Junowicz-Kockolaty looked into the process that divides fructose 1,6-diphosphate into the two triose phosphates in one paper. Previous work suggested that the split happened via 1,3-diphosphoglyceraldehyde + an oxidizing enzyme and cozymase.

Meyerhoff and Junowicz discovered that neither inorganic phosphates nor any other cozymase or oxidizing enzymes had an impact on the equilibrium constant for the isomerase and aldoses process. They also eliminated diphosphoglyceraldehyde as a potential glycolysis intermediary. By the 1930s, all of these components were in place, and Gustav Embden offered a comprehensive, step-by-step description of the glycolysis pathway. The very brief lifespan and low steady-state concentrations of the intermediates of the fast glycolytic reactions posed the greatest challenges in figuring out the specifics of the pathway. Meyerhof, Embden, and numerous other biochemists had successfully solved the glycolysis riddle by the 1940s. In the years that followed, more information about the separate pathway's control and interaction with other metabolic pathways came to light[3]–[5].

This equation appears to be out of proportion in terms of oxygen atoms, hydrogen atoms, and charges due to the use of symbols. The two phosphates (Pi) groups keep the atoms in equilibrium as follows: Each is present as a hydrogen phosphate anion ($[HPO4]_2$), which dissociates to produce 2H+ in total. When each attaches to an adenosine diphosphate (ADP) molecule, an oxygen atom is released, resulting in a total contribution of O_2. The disparity between ADP and ATP balances charges. ADP dissociates into its three hydroxyl groups in the cellular milieu, forming ADP3, which frequently forms an ionic link with Mg^2+ to form ADPMg. Except having four hydroxyl groups, ATP acts exactly like ATPMg2. When these variations along with the true charges on the two phosphate groups are taken together, the net charges of −4 on each side are balanced. For straightforward fermentations, the conversion of one glucose molecule to two pyruvate molecules results in a net output of two ATP molecules. The majority of cells will then perform additional processes to "repay" the NAD+ that was used, resulting in the end products of ethanol or lactic acid. To replenish NAD+, many microbes use inorganic substances as hydrogen acceptors.

Much more ATP is produced by cells engaged in aerobic metabolism, but not as a result of glycolysis. These additional oxygen processes make use of glycolysis' NADH + H+ and pyruvate. For every glucose molecule, eukaryotic aerobic respiration generates an extra 34 molecules of ATP, but the majority of these are produced using a process very dissimilar from substrate-level phosphorylation in glycolysis. Under hypoxic (low oxygen) circumstances, higher flux through the pathway comes from anaerobic respiration's poorer energy output per glucose than aerobic respiration, unless alternative sources of anaerobically oxidizable substrates, such as fatty acids, are discovered. The investment phase and the payout phase are the two stages of glycolysis. The energy is invested in the process as ATP, and the net synthesis of ATP and NADH molecules takes place in the payout phase. The payout phase results in the creation of 4 ATP from a total of 2 ATP in the investment phase, leaving a net total of 2 ATP. Substrate-level phosphorylation refers to the process of generating fresh ATP.

Investment phase: During this stage, glucose is given two phosphates. Hexokinase phosphorylates glucose to produce glucose-6 phosphate at the start of glycolysis. (G6P). In this stage, a phosphate molecule is transferred for the first time, and ATP is used for the first time. Furthermore, this is a permanent action. Because the glucose molecule cannot easily cross the cell membrane, this phosphorylation confines it inside the cell. The subsequent isomerization of G6P into fructose 6-phosphate by phosphoglucose isomerase follows. (F6P). The second phosphate is then added by phosphofructokinase (PFK-1). Fructose 1,6-bisphosphate is created when PFK-1 phosphorylates the F6P using the second ATP. The rate-limiting process is also irrevocable at this point. In the following stage, fructose 1,6-bisphosphate experiences lysis into two molecules, which are substrates for fructose-bisphosphate aldolase to transform it into dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P). The enzyme triosephosphate isomerase converts DHAP to G3P. Since DHAP and G3p change back and forth, they are in balance with one another (Figure. 1).

Payoff Stage:It is important to keep in mind that at the outset of this period, there are two 3-carbon sugars overall for every glucose molecule. By converting NAD+ into NADH, the enzyme glyceraldehyde-3-phosphate dehydrogenase converts G3P into 1,3-diphosphoglycerate. The 1,3-diphosphoglycerate then undergoes a phosphate group loss reaction with phosphoglycerate kinase to become 3-phosphoglycerate, which is then phosphorylated at the substrate level to produce ATP. At this moment, each 3-carbon molecule has generated one ATP, totaling two. Phosphoglycerate mutase converts 3-phosphoglycerate to 2-phosphoglycerate, which is then converted by enolase into phosphoenolpyruvate. (PEP). Pyruvate kinase converts PEP into pyruvate in the final stage and phosphorylates ADP into ATP through substrate-level phosphorylation, producing two additional ATP. This action cannot be undone. In total, 1 glucose molecule requires 2 ATP as an input, and 4 ATP, 2 NADH, and 2 pyruvate molecules are produced as a result.

Free energy change: The formula $G = G^{\circ\prime} + RT\ln Q$, where Q is the reaction quotient, can be used to determine the shift in free energy, or G, for each stage in the glycolysis pathway. Knowing the molecule amounts is necessary for this. The concentrations of NAD+ and NADH are not known for erythrocytes, but the rest of these numbers are. The oxidation of glyceraldehyde-3-phosphate is more advantageous because the ratio of NAD+ to NADH in the cytosol is about 1000. The real free energy change can be determined using the measured concentrations of each stage and the standard free energy changes.

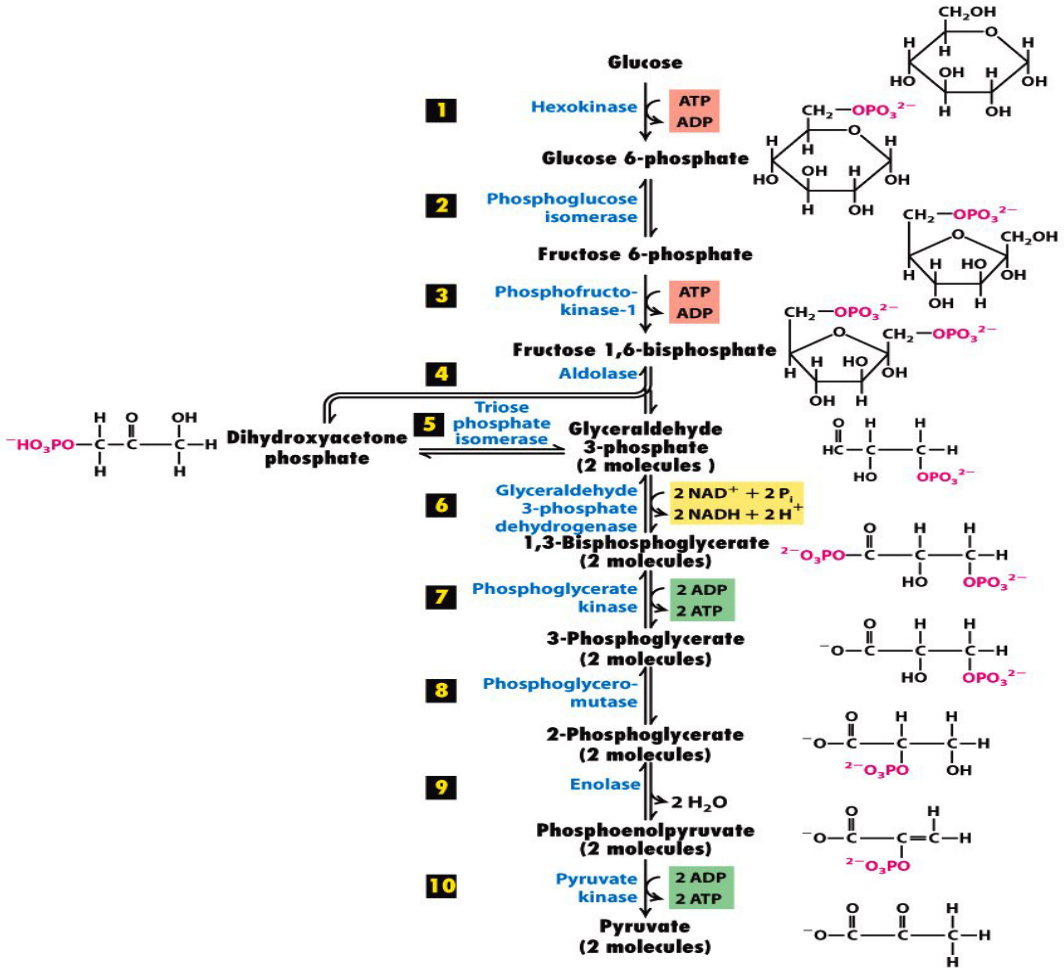

Figure 1: Glycolysis pathway: Daigrame showing the steps involved in the glycolysis pathway (Gpat India).

Pyruvic acid can be produced from glucose through the process of glycolysis, returned to carbs (such as glucose) through the process of gluconeogenesis, or transformed into fatty acids through the process of acetyl-CoA. It can be transformed into ethanol and used to build the amino acid alanine. When oxygen is present (aerobic respiration), pyruvic acid provides energy to living cells through the citric acid cycle, also known as the Krebs cycle; when oxygen is not present, it ferments to create lactic acid. An essential substance in biology is pyruvate. It is a byproduct of glycolysis, an anaerobic process for the breakdown of carbohydrates. One glucose molecule degrades into two pyruvate molecules, which are then used to produce additional energy in one of two ways. Pyruvate is transformed into acetyl-coenzyme A, which serves as the primary input for the Krebs cycle, a sequence of processes. Pyruvate is also changed to oxaloacetate by an anaplerotic reaction, which refills Krebs cycle intermediates and also uses oxaloacetate for gluconeogenesis. This net reaction converts pyruvate into acetyl CoA and CO_2. Acid is broken down anaerobically if there is not enough oxygen present, producing lactate in mammals and ethanol in plants and microbes. Pyruvate produced during glycolysis is fermented into lactate using the cofactor NADH and the enzyme lactate dehydrogenase. As an alternative, it undergoes alcoholic fermentation to become acetaldehyde and then ethanol.

$$2 \text{ pyruvate} + 2NAD^+ + 2CoA \rightarrow 2\text{Acettl CoA} + 2NADH + 2\ CO_2$$

A crucial node in the network of biochemical processes is pyruvate. Pyruvate can be transformed into ethanol, the amino acid alanine, fatty acids or energy through acetyl-CoA, carbs through gluconeogenesis, and fatty acids or energy. As a result, it combines several important biochemical functions.

There are three main enzymatic control sites within the glycolytic pathway. These include the phosphofructokinase, pyruvate kinase, and hexokinase processes. Energy demand within the cell, which is decided by regional markers like ATP and AMP, as well as energy demand within the organism as a whole, which can be affected by hormone signaling pathways, are key factors for controlling the pathway. We will also observe that the control of the pathway can change based on the sort of cell and the requirements of the cell.

Regulation of the glycolysis: The existence of various hexokinase enzymes in various cellular kinds is one of the main processes that control the regulation of the hexokinase phase in glycolysis (Figure. 2). In essence, these are proteins that, despite being expressed by various genes, serve the same purpose in the organism. They're referred to as isozymes. Isozymes can vary in their post-translational modifications, enzyme kinetics, expression patterns in various tissues, and ability to attach to various allosteric effectors. This enables the body to exert varying levels of control over the same processes in various parts of the body. Four significant hexokinase isozymes in mammals differ in subcellular sites and kinetics characteristics. As a result, hexoses can be phosphorylated differently based on the environment and a given physiological role. The hexokinases I, II, III, and IV are given these names. In addition to glucose, all hexokinases also accept other hexoses as substrates. Mannose, fructose, and 2-deoxyglucose are some of these. Hexokinase IV, which is unique to the liver and pancreas, is also frequently referred to as glucokinase[6], [7].

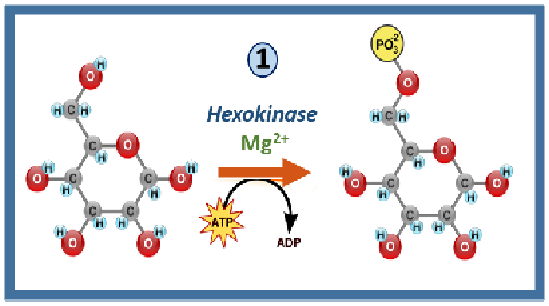

Figure 2: Regulation of the hexokinase: Diagram showing the regulation of the hexokinase (Biology libre text).

Glucose-6-phosphate (G6P) can take on several different destinies within the organism. Through glycolysis and aerobic breathing processes, it can be used as a source of energy. Animals that transform pyruvate into lactate can also endure brief periods of anaerobic respiration. To preserve homeostatic equilibrium, G6P can also be dephosphorylated in the liver and discharged once more into circulation. When releasing insulin and glucagon, the pancreas employs G6P as a sensor. The G6P can act as a fundamental component in anabolic mechanisms. Through the Pentose Phosphate Pathway, it can be transformed into ribose, which is then used to build nucleotide molecules. Hexosamines, which are necessary for the synthesis of glycoproteins and proteoglycans, can also be produced using it. Hexokinase I (HKI) is broadly dispersed throughout the body and is primarily expressed in red blood cells and cerebral tissue. This protein is restricted to the mitochondria of brain cells. This colocalization facilitates the effective linking of the Krebs cycle, oxidative phosphorylation, and glycolysis processes within the mitochondria. It also links HKI activity to energy load and oxidative phosphorylation because HKI primarily utilizes ATP produced in mitochondria

for its reaction mechanism. Additionally, HK association with the mitochondria has a cellular protective impact by lowering the likelihood of apoptosis or planned cell death.

On the other hand, Red Blood Cells (RBCs) are highly differentiated cells with a very limited lifetime. In people, they are changed every two weeks on average. RBCs are enucleated and do not have mitochondria, and thus, only produce ATP through the process of glycolysis. In this arrangement, the HKI protein is allowed to move around in the cytoplasm. Due to its low Km, HKI is active at low substrate amounts and has a strong affinity for glucose. The byproduct glucose-6-phosphate (G6P) inhibits it as well through the mechanism of negative feedback suppression. This negative feedback inhibition can be overridden by low to intermediate amounts of free inorganic phosphate. Skeletal muscle, heart muscle, and insulin-sensitive organs all express HKI and HKII. The use of G6P in energy production is believed to be primarily catabolic, but HKII may play a more important part in anabolic processes by supplying G6P for conversion to G1P and subsequent use in glycogenesis. The mitochondria's exterior layer is where HKI and HKII are found. However, only about 70% of HKII is linked with mitochondria, with the remainder of HKII fractionating with the cytosolic proteins, while 95% of HKI is. This may help to explain why HKII is not present in cerebral tissue and its increased involvement in anabolic glycogenesis processes in skeletal muscle. Additionally, tumor cells frequently overexpress HKII, and this is linked to increased death rates. Additionally, it has been connected to the formation of medication tolerance and metastasis mechanisms. Similar to HKI, HKII also has a low Km and is blocked by G6P, although this inhibition is not relieved by the presence of inorganic phosphate[8], [9].

Regulation of Phosphofructokinase-1: The first irreversible glycolysis process is the PFK1 reaction. It also stands for the pathway's determination to move. Fructose-6-phosphate (F6P) is phosphorylated to fructose-1,6-bisphosphate (F1,6BP), which commits the latter to proceed along the glycolytic route. At that time, it is useless for any other use. Contrarily, F6P could be transformed back into glucose-6-phosphate and utilized for a variety of functions. PFK1 is one of the most crucial control points in the glycolytic pathway due to the dedicated character of this phase (Figure. 3). The muscle (M), liver (L), and platelet (P) components are among the three kinds of subunits that make up the tetramer that makes up the PFK1 enzyme. (P). Depending on the sort of tissue it is found in, the PFK1 tetramer's structure varies. For instance, only the M isozyme is expressed by adult muscle, so the muscle PFK1 is only made up of homotetramers of M4. The L variant is primarily expressed in the liver and kidneys. Both the M and L components tetramerize at random in erythrocytes to produce M4, L4, and the three hybrid versions of the enzyme (ML3, M2L2, M3L). As a consequence, subunit makeup affects the kinetic and regulatory characteristics of the different isoenzyme pools.

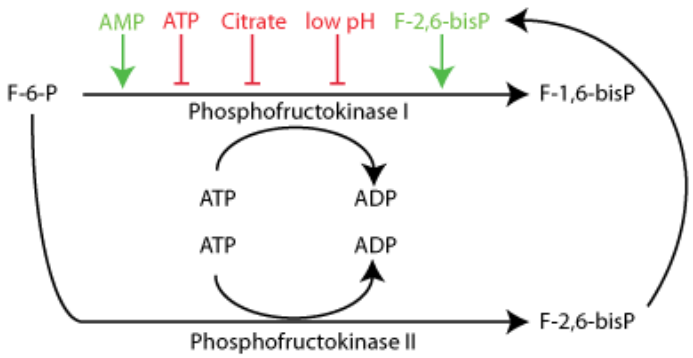

Figure 3: Regulation of the Phosphofructokinase-1: Diagram showing the regulation of the Phosphofructokinase-1 (education.med.nyu. edu).

The variations of glycolytic and gluconeogenic rates that have been found for various tissues are greatly influenced by tissue-specific alterations in PFK1 activity and isoenzymes content. This suggests that the cell is in a low-energy condition and that glycolysis and other forms of energy production are required. High ATP and citrate concentrations are examples of allosteric regulators. It should be noted that this enzyme uses ATP as a substrate, and ATP has the usual substrate binding site. It will function as an inhibitor when there is sufficient ATP to allow it to attach allosterically to the enzyme as well. The first Kreb's Cycle (Citric Acid Cycle) component, citrate, can also block PFK-1 allosterically.

High amounts of citrate suggest a high energy load within the cell and that no more pyruvate is required to produce energy through oxidative phosphorylation. Additionally, we'll see that fructose 2,6-bisphosphate primarily functions as a stimulator and modulator of PFK1 in liver cells. The PFK-2/FBPase-2 Enzyme is regulated in reaction to this communication, which in turn regulates the glycolytic pathway. This enzyme's action is managed by the PKA signaling chain. The phosphorylation of fructose 6-phosphate to fructose 2,6-bisphosphate is carried out by this enzyme. It should be noted that this fructose bisphosphate form is NOT the same as the bisphosphate form used in the glycolytic pathway. The PFK1 enzyme produces fructose 1,6-bisphosphate, which is necessary for the glycolytic process.

Since it is a distinct enzyme, PFK-2/FBPase-2 is not immediately connected to the glycolytic pathway. Fructose 2,6-bisphosphate can, however, function as an allosteric activator of the PFK1 enzyme, as we earlier pointed out. The PFK-2/FBPase-2 enzyme has two distinct functions: it can phosphorylate the 2'-position of fructose 6-phosphate using its kinase activity and eliminate the phosphoryl group from position 2' of fructose 2,6-bisphosphate to produce fructose 6-phosphate using its FBPase activity. When the protein is in the dephosphorylated form, the PFK-2 component is active, but the FBPase is not. Fructose 1,6-bisphosphate can be formed by PFK1 and fructose 2,6-bisphosphate to be produced by PFK-2 if there is a lot of fructose-6-phosphate presents (for example, if you recently consumed high fructose corn syrup in your sweet energy drink).

Fructose 2,6-bisphosphate will then interact with PFK1 and enhance its activity, turning fructose 6-phosphate into fructose-1,6-bisphosphate. However, you need to tone down the glycolytic pathway and stop fructose-2,6-bisphosphate from rapidly upregulating PFK1 during glucagon signaling. The PFK-2/FBPase-2 enzyme will be phosphorylated by Protein Kinase A to accomplish this and change its function. The phosphorylase activity is activated while the kinase activity is suppressed. When glucagon is present, PKA will phosphorylate the PFK-2/FBPase-2 enzyme, turning on the phosphatase activity while turning off the kinase activity. Fructose-6-phosphate is recovered by dephosphorylating fructose-2,6-bisphosphate. (F6P). The reverse isomerase process can then be used by F6P to restore glucose-6-phosphate. (G6P). Following the transfer of G6P to the rER for dephosphorylation, unbound glucose can then be released back into the bloodstream.

Pyruvate kinase: pyruvate kinase enzyme functions as a crucial regulating element within the system, just like PFK1. The pyruvate kinase enzyme is a tetramer made up of a variety of isozymes that are produced in various organs. The L form of pyruvate kinase is primarily found in the liver, the R form is primarily found in erythrocytes, the M1 form, which is primarily found in muscle and the brain, and the M2 form, is present in embryonic tissue and to some extent in the majority of adult tissues. The same gene locus gives birth to the L and R forms, as well as the M1 and M2 forms, which are both splice variations. As we begin with the activator, fructose 1,6-bisphosphate, we will concentrate on some of the basic regulatory processes shared by the majority of the isozymes of pyruvate kinase. It is known as a feedforward stimulus when FBP activates pyruvate kinase enzymes because FBP is an early

product in the same metabolic pathway. The attachment of FBP to the enzyme stimulates all of the isozymes, except the M1 type. Similar to this, ATP (or high energy load), the end product of the process, and high amounts of alanine block all known pyruvate kinase isozymes. Pyruvate can be produced from alanine in a single biochemical process. Pyruvate functions as a biochemical intermediary in the synthesis of alanine as a result. A large energy load within the cell is indicated by the presence of elevated amounts of alanine. As a result, the pyruvate kinase family of enzymes is negatively regulated by elevated amounts of alanine.

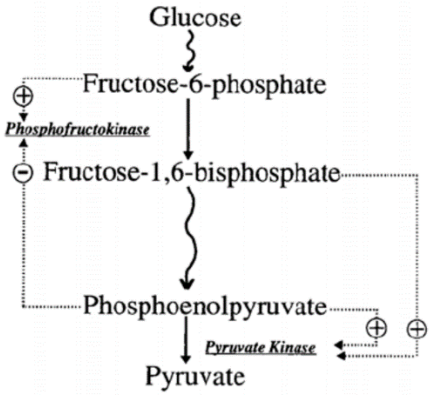

Figure 4: Regulation of the pyruvate kinase: Diagram showing the regulation of the pyruvate kinase (research gate).

The liver isozyme of Pyruvate Kinase is also downregulated during glucagon signaling, in a manner similar to the PFK-2/FBPase-2 enzyme's PFK-2 activity. Pyruvate Kinase is phosphorylated by Protein Kinase A, which suppresses the enzyme's action and stops phosphoenolpyruvate from being converted to pyruvate (Figure. 4). To guarantee that glucose resources will be taken from cellular use by the liver and released into the bloodstream to reestablish homeostatic blood glucose levels, the glycolytic pathway is dual-regulated during glucagon signaling.

DISCUSSION

Glycolysis is an old process that developed well before oxygen was prevalent in the Earth's atmosphere and is highly conserved among living species. The first biochemical route to be identified as glycolysis, also known as the Embden-Meyerhof-Parnas pathway. The Greek words "glykys," which means "sweet," and "lysis," which means "to split," are the origins of the term "glycolysis." This is the breakdown of one glucose molecule into two pyruvate molecules, which is the result of glycolysis. Pyruvate typically reaches the mitochondria where it is metabolized to acetyl-CoA, whereas when oxygen is not present, it is reduced to lactate. Without the need for molecular oxygen, glycolysis includes ten cytosolic processes and produces two ATP molecules. In comparison, the mitochondria's oxidative phosphorylation process produces 30 ATP molecules while requiring oxygen.

Increased glycolysis flow promotes the growth of cancer cells by supplying extra ATP energy and glucose-derived metabolic intermediates for nucleotide, lipid, and protein production. As a result, glycolysis and other metabolic processes that regulate cell proliferation might make good candidates for treatments and diagnostic techniques. In this context, monitoring glucose absorption and lactate excretion by cancer cells may help identify changes in glucose metabolism, and monitoring the activity of rate-limiting glycolytic enzymes may offer information on the metabolic control points. Furthermore, metabolomic research can produce sizable, combined databases to monitor changes in carbon flux through glycolysis and its

auxiliary anabolic pathways. These methods, which have been described here, can identify and measure the metabolic changes that underpin the proliferation of malignant cells.

If somatic evolution is the cause of carcinogenesis, prevalent elements of the cancer phenotype must have undergone active selection to give a sizable growth benefit. Upregulation of glycolysis, which results in higher glucose intake, is a nearly ubiquitous characteristic of primary and metastatic malignancies and can be seen with clinical tumor imaging. We suggest that sporadic hypoxia in pre-malignant lesions is adapted to by prolonged glucose metabolism to lactate even under aerobic circumstances. A microenvironmental acidity caused by glycolysis upregulation, however, necessitates the evolution of forms immune to acid-induced cell toxicity. A strong growth edge that encourages unrestricted proliferation and invasion is present in subsequent cell populations with upregulated glycolysis and acid tolerance.

The function of glycolysis in normal and cancer cells has been debated since Warburg noted that cancer cells show a high rate of glycolysis even in the presence of oxygen (aerobic glycolysis). Aerobic glycolysis and cancer are not caused by abnormalities in mitochondrial metabolism, but the benefits of increased glycolysis in cancer are still debatable. Aerobic glycolysis is used by a wide variety of cells, from microbes to lymphocytes, during fast proliferation, which indicates it may be crucial for promoting cell development. Here, we discuss the role of glycolysis in the biochemical functions of proliferating cells. We give a thorough breakdown of the metabolic processes needed to build a new cell and show how crucial glycolysis is for producing the carbons needed to produce biomass. We contend that the primary purpose of aerobic glycolysis is to sustain high concentrations of glycolytic intermediates to support anabolic processes in cells, explaining why higher glucose metabolism is advantageous in naturally proliferating cells[10].

The majority of cancer cells have increased glycolysis and rely heavily on this metabolic process to produce ATP as their primary source of energy. The Warburg effect is an occurrence that is regarded as one of the most basic metabolic changes during malignant transformation. In recent years, there are substantial progress in our knowledge of the underlying processes and the possible therapeutic consequences. Numerous potential pathways by which this metabolic alteration might occur during the development of cancer have been suggested by biochemical and molecular research. These pathways include oncogenic signaling, aberrant expression of metabolic enzymes, adaptation to a hypoxic tumor milieu, and mitochondrial abnormalities and dysfunction. The greater reliance of cancer cells on the glycolytic pathway for the production of ATP is significant because it provides a biochemical foundation for the development of therapeutic approaches to selectively destroy cancer cells by pharmacologically inhibiting glycolysis. In vitro and in vivo studies have revealed several small molecules that, alone or in conjunction with other treatment methods, show potential anticancer activity. The glycolytic inhibitors are especially effective against cancer cells that have mitochondrial problems or are exposed to hypoxic conditions, which are frequently linked to cellular resilience to traditional anticancer medications and radiation treatment. The discovery of innovative glycolytic inhibitors as a new class of anticancer drugs is expected to have widespread therapeutic uses because enhanced aerobic glycolysis is frequently observed in a wide range of human cancers and hypoxia is present in the majority of tumor microenvironments.

Aerobic glycolysis is described as the use of glucose above what is required for oxidative phosphorylation, even when there is enough oxygen for the entire process of converting glucose to carbon dioxide and water. Aerobic glycolysis is present in the typical human brain at rest and rises locally during increased neuronal activity; however, due to a predominant

energy-centric emphasis on the role of glucose as a substrate for oxidative phosphorylation, its numerous biological functions have received little attention. We used positron emission tomography to assess the regional distribution of aerobic glycolysis in 33 neurologically healthy young adults at rest as a first step in redressing this negligence. We demonstrate that previously well-described functional regions of the brain vary in their spread of aerobic glycolysis. Particularly, the medial and lateral parietal and prefrontal cortices exhibit a marked increase in aerobic metabolism. The amounts of aerobic glycolysis in the cerebellum and medial temporal lobes, however, are considerably lower than the brain mean. The rates of cerebral energy consumption are not directly correlated with the rates of aerobic glycolysis. For instance, sensory cortices have modest rates of aerobic glycolysis but high metabolic rates for glucose and oxygen intake. These remarkable regional differences in aerobic glycolysis in the healthy human brain offers a chance to investigate how various brain systems use glucose's diverse cell biology to support their functional specializations in both health and illness.

By eliminating dead cells through efferocytosis, resolving-type macrophages stop persistent inflammation. Although oxidative phosphorylation is believed to be the primary source of energy for these macrophages, new research points to a potential connection between efferocytosis and glycolysis. We looked into the molecular and cellular processes that underlie efferocytosis-induced macrophage glycolysis and its effects to learn more about this problem. In contrast to the glycolysis in pro-inflammatory macrophages, we discovered that efferocytosis stimulates a brief rise in macrophage glycolysis that is reliant on the swift stimulation of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 (PFKFB2). Compared to wild-type control mice, mice implanted with PFKFB2 bone marrow that is activation-defective show impaired thymic efferocytosis, increased thymic necrosis, and lower expression of the efferocytosis receptors MerTK and LRP1 on thymic macrophages. In vitro, mechanistic investigations showed that glycolysis triggered by the uptake of a first apoptotic cell supports continual efferocytosis through lactate-mediated upregulation of MerTK and LRP1. To maintain continuous efferocytosis in a lactate-dependent way, efferocytosis-induced macrophage glycolysis reflects a special metabolic process. This mechanism can be therapeutically improved to support efferocytosis and resolution in chronic inflammatory disorders because it differs from inflammatory macrophage glycolysis.

CONCLUSION

Glycolysis is crucial since it is the biochemical process in which glucose produces energy for cells. The most crucial source of energy for all living things is glucose. Most cells in the human body favor glucose as a source because red blood cells cannot use anything else. A mechanism by which cells partly break down glucose (sugar) using oxygen-free enzyme reactions. One process that organisms use to make energy is glycolysis. Only two total molecules of ATP are produced during glycolysis for every glucose molecule. Hexokinase, phosphofructokinase, and pyruvate kinase are the three major enzymes involved in glycolysis. The substance is converted to lactate through the action of lactate dehydrogenase. All forms of cellular metabolism begin with glycolysis, which is anaerobic and oxygen-free. The route will go on to the Krebs cycle and oxidative phosphorylation if oxygen is present.

REFERENCES:

[1] M. Rehberg, J. B. Ritter, and U. Reichl, “Glycolysis Is Governed by Growth Regime and Simple Enzyme Regulation in Adherent MDCK Cells,” *PLoS Comput. Biol.*, 2014, doi: 10.1371/journal.pcbi.1003885.

[2] M. M. Kutyna *et al.*, “Distinct Senescent Bone Marrow Microenvironment in Therapy-Related Myeloid Neoplasms,” *Blood*, 2021, doi: 10.1182/blood-2021-148215.

[3] Merlinta, “Hubungan pengetahuan tentang vaksin mr (measles rubella) dan pendidikan ibu terhadap minat keikutsertaan vaksinasi MR di puskesmas kartasura,” *Int. Rev. Immunol.*, 2018.

[4] L. T. Putri, “Strategi Komunikasi Pemasaran Brand Busana Muslim Dinda Firdausa di Media Online,” *fashion*, 2018.

[5] N. D. Handayani, “Tingkat Efektivitas Waktu Proses Rotating Biological Contactor (RBC) dalam Menurunkan Kadar BOD Limbah Cair Laundry,” *Int. Rev. Immunol.*, 2018.

[6] V. S. Friyani, “Hubungan Persepsi Terhadap Organisasi dengan Minat Mahasiswa Kedokteran Untuk Berorganisasi,” *Int. Rev. Immunol.*, 2018.

[7] A. Prabowo, E. Wuryanto, and N. Sukraeny, “TINGKAT KELELAHAN KERJA PERAWAT KAMAR BEDAH RSUP Dr. KARIADI SEMARANG,” *Int. Rev. Immunol.*, 2018.

[8] siti muthmainah, “ANALISIS PENETAPAN UJRAH BARANG GADAI DI PEGADAIAN SYARIAH CABANG INDRAMAYU,” *Int. Rev. Immunol.*, 2018.

[9] Y. Adhipratama, “Perancangan antarmuka pengguna dengan metode Lean UX,” *Int. Rev. Immunol.*, 2018.

[10] UFAPE, “PPC Engenharia de Alimentos,” *Int. Rev. Immunol.*, 2018.

CHAPTER 3

GLUCONEOGENESIS; REVERS THE GLYCOLYSIS

Dr. Manish Soni, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id- manishsoni@jnujaipur.ac.in

ABSTRACT:

The process of producing glucose from non-carbohydrate substrates is known as gluconeogenesis. The reverse of glycolysis, known as gluconeogenesis, occurs when a fasting condition needs a source of glucose. Gluconeogenesis reactions primarily take place in the liver and kidney cytoplasm. This paper covered the locations of the Glucoseneogenesis progenitor sites. We provided an overview of the control and gluconeogenesis response.

KEYWORDS:

Amino Acids, Citric Acid, Cori Cycle, Fatty Acid, Glucogenic Amino Acid.

INTRODUCTION

A biochemical process known as gluconeogenesis (GNG) produces glucose from specific carbon sources that are not carbohydrates. It is a universal mechanism that occurs in all living things, including fungi, bacteria, and other microorganisms. The process happens in many other species during fasting, starvation, low-carbohydrate diets, or vigorous activity. Substrates for gluconeogenesis in people can originate from any non-carbohydrate substances that can be transformed into pyruvate or glycolysis intermediates. These substrates include glycerol, odd-chain fatty acids (but not even-chain fatty acids; see below), and lactate from the Cori cycle from other parts of metabolism. For the breakdown of proteins, these substrates include glucogenic amino acids (but not ketogenic amino acids).

Acetone made from ketone bodies can also act as a substrate during extended fasting, giving a route from fatty acids to glucose. Although the liver produces the majority of gluconeogenesis, diabetes, and extended fasting raise the proportional input of the kidney to gluconeogenesis. Up until it is connected to the breakdown of ATP or GTP when the gluconeogenesis route is essentially exergonic. For instance, the route from pyruvate to glucose-6-phosphate needs 4 ATP and 2 GTP molecules to function naturally. These ATPs are generated from the breakdown of fatty acids through beta-oxidation[1], [2].

Precursors of gluconeogenesis: Lactate, glycerol (which is a component of the triglyceride molecule), alanine, and glutamine are the primary gluconeogenic constituents in people. They comprise more than 90% of the total gluconeogenesis. All stages of the citric acid cycle, including other glucogenic amino acids, can serve as substrates for gluconeogenesis by being converted to oxaloacetate. In general, gluconeogenesis in humans does not rise when gluconeogenic substrates are consumed in the diet. Propionate is the main gluconeogenic substance in ruminants. Propionate is produced by the -oxidation of odd-chain and branched-chain fatty acids in non-ruminants, including humans, and it serves as a (relatively small) fuel for gluconeogenesis. The enzyme lactate dehydrogenase transports lactate back to the liver, where the Cori cycle uses it to transform it into pyruvate. Pyruvate, the first specified substrate of the gluconeogenic pathway, can then be used to produce glucose.

Amino acids' carbon skeleton can join the cycle directly (as pyruvate or oxaloacetate) or tangentially (via the citric acid cycle) thanks to transamination or deamination. The

proportion of Cori cycle lactate to total glucose production rises with fasting time. In particular, the percentages of Cori cycle lactate that contributed to gluconeogenesis after 12, 20, and 40 hours of fasting by human participants were 41%, 71%, and 92%, respectively. In biochemistry, the issue of whether even-chain fatty acids can be converted into glucose in mammals has long persisted. Odd-chain fatty acids can be metabolized to produce acetyl-CoA, propionyl-CoA, and succinyl-CoA, the latter of which can be transformed into pyruvate and used in the process of gluconeogenesis. Contrarily, acetyl-CoA is the only product of the oxidation of even-chain fatty acids, and its incorporation into the gluconeogenic pathway necessitates the existence of a glyoxylate cycle (also known as a glyoxylate shunt), which generates four-carbon dicarboxylic acid intermediates. Malate synthase and isocitrate lyase are two of the enzymes that make up the glyoxylate shunt, which is found in fungi, plants, and microbes. The genes encoding both enzymatic functions have only been discovered in worms, where they are present as a single bi-functional enzyme, despite some accounts of glyoxylate shunt enzymatic activities being identified in animal tissues.

Other species, such as arthropods, echinoderms, and even some mammals, have been found to have genes coding for malate synthase alone (but not isocitrate lyase). Monotremes (like the platypus) and marsupials (like the opossum) have been discovered to have the malate synthase gene, but placental animals do not. It has not been proven that humans have a glyoxylate cycle, and it is generally believed that fatty acids cannot be immediately converted to glucose in people. The incorporation of labeled atoms from acetyl-CoA into citric acid cycle intermediates, which are interchangeable with those derived from other physiological sources, such as glucogenic amino acids, has been shown to result in carbon-14 ending up in glucose when it is supplied in fatty acids.

Since an equal two carbon atoms are released as carbon dioxide during the citric acid cycle, the 2-carbon acetyl-CoA obtained from the oxidation of fatty acids cannot create a net yield of glucose in the absence of other glucogenic sources. Acetone is one of the ketone bodies that are produced during ketosis from acetyl-CoA from fatty acids, and up to 60% of acetone may be metabolized in the liver to the pyruvate intermediates acetol and methylglyoxal. Thus, up to 11% of the gluconeogenesis that occurs during fasting may be accounted for by ketone bodies made from fatty acids. The route for gluconeogenesis requires ATP, which is produced during the breakdown of fatty acids.

The production of carbohydrates like glucose and glycogen from materials other than sugars is known as gluconeogenesis. Lactic acid to glucose metabolism is one illustration. From the lactic acid raised by a glycolytic shunt to produce glucose by glyconeogenesis, it moves through the following process: lactic acid is transformed into alanine and then transported into the liver. The liver uses glyconeogenesis to convert alanine back to lactic acid and then to glucose. However, the kidney is where some glyconeogenesis takes place. It is feasible to produce glucose by glyconeogenesis if enzymes are engaged in the glycolytic shunt, except for three one-way (irreversible) reactions. The three processes are: (1) Hexokinase converts glucose into G6P; (2) PFK-1 transforms F6P into F1,6BP; and (3) Pyruvate kinase transforms PEP into pyruvate.

The use of additional enzymes, glucose-6-phosphatase, and fructose-1,6-bisphosphatase, respectively, allows for the resolution of the opposite processes of (1) and (2). The third and final response, however, is more intricate. Pyruvate is first transported into mitochondria where it is catalyzed by pyruvate carboxylase and transformed into oxaloacetate with the aid of CO2 and ATP. Oxaloacetic acid is first converted to malate, which is then transported outside of mitochondria by the malate shuttle and converted back to oxaloacetate. Lastly, the enzyme phosphoenolpyruvate carboxylase converts oxaloacetate to PEP. In this process,

glucose is created from two lactic acid molecules. Six ATP molecules are required for glyconeogenesis to produce one glucose molecule.

1. The cytosol or mitochondria of the liver or kidney are where glucose synthesis begins.
2. Two pyruvate molecules must first carboxylate to create oxaloacetate. One ATP (energy) atom is needed for this.
3. Oxaloacetate is changed into malate by NADH so that it can be carried outside of the mitochondria.
4. When malate exits the mitochondria, it is converted back to oxaloacetate through oxidation.
5. Oxaloacetate is changed into phosphoenolpyruvate by the enzyme Phosphoenolpyruvate Carboxykinase (PEPCK).
6. Fructose 1,6-bisphosphate is produced from phosphoenolpyruvate by undoing the glycolytic pathways (Figure. 1).
7. In the process that releases inorganic phosphate, fructose-1,6-bisphosphate is changed to fructose-6-phosphate and is catalyzed by fructose-1,6-bisphosphatase.
8. Fructose-6-phosphate is changed into glucose-6-phosphate by the enzyme phosphoglucoisomerase (Figure. 1).
9. Inorganic phosphate is produced by glucose-6-phosphate, which then releases unbound glucose into circulation. An enzyme called glucose 6-phosphatase is at work here.

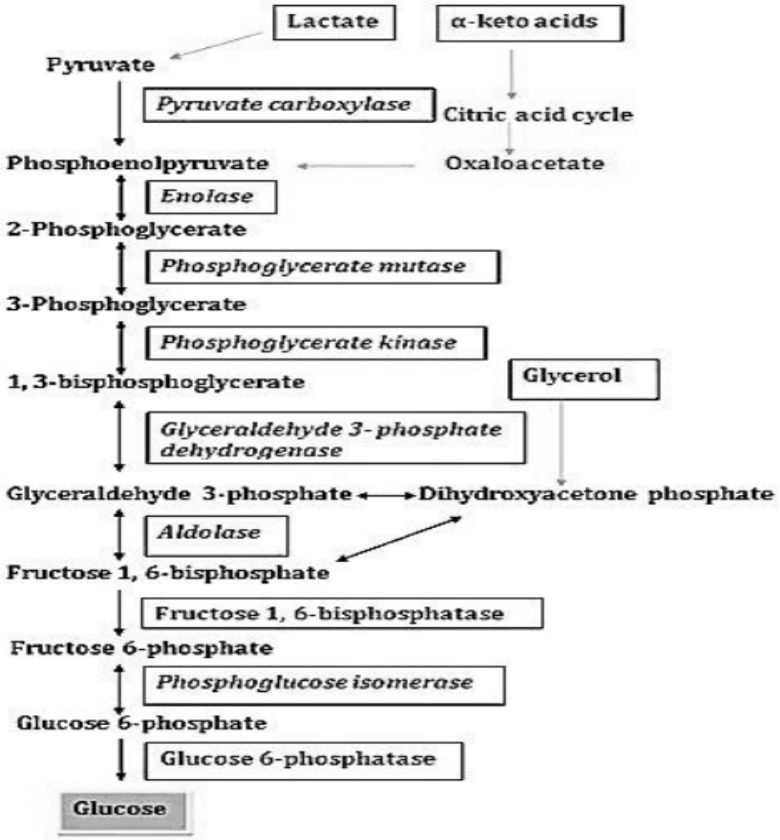

Figure 1: Gluconeogenesis pathway: Diagram showing the gluconeogenesis pathway (Biology ease).

Sites of the gluconeogenesis: The liver, the kidney, the gut, the muscle, and the liver were thought to be the only places in animals where gluconeogenesis took place, but more recent

research suggests that gluconeogenesis also takes place in the astrocytes of the brain. These tissues employ gluconeogenic antecedents that are somewhat unique. While the kidney preferentially uses lactate, glutamine, and glycerol, the liver preferentially uses these substances as well as glucogenic amino acids, particularly alanine. The biggest quantitative supply of substrate for gluconeogenesis, particularly for the kidney, is lactate from the Cori cycle. The kidney only uses gluconeogenesis, whereas the liver employs both glycogenolysis and gluconeogenesis to create glucose.

Following a meal, the kidney boosts gluconeogenesis while the liver switches to glycogen production. Glycerol and glutamine are primarily used by the gut. The primary substrate for gluconeogenesis in the bovine liver is propionate, and when glucose consumption rises, the ruminant liver may use gluconeogenic amino acids (like alanine) more frequently. When newborns and lambs transition from the predominant stage to the ruminant stage, the ability of the liver cells to use lactate for gluconeogenesis decreases. Very high rates of gluconeogenesis from propionate have been seen in sheep renal tissue. All animals only have one mitochondrion that can produce oxaloacetate from pyruvate and TCA cycle intermediates, and the cytoplasm contains the enzymes that change phosphoenolpyruvic acid (PEP) into glucose-6-phosphate.

PEP carboxykinase (PEPCK), an enzyme that connects these two aspects of gluconeogenesis by converting oxaloacetate to PEP, can be found in different places depending on the species. It can be equally distributed between the cytosol and mitochondria, as it is in humans, or completely within the mitochondria. There are specific transport proteins that move PEP across the mitochondrial membrane, but no such proteins move oxaloacetate across the membrane. Oxaloacetate must therefore be changed into malate or aspartate, removed from the mitochondrion, and then changed back into oxaloacetate in organisms that do not have intra-mitochondrial PEPCK[3]–[5].

Regulation of gluconeogenesis: Energy conservation is crucial for creatures, so they have developed mechanisms to control the metabolic processes that use and discharge the most energy. Seven of the ten stages in glycolysis and gluconeogenesis take place at or close to balance. Fructose-1,6-bP, glucose-6-P, and pyruvate all undergo very random conversions in the process of gluconeogenesis, which is why these reactions are tightly controlled. For the body to function properly, energy conservation is crucial. Gluconeogenesis is suppressed when there is an abundance of energy accessible. Gluconeogenesis is triggered when energy is needed. Acetyl-CoA controls how pyruvate is transformed into PEP. Acetyl-CoA exclusively activates pyruvate carboxylase (Figure. 2).

When acetyl-CoA quantities are high, organisms use pyruvate carboxylase to divert pyruvate from the TCA cycle because acetyl-CoA is a crucial molecule in the TCA cycle that generates a lot of energy. It is ideal to direct those metabolites toward storage or other required processes if the organism does not require more energy. The molecules AMP and fructose-2,6-bP adversely control and prevent the conversion of fructose-1,6-bP to fructose-6-P with the aid of fructose-1,6-phosphatase. These are the phosphofructokinase-receptor controllers of glycolysis (Figure. 2). AMP and fructose-2,6-bP both favorable control phosphoglucose kinase. Once more, the organism increases gluconeogenesis and reduces glycolysis when the energy levels generated are greater than what is required, or when there is a high ATP to AMP ratio. In contrast, an organism increases glycolysis and reduces gluconeogenesis when energy levels are lower than necessary, as seen by a low ATP-to-AMP ratio. Substrate level modulation regulates the process by which glucose-6-phosphatase converts glucose-6-P to glucose. Glucose-6-P is the molecule in charge of this kind of

control. Glucose-6-phosphatase becomes more active and produces more glucose as the amounts of glucose-6-P rise. As a result, metabolism cannot continue.

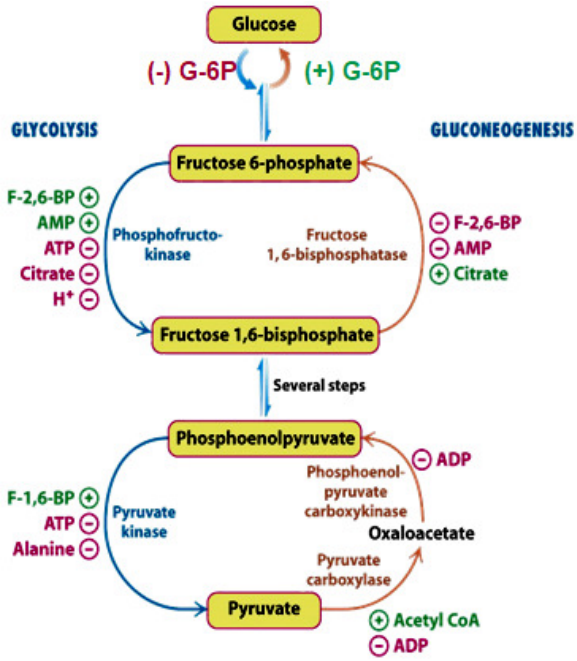

Figure 2: Gluconeogenesis regulation: Diagram showing the gluconeogenesis regulation (Quizlet).

DISCUSSION

From the perspectives of its roles and connections to general metabolism, the biochemical processes regulating it, and its changes in different physiologic and pathologic states, glucose synthesis is examined. Its activities are addressed in connection to renal ammonia production, the Cori cycle, the alanine cycle, and cerebral metabolism. The main physiological sources from which glucose is synthesized are listed. We describe gluconeogenesis control systems that seem to function under physiological circumstances. Particular attention is paid to the regulation of the following processes or enzymes: supply of substrate from peripheral tissues, substrate uptake by the liver, transport of metabolites across the mitochondrial membranes, pyruvate carboxylase, pyruvate dehydrogenase, the Krebs cycle, P-enol pyruvate carboxykinase, pyruvate kinase, fructose-1, 6-diphosphatase, P-fructokinase, glucose 6-phosphatase, glucokinase, phosphorylase, and glycogen synthetase. Also taken into account are the impacts of alterations in ATP, ADP, AMP, cyclic AMP, the [free NADH]/[free NAD+] ratio, Ca++, and H+. With specific reference to studies in men, modifications in gluconeogenesis in different circumstances are described. The search for endocrine and other variables causing changes in metabolism continues. The following scenarios are taken into account: prenatal and newborn growth, diabetes, and insulin therapy, pregnancy and breastfeeding, exercise, obesity, glucocorticoid insufficiency and excess, alcohol hypoglycemia, hypoxia, and fatty acid infusion. There are also descriptions of how specific diabetic medications affect gluconeogenesis. Regarding the potential functions and modes of action of glucagon, insulin, adrenal steroids, the sympathetic nervous system, and fatty acids in the regulation of gluconeogenesis in vivo, some broad inferences are made. The areas that require more study are listed.

For the preservation of steady blood glucose concentrations, the concerted control of cellular glucose uptake and endogenous glucose synthesis is essential. The liver greatly influences the

rate of hepatic glucose release by regulating the generation of de novo glucose (gluconeogenesis) and the breakdown of glycogen. (glycogenolysis). A substantial portion of the suppression of this metabolic pathway during the postprandial state can be ascribed to insulin. A variety of nutritional and hormonal cues communicate to change hepatic gluconeogenic flux. Here, we go over a few of the molecular processes by which insulin regulates hepatic gluconeogenesis, limiting the amount of glucose the liver produces and eventually preserving normoglycemia. One of the primary methods by which gluconeogenesis is controlled is at the level of transcriptional regulation of the important hepatic gluconeogenic genes PCK1 and G6PC, where several signaling pathways controlled by insulin overlap. An understanding of these insulin-moderated pathways is essential for therapeutic reasons because insulin fails to inhibit hepatic gluconeogenesis in people with impaired insulin signaling, such as insulin resistance in type 2 diabetes.

Since almost all ingested carbs are converted to volatile fatty acids in the rumen, gluconeogenesis, a continuous process, is crucial for ruminants. The only significant volatile fatty acid that adds to gluconeogenesis is propionate. The research of bovine gluconeogenesis involves a wide range of methodologies and analytical methods. By using a range of glucose labels and single-injection or continuous-infusion isotope dilution methods, glucose kinetics can be studied. Correcting for recycling of labels is an essential issue. The quantity of glucose plus -glucose polymers that enter the small intestine can be used to determine the absorption of glucose from the gut or it can be quantified by merging arterial-venous differences and portal blood flow rates. Using isotope diffusion methods, it is possible to quantify the production of propionate in the ruminoreticulum[6], [7].

Propionate with meticulous adjustments for the propionate carbon entering the citric acid cycle before absorption into glucose must be used to measure the conversion of propionate to glucose. To quantify the inputs of amino acids and other precursors to glucose, the same basic methods used with propionate must be used. The gluconeogenic processes and regulators can be better understood through in vitro investigations of gluconeogenic enzymes, and cellular, tissue, or organ preparations; however, these studies must be supported by in vivo tests. knowledge of some parts of ruminant gluconeogenesis has advanced significantly, but much more research will be needed to reach full knowledge. Since almost all ingested carbs are converted to volatile fatty acids in the rumen, gluconeogenesis, a continuous process, is crucial for ruminants. The only significant volatile fatty acid that adds to gluconeogenesis is propionate. The research of bovine gluconeogenesis involves a wide range of methodologies and analytical methods. By using a range of glucose labels and single-injection or continuous-infusion isotope dilution methods, glucose kinetics can be studied.

Correcting recycling labels is an essential issue. The quantity of glucose plus -glucose polymers that enter the small intestine can be used to determine the absorption of glucose from the gut or it can be quantified by merging arterial-venous differences and portal blood flow rates. Using isotope diffusion methods, it is possible to quantify the production of propionate in the ruminoreticulum. Propionate with meticulous adjustments for the propionate carbon entering the citric acid cycle before absorption into glucose must be used to measure the conversion of propionate to glucose. To quantify the inputs of amino acids and other precursors to glucose, the same basic methods used with propionate must be used. The gluconeogenic processes and regulators can be better understood through in vitro investigations of gluconeogenic enzymes, and cellular, tissue, or organ preparations; however, these studies must be supported by in vivo tests. knowledge of some parts of ruminant gluconeogenesis has advanced significantly, but much more research will be needed to reach full knowledge.

Does AMPK play a part in the regulation of hepatic gluconeogenesis, and could addressing AMPK in the liver be a successful type 2 diabetes treatment plan? This review aims to provide answers to these commonly posed concerns. Following a short overview of the characteristics of AMPK and various small-molecule AMPK activators, we discuss the diverse methods for regulating hepatic glucose production, primarily via gluconeogenesis. Critical discussion is held of the various experimental and genetic models that have been applied to reach findings about the function of AMPK in the regulation of hepatic gluconeogenesis. Also taken into account are the impacts of various anti-diabetic medications, especially metformin, on liver gluconeogenesis. We conclude that short-term antagonism of glucagon signaling and long-term improvement of insulin sensitivity through a decrease in hepatic lipid content are the primary effects of AMPK activation relevant to the regulation of hepatic gluconeogenesis.

Increasing hepatic glycogen synthesis and decreasing liver glucose production are efficient ways to manage hyperglycemia in type 2 diabetes mellitus (T2DM), but there aren't many drugs that have both of these characteristics. In this paper, we present coronarin A, a naturally occurring chemical extracted from the rhizomes of *Hedychium gardnerianum*, which inhibits gluconeogenesis while concurrently stimulating glycogen production in primary rat hepatocytes. In rat primary hepatocytes, we demonstrated that coronarin A (3, 10 M) dose-dependently promoted glycogen production along with elevated Akt and GSK3 phosphorylation. Pretreatment with the Akt inhibitor MK-2206 (2 M) or the PI3K inhibitor LY294002 (10 M) prevented coronarin A's ability to cause the production of glycogen.

In contrast, coronarin A (10 M) substantially decreased gluconeogenesis in rat primary hepatocytes along with elevated phosphorylation of MEK, ERK1/2, and -catenin and elevated TCF7L2 transcript expression. The coronarin A-suppressed gluconeogenesis was eliminated by pretreatment with either the ERK inhibitor SCH772984 (1 M) or the -catenin inhibitor IWR-1-endo (10 M). More significantly, we discovered that coronarin A controlled a crucial upstream protein called IRS1 to initiate PI3K/Akt/GSK3 and ERK/Wnt/-catenin signaling. mTOR and S6K1, the downstream target of mTORC1, were dephosphorylated by coronerin A (10, 30 M), which further blocked the serine phosphorylation of IRS1 and then boosted the tyrosine phosphorylation of IRS1[8]–[10].

The aggressive illness known as acute myeloid leukemia (AML) is marked by unfavorable prognoses and treatment resistance. Devimistat, a new substance, prevents the pyruvate dehydrogenase complex from working. The failure of phase III clinical study in AML patients using devimistat and chemotherapy suggests that AML cells can get around the metabolic suppression of devimistat. Unknown mechanisms allow AML cells to withstand PDH suppression.

Devimistat-treated AML cell lines or those lacking the crucial PDH component PDHA displayed diminished glycolysis and reduced glucose uptake as a result of decreased levels of the glucose transporters GLUT1 and hexokinase II. Increased susceptibility to 2-deoxyglucose was seen in both delimitate-treated and PDHA mutant cells, indicating dependence on lingering glycolysis. In devimistat-treated cells, the rate-limiting gluconeogenic enzyme phosphoenolpyruvate carboxykinase 2 (PCK2) was markedly elevated, and its inhibition enhanced susceptibility to devimistat. Devimistat was repelled by the gluconeogenic amino acids glutamine and asparagine in AML cells. Fatty acid oxidation, ATP citrate lyase (ACLY), and acyl-CoA synthetase short-chain family member 2 (ACSS2) were all significant non-glycolytic sources of acetyl-CoA that contributed to resilience. Last but not least, devimistat decreased the action of FASN. Together, these findings imply that PDH and glycolysis inhibition in AML cells are made up for by gluconeogenesis, which

maintains important glycolytic intermediates, fatty acid oxidation, ACLY, and ACSS2 for non-glycolytic acetyl-CoA synthesis. In AML, tactics to block these exit routes should be investigated.

CONCLUSION

The process of gluconeogenesis, which produces glucose, is crucial for preserving blood glucose levels during fasting. Beginning in the cell's organelles, glucose synthesis occurs. The pyruvate carboxylase enzyme uses one ATP molecule to carboxylate pyruvate in the first stage, converting it to oxaloacetate. The enzymes phosphoenolpyruvate carboxykinase, pyruvate carboxylase, glucose 6-phosphatase, and fructose 1,6-bisphosphatase, which are typically located in the liver, kidney, intestine, or muscle, catalyze the four irreversible stages of the gluconeogenesis pathway. Reversing glycolysis into gluconeogenesis involves three new processes and four new enzymes, which lowers the normal free energy. Four ATP, two GTP, and two NADH are required to produce each unit of glucose from two pyruvate molecules. Without access to carbs, the body can make glucose thanks to gluconeogenesis. Additionally, it aids in the utilization of fat tissue's glucose stores. These factors make gluconeogenesis significant.

REFERENCES:

[1] X. Zhang, S. Yang, J. Chen, and Z. Su, "Unraveling the regulation of hepatic gluconeogenesis," *Frontiers in Endocrinology*. 2019. doi: 10.3389/fendo.2018.00802.

[2] M. Hatting, C. D. J. Tavares, K. Sharabi, A. K. Rines, and P. Puigserver, "Insulin regulation of gluconeogenesis," *Annals of the New York Academy of Sciences*. 2018. doi: 10.1111/nyas.13435.

[3] J. Yip, X. Geng, J. Shen, and Y. Ding, "Cerebral gluconeogenesis and diseases," *Frontiers in Pharmacology*. 2017. doi: 10.3389/fphar.2016.00521.

[4] G. Grasmann, E. Smolle, H. Olschewski, and K. Leithner, "Gluconeogenesis in cancer cells – Repurposing of a starvation-induced metabolic pathway?," *Biochimica et Biophysica Acta - Reviews on Cancer*. 2019. doi: 10.1016/j.bbcan.2019.05.006.

[5] P. Sadiku *et al.*, "Neutrophils Fuel Effective Immune Responses through Gluconeogenesis and Glycogenesis," *Cell Metab.*, 2021, doi: 10.1016/j.cmet.2020.11.016.

[6] L. Agius, B. E. Ford, and S. S. Chachra, "The metformin mechanism on gluconeogenesis and AMPK activation: The metabolite perspective," *International Journal of Molecular Sciences*. 2020. doi: 10.3390/ijms21093240.

[7] R. P. Walker, Z. H. Chen, and F. Famiani, "Gluconeogenesis in Plants: A Key Interface between Organic Acid/Amino Acid/Lipid and Sugar Metabolism," *Molecules*. 2021. doi: 10.3390/molecules26175129.

[8] A. M. Shah and F. E. Wondisford, "Tracking the carbons supplying gluconeogenesis," *Journal of Biological Chemistry*. 2020. doi: 10.1074/jbc.REV120.012758.

[9] S. Yu, S. Meng, M. Xiang, and H. Ma, "Phosphoenolpyruvate carboxykinase in cell metabolism: Roles and mechanisms beyond gluconeogenesis," *Molecular Metabolism*. 2021. doi: 10.1016/j.molmet.2021.101257.

[10] H. Zheng *et al.*, "MicroRNA-185-5p inhibits hepatic gluconeogenesis and reduces fasting blood glucose levels by suppressing G6Pase," *Theranostics*, 2021, doi: 10.7150/THNO.46882.

CHAPTER 4

AN OVERVIEW OF THE GLYCOGEN METABOLISM

Prof. Kapilesh Jadhav, Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id-kapilesh@jnujaipur.ac.in

ABSTRACT:

Both carbohydrates and non-carbohydrate precursors provide glucose, which is then deposited in the form of glycogen in the cells. Glycogen metabolism is accomplished by two pathways; Glycogenesis and Glycogenolysis. The catabolism and anabolism of glycogen are covered in this paper. We also explained how the human body controls these glycogen metabolisms.

KEYWORDS:

Glucose Metabolism, Glycogen Metabolism, Glycogen Synthase, Glucose Phosphate, Glycogen Phosphorylase.

INTRODUCTION

The building blocks for glucose, such as lactate and alanine, are obtained from recently consumed carbs or gluconeogenic precursors. Simply put, glycogenolysis is the process by which glycogen is broken down for use as an energy source, primarily in the liver and skeletal muscle. Depending on the environment, there are two different routes for glycogen degradation. Skeletal muscle utilizes glucose-1-phosphate, which is produced when glycogen is broken down, as the sole source of energy for movement. Skeletal muscle undergoes glycogenolysis during vigorous exercise. Contrarily, liver glycogenolysis produces glucose for circulatory outflow. Nutritional deprivation is the catalyst for hepatic glycogenolysis, which is accompanied by a rise in hormones like glucagon**.**

Glycogenesis is the process by which glucose molecules are joined to the glycogen chains for storage, resulting in the production of glycogen. Glycogenesis in the human body begins to occur after the Cori cycle when the body is at rest. In the liver, the procedure typically takes place. It is crucial to remember that the peptide hormone insulin can trigger glycogenesis in response to the body's comparatively elevated glucose levels. Glycogenesis is the term used to describe the biochemical process that converts glucose, the simplest cellular sugar, into glycogen. The body is known to produce glycogen during the glycogenesis period to store these molecules for eventual use (for a time when the body does not have glucose readily accessible).

It's crucial to understand that glucose differs from fat which is eventually metabolized for energy. It is normal for the body to use glycogen reserves during meals, particularly when the blood glucose level has dropped. The body's cells are known to turn to their glycogen stores in this circumstance, going through a procedure that is the opposite of glycogenesis. Glycogenolysis is the broad name for this counter-process. Energy is needed for the metabolic process of glycogenesis (Figure. 1). It entails the following actions: the metabolism of glucose. The conversion of glucose to G-6-P is the first stage in the production of glycogen. In a prior part, it was discussed how hexokinases, including glucose kinase, catalyze this reaction[1], [2].

Production of glucose-1-phosphate: The phosphate group is moved from carbon 6 to carbon 1 on the glucose molecule by phosphoglucomutase during the second step. Glucose-l-phosphate is produced from G-6-P. Mg+ and glucose-1,6-bisphosphate are needed as cofactors for phosphoglucomutase; the word "bisphosphate" denotes the presence of two phosphates bound at separate sites of the same molecule. It is known as diphosphate (i.e., ADP) when the phosphates are joined by an anhydride link. The response can be stopped.

Activation of glucose: Uridine diphosphate glucose (UDPG) and pyrophosphate are produced when glucose-1-phosphate interacts with the high-energy molecule uridine triphosphate (UTP). (PPi). Uridine diphosphate-glucose pyrophosphorylase, also known as glucose-1-P uridyltransferase, is the enzyme that catalyzes the process. Pyrophosphatase works quickly to hydrolyze inorganic pyrophosphate. This reaction is practically permanent because of the instantaneous loss of pyrophosphate. The nucleotide sugar (UDPG) has the required reactivity to take part in glycogen synthesis thanks to the presence of glucose in it. To trigger glucose, UDP must be bound. Luis F. Leloir identified the function of nucleotide carbohydrates in metabolism, including UDPG.

Glucose is added to the polymer's skeleton: The UDPG-activated glucose is now moved to stored glycogen. The final glucose of a glycogen chain forms one glycosidic bond to carbon 4. Glycogen synthase, a glucosyl transferase that needs the existence of the preexisting glycogen polymeric structure and continuously adds glucose molecules via 14 bonds, catalyzes this process. Almost no reversal is possible in the response. Since glycogen synthase can only make 14 bonds, its activity can only linearly extend the length of existing glycogen branches by adding glucose molecules one at a time. The growth of branches. Amylo-(1,4)-(1,6)-glucan-transferase, also known as the branching enzyme, cuts a terminal section of at least six glucose molecules and inserts it with a 16 glycosidic bond on an adjacent chain after glycogen synthase has constructed a glycogen chain with 10 or more glucose residues (Figure. 1).

In this way, the budding enzyme and glycogen synthase work together to create glycogen. There are two equivalent types of glycogen synthase in tissues: b (inactive, phosphorylated) and a. (active, dephosphorylated). Only in the presence of overwhelming levels of G-6-P, which functions as a positive allosteric activator, does form b exhibit activity. Regardless of the presence of G-6-P, form an is active. Synthase B is converted by a Phosphatase into A. Through phosphorylation, the inactive a version is changed into b. The significance of phosphorylation as a method to control glycogen synthase and the glycogen pathway is demonstrated by this[3]–[5].

Glycogenin Preformed glycogen strands are necessary for glycogen synthase to function. Despite this, glycogen production can still take place when glycogen is completely absent. This relies on how glycogen, a starter protein, works. It accepts the first glucose molecule before it forms a glycosidic bond with a tyrosine-protein. UDPG serves as a glucose source during the autocatalytic process. Additionally, glycogenincatalyzes the addition of units one at a time to create a straight chain of six to seven glucose molecules connected by 14 bonds. The chain that is attached to glycogenin is still being affected by glycogen synthase and the branching enzyme, which gives the polymer its final distinctive structure. The amount of glycogen ppapers in the cell is dependent on the supply of glycogenin because each glycogen molecule is covalently attached to an initiator protein.

It takes energy for the endergonic process of adding glucose to glycogen. One molecule of ATP is used in the first phosphorylation process, which is present in all routes for the utilization of glucose. UTP, a substance with energy-rich links, is required in the process of

glucose activation. Two ATPs are used in the incorporation of one glucose molecule into glycogen. It seems pointless to use energy to retain glucose. Accumulating G-6-P would be more energy efficient because it is a metabolite that cannot exit the cell and needs less energy to produce. Under metabolic circumstances, it is not feasible to use G-6-P as an energy reserve. The quantity of scattered particulates in a solution determines its osmotic pressure, not its size. From an osmotic perspective, one molecule of G-6-P or any other solute is equal to one glycogen molecule, which is made up of thousands of glucose molecules. Therefore, the buildup of several G-6-P molecules would cause the osmotic pressure to rise, which might cause cell enlargement and disintegration. Large amounts of glucose molecules can be stored in glycogen with little impact on the osmolarity of the cell.

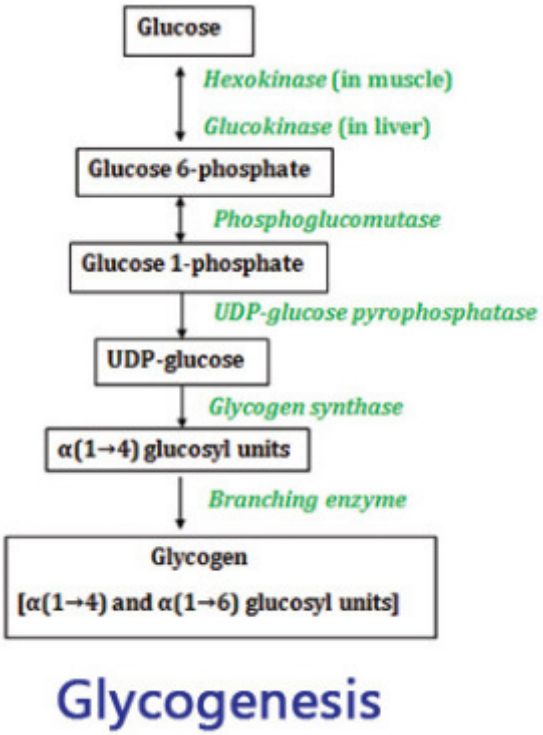

Figure 1: Glycogensis: Diagram showing the synthesis of the glycogen (Simple pharmaanotes).

Hormonal regulation glycogenesis: One of the primary types of regulation is the varied phosphorylation of glycogen synthase and glycogen phosphorylase. Enzymes govern this under the direction of hormonal action, which is controlled by a variety of variables. As a result, in contrast to allosteric systems of control, a wide variety of potential effectors are available. While glycogen synthase is blocked by phosphorylation, glycogen phosphorylase is triggered. The enzyme phosphorylase kinase changes glycogen phosphorylase from its less active "b" form to an active "a" form. Protein kinase itself activates and deactivates phosphoprotein phosphatase-1, the latter of which enzyme. Adrenaline itself activates the protein kinase an enzyme. A receptor protein that epinephrine attaches to causes adenylates cyclase to be activated. By binding to the regulatory component of protein kinase a, two molecules of cyclic AMP activate it, causing the catalytic subunit of protein kinase A to separate from the assembly and phosphorylate other proteins. The latter enzyme is responsible for the creation of cyclic AMP from ATP. The less active "b" version of glycogen phosphorylase can still be triggered by itself without undergoing a conformational change. As was already observed with phosphofructokinase regulation, 5'AMP functions as an allosteric activator while ATP is an inhibitor, helping to alter the rate of flux in reaction to energy

demand. Epinephrine suppresses glycogen synthase in addition to activating glycogen phosphorylase. The result of triggering glycogen phosphorylase is enhanced by this. A comparable process is used to accomplish this inhibition, in which protein kinase A phosphorylates the enzyme to reduce activity. Coordinated mutual management is the term used for this. For more details on the control of glycogenesis. Secondary mediators include cyclic AMP (cAMP) or calcium ions. A good illustration of negative influence is this. Phosphorylase kinase is stimulated by calcium ions. As a result, glycogen synthase is inhibited and glycogen phosphorylase is activated.

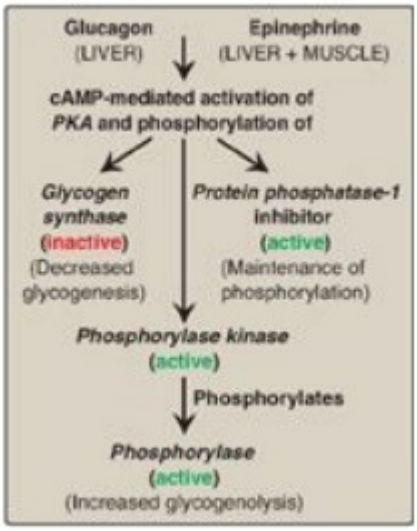

Figure 2: Regulation of the Glycogensis: Diagram showing the hormonal regulation of the glycogen (pharmacy 180).

-Glycogen (n) is broken down into glucose-1-phosphate and glycogen during the process of glycogenolysis. (n-1). The enzyme glycogen phosphorylase catabolizes glucose branches by sequentially removing glucose monomers through phosphorolysis. When cAMP binds to phosphorylase kinase, it transforms it into its active state, allowing it to catalyze the conversion of phosphorylase b to phosphorylase a, which starts glycogenolysis in the muscles. The general process for converting glycogen to glucose-1-phosphate is as follows: Glycogen (n residues) plus Pi is equivalent to glycogen(n-1 residues) plus glucose-1-phosphate. By substituting a phosphoryl group for the [1-4] linkage here, glycogen phosphorylase breaks the bond connecting a terminal glucose molecule to a glycogen branch. The enzyme phosphoglucomutase transforms glucose-1-phosphate into glucose-6-phosphate, which frequently winds up in glycolysis. Up to four residues before a branch of glucose with a bond, glucose residues from the branches of glycogen are phosphorylated. The leftover three of the four glucose molecules are then moved to the end of a different glycogen branch by the glycogen debranching enzyme. This reveals the branching point, which is then hydrolyzed by glucosidase, removing the branch's terminal glucose residue as a glucose molecule. Only in this instance is glucose-1-phosphate, not a glycogen product. Hexokinase then phosphorylates the glucose to produce glucose-6-phosphate[6], [7].

The signaling of the nerves or the production of hormones triggers the glycogenolysis pathway. Usually, it takes place when the body is under duress due to low blood sugar or an emergency. A human's body begins generating adrenaline or epinephrine when they are in a dangerous circumstance. This is known as the fight or flight reaction. The body prepares for a severe scenario and requires a lot of energy to be generated in the muscles during this

reaction, so stored glycogen is needed. The muscles will require glucose to consume and generate energy in this scenario. By releasing the glycogen, the body prepares the area, and the glycogenolysis enzymes begin to separate the bands right away. The muscle cells, or myocytes, use the units of glucose-1-phosphate and glucose-6-phosphate to break down and generate energy at a significant degree. This makes it easier for them to respond in an emergency. He will use the increased strength to battle or run away. Additionally, it happens when someone is abstaining and their blood sugar level falls. The liver's accumulated glycogen is transformed into glucose before being discharged into circulation. Glycogen is the musculature is broken down during the fight-or-flight reaction to prepare them for any action. Cells in various bodily regions send messages when the blood sugar level drops, causing the glycogenolysis enzymes to begin breaking it down to keep the level stable.

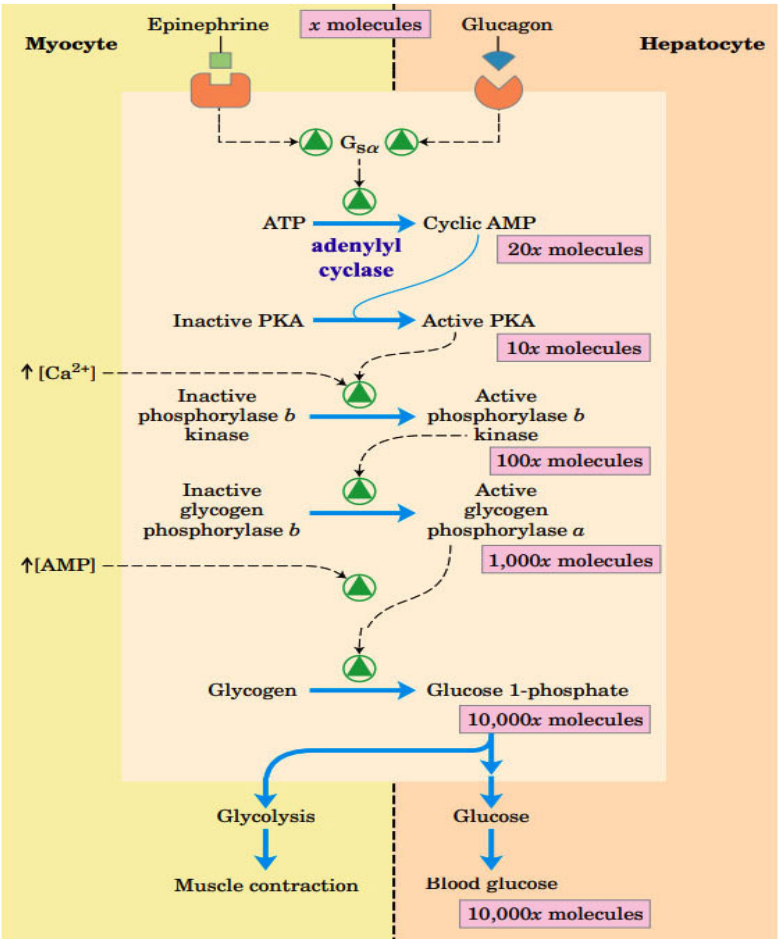

Figure 3: Regulation of the Glycoge metabolism: Diagram showing the hormonal regulation of the glycogen metabolism (Biotechnology MCQ).

The glycogen phosphorylase, also known as s phosphorylase, primarily controls how quickly muscles and the liver break down glycogen. In both its active and dormant states, phosphorylase is present. (phosphorylase b). The phosphorylases in muscles and liver cells are two different pools. Although they are expressed by different genes, muscle phosphorylase, and liver phosphorylase share a regulatory system. A high-energy phosphate group from ATP is added to the inactive version of phosphorylase B in the liver and muscle to initiate it. The phosphorylase kinase enzyme catalyzes the process, which produces phosphorylase an (an active form). In liver cells, glucagon controls the stimulation of phosphorylase B, and in muscles, adrenaline does the same. The hormones bind to the target

cell's cell membrane receptors and trigger the enzyme adenylate cyclase, which turns ATP into cAMP and raises cytosolic amounts of cAMP (2nd messenger in the cell).

The rate-limiting process in glycogenolysis is catalyzed by protein kinase A, which is further phosphorylated and activated by the increase in cAMP. Activated PKA then triggers phosphorylase kinase, which in turn activates phosphorylase b. The breakdown of glycogen is affected anabolically by insulin. The stimulation of protein phosphatase-1 enhances the dephosphorylation of several enzymes involved in the breakdown of glycogen, which is how it affects the liver and muscles. The main way that insulin affects glycogenolysis and glycogenesis is through the protein phosphatase-1. Insulin works on the muscle target tissues' insulin receptors to transport and activate the IRS-1, which in turn triggers the PI3K, PIP3, and mTORc1 complex pathway's downstream signaling. Insulin action causes the protein phosphatase-1 to become active. The phosphate family of enzymes, to which this enzyme belongs, catalyzes the breaking of phosphate by the addition of water molecules. On one side, glycogen synthase b (inactive form) is dephosphorylated and transformed into glycogen synthase a by the protein phosphatase-1, which catalyzes the elimination of phosphate from it. (Active form). The active glycogen synthase then encourages the production of glycogen in the muscles and liver. The protein phosphatase-1, on the other hand, catalyzes the removal of the phosphate group from the active form of glycogen phosphorylase, which results in its dephosphorylation and transforms it into glycogen phosphorylase b (inactive form). As a consequence, the liver and muscles' glycogenolysis is inhibited.

DISCUSSION

Almost every creature, from yeast to humans, stores carbohydrates as glycogen. The majority of human organs store glucose as glycogen, with the liver and muscle having the largest concentrations. In the 1850s, the French scientist Claude Bernard discovered a compound that resembled glucose in the liver and muscles and gave it the name glycogen, or "sugar former." In the 150 years since its discovery, scientists studying glycogen metabolism have made several advancements that are now regarded as major turning points in biology and cell communication. However, there are still many unanswered issues, and research keeps highlighting how intricately the management of glycogen metabolism is controlled. According to traditional classifications, the roles of glycogen appear straightforward: hepatic glycogen is broken down to release glucose into the bloodstream to feed other tissues, whereas muscle glycogen is degraded to produce ATP during increased energy demand. Recent research has shown that the functions of glycogen metabolism in energy sensing, the coordination of cellular reactions to hormonal cues, and the integration of metabolic pathways are significantly more complicated.

Glycogen, a branched polymer of glucose that is primarily held in the liver and skeletal muscle in the human body, provides glucose to the bloodstream during times of fasting and to the muscle cells during muscle contraction. Other tissues, including the brain, heart, kidney, adipose tissue, and erythrocytes, have been found to contain glycogen, but the role of this compound in these tissues is largely unclear. The process of converting glucose into the direct glucose donor for glycogen synthesis, uridine 5'-diphosphate-glucose, involves several reactions, including the entry of glucose into the cell through transporters, phosphorylation of glucose to glucose 6-phosphate, isomerization to glucose 1-phosphate, and formation of uridine 5'-diphosphate-glucose. A brief glucose chain is created by glycogenin, which is then lengthened by glycogen synthase. The glycogen branching enzyme inserts branch points at regular distances into the glycogen molecule.

Laforin and Malin are two proteins that are involved in the construction of glycogen, but it is unclear exactly what they do in people. Glycogen builds up in the skeletal muscle and liver mainly after exertion, as well as in the postprandial period. Two enzymes, glycogen phosphorylase, which produces glucose 1-phosphate from the linear chains of glycogen, and glycogen debranching enzyme, which untangles the branch points, carry out glycogenolysis, the decomposition of glycogen, in the cytosol. Glycogen breakdown in the lysosomes is facilitated by -glucosidase. The dephosphorylation of glucose 6-phosphate to glucose, which is required for free glucose to exit the cell, is catalyzed by the glucose 6-phosphatase pathway. Glycogen storage disorders are brought on by mutations in the genes that encode the enzymes involved in glycogen biosynthesis.

The basic routes for glycogen synthesis and glycogenolysis are the same in all organs, but the particular roles of the involved enzymes in various cell types vary. The glycogen-metabolizing enzymes in the liver have characteristics that allow the liver to function as a sensor of blood glucose and to store or mobilize glycogen in response to peripheral requirements. Glycogen is kept in the liver as a reservoir of glucose for extrahepatic tissues. Glucose is the main factor in liver glycogen accumulation; it inhibits glycogenolysis and actively encourages glycogen synthesis. Insulin, glucocorticoids, parasympathetic (vagus) nerve signals, and gluconeogenic components like fructose and amino acids are additional glycogenic triggers for the liver. The hormones noradrenaline and ATP, as well as glucagon, play a major role in the phosphorolysis of glycogen. Numerous glycogenolytic triggers, including adenosine, nucleotides, and NO, also have a secondary effect by causing non-parenchymal cells to secrete eicosanoids. Effectors frequently start glycogenolysis jointly using a variety of methods.

With rising exercise exertion, the use of carbohydrates as intramuscular glycogen stores and glucose supplied from plasma becomes an increasingly significant energy substrate for the active muscle. A study of the mobilization and production of glycogen by the actions of glycogen phosphorylase and glycogen synthase, respectively, is followed by an update on the molecular cues by which glucose transport is enhanced in the contracting muscle. The signaling relating to the well-described greater sensitivity of glucose transport to insulin in the post-exercise phase, which can result in an excess of intramuscular glycogen resynthesis post-exercise, is the final topic covered in this overview[8]–[10].

Microorganisms can use a wide range of nutrients and can adjust to ever-changing natural circumstances. To survive the temporary starvation circumstances that are prevalent in the ecosystem, many microorganisms, including yeast and bacteria, collect carbon and energy reserves. To guarantee the production of this homopolysaccharide, various sensing and communication mechanisms have developed in evolutionary remote species. Glycogen biosynthesis is one major method for such metabolic storage. Fundamentally speaking, there are some general parallels between the mechanisms of glycogen synthesis and degradation in yeast and bacteria. However, the control of these processes can occasionally be quite distinct, showing that they have independently developed to react best to each species' unique habitat circumstances.

This review seeks to emphasize the mechanisms that control glycogen metabolism in yeast and bacteria at both the transcriptional and post-transcriptional levels, with an emphasis on a few regions where the most progress has been made in recent years. We pay close attention to the different signaling networks that regulate the action of the glycogen storage enzymes in the yeast system. We also go over our most recent discoveries regarding the crucial part the vacuole plays in the breakdown of glucose. Particular focus is given to elements of the

genetic regulation of glycogen metabolism and its relationship to other biological processes in the instance of bacterial glycogen.

To sustain blood glucose balance between meals, liver glycogen is degraded to glucose after meals in reaction to a rise in blood glucose content in the portal vein as well as endocrine and neuroendocrine cues. Changes in the activities of phosphorylase and glycogen synthase during the daily cycle regulate the production and degradation of glycogen. By phosphorylating serine-14, phosphorylase is controlled. The liver phosphorylase (GPa) is only catalytically active in its phosphorylated state. The actions of phosphorylase kinase and phosphorylase phosphatase are necessary for the interconversion of GPa and GPb (unphosphorylated). The latter consists of a glycogen-targeting protein (G-subunit) from the PPP1R3 family and protein phosphatase-1. GPa dephosphorylation involves at least two of the six G-subunits (GL and PTG) expressed in the liver. The degree to which the allosteric effector's glucose, glucose 6-phosphate, and adenine nucleotides are present and how acetylated lysine residues are is what determines whether the phosphorylase is in a relaxed (R) or rigid (T) shape. The G-subunit, GL, encoded by the PPP1R3B gene is highly expressed in the liver and can operate as both a phosphorylase phosphatase and a synthase phosphatase. The C-terminus of the G-subunit contains an allosteric binding site for GPa that suppresses the activity of the synthase phosphatase. The control of glycogen production by glucose, its downstream metabolites, and extracellular cues like insulin and neurotransmitters involves GPa to GPb conversion as a significant upstream process.

CONCLUSION

Hepatic glycogen makes up the majority of glycogen, which is the body's backup carbohydrate. Muscles and the liver both produce glycogen. Continuously, -D-glucose joins to make glycogen. Two enzymes, glycogen phosphorylase, which produces glucose 1-phosphate from the linear chains of glycogen, and glycogen debranching enzyme, which untangles the branch points, carry out glycogenolysis, the decomposition of glycogen, in the cytosol. Glycogen breakdown in the lysosomes is facilitated by -glucosidase. The phosphorylation and dephosphorylation of regulating and metabolic enzymes control the breakdown of glycogen in the liver. Ca2+, cAMP, intracellular glucose levels, and possibly another mechanism in the case of insulin—all regulate the phosphorylation state.

REFERENCES:

[1] C. E. Zois and A. L. Harris, “Glycogen metabolism has a key role in the cancer microenvironment and provides new targets for cancer therapy,” *Journal of Molecular Medicine*. 2016. doi: 10.1007/s00109-015-1377-9.

[2] M. Hargreaves and L. L. Spriet, “Skeletal muscle energy metabolism during exercise,” *Nature Metabolism*. 2020. doi: 10.1038/s42255-020-0251-4.

[3] L. F. Obel *et al.*, “Brain glycogen - new perspectives on its metabolic function and regulation at the subcellular level,” *Frontiers in Neuroenergetics*. 2012. doi: 10.3389/fnene.2012.00003.

[4] M. A. Hearris, K. M. Hammond, J. M. Fell, and J. P. Morton, “Regulation of muscle glycogen metabolism during exercise: Implications for endurance performance and training adaptations,” *Nutrients*. 2018. doi: 10.3390/nu10030298.

[5] L. Wang *et al.*, “Bioinformatics Analysis of Metabolism Pathways of Archaeal Energy Reserves,” *Sci. Rep.*, 2019, doi: 10.1038/s41598-018-37768-0.

[6] B. L. Farah, P. M. Yen, and D. D. Koeberl, “Links between autophagy and disorders of glycogen metabolism – Perspectives on pathogenesis and possible treatments,” *Molecular Genetics and Metabolism*. 2020. doi: 10.1016/j.ymgme.2019.11.005.

[7] G. van de Werve and B. Jeanrenaud, "Liver glycogen metabolism: An overview," *Diabetes. Metab. Rev.*, 1987, doi: 10.1002/dmr.5610030104.

[8] A. F. Alghannam, M. M. Ghaith, and M. H. Alhussain, "Regulation of energy substrate metabolism in endurance exercise," *International Journal of Environmental Research and Public Health*. 2021. doi: 10.3390/ijerph18094963.

[9] E. A. Wong and Z. Uni, "Centennial Review: The chicken yolk sac is a multifunctional organ," *Poultry Science*. 2021. doi: 10.1016/j.psj.2020.11.004.

[10] J. Fradinho, L. D. Allegue, M. Ventura, J. A. Melero, M. A. M. Reis, and D. Puyol, "Up-scale challenges on biopolymer production from waste streams by Purple Phototrophic Bacteria mixed cultures: A critical review," *Bioresource Technology*. 2021. doi: 10.1016/j.biortech.2021.124820.

CHAPTER 5

CITRIC ACID CYCLE

Ms. Anu Singh, Assistant Professor
Department of Food Technology, Jaipur National University, Jaipur, India
Email Id-anu.singh@jnujaipur.ac.in

ABSTRACT:

The citric acid cycle is an important biochemical process that links the metabolism of proteins, fats, and carbohydrates. The cycle's processes are carried out by eight enzymes, which fully oxidize acetate (a two-carbon molecule) in the form of acetyl-CoA into two molecules of carbon dioxide and water. The citric acid cycle acts as a biochemical interaction center and a source of biosynthetic intermediates a route made of amphibole. Compounds, which will have substantial cataplerotic impacts on the cycle if any are drawn off for biosynthesis.The historical context, the reaction, control, and significance of the citric acid cycle were all covered in this paper.

KEYWORDS:

Amino Acid, Acetyl Coa, Citric Acid, Citrate Synthase, Kerbs Cycle.

INTRODUCTION

Han Krebs, who was born on August 25, 1900, in Hildesheim, is credited with developing the tricarboxylic acid (TCA) cycle. He was the son of Georg, a physician, and Alma, who would pass away from melancholy when Krebs was 19 years old. After finishing the gymnasium in Hildesheim in September 1918, Krebs entered the German army signal corps until the ceasefire of 11 November 1918. Krebs sought out opportunities to acquire biochemistry training after completing his internal medicine residency at Gottingen, Freiburg, Munich, and Berlin in December 1925. He had spent time in the hospitals and had concluded that medical study was essential. Bruno Mendel, an acquaintance of Albert Einstein, professor of physics at the University of Berlin, who in turn was a frequent dinner guest at the home of Otto Warburg's father, and who worked with Krebs in the Third Medical Clinic in Berlin, heard that Warburg was looking for an assistant and suggested Krebs. With the help of this network, Krebs was able to land his first paid job as Otto Warburg's aide at the Kaiser Wilhelm Institut für Biologie in Berlin-Dahlem. During this time, Krebs transitioned from being a doctor to a scientist. Otto Warburg was the most remarkable individual I have ever been directly involved with, according to Krebs (1981). Remarkable for being a high-caliber scientific prodigy, a fiercely autonomous, perceptive thinker, and an eccentric who molded his life resolutely and fearlessly following his ideas and principles[1]–[3].

The premier institution for conducting a scientific study during the period was the Kaiser Wilhelm Institute. Emil Fischer, Richard Willstater, Max von Laue, Fritz Haber, Otto Hahn, Lisa Meitner, and Michael Polanyl were also on the staff in addition to Warburg. Former Warburg pupil Otto Meyerhof, who worked in the same building, described the glycolytic process involved in the conversion of glucose to lactate. Among the biochemical students who attended Kaiser Wilhelm were Fritz Lipmann and Severo Ochoa, who would later be instrumental in demonstrating the function of coenzyme A in the TCA cycle. Krebs learned from Warburg the methods he had created, including the use of tissue slices, spectrophotometry, and manometry, in addition to the discipline and rigor necessary for scientific study. These methods most especially manometry were crucial in helping Krebs

develop the idea of a biochemical cycle, initially the urea cycle and then the TCA cycle. Understanding Krebs' work requires an awareness of how prevalent the Warburg adage "methods are everything" was at the time. There was no hypothetical grand scheme. This is the first time I ever thought you could anticipate in biology, said Krebs about his later work on the thermodynamics coupling of cellular redox and phosphorylation states. Krebs' early writings were firmly empiricist. He was able to gauge response times. In a metabolic pathway, he added different possible players, and if they raised the total rate, he deduced that they were a part of the pathway. Understanding the cycle requires a fundamental understanding of Krebs' instruction. Hans Krebs took 31 years to become a skilled autonomous scientific detective, according to his biographer Holmes, but it only took him 9 months to make one of the most important findings of his age in his field.

The Krebs cycle also referred to as the TCA cycle (tricarboxylic acid cycle), and the citric acid cycle (CAC) are molecular processes that release accumulated energy through the oxidation of acetyl-CoA obtained from carbs, fats, and proteins. Creates that respire (as opposed to creatures that decompose) use the Krebs cycle to produce energy, either through anaerobic or aerobic respiration. The cycle additionally supplies the reducing agent NADH and intermediates of a few amino acids that are needed in a variety of other processes. It may have evolved abiogenically and was one of the early components of metabolism given its prominent role in many biochemical pathways. Despite the term "cycle," molecules need not travel along a single path; at least three additional parts of the citric acid cycle have been identified. Citric acid, a tricarboxylic acid that is frequently referred to as citrate because the ionized form predominates at biological pH, is consumed and then produced by this series of processes to finish the cycle.

Acetate (in the form of acetyl-CoA) and water are consumed by the cycle, which then converts NAD+ to NADH and releases carbon dioxide. The electron transport route for oxidative phosphorylation receives the NADH produced by the citric acid cycle. The end outcome of these two interconnected processes is the oxidation of nutrients to create ATP, a type of useable chemical energy. The citric acid cycle takes place in the framework of the mitochondrion in mammalian cells. In prokaryotic cells, such as bacteria, without mitochondria, the citric acid cycle reaction chain is carried out in the cytoplasm, and the plasma membrane, rather than the inner membrane of the mitochondrion, is the site of the proton gradient for ATP synthesis. Three NADH, one FADH2, and one GTP make up the total output of energy-containing molecules from the citric acid cycle.

A vital biochemical process that links the metabolism of proteins, fats, and carbohydrates is the citric acid cycle. Eight enzymes carry out the cycle's processes, fully oxidizing the two-carbon acetate molecule present in the form of acetyl-CoA to produce two molecules each of carbon dioxide and water. The two-carbon organic product acetyl-CoA, which joins the citric acid cycle, is created through the catabolism of carbohydrates, lipids, and proteins. The cycle also changes one equivalent of flavin adenine dinucleotide (FAD) into one equivalent of FADH2, three equivalents of nicotinamide adenine dinucleotide (NAD+) into three equivalents of reduced NAD+ (NADH), and one equivalent of each of guanosine diphosphate (GDP) and inorganic phosphate (Pi) into one equivalent of guanosine triphosphate. (GTP). The oxidative phosphorylation process uses the NADH and FADH2 produced by the citric acid cycle to produce ATP, which is a powerful source of energy.

One of the main sources of acetyl-CoA is the glycolysis of carbohydrates, which produces pyruvate, which the pyruvate dehydrogenase complex then decarboxylates to produce acetyl-CoA as shown in the following chemical scheme: Acetyl-CoA, a byproduct of this process, catalyzes the citric acid cycle. Fatty acids can also be oxidized to produce acetyl-CoA. The

cycle's mechanical diagram is provided below: The two-carbon acetyl group from acetyl-CoA is transferred to the four-carbon donor molecule (oxaloacetate) to start the citric acid cycle, which results in the formation of a six-carbon compound.

After that, the citrate undergoes a sequence of molecular changes during which it loses two carboxyl groups as CO2. Instead of coming straight from acetyl-CoA, the carbons released as CO2 come from what was oxaloacetate. After the first shift of the citric acid cycle, the carbons given off by acetyl-CoA become a component of the carbon backbone of oxaloacetate. The citric acid cycle must be turned multiple times to lose the carbons given by acetyl-CoA as CO2. Although many of the compounds in the citric acid cycle are also used as precursors in the biosynthesis of other substances, they might not be wasted due to the citric acid cycle's involvement in anabolism.

The majority of the electrons made accessible by the cycle's oxidative stages are moved from NAD+ to NADH. In the citric acid cycle, three molecules of NADH are created for each acetyl group that enters. In mitochondria, the citric acid cycle involves several oxidation-reduction reactions. Additionally, after being transferred to the succinate dehydrogenase's FAD cofactor and reducing it to FADH2, the electrons from the succinate oxidation step are next transferred to the mitochondrial membrane's ubiquinone (Q), where they reduce it to ubiquinol (QH2), a substrate for Complex III's electron transfer chain. Oxidative phosphorylation produces 2.5 and 1.5 ATP molecules for each NADH and FADH2 created in the citric acid cycle, correspondingly. The four-carbon oxaloacetate has been regenerating after each cycle, and the cycle resumes. It involves eight steps. In the network of mitochondria, the Krebs cycle or TCA cycle occurs under oxygen conditions (Figure. 1)[4], [5].

1. **Step 1:** Coenzyme A is produced during the condensation of acetyl CoA with the 4-carbon molecule oxaloacetate to create 6C citrate. Citrate synthase is the catalyst for the process.
2. **Step 2:** Isocitrate, the isomer of citrate, is produced. This process is catalyzed by the protein aconitase.
3. **Step 3:** Isocitrate is dehydrogenated and decarboxylated in step 3 to produce 5C -ketoglutarate. The emission of CO2 in a molecular state. The process is catalyzed by isocitrate dehydrogenase. It is an enzyme that depends on NAD+. NADH is created from NAD+.
4. **Step 4:** In step 4, succinyl CoA, a 4C molecule, is created by the oxidative decarboxylation of -ketoglutarate. The enzyme complex known as -ketoglutarate dehydrogenase facilitates the process. NAD+ is changed to NADH, which results in the emission of one CO2 molecule.
5. **Step 5:** Succinate is created by succinyl CoA. The process is catalyzed by the succinyl CoA synthase enzyme. Along with that, GDP is phosphorylated at the substrate level to produce GTP. ATP is created when GTP moves its phosphate to ADP.
6. **Step 6:** In step 6, the enzyme succinate dehydrogenase converts succinate to fumarate. As a result, FAD is changed into FADH2.
7. **Step 7:** By adding one H2O, fumarate is transformed into malate. Fumarase is the enzyme that is catalyzing this process.

In step 8, malate is dehydrogenated to create oxaloacetate, which joins forces with an additional acetyl CoA molecule to initiate a new cycle. Removed hydrogens are transported to NAD+ to create NADH. The process is catalyzed by malate dehydrogenase. The pace of each reaction's progression in the citric acid cycle is influenced by several variables. Below is

a description of them: The abundance of the different substrates used in a reaction sequence is one of the factors that determine how it will proceed. The comparatively low quantity of OAA highlights its function in regulating Acetyl-CoA's entry into the cycle. The activity of the enzyme cycle would be regulated by regulating the pace of this process.

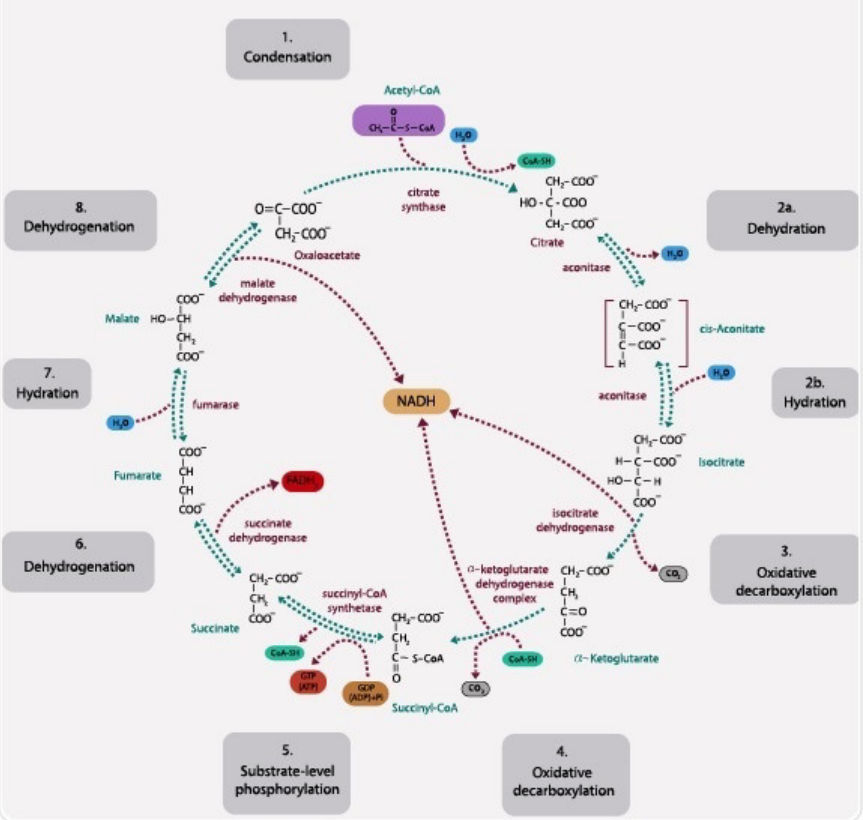

Figure 1: citric acid cycle: Diagram showing the steps involved in the citric acids cycle (New medical)

Level of enzymes: The relative proportions of the individual enzymes, including the distinctive dehydrogenases of the citric acid cycle, are consistent in all mitochondria from extremely diverse origins.The findings imply the existence of a genetic process that regulates the synthesis or incorporation of essential mitochondrial enzymes during mitochondriogenesis.A singular operon comprising every structural gene required to regulate enzyme biosynthesis may be the genetic process (Figure. 2).

Respiration regulation: In addition to the type and quantity of the compounds that must be oxidized, the respiration rate also relies on how closely phosphorylation is coupled to respiration.

The ratio [ADP]/[ATP] controls the rate of respiration in intact mitochondria because these cells are typical "tightly" linked. Respiration is encouraged when this percentage is elevated. Low ratios, or large ATP amounts, on the other hand, cause respiration to slow down. Due to the inverted electron flux they induce, additional ATP can even prevent respiration. The term "respiratory control" now refers to these occurrences.

Accessibility of Intermediates in Cycles: The proximity of the citric acid cycle's intermediates to acetyl-CoA affects the cycle's action as well.A mechanism for the admittance of some substrates and the rejection of others is provided by the mitochondrial membrane itself.

Here are a few instances: Although fumarate cannot be readily accessed by mitochondrial succinate dehydrogenase, succinate can. Additionally, the mitochondrial fumarase does not have unrestricted access to additional fumarate.

Ketosis: In diabetics, the liver produces more acetyl-CoA than can be cyclized by the Krebs cycle or other synthetic processes, resulting in a buildup of ketone bodies, acetoacetate, and acetone. Under these circumstances, the rate of the Krebs cycle slows down presumably due to hormonal action since ketone body formation (i.e., ketosis) is affected by hormones of the hypophysis and adrenal cortex.

Regulating enzyme activity: The citric acid cycle is regulated by three enzymes: citrate synthase, isocitrate dehydrogenase, and beta-ketoglutarate dehydrogenase.Adenosine triphosphate (ATP), NADH, acetyl CoA, and succinyl CoA all block citrate synthase.ADP activates isocitrate dehydrogenase, while ATP and NADH suppress it.Succinyl CoA and NADH block -Ketoglutarate dehydrogenase (Figure. 2)[6], [7].

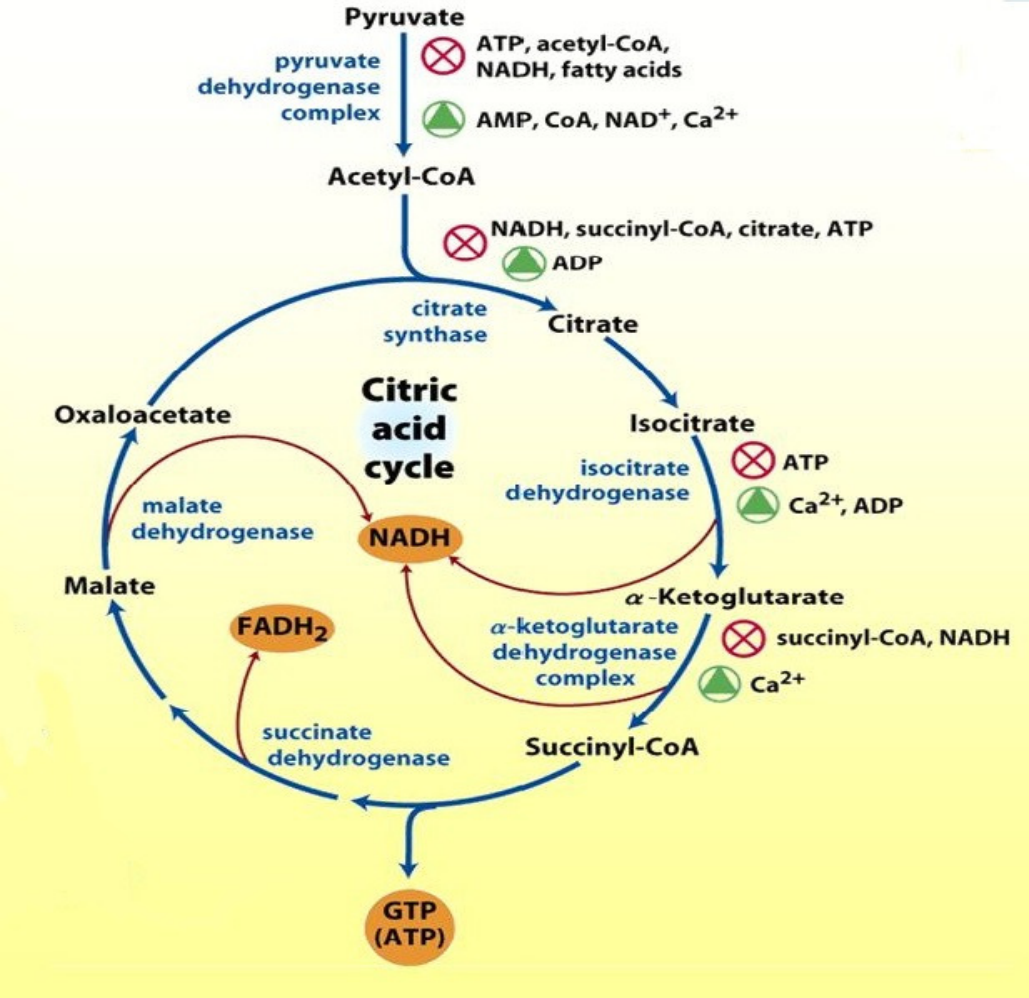

Figure 2: Regulation of the citric acid cycle: Diagram showing the regulation of the citric acids cycle (Bioscience notes)

Metabolites regulate allostery. The supply of substrates and product inhibition plays a significant role in the control of the citric acid cycle. Large quantities of metabolic energy could be lost in the excess creation of reduced coenzymes like NADH and ATP if the cycle were allowed to continue unabated. ADP, which is then transformed into ATP, serves as the

cycle's main final fuel. ADP deficiency results in precursor NADH building up, which can block a variety of enzymes. The citric acid cycle enzymes pyruvate dehydrogenase, isocitrate dehydrogenase, -ketoglutarate dehydrogenase, and citrate synthase are all inhibited by NADH, a byproduct of all dehydrogenases except succinate dehydrogenase. Pyruvate dehydrogenase is inhibited by acetyl-coA, whereas citrate synthase and alpha-ketoglutarate dehydrogenase are inhibited by succinyl-coA. Citrate synthase and -ketoglutarate dehydrogenase are both inhibited by ATP when evaluated in vitro with TCA enzymes; however, ATP levels do not differ by more than 10% in vivo between rest and intense exercise. For an allosteric effector whose concentration varies by less than 10%, there is no known allosteric process that can explain significant variations in response rate.

Citrate suppresses phosphofructokinase, an enzyme involved in glycolysis that catalyzes the production of fructose 1,6-bisphosphate, a precursor to pyruvate, and is used for feedback inhibition. Because of the reduction in the enzyme's substrate and the buildup of citrate, a continuously high rate of flux is prevented. Calcium regulates. Another control used in the citric acid cycle is calcium. During cellular stimulation, calcium concentrations in the mitochondrial matrix can increase to tens of micromolar values. The pyruvate dehydrogenase complex is then activated by activating pyruvate dehydrogenase phosphatase. Isocitrate dehydrogenase and -ketoglutarate dehydrogenase are also activated by calcium. This speeds up many of the cycle's stages' reactions, increasing the flux along the entire route. control of transcription. Recent research has shown a critical connection between citric acid cycle intermediates and the control of hypoxia-inducible genes.

Many of the intermediates in the citric acid cycle are used in the synthesis of key chemicals that have cataplerotic impacts on the cycle. The mitochondrion is unable to move acetyl-CoA outside of it. Citrate is taken out of the citric acid cycle and transported across the inner mitochondrial membrane into the cytoplasm to produce intracellular acetyl-CoA. There, ATP citrate lyase breaks it down into acetyl-CoA and oxaloacetate. The mitochondrion receives the oxaloacetate back as malate (and then converted back into oxaloacetate to transfer more acetyl-CoA out of the mitochondrion). The creation of cholesterol and the formation of fatty acids both depend on cytosolic acetyl-CoA. The synthesis of steroid hormones, biliary acids, and vitamin D can all be done using cholesterol as a starting point. Many non-essential amino acids get their carbon structures from stages in the citric acid cycle[8]–[10].

The alpha keto-acids produced from the stages of the citric acid cycle must obtain their amino groups from glutamate in a transamination process, which uses pyridoxal phosphate as a cofactor. In this process, glutamate is transformed into alpha-ketoglutarate, an intermediary in the citric acid cycle.

The intermediaries oxaloacetate, which produces aspartate and asparagine, and alpha-ketoglutarate, which produces glutamine, proline, and arginine, can supply the carbon skeletons for the production of amino acids. Aspartate and glutamine from this group of amino acids are combined with carbon and nitrogen elements from outside sources to create purines, which serve as the building blocks for DNA and RNA as well as ATP, AMP, GTP, NAD, FAD, and CoA. Aspartate is used to make some of the pyrimidines derived from oxaloacetate.

Thymine, cytosine, and uracil are the pyrimidines, and they also make up CTP, UMP, UDP, and UTP. They constitute the complementary bases to the purine bases in DNA and RNA. The succinyl-CoA intermediary of the citric acid cycle provides the bulk of the carbon atoms in porphyrins.

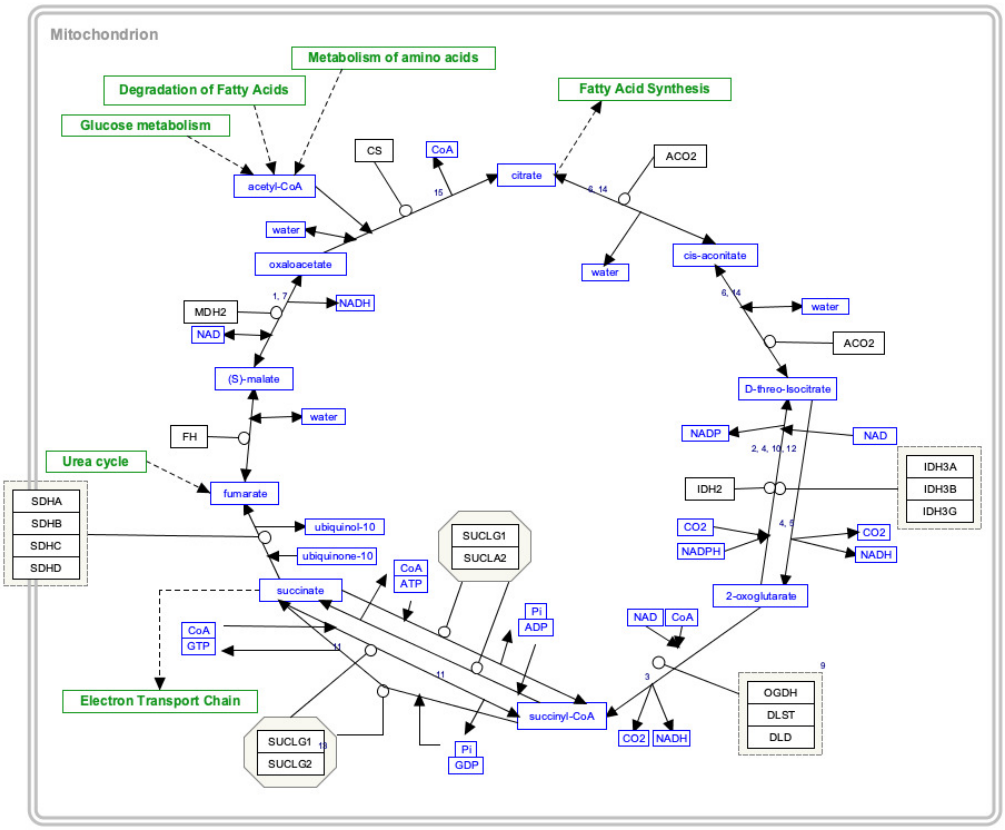

Figure 3: citric acid cycle synthesis of the other biomolecules: Diagram showing the role of the citric acid cycle intermediate in the synthesis of the other biomolecules (Wikipedia)

These compounds play a significant role in the development of hemoproteins like myoglobin, cytochromes, and hemoglobin. Oxaloacetate in the mitochondria is converted during gluconeogenesis into malate, which is then carried outside of the mitochondrion and converted back to oxaloacetate in the cytoplasm. The rate-limiting stage in the conversion of virtually all gluconeogenic precursors (including the glucogenic amino acids and lactate) into glucose by the liver and kidney is the decarboxylation of cytosolic oxaloacetate to phosphoenolpyruvate.

DISCUSSION

The last prevalent oxidative route for amino acids, fats, and carbs is the citric acid cycle. The body's primary biochemical route for supplying energy is this one. The most crucial link linking almost all of the separate metabolic networks is TCA. Introduction, regulation, and the energetics of the TCA cycle have all been covered in this overview paper. The goal of the current research was to examine the literature on the TCA cycle.In the muscles of rodents engaged in a running regimen, citrate synthase, and DPN-specific isocitrate dehydrogenase activity levels rose by a factor of two. Both the amount of cytochrome c and the degree of succinate dehydrogenase activity increased at the same time. While levels of -ketoglutarate dehydrogenase and mitochondrial malate dehydrogenase rose 50%, glutamate dehydrogenase activity jumped by about 35%. These results show that the mitochondrial citric acid cycle and citric acid cycle-related enzymes do not increase concurrently with the adaptive response of skeletal muscle to exercise, leading to a change in mitochondrial composition, unlike the components of the respiratory chain.

The exogenous citric acid (CA), an essential intermediate product of the tricarboxylic acid (TCA) cycle, can increase TCA cycle activity and initiate the branched operation of the TCA cycle, thereby supplying the energy needed for resilience to challenging circumstances. Less research has been done on how CA administration affects the TCA cycle-related metabolism when cadmium (Cd) is present. The development, Cd accumulation, antioxidant systems, and metabolic pathways of leaves and roots were examined by a potted soil experiment with Cd (50 mg/kg) and CA (4 mmol/L) interventions to examine the impacts of CA on the Cd tolerance of Dahurian wildrye grass (*Elymus dahuricus*). The findings indicated that Cd stress had a significant impact on development and caused clover stems and leaves to produce reactive oxygen, which in turn caused membrane peroxidation and activated the antioxidant defense system. Exogenous CA could effectively support an increase in root Cd content as well as effectively alleviate the inhibition of Cd stress on development and decrease the quantity of reactive oxygen by enhancing antioxidant capacities. The administration of CA raised the contents of sugars, sugar alcohols, and resistant compounds, and encouraged the breakdown of amino acids like -aminobutyric acid, according to metabolomic findings. (GABA). The TCA cycle activity, the biosynthesis of the shikimic acid pathway in leaves and roots, as well as the GABA shunt in roots, may have changed as a result of these modifications, which significantly increased the Cd resistance.

Transition metal ions in coal can hasten the coal's spontaneous combustion by lowering the activation energy of the coal-oxygen reaction during coal oxidation. By incorporating metal chelators into coal, the enzymatic activity of metal ions may be reduced. Citric acid, an eco-friendly metal chelating substance, was suggested as a coal spontaneous combustion inhibitor in this paper. To learn more about how citric acid inhibits coal spontaneous burning, experiments were conducted. The findings demonstrated that the oxidation of blocked coal samples produced significantly less CO, CO2, and oxygen usage than unprocessed coal. The crossing point temperature of inhibited samples rose by 9–12 °C compared to unprocessed coal, and the crossing point temperature's arrival time was postponed by 30–40 min. Additionally, the temperature of the experimental coal sample's distinguishing features after suppression was higher than that of unprocessed coal. When testing samples reached the fast combustion stage, the reaction enthalpy of the blocked coal samples was lower than that of the unprocessed coal. The amount of -CH3 and -CH2-groups in coal samples rose after inhibition, but the amount of -COOH and C=O groups dropped, according to Fourier Transform infrared spectroscopy (FTIR) studies. After being treated with citric acid, the coal sample's surface became smoother and had smaller pores, according to research done with a scanning electron microscope (SEM) Citric acid, in conclusion, effectively inhibits various kinds of coal.

The genetic process underlying the growth of prostate cancer remains a mystery despite extensive research. Recent studies revealed that lipids, particularly fatty acids, steroids, and hormones (like prolactin), as well as citric acid, play a major role in the initiation and spread of prostate cancer. Citric acid, however, is thought to be a potential biomarker for prostate cancer, making early detection of the condition feasible. This study's primary objective is to ascertain the levels of citric acid in three distinct materials. This is the first time that citric acid has been measured in three distinct matrices, to the best of our understanding tissue, urine, and blood. Patients with prostate cancer were sampled, as were members of a chosen comparison group individuals with benign prostatic hyperplasia. The fast fluorometric test was used for the results. Using chemometric techniques, the results were correlated with the histopathological data (Gleason score and Classification of Malignant Tumors (pTNM) staging scale) and the biochemical data (values of prostate-specific antigen, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, total cholesterol,

creatinine, and prolactin). For tissue samples, the findings showed a decreased level of citric acid in the instance of prostate cancer. The average analyte concentrations in blood and pee in the positive group seemed to be higher and more consistent. In the instance of urinary citric acid, this tendency was statistically significant. Furthermore, a substantial inverse relationship between citric acid concentration and tumor grade was found. In cancer patients, there was an especially strong negative association between total cholesterol, high-density lipoprotein, and prolactin. On the other hand, only in the control group was an unfavorable correlation between low-density lipoprotein and prolactin amounts seen. On the premise of the findings, one may surmise that hormones, in particular prolactin, have an impact on the emergence of prostate cancer. The current study enabled us to confirm the viability of citric acid as a possible prostate cancer indicator.

The process of cellular metabolism produces ATP, which is needed to power important cellular processes. Recent studies, however, demonstrate that citric acid cycle intermediates specifically target essential cellular processes of the natural defense system. Important myeloid cell activities during infection and inflammation are mediated or controlled by succinate, itaconate, citrate, and fumarate. The regulatory roles of intermediates from the citric acid cycle in myeloid cells are covered in this overview along with possible practical uses, significant mechanistic issues, and upcoming research paths.

CONCLUSION

The Krebs cycle also referred to as the tricarboxylic acid cycle or the citric acid cycle, is at the core of cellular metabolism and is essential for both the creation of biomolecules and energy. It completes the task of breaking down sugar that was begun during glycolysis and powers ATP synthesis in the process. Each cycle shift in the citric acid cycle involves the regeneration of all the intermediates, including citrate, iso-citrate, alpha-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. Oxaloacetate and alpha-ketoglutarate, which are CAC (citric acid cycle) intermediates, act as a forerunner for the synthesis of various amino acids, including asparagine, aspartate, glutamine, arginine, and proline. Additionally acting as an early precursor when nucleotides are being created, oxaloacetate.

REFERENCES:

[1] D. Roosterman and G. S. Cottrell, “Rethinking the citric acid cycle: Connecting pyruvate carboxylase and citrate synthase to the flow of energy and material,” *International Journal of Molecular Sciences*. 2021. doi: 10.3390/ijms22020604.

[2] R. Pesi, F. Balestri, and P. L. Ipata, “Metabolic interaction between urea cycle and citric acid cycle shunt: A guided approach,” *Biochem. Mol. Biol. Educ.*, 2018, doi: 10.1002/bmb.21099.

[3] H. Oexle, E. Gnaiger, and G. Weiss, “Iron-dependent changes in cellular energy metabolism: Influence on citric acid cycle and oxidative phosphorylation,” *Biochim. Biophys. Acta - Bioenerg.*, 1999, doi: 10.1016/S0005-2728(99)00088-2.

[4] G. Czibik, V. Steeples, A. Yavari, and H. Ashrafian, “Citric acid cycle intermediates in cardioprotection,” *Circ. Cardiovasc. Genet.*, 2014, doi: 10.1161/CIRCGENETICS.114.000220.

[5] J. J. Van Hellemond, F. R. Opperdoes, and A. G. M. Tielens, “The extraordinary mitochondrion and unusual citric acid cycle in Trypanosoma brucei,” *Biochem. Soc. Trans.*, 2005, doi: 10.1042/BST20050967.

[6] W. He *et al.*, "Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors," *Nature*, 2004, doi: 10.1038/nature02488.

[7] S. Hallan *et al.*, "Metabolomics and Gene Expression Analysis Reveal Down-regulation of the Citric Acid (TCA) Cycle in Non-diabetic CKD Patients," *EBioMedicine*, 2017, doi: 10.1016/j.ebiom.2017.10.027.

[8] J. L. Santos *et al.*, "Circulating citric acid cycle metabolites and risk of cardiovascular disease in the PREDIMED study," *Nutr. Metab. Cardiovasc. Dis.*, 2021, doi: 10.1016/j.numecd.2021.01.002.

[9] J. Struyf, "Seasonal and Zodiac Sign Properties of the Citric Acid Cycle and the Grouping of Biochemically Important Functional Groups," *World J. Chem. Educ.*, 2020, doi: 10.12691/wjce-8-3-4.

[10] S. Cho, N. Song, J. Y. Choi, and A. Shin, "Effect of citric acid cycle genetic variants and their interactions with obesity, physical activity and energy intake on the risk of colorectal cancer: Results from a nested case-control study in the UK biobank," *Cancers (Basel).*, 2020, doi: 10.3390/cancers12102939.

CHAPTER 6

PENTOSE PHOSPHATE PATHWAY

Dr. Sunita Rao, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id-sunita.rao@jnujaipur.ac.in

ABSTRACT:

Because it includes some glycolytic pathway processes and serves as a conduit for glycolysis, the pentose phosphate pathway is also known as the HMP shunt. An additional method of using glucose is the pentose phosphate route. It is divided into an aerobic and an anaerobic component. Because of this, the PPP can function as a circuit and a route simultaneously. The function of the pentose phosphate pathway in metabolism was covered in this paper.

KEYWORDS:

Carbons Molecule, Non-Oxidative, Oxidative Phase, Phosphate Pathway, Ribulose Phosphate.

INTRODUCTION

According to evolutionary theory, the biochemical processes that make up the PPP is very ancient and appear to have existed alongside life from the very beginning of development. Metal-catalyzed enzyme-free processes similar to the PPP are seen in a primordial Archean ocean reaction environment reconstruction. This suggests the pre-enzymatic genesis of the PPP's fundamental structure and its possible derivation from chemically constrained pre-biotic metal-catalyzed sugar phosphate interconversions. The contemporary cellular PPP however is catalyzed by sophisticated enzymes, except for one step, the interconversion of 6-phosphoglucose-δ-lactone to 6-phosphogluconate, which is still regarded as at least partially spontaneous. The PPP is divided into two biochemical divisions known as the oxidative and non-oxidative PPP by these enzymatic processes.A metabolic route that runs adjacent to glycolysis is the pentose phosphate pathway, also known as the phosphogluconate pathway, the hexose monophosphate shunt, and the HMP Shunt[1], [2].

It produces ribose 5-phosphate, a precursor for the production of nucleotides, pentose (5-carbon carbohydrates), and NADPH. Even though the pentose phosphate pathway does entail the oxidation of glucose, it plays more catabolic than catabolic function. The route is particularly crucial for red blood cells (erythrocytes). The route is divided into two different stages. The oxidative phase, where NADPH is produced, is the first. The non-oxidative production of 5-carbon carbohydrates is the second. The pentose phosphate route is primarily carried out in the cytosol of most animals; in plants, the majority of processes happen in the plastids. The term "oxidative" used to describe this period derives from the combustion process. A paper breaks down during oxidation when it sheds at least one electron. There are two irrevocable stages in this phase:

Step 1: The first step is the oxidation of glucose-6-phosphate to the lactone. As a result of this process, NADP yields NADPH. As glucose-6-phosphate is metabolized, the superscripts + + start superscript, plus, and end superscript are diminished. After glucose-6-phosphate is oxidized, a separate process, mediated by a different enzyme, utilizes water to create 6-phosphogluconate, the linear product.

The high energy electron shuttle, NADH, described in the sections on cellular respiration, is comparable in structure and operation to NADPH. Similar to NADH, NADPH uses its extra phosphate group to give electrons within the cell. After giving up one of its electrons, NADPH is said to have been oxidized (oxidation is the loss of an electron), and it is now represented by the symbols NADP + + start superscript, plus, end superscript. NADPH is frequently used in reactions that create compounds and is present in large amounts in cells, making it easily accessible for these kinds of reactions.

Step 2: Next, a carbon atom is eliminated (cleaved), releasing CO 2 2 start subscript, 2, end subscript. Once more, NADPH is produced by reducing NADP $^{++}$ start superscript, plus, end superscript to NADPH using the electrons released during this cleavage. The name of this brand-new 5-carbon compound is ribulose-5-phosphate.

The period that isn't oxidative: Because these processes can be reversed, the non-oxidative period is very useful. As a result, various molecules from various regions of the non-oxidative phase can join the pentose phosphate route and transform up until the first molecule of the non-oxidative phase (ribulose-5-phosphate). The oxidative step also yields ribulose-5-phosphate, a forerunner to the sugar that goes into making DNA and RNA.

Step 3: It is possible to transform ribulose-5-phosphate into two distinct 5-carbon compounds. The component we will concentrate on is ribose-5-phosphate, a sugar that is used to build DNA and RNA. Since the next step's carbon total is the same, ribulose-5-phosphate is not split.

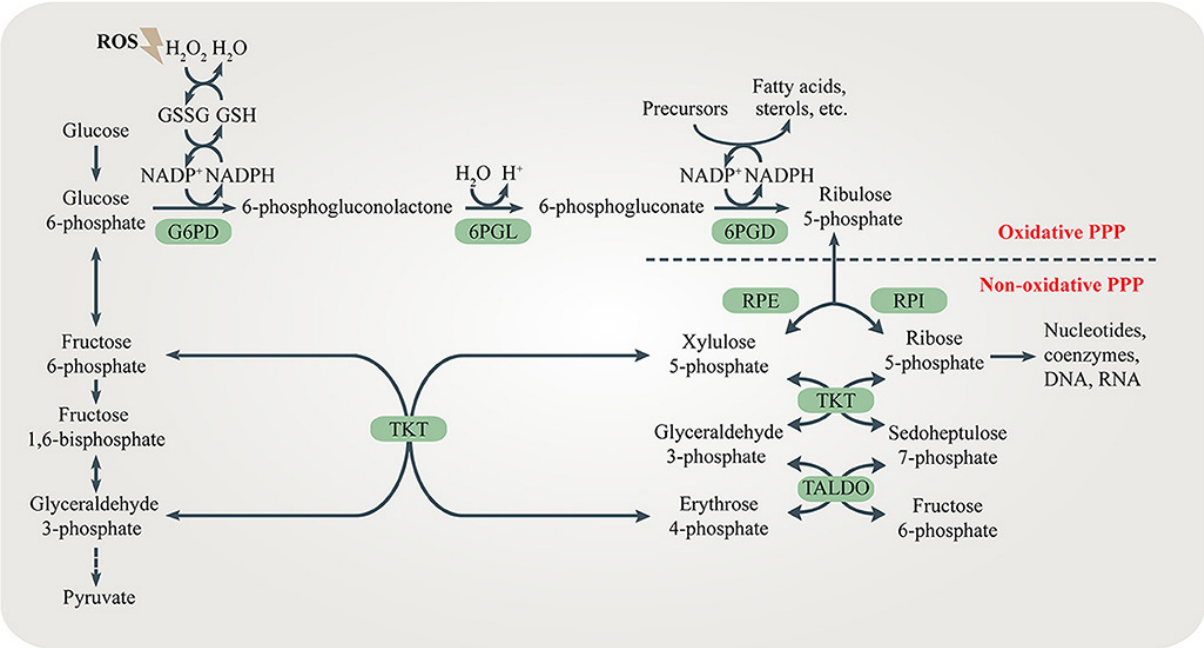

Figure 1: Oxidative phase and the non-oxidative phase: Diagramed showing the Oxidative phase and the non-oxidative phase of the pentose phosphate pathway (Frontier).

Step 4: Depending on the requirements of the cell, the remainder of the cycle consists of various choices. One 10-carbon molecule is created by combining the ribose-5-phosphate from step 3 with another molecule of the compound. Ribose-5-phosphate that is in excess and may not be required for nucleotide biosynthesis is transformed into other carbohydrates that the cell can use for metabolism. The 10-carbon molecule undergoes interconversion to form the 3- and 7-carbon molecules. If necessary, the 3-carbon product can be transported to the

glycolysis process. Having said that, keep in mind that during this period, we can also move back up to another ppaper. So that 3-carbon molecule from glycolysis could also be transported over and converted into ribose-5-phosphate for DNA and RNA synthesis.

Step 5: The 3-carbon and the 7-carbon molecules that underwent interconversion in step 4 above undergo another interconversion to form new 4-carbon and 6-carbon molecules. While the 6-carbon molecule can be used in glycolysis, the 4-carbon molecule serves as a precursor for amino acids. The same step-by-step turnaround that occurred in choice 4 can also occur here. Similar to how glycolysis occurs in the cytoplasm of the cell, the pentose phosphate pathway also occurs there. The ribose-5-phosphate sugar, which is necessary for the synthesis of DNA and RNA, and the NADPH molecules, which aid in the synthesis of other molecules, are the two most significant byproducts of this process.

An additional method of using glucose is the pentose phosphate route (PPP). It is divided into an aerobic and an anaerobic component. Because of this, the PPP can function as a circuit and a route simultaneously. Ribulose-5-phosphate, carbon dioxide (CO_2), and decreased nicotinamide adenine dinucleotide phosphate are the products of its aerobic component. (NADPH). Out of one molecule of glucose, two molecules of NADPH and one molecule of ribulose-5-phosphate are created. The result of the anaerobic change of ribulose-5-phosphate is fresh glucose-6-phosphate rather than energy. Other glycolytic pathways, like glycolysis or the HBP, may return as a result. Six molecules of glucose-6-phosphate are converted into five molecules, 12 NADPH, and six molecules of CO_2 in total through a sequence of PPP processes. All of the processes in the PPP are depicted in a system.

The primary functions of NADPH are glutathione reduction, pyruvate oxidation to malate, and fatty acid production. Ribose-5-phosphate, which is used for the synthesis of nucleotides and nucleic acids, is readily transformed from ribulose-5-phosphate, the byproduct of the aerobic portion of PPP. As a result, based on the specific requirements of a cell's metabolism, the PPP connects anaplerosis, nucleotide synthesis, and antioxidant defense. Glucose-6-phosphate is used by the PPP, glycolysis, and the hexosamine biosynthetic system. The NADP+-dependent glucose-6-phosphate dehydrogenase oxidizes this substrate twice, first to 6-phospho-glucon-lacton as an intermediary and then to 6-phosphogluconate by gluconolactonase. The 6-phosphogluconate dehydrogenase, which is NADP+-dependent, then transforms 6-phosphogluconate into ribulose-5-phosphate. The intermediate 3-keto-6-phosphogluconate is thus fragile. The PPP's final three processes yield two molecules of NADPH[3]–[5].

The subsequent anaerobic section of PPP enables the translation of ribulose-5-phosphate to glycolysis intermediates. Therefore, a cycle rather than a straight route may be preferred to represent the PPP. Ribose-5-phosphate and xylulose-5-phosphate can be created by isomerizing and epimerizing ribulose-5-phosphate. Their interaction with seduheptulose-7-phosphate and glyceraldehyde-3-phosphate is catalyzed by the enzyme transketolase. Transaldolase catalyzes the synthesis of erythrose-4-phosphate and fructose-6-phosphate in the latter two. Fructose-6-phosphate and glyceraldehyde-3-phosphate are produced in a concluding interaction between erythrose-4-phosphate and another molecule of xylulose-5-phosphate, which can enter glycolysis right away.

The PPP enzymes' ability to modify enzymatic activity and promote cellular development through allosteric regulation by their metabolic products. The PPP can function in various states depending on cellular requirements and outside signals. TKT and TALDO reversely route G3P and F6P from glycolysis to the nonoxidative PPP in quickly dividing cells to produce RNA and DNA precursors from R5P. Hexose phosphate isomerase uses glycolysis

to change F6P into G6P during reactive stress and then moves it back to the PPP to keep NADPH levels stable. Similarly to this, the PPP products can be moved back into the glycolytic pathway when there is a large ATP requirement. Other pathways than the PPP sustain NADPH limits, which are crucial to reduce oxidative stress and stopping cell death when glucose is limited. One of these routes is controlled by the stimulation of AMP-activated protein kinase, which prevents fatty acid synthesis from using up NADPH. Malic enzyme and cytoplasmic isocitrate dehydrogenase both act simultaneously to promote fatty acid oxidation, which increases the amount of NADPH molecules produced. A high NADPH/NADP+ ratio is maintained in colorectal cancer by upregulating the malic and isocitrate dehydrogenase enzyme flux to counteract a rise in NADP+ concentration caused by the loss of G6PD activity. The proapoptotic Phorbol-12-myristate-13-acetate-induced protein 1, also known as NOXA, is one of many proteins that play a role in favorably modulating the flux into the PPP in many cancers. Interestingly, NOXA served two purposes. When glucose is present, NOXA makes it easier for the PPP to be used to support life, but when glucose is limited, NOXA is dephosphorylated and acts as a proapoptotic protein.

The PPP's reaction to oxidative stress serves as a model for understanding the fast metabolic as well as transcriptional control of the metabolic network. As stated, under typical growth conditions, other NADP-oxidizing enzymes in yeast take over G6PDH's NADPH-producing function. However, after exposure to hydrogen peroxide (H_2O_2), the NADP+/NADPH ratio falls, making G6PDH null cells extremely susceptible to oxidants Indeed, when cells are subjected to the oxidant, the PPP's activity is quickly increased. Gene regulation and metabolic pathways collaborate to cause this metabolic change (Figure. 2). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase (PK) are two glycolysis enzymes that are inactivated in the first seconds after an oxidative burst, causing a halt in glycolysis while the flow of the P. This rapid reaction lasts for a short period a few seconds to minutes after which transcriptional responses take over and sustain greater PPP activity by upregulating enzymes and post-translational modifications, such as those that boost G6PDH activity. This stringent rule appears to serve two purposes. It reduces carbon depletion from CO_2 generation and avoids an excess of NADPH and PPP intermediates during normal development. When duress circumstances arise, it also promotes a quick cellular reaction.

It appears that various processes are required for the PPP flow to profit from the temporal inhibition of glycolysis. For instance, chemical oxidation quickly renders GAPDH inactive, which is well correlated with the increase in PPP molecule amounts seen within a few seconds. Allosteric regulation is one of the additional processes supporting the inhibition of glycolytic enzymes. The glycolytic intermediary phosphoenolpyruvate (PEP) feedback inhibits trisphosphate isomerase (TPI), which maintains a greater activity of the PPP. PEP is the fuel for pyruvate kinase, which is also allosterically regulated. Post-translational changes that influence G6PDH activity appear to be a third method for accelerating PPP activation. Phosphorylation and acetylation boost G6PDH activity in human and *Xenopus laevis* cells during the stress reaction, preventing this enzyme from becoming rate-limiting.

It is mechanistically linked to steady-state adaptation to physiological circumstances that are associated with higher reactive oxygen species (ROS) generation that the glycolytic/PPP transition occurs during oxidative stress. A key regulating role is played in this situation by PK and its feedback regulatory effect on TPI and other metabolic enzymes. When cells respire quickly, the activity of PK is decreased in budding yeast, and less active isoforms like PKM2 in humans and PYK2 in yeast are produced. The resulting buildup of PEP inhibits several glycolytic enzymes, including the redox regulator TPI, through feedback suppression, and the flow through the PPP rises. When disrupted, TPI inhibition by PEP caused protein

oxidation and mitochondrial damage in respiring cells and was necessary to avoid oxidative stress and oxidative damage.

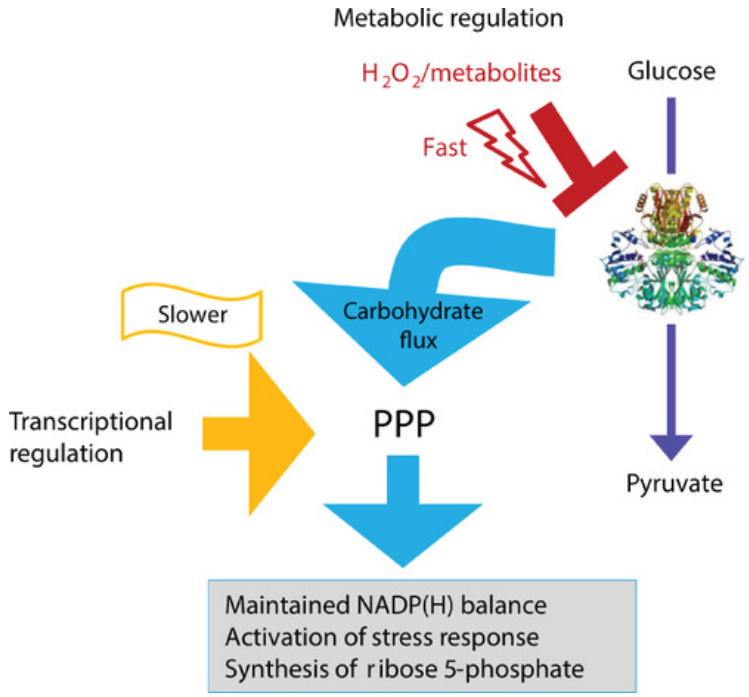

Figure 2: Regulation of the pentose phosphate pathway: Diagramed showing the regulation of the pentose phosphate pathway during Oxidative stress (Online library).

DISCUSSION

The production of ribonucleotides is dependent on the pentose phosphate pathway (PPP), which diverges from glycolysis at the first committed stage of glucose metabolism and is a significant source of NADPH. Fatty acid production and the scavenging of reactive oxygen species both require and utilize NADPH. (ROS). To satisfy their anabolic needs and fight oxidative stress, glycolytic cancer cells need the PPP, which is crucial in this regard. Recently, it was discovered that several neoplastic tumors had changed over time to help glucose enter the PPP. The basic roles of the PPP, its regulation in cancer cells, and its significance for cancer cell metabolism and survival are all outlined in this overview.

A significant source of reducing power and biochemical precursors for biosynthetic processes is the oxidative pentose phosphate route. The cytosol and plastids both contain some, if not all, of the pathway's enzymes, though the exact location of their functions differs. Metabolism may be made more difficult by the pathway's perceived exclusion from the cytosol. However, the activities of a family of plastid phosphate translocators allow for the interchange of intermediates between the cytosol and the plastids, partially offsetting these difficulties. The broad distribution of numerous genes encoding each of the enzymes of the oxidative pentose phosphate pathway is confirmed by molecular analysis. The dynamic characteristics of the activity that catalyzes a particular process may be matched to the metabolic needs of a specific tissue through differential expression of these isozymes. Recent advances in the use of -steady-state labeling techniques allow us to verify this theory. These techniques allow for the quantification of flow through metabolic networks and the differentiation of cytosolic and plastidic glucose oxidation pathways.

Numerous human cancers have substantial changes to energy metabolism that give cancer cells several benefits, including the encouragement of biosynthesis, the production of ATP,

detoxification, and support for fast growth. One important route for the metabolism of glucose is the pentose phosphate pathway (PPP). A crucial reductant in anabolic processes, nicotinamide adenine dinucleotide phosphate (NADPH), is produced by the PPP by directing glucose flow to its oxidative branch. It is now understood that the PPP regulates cancer cell development by providing cells with NADPH for ribose biogenesis, reductive biosynthesis, and the elimination of intracellular reactive oxygen species in addition to ribose-5-phosphate.

As a result, changes to the PPP directly affect cell growth, longevity, and aging. Furthermore, new research has demonstrated that a variety of elements, such as tumor suppressors, oncoproteins, and intracellular metabolites, control the PPP ontogenetically and/or metabolically. Cancer development and longevity are significantly impacted by PPP flux dysregulation. Therefore, it will help create therapeutic approaches that target this pathway to have a better grasp of how the PPP is reprogrammed and the process underpinning the equilibrium between glycolysis and PPP flux in cancer.

For a very long time, almost solely, the pentose phosphate pathway one of the primary antioxidant cellular defense systems was associated with its function as a source of reducing power and ribose phosphate for the cell. Along with this "traditional" association, this metabolic cascade's functions in the cell cycle, apoptosis, differentiation, motility, angiogenesis, and the reaction to anti-tumor treatment have all come into focus in recent years. The pentose phosphate pathway is now a very intriguing target in tumor cells as a result of these discoveries. This review summarizes the most recent findings linking the pentose phosphate pathway's activity to various aspects of tumor metabolism, including cell proliferation and death, tissue invasion, angiogenesis, and therapy resistance, and it discusses the potential utility of drugs that modulate the pathway as tumor therapies[6], [7].

With the aid of transient studies in a continuous culture of Saccharomyces cerevisiae, the in vivo kinetics of the pentose phosphate pathway have been investigated. After disrupting the steady state with a surge of glucose, rapid sampling was carried out using a specific sampling tool. The monitoring period lasted for 120 s following the heartbeat. The changing impact of protein biosynthesis can be disregarded during this brief period. Enzymatic assays and HPLC were used to measure the relevant molecules (glucose 6-phosphate, NADP, NADPH, 6-phosphogluconate, and MgATP2). The determination of kinetic rate formulae and factors for in vivo circumstances was then done using the experimental data.

The in vivo diagnostic confirms an ordered Bi-Bi pathway with noncompetitive inhibition by $MgATP_2$ for the enzyme glucose-6-phosphate dehydrogenase, which is consistent with the findings from *in vitro* studies. An ordered Bi-Ter pathway with competitive suppression by $MgATP_2$ has been discovered for 6-phosphogluconate dehydrogenase. The inhibitory impact disappears and the flux through the pentose phosphate pathway rises as a result of the abrupt drop in $MgATP_2$ concentration following the pulse of glucose. During the dynamic time, this regulatory event ensures the flux equilibrium through the pentose phosphate pathway and glycolysis.

From glucose 6-phosphate (G6P), the pentose phosphate pathway (PPP) diverges, yields NADPH and ribose 5-phosphate (R5P), and diverts carbons back to the glycolytic or gluconeogenic pathway. It has been shown that the PPP plays a significant role in regulating cellular reduction-oxidation (redox) balance and metabolism. There is evidence that PPP enzymes are involved in numerous chronic illnesses. We'll talk about the PPP's involvement in cancer and type 2 diabetes in this overview.

Many processes have been identified that support cancer cell survival when nutrients are scarce. Long noncoding RNAs (lncRNAs) have become important actors in the development

of colon cancer (CRC), but it is unclear how these lncRNAs affect CRC development when nutrients are scarce. We discovered LINC01615, a lncRNA that was markedly elevated in reaction to serum starvation. LINC01615 can help CRC cells adjust to serum-depleted environments and improve cell viability in those environments. Under serum starvation, LINC01615 triggered the pentose phosphate pathway (PPP), which resulted in increased nucleotide and lipid synthesis and reduced ROS generation. The main rate-limiting enzyme of the PPP is glucose-6-phosphate dehydrogenase (G6PD), and LINC01615 increased G6PD expression by preferentially binding with hnRNPA1 and promoting G6PD pre-mRNA splicing. The degradation of METTL3 caused by serum starvation was also discovered to enhance the stability and level of LINC01615 in an m6A-dependent way. In PDO and PDX models, LINC01615 reduction and oxaliplatin produced outstanding anticancer effects. Overall, our findings offered a possible therapeutic target for CRC and showed a new adaptive survival strategy allowing tumor cells to endure under nutrient supply limitations[8]–[10].

CONCLUSION

The pentose phosphate pathway diverges from the glucose 6-phosphate, generates NADPH and ribose 5-phosphate (R5P), and redirects carbons directly to the glycolytic or gluconeogenic system. The pentose phosphate is composed of two significant stages. The phase of oxidation (irreversible) portion is not oxidized (reversible) it has been shown that the PPP plays a significant role in regulating cellular reduction-oxidation (redox) balance and metabolism. NADPH provides high-energy electrons for reduced metabolism as well as antioxidant defense.

The oxidative pentose-phosphate pathway, malic enzyme 1, and isocitrate dehydrogenase 1 converts cytosolic NADP to NADPH. An essential part of cellular respiration is the pentose phosphate pathway. It is crucial for maintaining carbon homeostasis, providing the building blocks for the production of nucleotides and amino acids, providing reducing molecules for anabolism, and preventing oxidative stress.

REFERENCES:

[1] T. Ge, J. Yang, S. Zhou, Y. Wang, Y. Li, and X. Tong, “The Role of the Pentose Phosphate Pathway in Diabetes and Cancer,” *Frontiers in Endocrinology*. 2020. doi: 10.3389/fendo.2020.00365.

[2] N. Ghanem, C. El-Baba, K. Araji, R. El-Khoury, J. Usta, and N. Darwiche, “The Pentose Phosphate Pathway in Cancer: Regulation and Therapeutic Opportunities,” *Chemotherapy*. 2021. doi: 10.1159/000519784.

[3] L. Jin and Y. Zhou, “Crucial role of the pentose phosphate pathway in malignant tumors (review),” *Oncol. Lett.*, 2019, doi: 10.3892/ol.2019.10112.

[4] P. Jiang, W. Du, and M. Wu, “Regulation of the pentose phosphate pathway in cancer,” *Protein Cell*, 2014, doi: 10.1007/s13238-014-0082-8.

[5] I. Giacomini, E. Ragazzi, G. Pasut, and M. Montopoli, “The pentose phosphate pathway and its involvement in cisplatin resistance,” *International Journal of Molecular Sciences*. 2020. doi: 10.3390/ijms21030937.

[6] A. Masi, R. L. Mach, and A. R. Mach-Aigner, “The pentose phosphate pathway in industrially relevant fungi: crucial insights for bioprocessing,” *Applied Microbiology and Biotechnology*. 2021. doi: 10.1007/s00253-021-11314-x.

[7] A. Stincone *et al.*, "The return of metabolism: Biochemistry and physiology of the pentose phosphate pathway," *Biol. Rev.*, 2015, doi: 10.1111/brv.12140.

[8] J. Baardman *et al.*, "A Defective Pentose Phosphate Pathway Reduces Inflammatory Macrophage Responses during Hypercholesterolemia," *Cell Rep.*, 2018, doi: 10.1016/j.celrep.2018.10.092.

[9] D. Bojkova *et al.*, "Targeting the pentose phosphate pathway for sars-cov-2 therapy," *Metabolites*, 2021, doi: 10.3390/metabo11100699.

[10] M. A. Keller, A. V. Turchyn, and M. Ralser, "Non-enzymatic glycolysis and pentose phosphate pathway-like reactions in a plausible Archean ocean," *Mol. Syst. Biol.*, 2014, doi: 10.1002/msb.20145228.

CHAPTER 7

FRUCTOSE AND GALACTOSE METABOLISM DISORDERS

Dr. Manish Soni, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id- manishsoni@jnujaipur.ac.in

ABSTRACT:

Fructose and galactose, two types of carbohydrates that do not have their metabolic route, are broken down into glycolytic molecules and then incorporated into the glycolysis process to produce pyruvate and ATP. Galactose and fructose are taken in the organism by the liver for metabolism by the transport system. In this paper, we outlined the roles of the galactose and fructose metabolisms as well as the consequences of any malfunctions.

KEYWORDS:

Classic Galactosemia, Fructose Phosphate, Glucose Phosphate, Galactose Metabolism, Galactose Phosphate.

INTRODUCTION

Monosaccharides are joined into double sugars through a condensation process, which only requires the removal of a water molecule from the functional groups. A form of an enzyme called disaccharides is used in the process of digestion to separate a double sugar into its two monosaccharides. While constructing the bigger sugar expels a water molecule, destroying it absorbs a water molecule. These processes are essential for respiration. The widely used sugar sucrose Fru is the primary source of fructose in the majority of Western cultures. In the US, ketchup, and bread are just two examples of commercial food items that contain high fructose corn syrup. During commercial preparation, this syrup's Glc is converted to Fru using D-xylulose ketol-isomerase and glucose isomerase. Monomeric Fru and Glc mixtures are 1.3 times sweeter than equivalent quantities of sugar. Monomeric fructose and sucrose are also present in large quantities in fruits and veggies. Sucrose makes up about half of the total weight of apricots. Daily fructose consumption can reach 100g, particularly in communities that consume large amounts of sucrose and high-fructose corn syrup[1]–[3].

Facilitative glucose transporter 5 (GLUT5, Slc2a5) transports fructose selectively and passively across the gut apical membrane before GLUT2 transports it from the cytosol to the blood. (Slc2a2). The GLUT family in people (gene family Slc2a) has 14 individuals with different tissue expressions, substrate affinities, and specificities. In addition to fructose, GLUT2 also transfers glucose and galactose, with fructose having a Km that is more than five times greater than GLUT5. Normally, GLUT2 is primarily found in the liver, pancreas, gut, kidney, and brain, while GLUT5 is primarily expressed in the small intestine and kidney.GLUT7, GLUT8, GLUT9, and GLUT11 are additional GLUTs that can transport fructose and glucose, though new research revealed that neither GLUT7 nor GLUT9 transport fructose.

The three transporters that GLUT2 is most closely linked to are GLUT1 (expressed universally), GLUT3 (primarily in neuronal cells), and GLUT4 (an insulin-dependent transporter in skeletal muscle and adipocytes). These transporters do not transfer fructose but rather glucose, dehydroascorbate, glucosamine, galactose, and mannose as their substrates. Since there aren't many particular GLUT inhibitors available due to the genetic homology among GLUTs, pharmaceutically addressing one GLUT can be difficult because it may also

impact several other GLUTs. This makes it crucial to comprehend ligand identification in the GLUT family. Specific inhibition of GLUT5 appears to be the option with the fewest potential side effects when attempting to reduce the absorption of dietary fructose in the intestines; GLUT2 inhibition in the intestines is desirable but in pancreatic beta cells can result in insulin sensitivity and ultimately diabetes. The major facilitator superfamily (MFS) of transporters includes the GLUT family.

The MFS fold, which is shared by GLUTs, is made up of 12 transmembrane helices arranged into two regions of six helices each, the N- and C-halves, connected by a pseudo-twofold axis of symmetry that runs parallel to the cell membrane. The center polar cavity between the N- and C-domains is home to the substrate binding location (Figure .1). The substrate cavity is alternately exposed to each side of the membrane through four major conformations of the transporter, which are outward-facing, each with two states: open (no substrate), and occluded (no substrate). This means that during a cycle of transport, the transporter binds the substrate while in the outward-facing open conformation, moves into the outward-facing occluded conformation, then changes to the inward-facing occluded conformation, and finally releases the substrate while in the inward-facing open conformation.

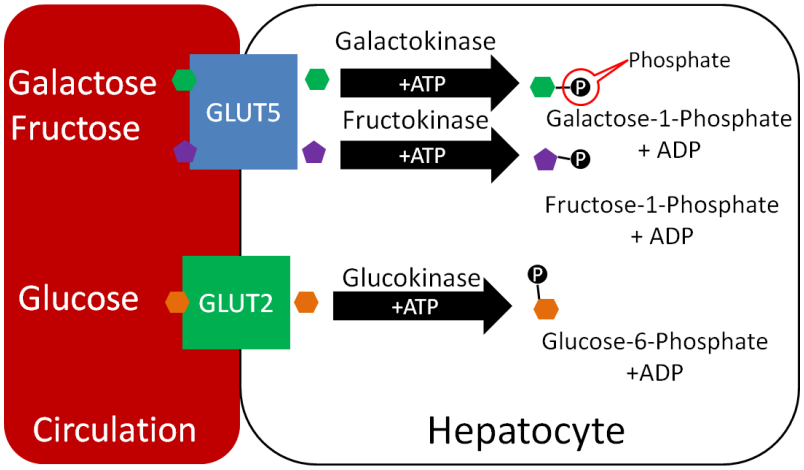

Figure 1: Absorption of the fructose: Diagramed showing the absorption of the fructose (Course. Lumen).

The suggested models based on GLUT crystal structures disregard the potential that at least some GLUT members may act as oligomers, and the mechanistic specifics of what enables the changes among the various conformational states are still being worked out. Both the inward- and outward-facing conformations of mammalian GLUT5 are known, and the fructose transport mechanism proposed postulates that two bent helices in the C-domain, transmembrane helix (TM) 7 and TM 10, play a crucial role in coupling fructose binding to conformational transitions between open and occluded states. Although GLUT2's structural determination has been elusive thus far, it shares 52–55% of its amino acid sequence with GLUT1 and GLUT3, whose crystal structures each catch one of the outward- and one of the inward-facing conformations, respectively, making it possible to simulate GLUT2's structure with ease.

Additionally, the substrate binding regions of GLUT1-4 share conserved amino acid sequences. The availability of GLUT crystal structures provides a strong foundation for understanding substrate diversity among GLUTs and has made it possible to conduct in silico high-throughput ligand screening to find new GLUT inhibitors. How, then, does GLUT5 distinguish so clearly between the two hexoses while GLUT2's substrate binding region recognizes both fructose and glucose? Are there any variations in the substrate binding sites

of GLUT2 and GLUT5 that could be used to develop new drugs? The molecular variety of glucose and fructose in solution and when attached to proteins is examined first. In water, both hexoses adopt the pyranose or furanose conformations, with the pyranose form dominating for both glucose and fructose (67.5% pyranose, 31.5% furanose, and 0.8% open-chain), and the furanose form being much more common for fructose than glucose. By September 2017, the Protein Data Bank had 759 protein structures with -glucopyranose, 56 with -fructofuranose, and 9 with -fructopyranose. When searching the Protein Data Bank for protein crystal structures liganded with glucose or fructose, we discovered that, as in solution, glucopyranose is the dominant conformation. The positions of the hydroxyl groups vary significantly more between fructofuranose and glucopyranose when compared to fructopyranose than between the pyranose types of hexoses. Although GLUT1 can transfer epimers of the C3, C4, and C5 fructofuranose moiety, research on GLUT1 and GLUT2 homologs suggests that the transporter attaches fructose in the furanose conformation and glucose in the pyranose form. It's conceivable that some fructose-transporting GLUTs can bind both the pyranose and furanose forms, whereas other GLUTs favor one conformer over the other.

The three enzymes fructokinase, fructose-bisphosphate aldolase B, and adenosine triphosphate (ATP)-dependent dihydroxyacetone kinase provide the most comprehensive understanding of fructose biosynthesis (or triokinase). Only rodent's and humans' livers and kidneys contain all three of these. Through fructokinase, fructose is quickly transformed in the liver to fructose 1-phosphate. Aldolase B then breaks down fructose 1-phosphate into the trioses dihydroxyacetone phosphate and glyceraldehyde. The liver uses aldolase B for typical glycolysis (glucose metabolism)[4]–[6].

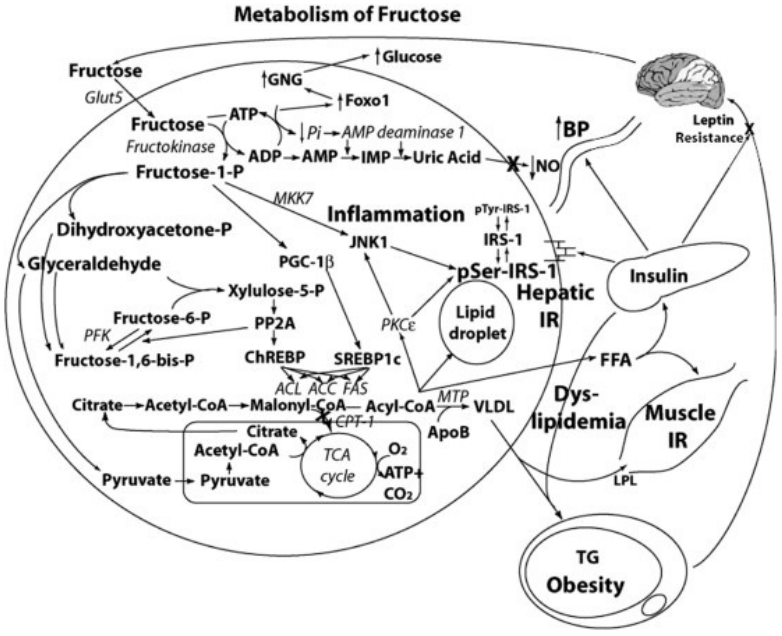

Figure 2: Fructose biosynthesis: Diagramed showing Fructose biosynthesis (Science direct.com).

Triokinase then uses glyceraldehyde to create glyceraldehyde-3-phosphate. There haven't been any rate-limiting stages in fructose metabolism up until this point, so there is a greater quantity of substrate going to metabolic pathways from triose phosphate; i.e. glycolysis, glycogenesis, gluconeogenesis, lipogenesis, and fatty acid esterification. Next, pyruvate is converted from glyceraldehyde-3-phosphate by the rate-limiting enzyme pyruvate kinase. The greater flow of pyruvate into the Krebs cycle is caused by fructose's enhancement of

pyruvate kinase activation. Fructose metabolism should be noticed because it does not encourage an insulin reaction (Figure .2).

A fructose product is fructose-1-phosphate. It is primarily produced by liver fructokinase, though tiny quantities are also produced in the mucosa of the small intestine and the proximal epithelial of the renal tubule. It is a crucial metabolic intermediary for glucose. Fructose entering cells is rapidly converted to fructose 1-phosphate due to fructokinase's high Vmax. It typically builds up in the liver in this state before being further converted by aldolase B. (the rate-limiting enzyme of fructose metabolism). It is changed into glyceraldehyde and dihydroxyacetone phosphate by aldolase B. Triose kinase then phosphorylates glyceraldehyde to produce glyceraldehyde 3-phosphate. Thus, fructose metabolism primarily yields glycolysis intermediates. This indicates that after fructose is metabolized, it follows the same course as glucose. Pyruvate, which is the result of glycolysis, can then go through gluconeogenesis, start the TCA cycle, or be retained as fatty acids.

N-acetylgalactosamine is the main type of galactose present in these intricate biomolecules. (GalNAc). Instead of coming from nutrition, galactose in food produces galNAc. The disaccharide lactose, which is present in dairy goods, is the main source of galactose in the human diet. Between 2% and 8% of milk, proteins are made up of this sugar. A disaccharide of glucose and galactose is lactose. When lactose is consumed, the digestive enzyme complex known as -galactosidase causes it to be converted into glucose and galactose. A GPI linkage holds the enzyme complex to the exterior of gut brush border cells. The -galactosidase complex has two enzymatic activities; one hydrolyzes the -glycosidic linkage in lactose (releasing glucose and galactose), and the other hydrolyzes the -glycosidic bond tying galactose or glucose to ceramide in ingested glycolipids. The same sodium (Na+)-dependent glucose transporter (SGLT1) that is in charge of absorbing glucose also absorbs galactose by gut enterocytes. Similar to how glucose and fructose enter the circulation from gut enterocytes through GLUT2-mediated transport. N-acetylgalactosamine is the main type of galactose present in these intricate biomolecules. Instead of coming from nutrition, galactose in food produces galNAc. The disaccharide lactose, which is present in dairy goods, is the main source of galactose in the human diet.

Between 2% and 8% of milk, proteins are made up of this sugar. A disaccharide of glucose and galactose is lactose. When lactose is consumed, the digestive enzyme complex known as -galactosidase causes it to be converted into glucose and galactose. A GPI linkage holds the enzyme complex to the exterior of gut brush border cells. The -galactosidase complex has two enzymatic activities; one hydrolyzes the -glycosidic linkage in lactose (releasing glucose and galactose), and the other hydrolyzes the -glycosidic bond tying galactose or glucose to ceramide in ingested glycolipids. The same sodium (Na+)-dependent glucose transporter (SGLT1) that is in charge of absorbing glucose also absorbs galactose by gut enterocytes. Similar to how glucose and fructose enter the circulation from gut enterocytes through GLUT2-mediated transport. D-galactose is catabolized through a biochemical route called the Leloir pathway. It was first characterized by Luis Federico Leloir, after whom it is called. Leloir pathway enzymes and intermediaries in galactose metabolism

Since this is the active state in the pathway, galactose mutarotase aids in the conversion of -D-galactose to -D-galactose in the first stage. Galactokinase then phosphorylates -D-galactose to produce galactose 1-phosphate (Figure .3). The third stage involves the conversion of galactose 1-phosphate to UDP-galactose by the enzyme D-galactose-1-phosphate uridylyltransferase using UDP-glucose as the source of uridine diphosphate. To facilitate the transferase process, UDP-galactose 4-epimerase recycles UDP-galactose to

UDP-glucose. D-glucose 1-phosphate is additionally changed into D-glucose 6-phosphate by phosphoglucomutase.

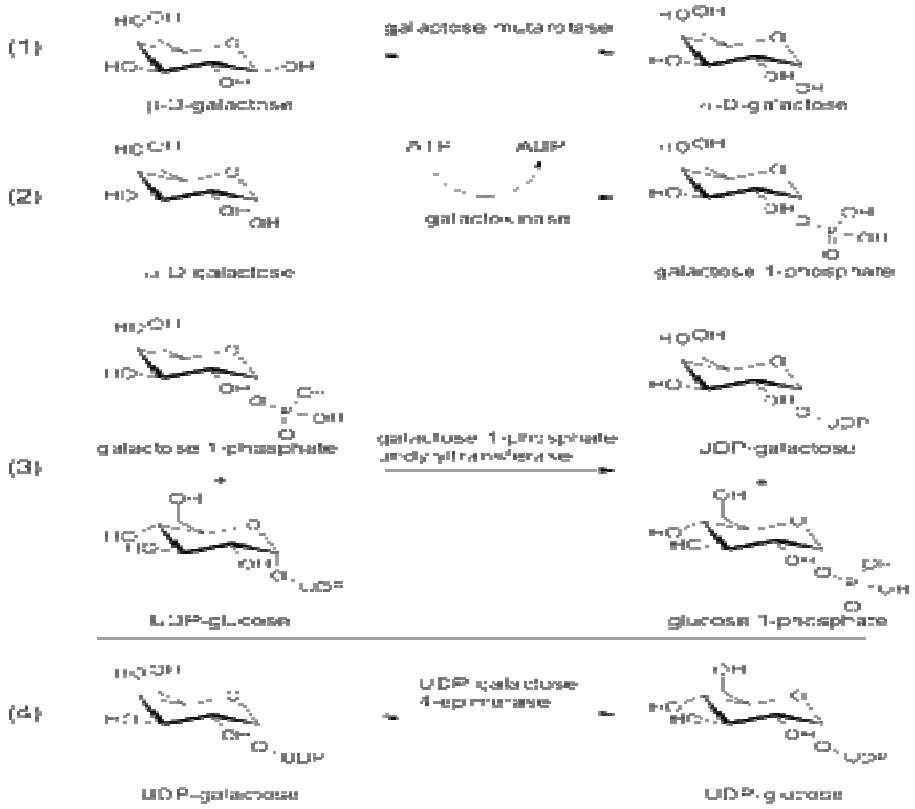

Figure 3: Galactose biosynthesis: Diagramed showing Galactose biosynthesis (Wikipedia).

Poor eating habits, neonatal failure to flourish, bleeding issues, and E. coli-associated sepsis in neglected newborns are all symptoms of classic galactosemia. Infants with typical galactosemia experience sickness and diarrhea right away after consuming lactose from breast milk or lactose-containing formula. Infants are occasionally incorrectly labeled as lactose intolerant due to the symptoms' fast development. However, there is a significant clinical difference between typical lactose sensitivity and classic galactosemia. In most US states, infants are screened for typical galactosemia, greatly lowering the risks for those who are born with this condition. Erythrocyte GALT activity and galactose-1-phosphate levels are typically measured in prenatal tests for galactosemia, with the former being substantially decreased or missing and the latter being high. Some prenatal tests only measure total blood galactose levels, and this assay may not be adequate to identify any abnormality in babies suffering from a clinical or biochemical variant of type 1 galactosemia.

It may be required to validate a diagnosis with a genetic test for GALT variant alterations in some instances of clinical or biochemical variant type 1 galactosemia. When lactose or meals containing galactose are consumed by infants with classic galactosemia, clinical symptoms can include impaired liver function (which, if untreated, can result in severe cirrhosis), hypoglycemia, hyperbilirubinemia, elevated blood galactose, hyper galactosemia, hyperchloremic metabolic acidosis, urinary galactitol excretion, and hyper aminoaciduria. If left untreated, these galactosemias can progress to cause catastrophic liver failure, brain injury, and blindness. Galactose should be eliminated from the diet. Blindness results from an NADPH-dependent aldose reductase that is found in brain tissue and the lens of the eye converting flowing galactose to the sugar alcohol galactitol. This enzymatic action has no

pathological consequences at typical galactose concentrations in the blood. However, a high level of galactitol in the lens produces osmotic edema, which leads to the development of cataracts and other undesirable eye conditions.

Eliminating lactose from the food is the main therapy for newborns with classic galactosemia, whose erythrocyte GALT activity is 10% of normal. All other milk products, including breast milk, are included in this lactose-removal plan. GALT-deficient people show urinary galactitol excretion and consistently increased erythrocyte galactose-1-phosphate levels, even when eating a diet low in galactose. Furthermore, even when dietary galactose is strictly limited for the rest of their lives, many people with classic galactosemia can experience serious long-term complications like speech impairment, cognitive decline, and in female patients, the possibility of premature follicular atresia leading to ovarian insufficiency and sterility. It is advised that people with typical galactosemia receive regular testing for the buildup of erythrocyte galactose-1-phosphate, increased urinary galactose output, developmental delay, speech issues, and cataract development due to these chronic clinical consequences.

Galactosemia Type 2: Variety 2 galactosemia, also known as the second variety, is caused by a galactokinase deficit. (GALK1). With an incidence of only 1 in 100,000 live births, this type of galactosemia is quite uncommon. Similar to babies with classic galactosemia, infants with GALK deficiency who continue to ingest a milk-based diet develop unusually high amounts of galactose in their blood and tissues. Similar to patients with typical galactosemia, those with GALK loss frequently exhibit cataracts, which disappear when galactose is restricted from the diet. Patients with GALK deficiency who adhere to a diet low in galactose, however, do not suffer any known long-term consequences, in contrast to those with typical galactosemia. The primary cause of the complications, besides cataracts, seen in patients with classic galactosemia and the more uncommon severe form of GALE deficiency is not the accumulation of galactose, but rather galactose-1-phosphate (Gal-1P), or possibly some metabolic derivative Gal-1P, according to this difference, which is quite significant both biochemically and clinically.

Galactosemia Type 3: A lack of UDP-galactose-4-epimerase causes type 3 galactosemia, the third disease of galactose biosynthesis. This deficiency has been identified in two distinct types. The more prevalent deficiency is generally benign and only affects red and white blood cells. Only 8 instances of the other type of GALE deficiency are known, and it is marked by severe enzyme impairment that affects numerous organs and causes symptoms resembling those of GALT deficiency[7], [8].

Lack of fructose 1-phosphate aldolase (aldolase B): Hereditary fructose intolerance is a clinical condition brought on by this deficit. Autosomal defective inheritance is thought to affect 1 in 20,000 newborns. When infants consume fructose, fructose 1-phosphate builds up and causes hypoglycemia, sickness, vomiting, stomach discomfort, sweating, tremors, disorientation, sleepiness, convulsions, and coma. Infants are healthy before this happens. Cirrhosis, mental decline, and proximal renal tubule acidosis with urine loss of phosphate and glucose can all result from prolonged consumption.

The signs of a fructose 1-phosphate aldolase deficiency are suggestive of the diagnosis, which is verified by DNA testing. Prior confirmation tests involved liver biopsy or 200 mg/kg IV sugar infusions to induce hypoglycemia. Direct DNA sequencing can also be used to diagnose and identify heterozygous bearers of defective gene. Fructose therapy over the short run Hypoglycemia is caused by a 1-phosphate aldolase deficiency; the long-term

therapy is a nutritional restriction of fructose, sucrose, and sorbitol. Many people acquire an innate dislike of foods high in fructose. An excellent prognosis is possible with therapy.

Deficiency in fructokinase: The benign increase of blood and urinary fructose levels is brought on by this deficit. Autosomal defective inheritance occurs in about 1 in 30,000 pregnancies. When a non-glucose-reducing compound is found in pee, the disease, which has no symptoms, is unintentionally identified.

Fructose-1,6-biphosphatase deficiency: This deficit impairs gluconeogenesis and causes ketosis, metabolic acidity, and fasting hypoglycemia. For newborns, this deficit can be deadly. Autosomal defective inheritance; frequency not known. Episodes may be brought on by febrile sickness. Oral or intravenous glucose is used to treat fructose-1,6-biphosphatase insufficiency acutely. Age usually leads to a rise in fasting tolerance.

DISCUSSION

Performance may be impacted by differences in the glycaemic and insulinemic reactions to various carbs. The goal of the current research was to ascertain the effects of pre-exercise ingestion of glucose (GLU), galactose (GAL), and trehalose (TRE) on metabolic reactions during exercise and at rest, as well as on performance during a time trial (TT). Three exercise trials were performed by eight skilled male riders over at least three days. The participants drank 500 ml of a beverage containing 75 g of either glucose, galactose, or trehalose 45 minutes before the commencement of the activity. The exercise trials included a [mean (SEM)] 702 (25) kJ TT shortly after 20 min of submaximal steady-state exercise (SS) at 65% of maximal power production. In comparison to GAL and TRE, the plasma glucose content in GLU was markedly higher at 15 minutes postprandial (P 0.05). This was accompanied by an increase in plasma insulin concentration that was more than twice as large in GLU as it was in GAL and TRE (118% and 145%, respectively).

Four GLU subjects and one TRE subject experienced return hypoglycemia (plasma glucose content less than 3.5 mmol/l) during SS exercise. There were no variations in TT results among the three attempts. Trehalose and galactose consumption were previous to exercise decreased the frequency of rebound hypoglycemia and decreased plasma glucose and insulin responses. Galactose and trehalose pre-exercise consumption resulted in attenuated insulin and glucose responses both at rest and during exercise, but there was no change in TT performance compared to pre-exercise administration of glucose. An uncommon autosomal hereditary genetic flaw is glucose-galactose malabsorption. The movement and uptake of glucose and galactose in the gut are affected by a mutation in the glucose sodium-dependent transporter-1 gene. Dehydration and hyperosmotic diarrhea are caused by unabsorbed galactose, glucose, and salt that remain in the gut as a result of the SGLT-1 defect.

Patients with glucose-galactose malabsorption frequently rely heavily on fructose as their main food supply. This investigation seeks to review all published papers on hereditary fructose and glucose malabsorption. Thirteen studies were found and examined after 100 published studies were evaluated for this study's criteria. According to studies, eating a lot of high-fructose foods can have a variety of negative impacts on one's health, including potentially fatal ones. None of the published studies analyzed in this study specifically addressed or explored the negative impacts of individuals with congenital glucose-galactose malabsorption consuming fructose as their main source of carbohydrates[9], [10].

Understanding the pathogenic processes causing obesity and diabetes mellitus may be aided by knowledge of normal hepatic glucose metabolism. Additionally, glycosylation processes and the metabolism of fatty acids are linked to hepatic glucose metabolism. Through the

portal vein, the liver gets dietary carbohydrates straight from the stomach. To ensure that enough glucose penetrates the cell to be processed, glucose kinase phosphorylates glucose to glucose 6-phosphate inside the hepatocyte. Numerous biochemical routes are open to glucose 6-phosphate. The majority of glucose 6-phosphate is used to make glucose by way of the production of glucose 1-phosphate and UDP-glucose during the postprandial phase. UDP-glucose is used in very small quantities to create the donors of monosaccharide molecules for glycosylation, UDP-glucuronate, and UDP-galactose. The formation of fructose 6-phosphate, which can either begin the hexosamine pathway to create UDP-N-acetylglucosamine or continue the glycolytic route to make pyruvate and then acetyl-CoA, is a second pathway of glucose 6-phosphate metabolism. When there is extra glucose within the hepatocyte, acetyl-CoA may join the tricarboxylic acid (TCA) cycle to be oxidized or may be transferred to the cytosol to make fatty acids. Finally, the pentose phosphate pathway allows glucose 6-phosphate to generate NADPH and ribose 5-phosphate. Glycosylation is a post-translational change of proteins and lipids that controls their function. Glycosylation intermediates are produced by glucose metabolism. Impaired glycosylation is linked to phosphoglucomutase (PGM)-1 and PGM-3 congenital impairment. The liver also creates glucose for other organs to use, either from the breakdown of glycogen or from de novo synthesis using mainly lactate and alanine. (gluconeogenesis).

It has been determined how *Staphylococcus aureus* breaks down D-galactose 6-phosphate. Galactose 6-phosphate undergoes isomerization to become tagatose 6-phosphate, which is then phosphorylated with adenosine 5′-triphosphate and degraded into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. These processes are catalyzed by inducible isomerase, kinase, and aldolase, which differ from their counterparts in glucose 6-phosphate metabolism.

CONCLUSION

The liver is where fructose and galactose are transformed into glucose after being transported by the blood to the organ. Through fructokinase, fructose is quickly transformed into fructose 1-phosphate. Aldolase B then breaks down fructose 1-phosphate into the trioses dihydroxyacetone phosphate and glyceraldehyde. The liver uses aldolase B to carry out regular metabolism (glucose metabolism). The lower gut receives fiber undamaged because the human body lacks the enzymes necessary to break down fiber into single sugars for absorption. Glucose is the primary type of carbohydrate circulating in the circulation after these have been ingested and passed through the liver. The main monosaccharide that the body uses for energy is glucose. With a well-established function in energy transport and the galactosylation of complicated molecules, galactose is essential for human metabolism, and there is growing evidence for additional roles.

REFERENCES:

[1] P. S. Kishnani and Y. T. Chen, “Disorders of Carbohydrate Metabolism,” in *Emery and Rimoin’s Principles and Practice of Medical Genetics and Genomics: Metabolic Disorders*, 2020. doi: 10.1016/B978-0-12-812535-9.00004-2.

[2] Z. Xiang *et al.*, “Revealing hypoglycemic and hypolipidemic mechanism of Xiaokeyinshui extract combination on streptozotocin-induced diabetic mice in high sucrose/high fat diet by metabolomics and lipidomics,” *Biomed. Pharmacother.*, 2021, doi: 10.1016/j.biopha.2021.111219.

[3] G. Wang *et al.*, “The protective effects of walnut green husk polysaccharide on liver injury, vascular endothelial dysfunction and disorder of gut microbiota in high fructose-induced mice,” *Int. J. Biol. Macromol.*, 2020, doi: 10.1016/j.ijbiomac.2020.06.055.

[4] I. Endreffy, G. Bjørklund, M. A. Urbina, S. Chirumbolo, M. D. Doşa, and F. Dicső, "High Levels of Glycosaminoglycans in the Urines of Children with Attention-Deficit/Hyperactivity Disorder (ADHD)," *J. Mol. Neurosci.*, 2020, doi: 10.1007/s12031-020-01496-w.

[5] D. Corps Fernández, A. R. Valbuena Parra, J. M. Mesa Latorre, and J. D. García Díaz, "Enfermedades por almacenamiento de glucógeno y otros trastornos hereditarios del metabolismo de los hidratos de carbono," *Med.*, 2016, doi: 10.1016/j.med.2016.09.021.

[6] D. Abukawa, T. Ohura, and Y. Tazawa, "Disorders of fructose and galactose metabolism," *Ryōikibetsu shōkōgun shirīzu.* 1995.

[7] P. S. Kishnani and Y.-T. Chen, "Chapter 87 – Defects in Metabolism of Carbohydrates," *Nelson Textb. Pediatr.*, 2016.

[8] W. E. Winter, J. P. R. Pelletier, and N. S. Harris, "Chapter 34 - Carbohydrate disorders," in *Contemporary Practice in Clinical Chemistry (Fourth Edition)*, 2020.

[9] A. Olgac, L. L. Tumer, A. Inci, B. Karaoglu, I. Okur, and F. S. Ezgu, "Bone mineral density and vitamin D status in inborn errors of metabolism," *J. Inherit. Metab. Dis.*, 2016.

[10] D. Corps Fernández, A. R. Valbuena Parra, J. M. Mesa Latorre, and J. D. García Díaz, "The glycogen storage diseases and other inherited disorders of carbohydrates' metabolism," *Med.*, 2016, doi: 10.1016/j.med.2016.09.021.

CHAPTER 8

FAT DIGESTION, MOVEMENT, AND TRANSPORTATION

Mr. Rahul Saxena, Associate Professor
Department of Food Technology, Jaipur National University, Jaipur, India
Email Id-rahulsaxena@jnujaipur.ac.in

ABSTRACT:

Fat molecules are broken down into triglycerides and fatty acids, and then they are absorbed in the stomach. In the presence of the lipase enzyme, fatty acids are broken down in the fats and lipids in the gut. The protein known as the lipoprotein is linked with these lipid molecules based on their molecular weight. This paper summarized the mechanism of fat transport and adsorption in the human system.

KEYWORDS:

Acid Metabolism, Density Lipoprotein, Fatty Acids, Lipid Digestion, Small Intestine.

INTRODUCTION

The term "fatty acid metabolism" refers to a group of metabolic procedures containing or strongly associated with fatty acids, a family of molecules included in the category of lipid macronutrients. The two major categories of these processes are (1) catabolic processes, which produce energy, and (2) anabolic processes, which use them as the building blocks for other substances. Fatty acids are broken down during metabolism to create energy, primarily in the form of adenosine triphosphate (ATP). When fully oxidized to CO_2 and water by beta-oxidation and the citric acid cycle, fatty acids produce the most ATP per gram when compared to the other macronutrient groups (carbohydrates and protein). Thus, in most mammals and, to a lesser degree, in plants, fatty acids (primarily in the form of triglycerides) serve as the primary form of fuel storage.

In the process of anabolism, undamaged fatty acids serve as vital building blocks for phospholipids, second messengers, hormones, triglycerides, and ketone bodies. For instance, phospholipids build the phospholipid bilayers from which all of the fatty acid-based cell membranes are made. The plasma membrane and other cell membranes, including those covering the nucleus, the mitochondria, the endoplasmic reticulum, and the Golgi apparatus, are made of phospholipids. Fatty acids are altered to create second messengers and local hormones in another sort of anabolism. The most well-known of these local hormones are undoubtedly the prostaglandins created from arachidonic acid held in the cell membrane[1]–[3].

Digestion and assimilation of lipids present some unique difficulties. In contrast to proteins and carbs, triglycerides are big molecules that are not water-soluble. As a result, when in a watery environment like the digestive system, they prefer to group in big droplets. The digestive process must break down those big fat droplets into smaller droplets before using lipases to enzymatically break down lipid molecules (Figure. 1). The small intestine performs the majority of the enzymatic breakdown of fats, with some assistance from the mouth and stomach. Once again requiring special treatment because lipids are not water-soluble and do not combine with the watery blood, the byproducts of lipid digestion are then taken into circulation and carried throughout the body. The lipid breakdown process is initiated in the pharynx by a few events. By physically breaking food down into smaller pieces, chewing also combines the food with saliva. Lingual lipase, which means "relating to the tongue," is an

enzyme generated by cells on the tongue that starts the enzymatic breakdown of lipids by separating the individual fatty acids from the glycerol backbone. Mixing and churning in the gut aids in the distribution of food and lipid molecules. Another lipase, termed gastric lipase ("gastric" means about the stomach), is produced by stomach cells and aids in the enzymatic breakdown of lipids. Additionally, ingested lingual lipase from food and saliva continues to function in the gut. But when combined, these two lipases contribute little to the breakdown of fat, and the majority of enzymatic processing takes place in the small intestine.

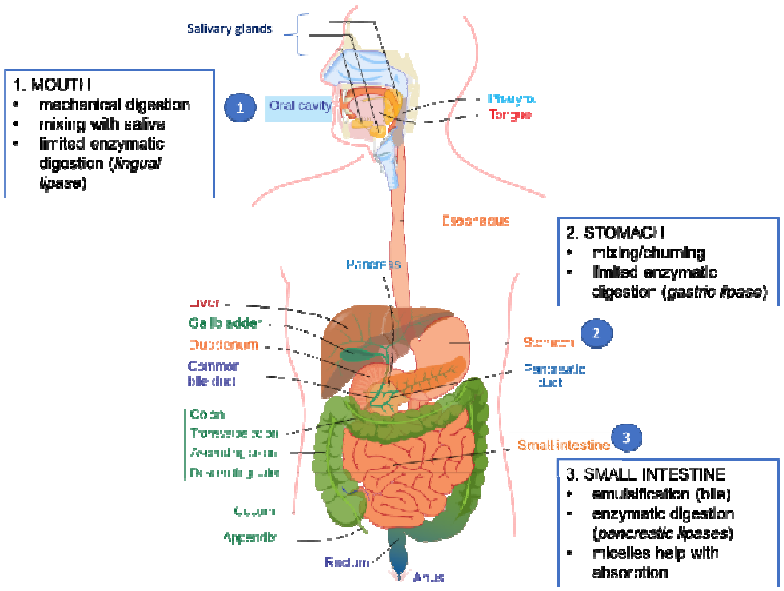

Figure 1: Lipid digestion: Diagramed showing the overview of Lipid digestion in the gastrointestinal tract (Open Oregon).

The majority of the food fats are undigested and clumped together in big droplets as the stomach contents pass into the small intestine. The duodenum, the first part of the small intestine, receives the discharge of bile, which is produced in the liver and kept in the gallbladder. Bile salts are drawn to both lipids and water because they have both a hydrophobic and a hydrophilic side. Because of this, they function as efficient emulsifiers, breaking big fat globules into smaller droplets. Emulsification increases the surface area for the action of digestive enzymes, making fats more available to them (Figure. 1). To enzymatically process lipids, the pancreas secretes pancreatic lipases into the small intestine. Fatty acids, monoglycerides (glycerol backbone with one fatty acid still connected), and some unbound glycerol are all products of the breakdown of triglycerides. The metabolic digestion of cholesterol and fat-soluble micronutrients is not necessary.

Fatty acids, monoglycerides, glycerol, cholesterol, and fat-soluble vitamins are the next byproducts of fat digestion that must reach the bloodstream to be utilized by cells all over the body. Bile once more aids in this procedure. Bile salts create micelles, which are structures that gather around the byproducts of fat digestion and assist fats in getting near the microvilli of intestinal cells so they can be ingested. The byproducts of fat breakdown spread across the intestinal cell membrane, and recycled bile ions continue the process of emulsifying fat and creating micelles. Long-chain fatty acids, monoglycerides, fat-soluble vitamins, and cholesterol are bigger lipids that require assistance with absorption and transport to circulation. Short- and medium-chain fatty acids and glycerol can be taken straight into the bloodstream once inside the intestinal cell. In the digestive cell, long-chain fatty acids and

monoglycerides recombine into triglycerides, which are then combined with cholesterol and fat-soluble vitamins to form transporters known as chylomicrons. The biggest chylomicrons with the most lipids (mostly TAGs) in their inner compartment are those with the lowest density. Very large-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) are characterized by shrinking size, less enclosed lipids, and rising density (Figure .2). Lipoproteins, except chylomicrons, could be categorized as nanoppapers because their sizes usually range from 1 to 100 nm. Similar to how milk, which is also hazy, is an emulsion of lipid/protein ppapers, larger lipoproteins, and chylomicrons create emulsions in the blood. When levels of chylomicrons are extremely high, the serum of individuals with high levels of lipids (hyperlipidemia) can appear milky white, particularly after consuming meals high in TAGs. Chylomicron and/or LDL amounts are most likely elevated in the creamy white plasma at the top (lower density). Red blood cells make up the majority of the bottom stratum. Apolipoprotein A-I is the main protein in HDL, a lipoprotein that guards against arterial illness. The apo A-I's more hydrophobic sides face inward, allowing for interactions with the core's hydrophobic lipids.

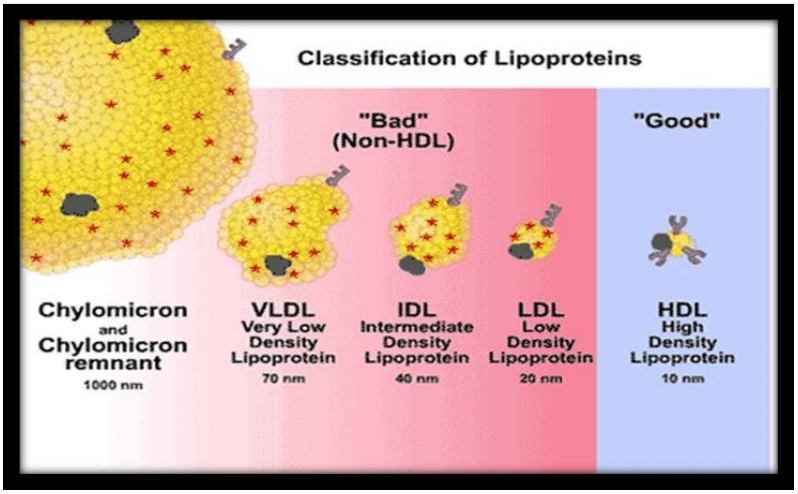

Figure 2: Lipoprotein: Diagramed showing the different types of lipoprotein (Research gate).

It is most likely a normal early lipoprotein. It will help you visualize how proteins cover the ppaper's exterior. Most likely cylindrical are mature lipoproteins. 200 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholines (POPC) molecules, 20 cholesterol molecules, and one duplicate of apolipoprotein A-I make up this embryonic HDL in the model. All lipoproteins, except HDL, are beta-lipoproteins because they all contain the apo-B protein. Any lipoprotein having apo B100 was produced in the liver because it is a constant component of VLDL (i.e., it cannot be exchanged with other lipoproteins) and its metabolic derivatives. There are interchangeable proteins in lipoproteins. Contrarily, the small intestine's enterocytes create apo B-48, a protein that is 48% smaller than apo B100 and thus uniquely identifies the lipoproteins (chylomicrons and chylomicron fragments) that were put together there. Apo B100 has a molecular weight of 555K and more than 4500 amino acids.

Except for an early stop codon that results in the shortened truncated apo B-48, the gene for the intestinal apo B48 is the same as the gene for apo B100. The Apo proteins attach to particular cell receptors, which may facilitate lipoprotein absorption. For instance, the apo B-100 protein of chylomicrons, which is located away from the apo B48 protein, is where the LDL receptors attach. Additionally, it attaches ApoE, which is mainly located on HDL and VLDL, though some are also found in LDL. The ApoB/ApoE receptor is another name for

the LDL receptors. LDL makes up more than 90% of the apoB-containing ppapers in circulation. Additionally, chylomicrons are only found in the blood after ingestion. For the metabolism of lipoproteins, some apoproteins can serve as cofactors and inhibitors. Chylomicrons become water-soluble due to this outer barrier, allowing them to move through the body's watery habitat. Small intestine chylomicrons first enter lymphatic capillaries before being transported to circulation (Figure. 3).

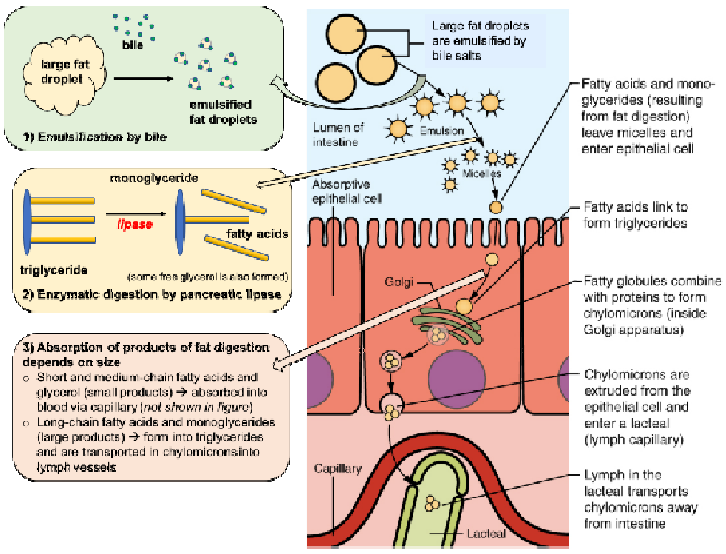

Figure 3: Lipid absorption in the small intestine: Diagramed showing the overview of Lipid digestion and adsorption in the small intestine (Open Oregon).

A baby's food should contain lipids in large quantities. Similar to whole cow's milk, breast milk has about 4% fat. Whether breastfed or given formula, a baby gets about half of its energy from fat, which is crucial for brain growth. However, bile and pancreatic enzyme secretion, which are vital components of lipid metabolism in later children and adults, are produced in minimal amounts in newborns.Infants can successfully process fat thanks to a few unique modifications. First off, they are born with a lot of oral and stomach lipases. Compared to adults, babies are much more dependent on these enzymes. Second, the lipase enzymes in breast milk are triggered in the infant's tiny intestine. In other words, the mother produces lipases and gives them to the infant through breast milk to aid in the digestion of milk lipids. Young babies can effectively process fat and benefit from its nutritional content for growth and mental development thanks to enhanced activity of lingual and gastric lipases and lipases found in breast milk. According to studies, premature babies given breast milk as opposed to the formula have more effective fat absorption. Even pasteurized breast milk, which is used to nourish infants in hospitals when given breast milk, is a little more difficult to digest because heat denatures the lipases. (Pasteurized breast milk and formula can still be digested by infants; they just do so less effectively and receive less of the end products of triglyceride metabolism[4], [5].

DISCUSSION

Body fat and diet are necessary for survival. The recognition, intake, digestion, absorption, and elimination of fat are all controlled by fatty acids. Although the entire body is directly affected, a large portion of this control is caused by impulses that come from the oral cavity. Although olfactory and gustatory components are supported by growing electrical, behavioral, and clinical data, textural cues for nutritional fat still predominate. Long-chain unsaturated, but possibly also saturated, fatty acid orosensory stimulation causes a variety of

cephalic phase reactions, such as the release of gastric lipase, the secretion of pancreatic digestive enzymes, the mobilization of lipids from the previous meal stored in the intestine, the secretion of pancreatic endocrine hormones, and, most likely indirectly, changed lipoprotein lipase activity. These mechanisms work together to affect postprandial lipemia. There is early proof of significant individual variation in the "taste" of fat, which may have unfavorable health effects. Further investigation is required to rule out the chance that systemic response to fat causes fat taste sensitivity.

Along with proteins, lipids, and their component fatty acids make up the majority of fish's organic matter. They are important sources of metabolic energy for development, including breeding, and mobility, including migration. Furthermore, the fatty acids in fish lipids are abundant in n-3 long-chain, highly unsaturated fatty acids (n-3 HUFA), which play crucial roles in crucial physiological processes and are especially significant in fish and the human diet. Indeed, seafood is the best provider of these essential nutrients for humans. Fish lipids have therefore attracted attention for a very long time due to their distinctive properties and quantity. This review aims to summarize the current state of our understanding of various aspects of the fundamental biochemistry, metabolism, and roles of fatty acids and the lipids they make up in a fish while, whenever possible, connecting that understanding to both wild and farmed fish. In doing so, it draws attention to the areas that need more in-depth study as well as the growing use of molecular technologies in fish lipid metabolism, which will facilitate advancements in molecular biological and genetic methods like genetics and proteomics.

Although not the only variable that affects the fatty acid composition of ruminant milk and flesh, fatty acid metabolism in rumen plays a significant role. Lipoproteins carry ingested lipids from the small intestine to an animal's various tissues and organs, where they undergo biochemical changes. Because of this, even though ruminants primarily absorb long-chain saturated fatty acids, some particular isomers are found in milk and flesh but not in feeds. Although the effects of many of them on human health are still not fully known, some have been shown to have positive health benefits in the prevention of lifestyle diseases and are thus desirable in food from the perspective of the mindful consumer[6]–[8].

Beyond their energy levels and fatty acid (FA) profiles, lipids are now being examined from a nutritional perspective. Dietary FA is the fundamental component of a wide range of more complex molecules, including triacylglycerols (TAG) and phospholipids (PL), which are organized in supramolecular frameworks and exhibit a variety of temperature behaviors. Typically, they are incorporated into intricate dietary matrices. Recent studies have shown that the lipids' supramolecular and molecular shapes, as well as whether they are liquid or solid at body temperature, have an impact on the digestion and absorption of ingested FA. The purpose of this review is to highlight recent findings regarding the effects of

(i) the intramolecular structure of TAG;
(ii) (ii) the type of lipid molecules carrying FA;
(iii) (iii) the supramolecular organization and physical state of lipids in native and formulated food products; and
(iv) (iv) the food matrix on FA digestion, absorption, and metabolism.
(v) To build a more trustworthy body of evidence and incorporate this information into upcoming nutritional guidelines, more work needs to be done right away. Additionally, it is possible to envision novel lipid formulations that take into consideration the health advantages of either the original or recomposed structures of lipids.

Dietary lipids (triacylglycerols, cholesteryl esters, and phospholipids) as well as endogenous lipids from bile (phospholipids and cholesterol) and shed intestinal epithelial cells are among the lipids that reach the digestive system. In this paper, we provide a thorough review of intestinal lipid digestion, uptake, and intracellular resynthesize in addition to their packaging into pre-chylomicrons in the endoplasmic reticulum, modification in the Golgi apparatus, and exocytosis into the lamina propria and subsequently to lymph. We also talk about the intestinal HDL and VLDL release, cytosolic lipid ppapers, and fatty acid oxidation as other outcomes of intestinal lipids. We also emphasize the relevance of these discoveries to human illness and the advancement of therapies that target lipid metabolism. We conclude by examining the newly discovered function of gut bacteria in controlling intestinal lipid metabolism and outlining important research issues.

Meat quality is influenced by the amount of intramuscular fat (IMF), which differs between pig types. The fundamental processes of the various IMF depositions in the various genetic backgrounds of pigs have not yet been completely clarified. In theory, lipid metabolism plays a role in the variance of IMF concentration. SREBP-1c and FAS, fatty acid transporting genes H-FABP and A-FABP, fatty acid oxidative gene CPT-1B, fatty acid oxidative gene and lipolytic gene ATGL and HSL, as well as the desaturated fatty acid gene, were among the genes and proteins whose expression levels and enzyme activities were examined. (SCD). Samples of the longissimus muscle were taken from slender Landrace pigs and obese Wujin pigs. The average daily gain of Wujin piglets was discovered to be smaller than that of Landrace pigs. Adipocyte diameter, IMF concentration, and PUFA proportion were all higher in Wujin pigs than in Landrace pigs. Wujin pigs showed greater amounts of FAS, SREBP-1c, SCD, A-FABP, and H-FABP gene expression compared to Landrace pigs, while CPT-1B, HSL, and ATGL gene expression was lower. Wujin pigs had greater levels of mRNA, protein expression or anabolic enzyme activities, fatty acid transportation, desaturation, and reduced levels of catabolism overall. As a result, the greater capacity of lipogenesis and fatty acid transportation and the lower capacity of lipolysis may account for the higher IMF concentration in fatty swine[9], [10].

The phrase "fat burner" refers to dietary products that make claims about having the ability to quickly increase fat metabolism or energy consumption, reduce fat absorption, boost weight loss, increase fat oxidation during exercise, or in some other way bring about long-term changes that favor fat metabolism. These supplements frequently include several components, each of which has a different suggested mode of action. It is frequently asserted that the mixture of these ingredients will have additive effects. The most common supplements are caffeine, carnitine, green tea, conjugated linoleic acid, forskolin, chromium, kelp, and fucoxanthin. Many supplements are said to enhance or improve fat metabolism. The research supporting some of these nutrients is succinctly outlined in this overview. The material that is currently accessible provides evidence for the fat metabolism-enhancing effects of caffeine and green tea. There is insufficient proof for many other supplements, even though some show some potential. The roster of supplements is industry-driven and is probably going to expand faster than the body of empirical evidence supporting them.

CONCLUSION

Fat and other dietary components are separated in the gut. Bile emulsifies lipids in the intestinal tract while enzymes break them down. The lipids are absorbed by the gastrointestinal walls. Chylomicrons are big lipoprotein structures made of long-chain fatty acids that carry lipids through the lymphatic system. Adipose triglyceride lipase aids in the release of fat in adipose tissue. Micelles deliver free fatty acids and monoglycerides, the end products of lipid digestion, to the walls of the digestive system for assimilation. The digestive

system begins to overcome a minor obstacle as stomach contents move into the small intestine, namely, combining the divided lipids with its watery secretions. Fatty acids from adipose cells are produced during moments of duress when the body needs energy and are mobilized for use. When blood glucagon and epinephrine levels rise, these hormones attach to specific binding sites on the outside of adipose cells to start the process. Energy, development, and membrane formation are three critical requirements of cancer cells that can be supported by fatty acid metabolism. Additionally, fatty acid oxidation, a major source of nicotinamide phosphate, catabolizes fatty acids.

REFERENCES:

[1] N. Yang *et al.*, "Interfacial behaviour of ß-lactoglobulin aggregates at the oil-water interface studied using particle tracking and dilatational rheology," *Soft Matter*, 2021, doi: 10.1039/d0sm01761b.

[2] P. Tso and J. A. Balint, "Formation and transport of chylomicrons by enterocytes to the lymphatics," *American Journal of Physiology - Gastrointestinal and Liver Physiology*. 1986. doi: 10.1152/ajpgi.1986.250.6.g715.

[3] H. Meng *et al.*, "Knockout of zebrafish colony-stimulating factor 1 receptor by CRISPR/Cas9 affects metabolism and locomotion capacity," *Biochem. Biophys. Res. Commun.*, 2021, doi: 10.1016/j.bbrc.2021.02.122.

[4] H. I. Friedman and B. Nylund, "Intestinal fat digestion, absorption, and transport. A review," *American Journal of Clinical Nutrition*. 1980. doi: 10.1093/ajcn/33.5.1108.

[5] S. Shaheen *et al.*, "Evaluation of nutraceutical potential of five selected wild edible plants of lahore, pakistan," *Pakistan J. Agric. Sci.*, 2020, doi: 10.21162/PAKJAS/20.9768.

[6] B. O. Schneeman and D. Gallaher, "Effects of Dietary Fiber on Digestive Enzyme Activity and Bile Acids in the Small Intestine," *Proc. Soc. Exp. Biol. Med.*, 1985, doi: 10.3181/00379727-180-42197.

[7] X. Y. Fu, Z. Li, N. Zhang, H. T. Yu, S. R. Wang, and J. R. Liu, "Effects of gastrointestinal motility on obesity," *Nutr. Metab.*, 2014, doi: 10.1186/1743-7075-11-3.

[8] A. S. Pavithra, R. Gayatri Devi, and A. Jyothipriya, "Effect of orange on constipation patients," *Drug Invent. Today*, 2019.

[9] M. J. Lehane, "Transcellular absorption of lipids in the midgut of the stablefly, Stomoxys calcitrans," *J. Insect Physiol.*, 1977, doi: 10.1016/0022-1910(77)90121-4.

[10] E. J. W. BARRINGTON, "GASTRIC DIGESTION IN THE LOWER VERTEBRATES," *Biol. Rev.*, 1942, doi: 10.1111/j.1469-185X.1942.tb00429.x.

CHAPTER 9

ALPHA, BETA, AND OMEGA OXIDATION OF THE FATTY ACIDS

Dr. Sunita Rao, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id-sunita.rao@jnujaipur.ac.in

ABSTRACT:

The process of breaking down fatty acids is called fatty acid catabolism or oxidation of fatty acids. A significant quantity of energy is produced during the exothermic reaction known as fatty acid oxidation. The mitochondria and brain cells both undergo fatty acid oxidation reactions. The oxidation of fatty acids can occur in three different ways: beta, alpha, and omega. The biological system of fatty acids undergoes beta-oxidation during oxidation. We quickly addressed the various oxidation pathways for fatty acids in this paper.

KEYWORDS:

Acyl CoA, Alpha Oxidation, Beta Oxidation, Fatty Acids, Omega Oxidation.

INTRODUCTION

Similar to how glucose is broken down, the fatty acids produced during the breakdown of triglycerides and other lipids are also gradually converted into usable energy. Nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide are needed for some of these reactive processes (FAD). Along with the enzymes of the citric acid cycle, the electron transport chain, and oxidative phosphorylation, the fatty acid catabolism-related enzymes are housed in the mitochondria. The effective utilization of energy held in fatty acids and other molecules is made possible by this localization of enzymes in the mitochondria, which is of paramount significance [1]. Using membrane transporters, fatty acids can be transported by membranes either directly or indirectly. The majority of fatty acids, however, have 14 or more carbons and must be transported across membranes to reach the mitochondrial matrix. This is accomplished in a straightforward but effective way. One of the ACSLs in the exterior mitochondrial membrane starts the transfer. Alternatively, mitochondrial glycerol-3-phosphate acyltransferase can use the formed acyl-CoAs to synthesize lysophosphatidic acid, the first stage in the glycerol-3-phosphate pathway, by transporting them into the mitochondrial matrix and oxidizing them to generate ATP. A membrane-bound CPT-I uses acyl-CoA which is intended for mitochondrial oxidation as its substrate to catalyze the synthesis of acyl-carnitine from acyl-CoA (Figure .1).

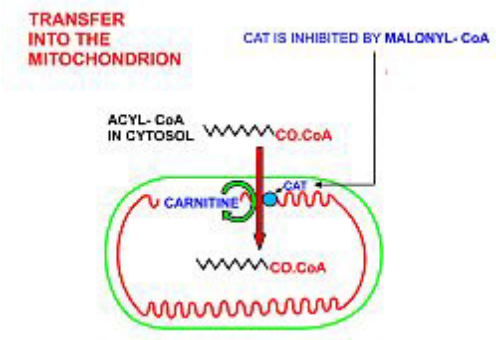

Figure 1: Fatty acid Transport: Daigrame shows the transport of fatty acids (Wikipedia).

Carnitine: acylcarnitine translocase, a protein that transfers both carnitine and acyl-carnitine to the cytosol and the mitochondrial matrix, respectively, receives acyl-carnitine after it crosses the outer mitochondrial membrane and reaches the interior mitochondrial membrane. When acyl-carnitine is present in the mitochondrial matrix, CPT-II catalyzes the reversible transfer of carnitine to CoA to replenish acyl-CoA, which then moves into the -oxidation pathway. It is significant to remember that the rate-limiting stage for the oxidation of fatty acids in mitochondria is the carnitine-mediated entrance mechanism. Three major stages make up the oxidation of mitochondrial fatty acids. Starting at carbon atom 3, the -carbon of the fatty acyl chain, fatty acids experience oxidative removal of consecutive two-carbon units as acetyl-CoA in the first stage. Acyl-CoA dehydrogenase, enoyl-CoA hydratase, hydroxy acyl-CoA dehydrogenase, and ketoacyl-CoA thiolase all play a role in the process. In seven cycles of this oxidative process, a 16-carbon fatty acid called palmitic acid loses two carbons to acetyl-CoA, producing a total of eight molecules.

The citric acid cycle's second phase, which also takes place in the mitochondrial matrix, includes oxidizing acetyl-CoA to CO2. The electron transport chain uses the flavin adenine dinucleotide and nicotinamide adenine dinucleotide (NADH) generated during -oxidation to make ATP in the third step. By way of the mitochondrial respiration chain, electrons from the first two stages are transferred to O2, supplying the necessary energy for oxidative phosphorylation, which produces ATP. A palmitic acid molecule can be completely oxidized to produce 129 ATP molecules as the overall output. Some fatty acids that are found in nature have an unusual amount of carbon atoms. In each round of the oxidative process, two carbons are removed as acetyl-CoA, further oxidizing these fatty acids. However, acetyl-CoA and propionyl-CoA are the byproducts of the thiolytic cleavage of fatty acids with an uneven amount of carbon atoms. To fully metabolize propionyl-CoA into succinyl-CoA, which can then be fully metabolized into oxaloacetate, a precursor for gluconeogenesis, three additional catabolic processes are required [2].

The oxidation of fatty acids can occur in one of three ways: The most frequent method of oxidizing fatty acids is called alpha oxidation. Energy is created when fatty acids are broken down into acetyl CoA, which is then transformed into carbon dioxide and water. It takes place in the peroxisomes. Single carbon atoms are eliminated from the carboxyl end through the process of beta-oxidation. The fact that phytanic acids have a methyl group that prevents -oxidation makes this route crucial for the oxidation of those acids. Fatty acids undergo methyl group removal during -oxidation, which is followed by beta-oxidation. In some species, this alternative route to beta-oxidation occurs. To learn more about omega oxidation, read this text [3]. Alpha Oxidation Process: The main target of alpha oxidation is phytanic acid, a branched-chain fatty acid that is acquired by people from the fat of ruminant mammals, dairy products, and plant materials. Chlorophyll, which is found in plant elements, causes people to produce phytanic acid. The following are the stages involved in phytanic acid's alpha oxidation:

Phytanoyl-CoA is created when phytanic acid first joins forces with CoA. Phytanoyl-CoA dioxygenase uses Fe2+ and O2 to oxidize the phytanoyl-CoA to 2-hydroxy phytanoyl-CoA. In the presence of 2-hydroxyphytanoyl-CoA lyase, 2-hydroxy phytanoyl-CoA is broken to produce pristanal and formyl-CoA. Aldehyde dehydrogenase causes pristanal to oxidize and produce pristanic acid. After that, the pristanic acid may go through beta-oxidation (Figure. 2). Simpler fatty acid types are produced by alpha oxidation and can then go through beta-oxidation. For instance, phytanic acids cannot undergo beta oxidation because they contain a methyl group. By decarboxylating the methyl molecule, alpha oxidation eliminates it, allowing beta-oxidation to continue. It generates intermediary hydroxy fatty acids that can be

used to synthesize cerebrosides and sulfatides, like cerebronic acid. Odd-chain fatty acids are created during the decarboxylation of fatty acids and can be used to create sphingolipids [4].

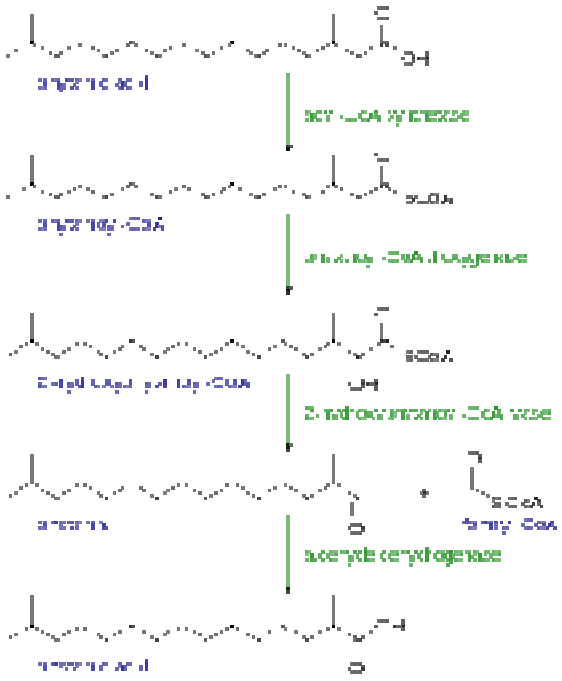

Figure 2: Alpha oxidation: Diagram showing the steps involved in Alpha oxidation (Wikipedia).

The long carbon chains of the fatty acids (in the form of acyl-CoA molecules) are then cut by beta-oxidation in the mitochondrial matrix into a series of two-carbon (acetate) units. These acetate units combine with coenzyme A to form molecules of acetyl CoA, which condense with oxaloacetate to form citrate at the "beginning" of the citric acid cycle. It is useful to think of this reaction as the "starting point" of the cycle because it introduces the fuel, acetyl-CoA, which will be released as CO2 and H2O during each cycle turn, and oxidative phosphorylation that follows. A significant amount of energy will also be captured in the form of ATP.

The stages in beta-oxidation are, in brief, as follows: Acyl-CoA dehydrogenase dehydrogenation, producing 1 $FADH_2$. Enoyl-CoA hydratase-mediated hydration. Using 3-hydroxy acyl-CoA dehydrogenase to dehydrate, 1 NADH + H+ is produced. Thiolase cleavage results in the production of 1 acetyl-CoA and a fatty acid that has undergone a 2-carbon reduction (forming a new, shortened acyl-CoA). Once the fatty acid has been fully reduced to acetyl-CoA, or, in the case of fatty acids with odd amounts of carbon atoms, acetyl-CoA and one molecule of propionyl-CoA per fatty acid molecule, this beta-oxidation process is repeated. In oxidative phosphorylation, each beta oxidative cut of an acyl-CoA molecule ultimately results in the production of 5 ATP molecules. The mitochondrion's citric acid cycle is where the acetyl-CoA created by beta-oxidation enters. It does this by joining with oxaloacetate to create citrate. When combined with oxidative phosphorylation, this causes the acetyl-CoA to completely burn off into CO2 and water. Per acetyl-CoA molecule that is oxidized, the energy produced during this process is collected as 11 ATP molecules and 1 GTP molecule. Acetyl-CoA undergoes this destiny wherever beta-oxidation of fatty acids happens, except in the liver under specific conditions.

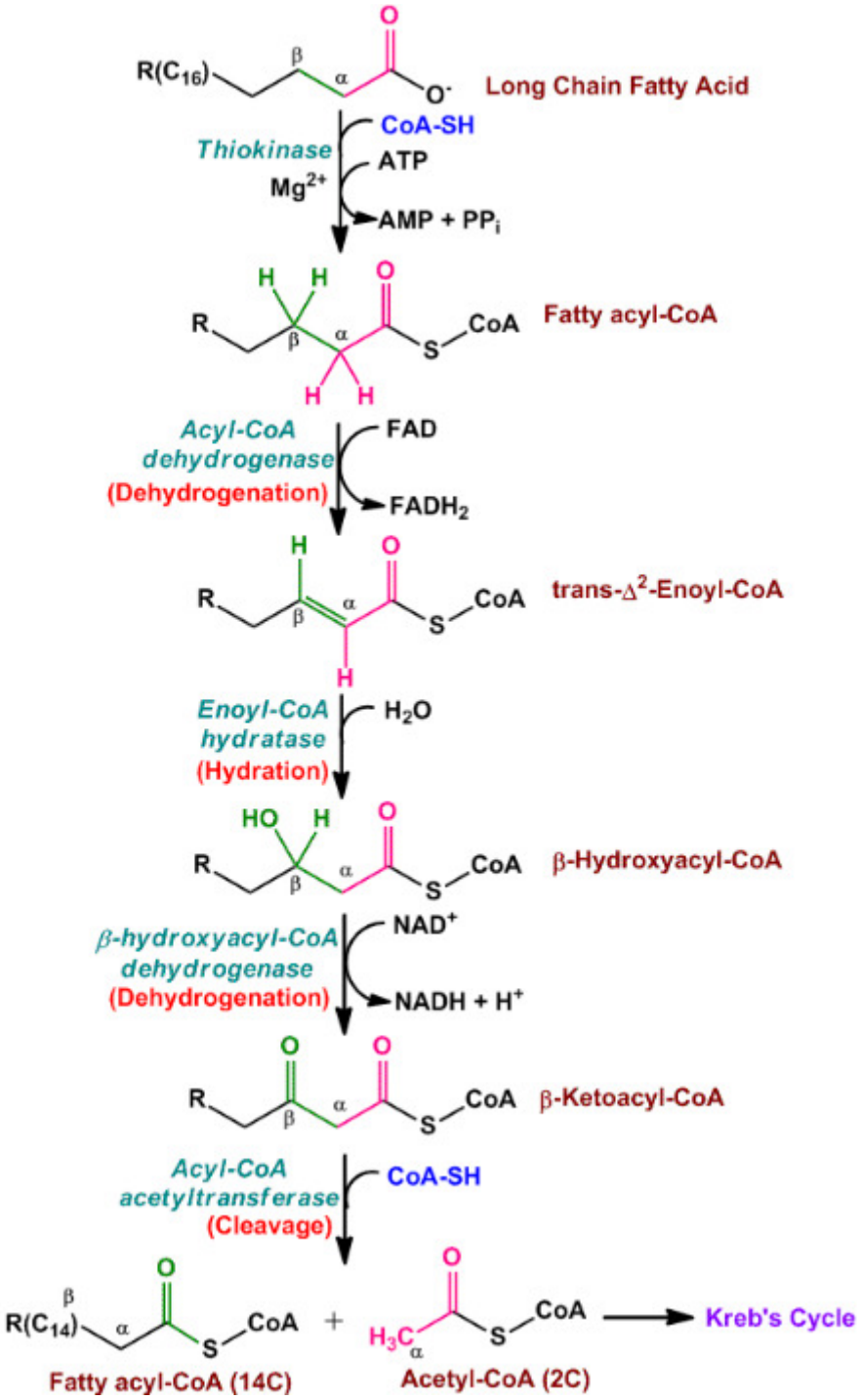

Figure 3: Beta oxidation: Diagram showing the steps involved in Beta oxidation (Simple pharmanotes).

Propionyl-CoA is then successively transformed into succinyl-CoA by the enzymes methyl malonyl-CoA mutase (MCM), which is dependent on vitamin B12, and propionyl-CoA carboxylase, which is dependent on biotin. Succinyl-CoA is first changed into malate, then into pyruvate, and after that, it is delivered to the matrix to begin the citric acid cycle (Figure. 3).

Oxaloacetate can be completely or partly redirected into the gluconeogenic pathway in the liver under certain conditions, including fasting, starvation, a low-carbohydrate diet, protracted intense exercise, and untreated type 1 diabetes. In these conditions, oxaloacetate is

hydrogenated to malate, which is then taken out of the liver cells' mitochondria and changed into glucose in the cells' cytosol before being discharged into circulation. As a result, when substantial gluconeogenesis has been induced by low (or nonexistent) insulin and high glucagon concentrations in the blood, oxaloacetate is not accessible in the liver for condensation with acetyl-CoA. Acetyl-CoA is redirected under these circumstances to the production of acetoacetate and beta-hydroxybutyrate.

Some mammal species go through a process called omega oxidation, also known as -oxidation, to metabolize fatty acids. It is a different route to beta-oxidation that uses the oxidation of the carbon instead of the carbon. The carbon most distant from the carboxyl group of the fatty acid. When oxidation is impaired, the process which is typically a secondary catabolic route for medium-chain fatty acids (10–12 carbon atoms) becomes more significant.

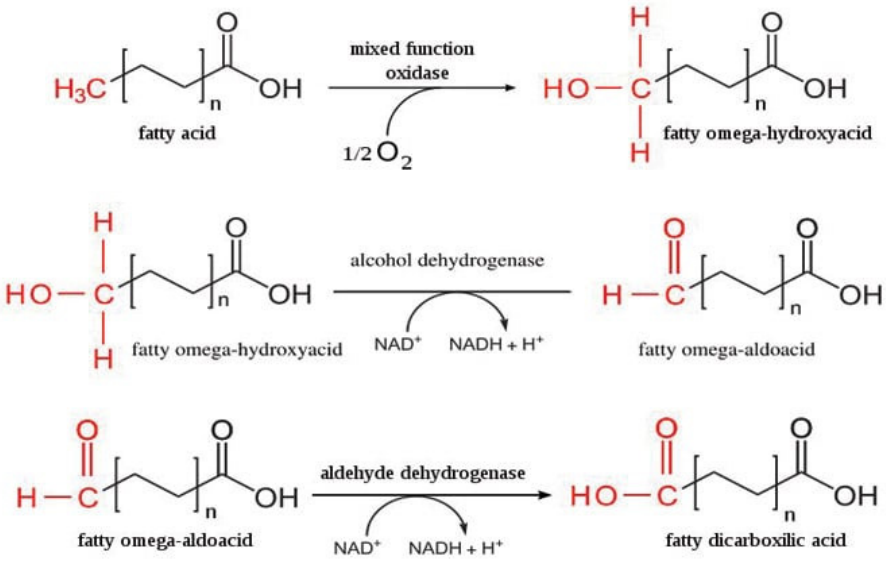

Figure 4: Omega oxidation: Diagram showing the steps involved in Omega oxidation (Microbe notes).

In contrast to oxidation, which occurs in the mitochondria, oxidation occurs in the smooth ER of liver and renal cells in mammals. The following are the stages in the process: Mixed-function hydroxylation oxidaseThe hydroxyl molecule is first added to the carbon in the first stage. The oxygen for the group is provided by molecular oxygen in a complicated reaction carried out by electron donor NADPH. Alcohol dehydrogenase for oxidationThe hydroxyl group is then oxidized by NAD+ to an aldehyde in the following stage (Figure.4). Aldehyde oxidase dehydrogenaseNAD+'s oxidation of the aldehyde molecule into a carboxylic acid is the third stage. This process results in the formation of a fatty acid with carboxyl groups at both ends [5].

Either end of the fatty acid can be joined to coenzyme A after these three procedures. Following that, the molecule can infiltrate the mitochondrion and go through oxidation. Adipic acid and succinic acid are the byproducts of subsequent oxidations; succinic acid can participate in the citric acid cycle. A hydroxy residue is added to the omega carbon of short, middle, and long-chain unsaturated or saturated fatty acids during the first stage of -oxidation, which can be used to create or inactivate signaling molecules. Arachidonic acid, also known as eicosatetraenoic acid, is metabolized by a subgroup of Cytochrome P450 (CYP450) microsome-bound -hydroxylases to 20-hydroxyeicosatetraenoic acid in people. In animal and cellular model systems, 20-HETE has a variety of activities, including

constriction of blood vessels, alteration of salt and water reabsorption by the kidney, and stimulation of the growth of cancer cells. Genetic studies in people suggest that 20-HETE causes hypertension, myocardial infarction, and brain stroke 20-Hydroxyeicosatetraenoic acid. Members of the CYP4A and CYP4F subfamilies of the cytochrome P450 superfamily, specifically CYP4A11, CYP4F2, and CYP4F3, are thought to be the main cytochrome P450 enzymes in charge of making 20-HETE in the majority of tissues. In a smaller variety of tissues, CYP2U1 and CYP4Z1 add to the synthesis of 20-HETE. The cytochrome ω-oxidases including those belonging to the CYP4A and CYP4F sub-families and CYPU21 also ω-hydroxylate and thereby reduce the activity of various fatty acid metabolites of arachidonic acid including LTB4, 5-HETE, 5-oxo-eicosatetraenoic acid, 12-HETE, and several prostaglandins that are involved in regulating various inflammatory, vascular, and other responses in animals and humans. The suggested functions of the cytochromes in modulating inflammatory reactions and the documented correlations of specific CYP4F2 and CYP4F3 single nucleotide variants with human Crohn's disease and Celiac disease, respectively, may be explained by this hydroxylation-induced inactivation.

DISCUSSION

The maritime atmosphere has been studied using specific organic molecules as tracers of biotic and human-caused source inputs1–8. To pinpoint the origins of the organic compounds in aerosols, this tracer method employs distinct molecular signatures or fingerprints for different lipid compound classes of marine and terrestrial plants9. Many of these materials, though, are unstable and go through transition processes. For instance, distant marine aerosols have not consistently contained unsaturated fatty acids, which are important components of marine and land plants. Unsaturated fatty acid transformation in the environment is not yet fully understood by the process involved. The finding of a homologous sequence of Beta-oxocarboxylic acids (C4-C14; C9 being the highest) in marine aerosols are described in this study. According to our hypothesis, these substances are byproducts of the decomposition of unsaturated fatty acids in the aquatic environment. We propose a photo-induced oxidative reaction scheme for unsaturated fatty acids in the marine atmosphere and surface saltwater based on the finding of -oxoacids and new aerosol, seawater, and rain data for mono- and dicarboxylic acids [6]. The acetyl-CoA carboxylases ACC1 and ACC2 produce malonyl-coenzyme A (malonyl-CoA), which is a crucial molecule in the control of energy balance. Here, we demonstrate that rodents with the Acc2 / mutation have a typical lifespan, a greater rate of fatty acid oxidation, and less fat. Malonyl-CoA levels in the heart and muscle of Acc2-deficient animals were 10- and 30-fold lower than those in the normal type, respectively. The soleus muscle of the Acc2 / mice oxidized fatty acids at a rate that was 30% greater than that of wild-type mice and was unaffected by the addition of insulin; in contrast, the wild-type muscle oxidized fatty acids at a rate that was 45% lower after the addition of insulin. In comparison to wild-type mice, the mutant animals acquired 50% less fat in their adipose tissue. These findings suggest that, in the setting of regular caloric consumption, pharmacological modulation of ACC2 may result in a reduction in body fat. We present a new method for -oxidizing polyunsaturated fatty acids (the reductase-dependent route). In addition to 3-cis-2-trans-enoyl-CoA isomerase and the enzymes required for the oxidation of saturated fatty acids, this pathway also necessitates the participation of a 2,4-dienoyl-CoA reductase that is NADPH-dependent. The initial route, which used the auxiliary enzyme 3-hydroxy acyl-CoA epimerase, is inactive in mitochondria but might have a small function in -oxidation systems that are not mitochondrial [8]. The primary route for the fatty acid breakdown in the human body is mitochondrial fatty acid -oxidation (FAO), which is crucial for preserving energy balance. When the availability of glucose is limited in the postabsorptive and fasted phases, fatty acids are an essential energy source. FAO is the

primary energy source for the heart, skeletal muscle, and kidney even when glucose is widely accessible. FAO is facilitated by several enzymes, transporters, and other proteins. The majority of the genes that code for these proteins have documented recessively hereditary defects. The clinical presentation of these disorders may include hypoketotic hypoglycemia, (cardio) myopathy, arrhythmia, and rhabdomyolysis and illustrates the importance of FAO during fasting and in hepatic and (cardio) muscular function. We outline the current state of knowledge regarding the biochemistry and metabolic operations of the FAO in this overview, as well as the pathophysiological processes connected to FAO diseases [9]. To maintain contractile activity in the heart and oxidative skeletal muscle, fatty acids are a significant fuel source. The uptake and -oxidation of fatty acids must be coordinatedly controlled to guarantee a sufficient, but not excessive, supply for mitochondrial Beta-oxidation to satisfy the energy requirements of these muscles. However, a mismatch between fatty acid uptake and -oxidation may be a factor in insulin intolerance in the muscle. An intracellular signaling cascade that eventually results in insulin-mediated changes in several cellular processes, including an increase in glucose transport, is triggered by the binding of insulin to its receptor and activation of the receptor's intrinsic protein tyrosine kinase activity. This insulin signaling route may change as a result of an accumulation of fatty acids and lipid molecules (such as long-chain acyl CoA, diacylglycerol, triacylglycerol, and/or ceramide).

Due to lipid buildup and inhibition of one or more stages in the insulin-signaling pathway, it is assumed that an imbalance between fatty acid uptake and oxidation is the main cause of insulin resistance in obesity and diabetes. Therefore, lowering the absorption of fatty acids by muscle can enhance insulin sensitivity. However, there is debate over the possible benefit of boosting fatty acid -oxidation in the heart or skeletal muscle to avoid cytoplasmic lipid buildup and reduce insulin resistance. While greater fatty acid -oxidation may reduce cytoplasmic lipid buildup, it can also slow down the metabolism of glucose in the muscle, and inadequate fatty acid oxidation may also be a factor in insulin resistance. In this overview, we address the possible pathways by which changes in fatty acid uptake and oxidation contribute to insulin resistance as well as the potential therapeutic benefits of targeting fatty acid uptake and oxidation in the treatment of insulin resistance [10]. An RNA transcript incorrectly labeled as non-coding encodes a conserved, protein-enriched in muscle called micro peptide regulator of -oxidation (MOXI). When MOXI interacts with the mitochondrial trifunctional protein, an enzyme complex that is essential for fatty acid -oxidation, it localizes to the interior mitochondrial membrane. In contrast to transgenic MOXI overexpression, isolated cardiac and skeletal muscle mitochondria from MOXI knockout animals show a reduced capacity to process fatty acids. Additionally, compared to wild-type (WT) mice, hearts from MOXI knockout mice selectively oxidize carbs over fatty acids in an isolated perfused cardiac system. The significant decrease in exercise ability seen in MOXI knockout mice emphasizes the importance of MOXI in metabolic regulation. The functional analysis of MOXI highlights the regulatory potential of additional unidentified micro peptides and sheds light on how mitochondrial metabolism and energy balance are regulated [11].

CONCLUSION

A fatty acid is converted into two units of acetyl coenzyme A during the process of fatty acid oxidation. The mitochondria use these chemicals to start producing energy. The beta-oxidation of fatty acids that occurs inside mitochondria involves the removal of two carbon atoms from acyl-CoA at the carboxyl end in the form of acetyl-CoA. A secondary oxidation process that takes place in peroxisomes is alpha oxidation. Between C1 and C2, the link is disrupted, releasing CO2 once every cycle. Another secondary oxidation process that takes

place in the ER is called omega oxidation. The methyl terminus of the molecule serves as the reaction's active region. Alpha oxidation primarily occurs in the brain and liver, in which one atom of carbon is lost in the form of a carbon dioxide molecule, while beta oxidation primarily occurs in the mitochondrial matrix, where two carbon units are released as acetyl CoA per cycle. This is the main distinction between alpha and beta-oxidation. Peroxisomes in humans use alpha-oxidation to convert nutritional phytanic acid, which cannot go through beta-oxidation because of its -methyl branch, into pristanic acid. Then, prostatic acid can pick up acetyl-CoA and undergo beta-oxidation, resulting in propionyl-CoA.

REFERENCES:

[1] "9.4: Oxidation of Fatty Acids," *Chemistry LibreTexts*, Jul. 15, 2020. https://chem.libretexts.org/Courses/Brevard_College/CHE_301_Biochemistry/09%3A_Metabolism_of_Lipids/9.04%3A_Oxidation_of_Fatty_Acids (accessed Mar. 23, 2020).

[2] R. Lehner and A. D. Quiroga, "Paper 5 - Fatty Acid Handling in Mammalian Cells," in *Biochemistry of Lipids, Lipoproteins and Membranes (Sixth Edition)*, N. D. Ridgway and R. S. McLeod, Eds. Boston: Elsevier, 2016, pp. 149–184. doi: 10.1016/B978-0-444-63438-2.00005-5.

[3] "Omega Oxidation of Fatty Acids," *BYJUS*. https://byjus.com/neet/omega-oxidation-of-fatty-acids/ (accessed Mar. 23, 2020).

[4] "Alpha Oxidation of Fatty Acids," *BYJUS*. https://byjus.com/neet/alpha-oxidation-of-fatty-acids/ (accessed Mar. 23, 2020).

[5] "Omega oxidation," *Wikipedia*. Dec. 13, 2020. Accessed: Mar. 23, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Omega_oxidation&oldid=994006019

[6] K. Kawamura and R. B. Gagosian, "Implications of ω-oxocarboxylic acids in the remote marine atmosphere for photo-oxidation of unsaturated fatty acids," *Nature*, vol. 325, no. 6102, Art. no. 6102, Jan. 1987, doi: 10.1038/325330a0.

[7] L. Abu-Elheiga, M. M. Matzuk, K. A. H. Abo-Hashema, and S. J. Wakil, "Continuous Fatty Acid Oxidation and Reduced Fat Storage in Mice Lacking Acetyl-CoA Carboxylase 2," *Science*, vol. 291, no. 5513, pp. 2613–2616, Mar. 2001, doi: 10.1126/science.1056843.

[8] H. Schulz and W.-H. Kunau, "Beta-oxidation of unsaturated fatty acids: a revised pathway," *Trends Biochem. Sci.*, vol. 12, pp. 403–406, Jan. 1987, doi: 10.1016/0968-0004(87)90196-4.

[9] S. M. Houten, S. Violante, F. V. Ventura, and R. J. A. Wanders, "The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders," *Annu. Rev. Physiol.*, vol. 78, no. 1, pp. 23–44, 2016, doi: 10.1146/annurev-physiol-021115-105045.

[10] L. Zhang, W. Keung, V. Samokhvalov, W. Wang, and G. D. Lopaschuk, "Role of fatty acid uptake and fatty acid β-oxidation in mediating insulin resistance in heart and skeletal muscle," *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids*, vol. 1801, no. 1, pp. 1–22, Jan. 2010, doi: 10.1016/j.bbalip.2009.09.014.

[11] C. A. Makarewich*et al.*, "MOXI Is a Mitochondrial Micropeptide That Enhances Fatty Acid β-Oxidation," *Cell Rep.*, vol. 23, no. 13, pp. 3701–3709, Jun. 2018, doi: 10.1016/j.celrep.2018.05.058.

CHAPTER 10

PEROXISOMAL AND MITOCHONDRIAL BETA OXIDATION

Dr. Manish Soni, Assistant Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id- manishsoni@jnujaipur.ac.in

ABSTRACT:

Beta oxidation is the process of the breakdown of the fatty acids molecules which occurs in the mitochondria and the peroxisomes.In human cells, peroxisomes perform a variety of metabolic tasks, including the process of very long-chain fatty acids, oxidization of branched-chain fatty acids, production of digestive acids and ether-linked phospholipids, and elimination of reactive oxygen species. Peroxisomal beta-oxidation doesn't rely on the energy requirements of the cells, whereas mitochondrial beta-oxidation does. The mitochondrial route is associated with degradation as well as the production of energy, whereas peroxisomal beta-oxidation plays a key role in pathways for biosynthesis. In this paper, we summarized the difference between peroxisomal and mitochondrial beta-oxidation.

KEYWORDS:

Beta Oxidation, Fatty Acids, Long Chain, Mitochondrial Oxidation, Peroxisomal Oxidation.

INTRODUCTION

A wide variety of physically and functionally distinct, membrane-enclosed organelles are present in eukaryotic cells. Such compartmentalization offers several distinct advantages, such as the ability to perform particular functions without interfering with other cellular processes, the creation of distinct local environments that facilitate particular metabolic functions, the sequestering of reaction intermediates and potentially toxic metabolites, and more. However, because they are not autonomous beings, cell organelles must engage and actively converse with other subcellular spaces to carry out their functions. With an emphasis on mammals, this review offers a thorough summary of what is presently understood about the functional and structural relationships between peroxisomes and mitochondria [1].

A peroxisome is a cell membrane. Almost all eukaryotic cells contain a membrane-bound organelle and form of the micro body, in their cytoplasm. The reactive cells are called peroxisomes. Molecular oxygen is frequently used as a co-substrate before hydrogen peroxide (H_2O_2) is produced. The production and recycling of hydrogen peroxide are how peroxisomes get their moniker. They play important functions in the reduction of reactive oxygen species and lipid biosynthesis. Peroxisomes are involved in the reduction of reactive oxygen species, specifically hydrogen peroxide, and the biosynthesis of plasmalogens, which are ether phospholipids essential for the healthy operation of mammalian brains and lungs. They also catabolize very long-chain fatty acids, branched-chain fatty acids, bile acid intermediates (in the liver), D-amino acids, and polyamines. They also have 10% of the overall activity of two enzymes in the pentose phosphate pathway, which is critical for energy production (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase). The role of peroxisomes in the production of isoprenoids and cholesterol in mammals is hotly contested. The glyoxylate cycle ("glyoxysomes") in the germination of seeds, photorespiration in leaves, glycolysis in trypanosomes, and methanol and/or amine oxidation and assimilation in some yeasts are additional recognized activities of peroxisomes

[2]. Similar to peroxisomes, mitochondria are active organelles that constantly modify their quantity, shape, and function in response to the surroundings. These structures are crucial for the production of adenosine triphosphate (ATP), the -oxidation of fatty acids, the production of ketone bodies, and the synthesis of iron-sulfur clusters in animals. Additionally, mitochondria function as critical nodes in cellular signaling networks that have a significant effect on a variety of biological processes, including gene expression, immune reactions, cell differentiation, and cell death. In light of this, it is not unexpected that mitochondrial dysfunction has been suggested to play a role in the etiology of numerous metabolic diseases.

Similar to peroxisomes, mitochondria are dynamic organelles that continuously adapt their size, structure, and function to their environment. Adenosine triphosphate synthesis, fatty acid -oxidation, ketone body synthesis, and the synthesis of iron-sulfur groups in mammals all depend on these structures. Furthermore, mitochondria serve as essential components in cellular communication networks that significantly influence a range of biological processes, such as gene translation, immune responses, cell differentiation, and cell demise. This makes it understandable why mitochondrial dysfunction has been implicated in the pathogenesis of numerous metabolic illnesses. Adipocyte-isolated fatty acid transport protein and very-long-chain fatty acyl-CoA synthase share 40% of their amino acid sequences.

Pristinic acid and other branched-chain fatty acids are primarily Beta oxidized in peroxisomes. Peroxisomes, mitochondria, and the endoplasmic reticulum are capable of activating branched-chain fatty acids produced from isoprenoids, presumably through the long-chain acyl-CoA synthetases found in these organelles. Uncertainty surrounds the presence of branched-chain acyl-CoA synthase in peroxisomes. Only the endoplasmic reticulum can generate dicarboxylic acids, prostaglandins, and the C27 bile acid precursors, di- and trihydroxycholestanoic acids. Trihydroxycoprostanoyl-CoA synthetase, a distinct enzyme found only in the liver, activates the bile acid intermediates that are created from cholesterol. Dicarboxylic acids and prostaglandins cannot be triggered by mitochondria or peroxisomes, which suggests that another enzyme than long-chain acyl-CoA synthetase is the proper substrate for these compounds.

Long-chain acyl-CoA esters are transported by a carnitine-dependent process through the inner membrane of the mitochondria and into the matrix, which is where the -oxidation events take place. To get to the mitochondrial matrix, short- and medium-chain fatty acid esters don't need a special delivery system. Because the peroxisomal membrane lacks CPT I and carnitine translocase, long-chain acyl-CoAs can enter the peroxisome matrix without the need for carnitine. In essence, the spread of amphiphilic fatty acyl-CoAs may be aided by the peroxisomal membrane's general permeability. Both carnitine octanoyl-transferase and carnitine acetyltransferase are found in the matrix of peroxisomes, but it is unknown how they work in peroxisomal -oxidation. Adrenoleukodystrophy protein (ALDP), a member of the ABC transporter family, and peroxisomal membrane protein (PMP) 70 are also present in peroxisomal membranes, but it is unknown whether or not they are involved in the transfer of fatty acids or fatty acyl-CoA [3].

Although short-, medium-, and long-chain fatty acids are predominantly catabolized by mitochondrial Beta-oxidation, several less-common carboxylates with various chemical structures are primarily degraded by peroxisomal -oxidation. VLCFAs (>C20), 2-methyl-branched fatty acids, dicarboxylic acids, prostanoids, and the C27 bile acid intermediates—which undergo beta-oxidation to become the mature C24 bile acid intermediates—are the substrates for peroxisomal -oxidation. Peroxisomal fatty acid oxidation follows a similar four-step process to mitochondrial oxidation: (a) an oxidation reaction in which the acyl-CoA is desaturated to a 2-trans-enoyl-CoA; (b) a hydration reaction in which the enoyl-CoA is

converted to a 3-hydroxy acyl-CoA; (c) a subsequent oxidation step in which the hydroxy intermediate is dehydrogenated to a 3-ketyl-CoA; and (d) thiolytic clear shorter than the initial molecule's carbon atoms, which can rejoin the spiral for the following round of -oxidation. An H_2O_2 catalyst catalyzes the initial process. A bifunctional protein performs the second and third stages (hydration and dehydrogenation), and a thiolase performs the fourth and final phase (thiolytic cleavage), all of which result in the production of acyl-CoA oxidase. In the last five years, it has become clear that most animals possess two or more acyl-CoA oxidases, two distinct bifunctional proteins, and two distinct thiolase.

Two distinct -oxidation pathways have been suggested to function within the peroxisome, mostly based on the substrate specificity of the oxidase that starts the first and rate-limiting phase (Figure 1). The classical route typically uses straight-chain saturated fatty acyl-CoAs as substrates, whereas the second -oxidation pathway, which was recently identified, works on 2-methyl-branched fatty acids and the intermediates of bile acids. In the L-hydroxy-specific classical -oxidation spiral, AOX catalyzes the first reaction, the dehydrogenation of acyl-CoA esters to their corresponding trans-2-enoyl-CoAs, while the second and third reactions, the hydration and dehydrogenation of enoyl-CoA esters to 3-ketoacyl-CoA, are carried out by a single enzyme, enoyl-CoA hydrate. 3-ketoacyl-CoA thiolase, the third enzyme in this conventional system, cleaves Acyl-CoA that is two carbon atoms shorter than the initial molecule and capable of reentering the -oxidation spiral are produced when 3-ketoacyl-CoA transforms into acetyl-CoA.

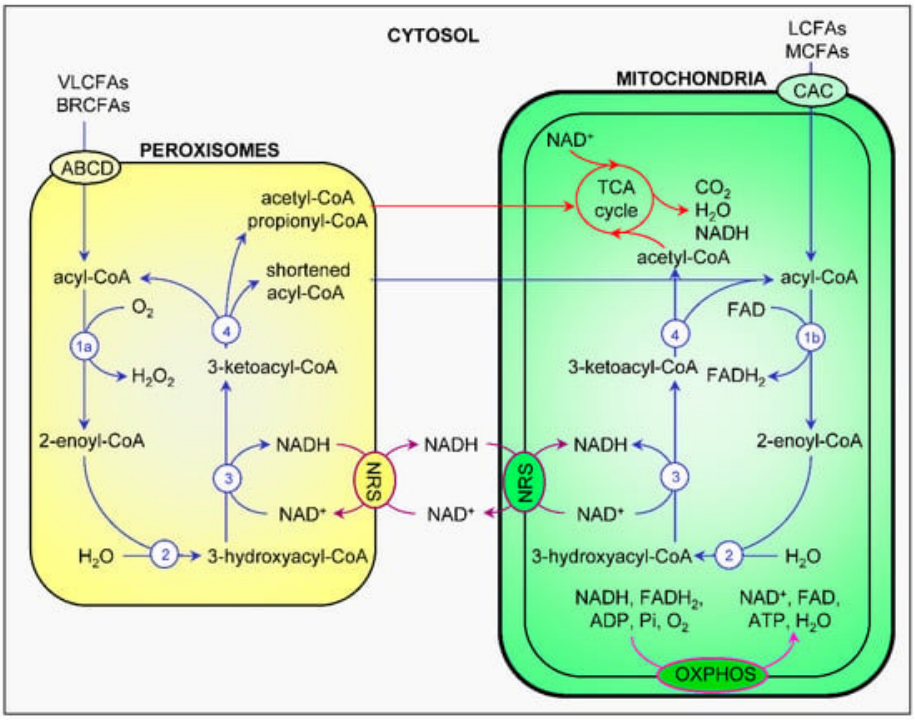

Figure 1: Beta oxidation: Diagram showing the Beta oxidation pathway in the mitochondria and the peroxisomes (MDPI).

In the liver of rats and mice, peroxisome proliferators and other biological PPAR ligands can powerfully activate all of the enzymes of this classical pathway. Humans' branched-chain acyl-CoA oxidase catalyzes the dehydrogenation of acyl-CoA esters to their respective trans-2-enoyl-CoAs in the second, D3-hydroxy-specific -oxidation pathway. D-3-hydroxy acyl-CoAs are then converted to 3-ketoacyl-CoAs by the newly discovered D-3-hydroxy acyl-CoA dehydratase/D-3-hydroxy acyl-CoA dehydrogenase (also known as multipurpose

protein-2). Sterol carrier protein (SCP)x, the second system's third enzyme, has thiolytic action in its N-terminal region. In rats, either trihydroxy coprostanol-CoA oxidase, which acts on bile acid intermediates, or pristinely-CoA oxidase, which promotes pristanic acid breakdown, perform the first desaturation step in this D-3-hydroxy-specific -oxidation spiral. However, the first and rate-limiting step of this second -oxidation pathway is only carried out by one enzyme in humans: the branched chain. New data firmly suggests that after the initial first desaturation phase catalyzed by specific oxidase, the division between two peroxisomal Beta-oxidation pathways may not be that rigid. It appears that either L-PBE or D-PBE can process the L- and D-hydroxy intermediates produced in the two -oxidation systems to varying degrees.

The majority of carbon-saturated fatty acids (SFAs) are broken down by mitochondrial Beta-oxidation. Unsaturated fatty acids (UFAs), branched-chain fatty acids (BCFAs), and very long-chain fatty acids (VLCFAs) require various oxidation processes, such as isomerization, alpha-oxidation Beta oxidation, omega-oxidation, and the oxidation process in peroxisomes. Peroxisomal -oxidation involves four consecutive processes, just like -oxidation in mitochondria. Although the reactions are similar, the differences in the catalytic proteins, electron transport chains, and orientations of the metabolites between mitochondria and peroxisomes indicate that studies on mitochondria cannot be applied to peroxisomes (Figure .1).VLCFAs enter the peroxisome as acetyl-CoA, initiate the second oxidation by starting the first oxidation, go through hydrolysis, go through thiolysis, and eliminate two C atoms. The cycle will be repeated if VLCFAs are still present until the number of carbon chains is less than 18, at which point the products enter the mitochondria to continue the metabolic process.

Although peroxisomal -oxidation contributes to fat metabolism, when faced with issues related to fat metabolism, particularly cancer, researchers frequently turn to mitochondria for answers. Consider prostate cancer (PCa) as an illustration. Numerous studies have shown that the development of PCa is correlated with free FAs and oxidative stress in the body, but the majority of studies concentrate on controlling mitochondria to prevent or cure PCa; as a result, peroxisomes have not yet been given significant consideration. Peroxisome Beta -oxidation can be seen as a potential focus in many issues, but it is never taken into consideration. Nowadays, the majority of research focuses on the relationship between peroxisomes and mitochondria, while less research has been done on its autonomous function. The oxidation of VLCFAs in fatty acid oxidation (FAO) is a characteristic of peroxisomes that varies from mitochondria.

Although the function of peroxisomes has been taken into consideration, the mitochondrial -oxidation pathway has long been thought to play a crucial part in lipid degradation. Any blockage of the oxidative pathway results in an increase in the amount of lipid present in tissues. Many illnesses can develop as a result of the body's vital components, VLCFAs, being dysregulated. Studies on people have demonstrated that the buildup of VLCFAs is the primary cause of several brain conditions, including Alzheimer's disease, multiple sclerosis, and dementia. Additionally, VLCFAs have been linked to ichthyosis, myopathy, and demyelination in investigations of sexually spread diseases. A deadly neurodegenerative pattern, including childhood-onset cerebral adrenoleukodystrophy (CCALD) and adrenomyeloneuropathy (AMN), is caused by VLCFAs accumulating in the plasma and tissue of patients. The weaker phenotype known as AMN is marked by an axonopathy that worsens over time. As a consequence, VLCFAs are not only an essential component of the body but also a substance that, in the event of in vivo dysregulation, may have powerful toxic effects.

Free FAs are primarily attached to fatty acid-binding proteins in living creatures and are typically present in low concentrations. In this instance, the main mode of FAO is mitochondrial -oxidation, and FAs are typically generated in vivo by the breakdown of deposited fat and essentially do not contain VLFCAs. ACOX1 works to ensure that peroxisomes can preferentially process VLCFAs that are not suitable for the internal environment by activating the transport capacity through the transporter as soon as the toxic VLCFAs enter the body or are in a free state. The body rarely experiences the euphoria brought on by VLCFAs due to this prompt processing system. Of course, this might also contribute to the ease with which peroxisomal Beta-oxidation is disregarded.

Additionally, peroxisomal -oxidation plays a crucial part in overcoming reactive stress. Highly active molecules like ROS and reactive nitrogen species (RNS) produce an excessive amount of free radicals when the body is exposed to various detrimental stressors, and the degree of oxidation surpasses the antioxidant capacity of cells to eliminate oxides. Tissue injury results from an imbalanced oxidative and antioxidant system. This is connected to the ratio of FADH2/NADH (F/N) joining the electron transport chain, which is engaged in the transfer of free hydrogen ions and electrons. In essence, the concentration and F/N ratio will be impacted by the FA carbon chain length, which will then have an impact on ROS generation. The ROS produced when VLCFAs are processed in mitochondria can seriously exacerbate oxidative damage there. The incidence of oxidative stress was significantly decreased by specific oxidation products produced during peroxisomal Beta-oxidation (Figure 2).

The high-energy electrons stored in FADH2 are immediately moved from O2 to H2O2 in peroxisomes, where they are then broken down into H2O and O2. So, by decreasing the quantity of -oxidation in mitochondria, the oxidation of VLCFAs in peroxisomes can lower the F/N ratio and lower the production of ROS. Naturally, ATP is lost during peroxisomal Beta-oxidation, and the energy transported by FADH2 is instead used to generate heat energy instead of ATP. However, studies have shown that peroxisomes speed up the breakdown of FAs in BAT under cold stress circumstances to aid in the body's rapid adaptation, proving that this heat loss is not a physiologically useless action. Peroxisomal Beta-oxidation is a crucial biochemical process during nonshivering thermogenesis as a result. There is an aural stimulus as a result. ROS generation rises and leads to oxidative damage in an excessively noisy environment, but peroxisomes can also control the disorders brought on by these stressors through -oxidation.

According to recent studies, PEX5 activates the oxidative feedback control system. Lipid ppapers have a further unexplored impact. (LDs). From microbes to humans, LDs are lipid-storing structures that serve as a source of metabolic energy for a variety of cellular functions, including membrane synthesis and molecular communication. According to research, after development, LDs and peroxisomes are produced in the same region of the endoplasmic reticulum with a near subcellular location, suggesting a potential for contact between the two organelles. It has been discovered by researchers that during times of food deprivation, peroxisomes can travel to and touch LDs with the aid of kinesin KLFC3, and after doing so, transport lipids from lipid droplets into the Beta-oxidation process more rapidly to promote their degradation and maintain energy balance.

The most recent study confirms this finding and provides a more detailed account of "starvation," which is a state that develops to defend the body from ROS because starvation raises both fatty acid peroxidation and ROS production. Peroxisomes are essential in ruminants, particularly milking cows, which are mammals. In contrast to other non-ruminant animals where FAO is primarily found in the mitochondria (76%), ruminants have

mitochondria and peroxisomes where FAO is found (about 50% in each organelle). Numerous studies have concentrated on the various lactation phases and butterfat percentage of dairy cows because dairy animals are crucial. Today, consumers make decisions based on goods that are known to improve health or avoid disease as well as the nutritional value of the food. The quantity of VLCFAs in milk is a worry in this respect.

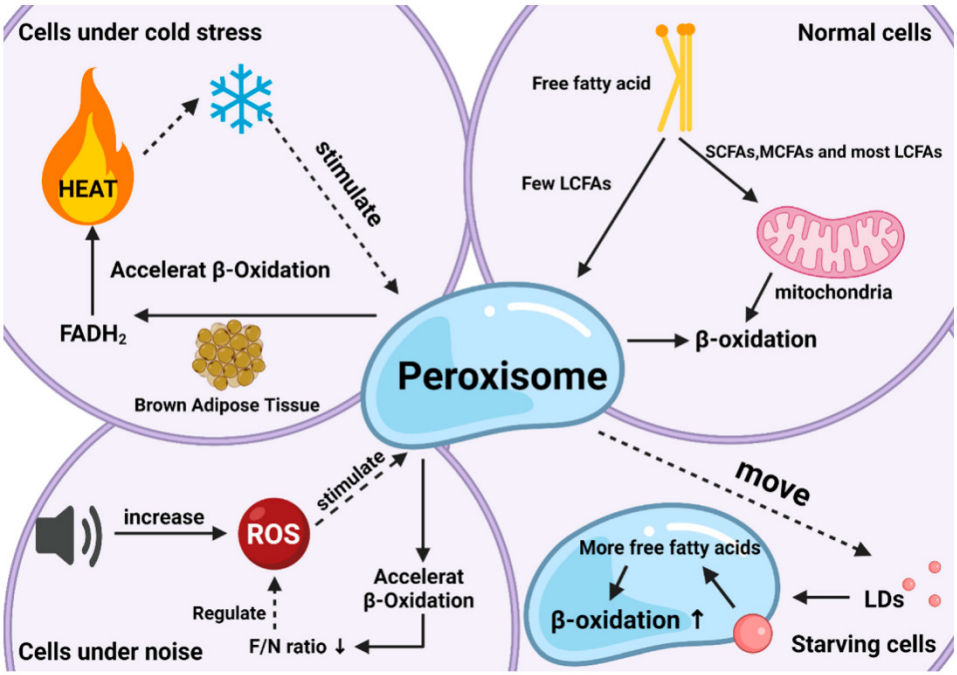

Figure 2: Role of the peroxisomal Beta oxidation: Diagram showing the function of the Beta oxidation in response to different adverse conditions (encyclopedia.pub).

Through the synthesis of ELOVL protein and the usage of peroxisomes, dairy cows have a full pathway for the synthesis and utilization of VLCFAs. Due to their high metabolic rates and physiological adaptation to extensive farming, high-producing dairy cows experience continuous oxidative stress in the field. Dairy cows' bodies go through several intricate biochemical changes during the postpartum period, with ketosis being one of them frequently. Fat accumulation in the liver frequently occurs in conjunction with ketosis. The primary idea is that some NEFAs enter the ketone body synthesis route to produce ketone bodies after excess NEFAs (nonesterified fatty acids, fatty acids above C10, primarily VLCFAs) enter the liver. The results of the study revealed higher levels of ROS and higher oxidant indices in the mammary epithelial cells of ketotic cows, suggesting an elevated oxidative stress state.

Although there isn't direct evidence linking the occurrence of ketosis to peroxisomes, numerous studies have demonstrated that factors related to peroxisomal -oxidation play a role in the development and management of the condition. These factors include PPAR, AMPK, and the presence of VLCFAs. As a result, it was assumed that the relationship between peroxisomes and ketosis was very likely additionally, mastitis and breast edema in milking heifers can both be linked to oxidative stress. Although some research used extrinsic medications to address oxidative stress-related illnesses, it was thought that the harm resulting from oxidative stress could be reduced by naturally controlling the rate of peroxisomal Beta–-oxidation. Unfortunately, there are currently very few studies that have connected these illnesses to peroxisomal Beta–oxidation [4].

DISCUSSION

One prevalent method for allowing rival paths is the sequestration of processes into separate organelles and spaces within the cell. The colocalization of a pathway guarantees that intermediates between enzymatic steps are channeled through substrates to increase kinetics and output while lowering human toxicity. Organelle architecture is driven by catalysis, which results in two connected properties: compartment permeability and its innate chemical environment. The proteinaceous structures known as bacterial microcompartments (BMCs) are effectively divided into cargo and shell proteins. Viral ppapers, sizable enzyme complexes like lumazine synthase, the ribonucleoprotein vault complex, and the icosahedral encapsulation complex are examples of other self-assembling protein complexes that could be modified to serve as an organelle. Utilizing the membranous organelles that make up eukaryotes' inherent metabolic order is an option for protein-based complexes. *De novo* design and construction of a complete organelle-like structure is a more ambitious field of study [5].

In mitochondrial fatty acid (FA) beta-oxidation, a critical process is catalyzed by medium-chain acyl-CoA dehydrogenase (MCAD). We evaluated the amounts of MCAD mRNA in animals given inhibitors of mitochondrial long-chain FA import to investigate the possible involvement of FAs and their metabolites in the control of MCAD gene expression. When carnitine palmitoyltransferase I antagonists were given to mice or rats, the steady-state MCAD mRNA levels increased tissue-restrictedly. This was a transcriptional impact mediated by the peroxisome proliferator-activated receptor, as shown by HepG2 cell cotransfection studies with MCAD promoter reporter plasmids. (PPAR). An in vivo interaction was confirmed when the activity was traced to a nuclear receptor response element that worked in a foreign promoter environment and specifically bound immunoreactive PPAR in rat liver nuclear extracts. Exogenously added FA and fabric acid compounds also triggered PPAR-mediated transactions of this promoter and element. It is uncommon for this distinct metabolic process to alter PPAR transactivation, which suggests that intracellular FA metabolites that build during such inhibition can control MCAD expression and are probable candidates for PPAR ligands. These findings suggest that the PPAR has a wider function to play in controlling FA biosynthesis [6].

In whole liver homogenates, mitochondrial and peroxisomal fatty acid metabolism were examined. In contrast to peroxisomal oxidation, which increased more gradually and continued to climb until it reached maximum activity in the absence of albumin, the oxidation of 0.2 mM palmitoyl-CoA or oleate by mitochondria increased rapidly with rising molar substrate: albumin ratios and became saturated at ratios below. The latter situation resulted in a significant depression of mitochondrial oxidation. Except when albumin was missing, peroxisomal oxidation was lower than mitochondrial oxidation in homogenates from the normal liver. Peroxisomal oxidation is distinct from mitochondrial oxidation in that it does not result in ketones, is cyanide-insensitive, is not reliant on carnitine, and is not blocked by (+)-octanoyl carnitine, malonyl-CoA, or 4-pentanoate. CoASH amounts that were best for peroxisomal oxidation hindered mitochondrial oxidation. Triton X-100 promoted peroxisomal oxidation in the presence of albumin, but freeze-thawing had no effect. Both methods reduced mitochondrial oxidation. Treatment with clofibrate boosted peroxisomal and mitochondrial oxidation by 2- and 6- to 8-fold, respectively. Diabetes and malnutrition did not affect peroxisomal oxidation. Hepatocytes from normal rats had their fatty acid metabolism significantly suppressed by cyanide and (+)-octanoyl carnitine. (+)-Octanoylcarnitine had less of an inhibitory effect on the hepatocytes of clofibrate-treated rodents, which had a 3- to 4-fold rise in fatty acid oxidation. Hepatocytes from treatment

animals that were oxidizing fatty acids produced hydrogen peroxide at a rate that was significantly greater than that of normal hepatocytes. It was determined that the peroxisomes' input to fatty acid oxidation was less than 10% in cells from both control- and clofibrate-treated animals, assuming that all of the H2O2 generated during fatty acid oxidation was the result of peroxisomal oxidation [7].

It has been proposed that one significant method by which diets high in fish oil reduce plasma triacylglycerol levels is decreased diacylglycerol acyltransferase (DGAT) activity, which in turn results in decreased triacylglycerol synthesis within hepatocytes. By increasing mitochondrial fatty acid oxidation and reducing the supply of fatty acids for triacylglycerol production, eicosapentaenoic acid (EPA), rather than docosahexaenoic acid (DHA), is thought to reduce plasma triacylglycerol. The various metabolic characteristics of EPA and DHA have been investigated in rat liver parenchymal cells and separated rat liver organelles to help comprehend the triacylglycerol-lowering process of fish oil. In isolated rat liver microsomes, EPA-CoA was a weaker substrate than DHA-CoA for DGAT, and the triacyl[3H]glycerol/diacyl[3H]glycerol ratio was significantly reduced when EPA was present. In rat liver parenchymal cells, the distribution of [1-14C]palmitic acid changed from inclusion into secretory glycerolipids toward oxidation in the presence of EPA (but not DHA).

In separated peroxisomes, purified mitochondria, and particularly in parenchymal cells of the rat liver, EPA was oxidized to a much higher degree than [1-14C] DHA. We postulated that both mitochondria and peroxisomes are involved in EPA oxidation, whereas peroxisomes are the primary site of DHA oxidation because the oxidation of EPA was more efficient and sensitive to the CPT-I inhibitor, etomoxir when measured in a mixture of both. In both the presence and lack of malonyl-CoA, EPA treatment in rats decreased plasma triacylglycerol and boosted liver mitochondrial fatty acid oxidation and carnitine palmitoyltransferase (CPT)-I activity. CPT-I's mRNA levels were only raised by EPA treatment, but DHA treatment had a greater impact on peroxisomal fatty acyl-CoA oxidase and fatty acid binding protein levels than EPA therapy did. In conclusion, cellular structures' choice of substrate is impacted by EPA and DHA. The current research provides significant evidence in favor of the idea that EPA, not DHA, reduces plasma triacylglycerol by promoting mitochondrial fatty acid oxidation [8].

The subcellular structures known as peroxisomes play a crucial part in cellular respiration. The presence of a collection of genetic diseases in which one or more peroxisomal processes are impaired highlights the significance of peroxisomes for people. The majority of these processes involve lipid metabolism, including fatty acid - and -oxidation. Here, we provide an overview of our present understanding of peroxisomal fatty acid oxidation with a focus on the following: (1) the substrates β-oxidized in peroxisomes; (2) the enzymology of the α- and β-oxidation systems; (3) the permeability properties of the peroxisomal membrane and the role of the different transporters therein; (4) the interaction with other subcellular compartments, including the mitochondria, which are the ultimate site of NADH reoxidation and full degradation of acetyl-CoA to CO2 and water; and (5) the different disorders of peroxisomal α- and β-oxidation [9].

In Saccharomyces cerevisiae, we have defined the function of YPR128cp, the orthologue of human PMP34, in fatty acid biosynthesis and peroxisomal growth. YPR128cp is found in the peroxisomal membrane and is a member of the mitochondrial carrier family (MCF) of solute transporters. When the medium-chain fatty acid (MCFA) laurate is used as a sole carbon source, yeast growth is impaired due to YPR128c gene disruption, in contrast to long-chain fatty acid (LCFA) oleate, which promotes normal development. Comparable activities were

observed in the corresponding lysates, even though MCFA but not LCFA -oxidation activity was significantly decreased in intact ypr128c mutant cells compared to intact wild-type cells. These findings suggest that MCFA -oxidation-specific transport is compromised in ypr128c cells. We investigated whether YPR128cp is an ATP carrier because MCFA -oxidation in peroxisomes needs both ATP and CoASH for activation of the MCFAs into their respective coenzyme A esters. To quantify ATP consumption within peroxisomes, we have used firefly luciferase which has been specifically tailored to peroxisomes. We demonstrate that, compared to wild-type cells, peroxisomal luciferase activity was significantly lower in intact ypr128c mutant cells, but similar in extracts of both cell types. We conclude that the transfer of ATP across the peroxisomal membrane is most likely mediated by YPR128cp [10].

CONCLUSION

The peroxisomal beta-oxidation procedure occurs by the VLCFAs enter the peroxisome as -CoA, initiating the second oxidation by starting the first oxidation, going through hydrolysis, going through thiolysis, and eliminating two C atoms. Peroxisomal beta-oxidation does not depend on the energy requirements of the cells, whereas mitochondrial beta-oxidation does. The mitochondrial route is associated with catabolism and energy generation, while peroxisomal beta-oxidation is primarily engaged in pathways for biosynthesis. Hydrogen peroxide, produced by peroxisomal oxidation, can be converted by catalase into water and oxygen. The hereditary anomalies Zellweger syndrome and adrenoleukodystrophy can make it difficult to break down very long-chain fatty acids.

REFERENCES:

[1] M. Fransen, C. Lismont, and P. Walton, “The Peroxisome-Mitochondria Connection: How and Why?,” *Int. J. Mol. Sci.*, vol. 18, no. 6, Art. no. 6, Jun. 2017, doi: 10.3390/ijms18061126.

[2] “Peroxisome,” *Wikipedia*. Mar. 20, 2020. Accessed: Mar. 23, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Peroxisome&oldid=1145660041

[3] J. K. Reddy and T. Hashimoto, “PEROXISOMAL β-OXIDATION AND PEROXISOME PROLIFERATOR–ACTIVATED RECEPTOR α: An Adaptive Metabolic System,” *Annu. Rev. Nutr.*, vol. 21, no. 1, pp. 193–230, 2001, doi: 10.1146/annurev.nutr.21.1.193.

[4] “Peroxisomal β-Oxidation.” https://encyclopedia.pub/entry/25137 (accessed Mar. 23, 2020).

[5] T. Chaijarasphong and D. F. Savage, “Sequestered: Design and Construction of Synthetic Organelles,” in *Synthetic Biology*, John Wiley & Sons, Ltd, 2018, pp. 279–306. doi: 10.1002/9783527688104.ch14.

[6] T. Gulick, S. Cresci, T. Caira, D. D. Moore, and D. P. Kelly, “The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression.,” *Proc. Natl. Acad. Sci.*, vol. 91, no. 23, pp. 11012–11016, Nov. 1994, doi: 10.1073/pnas.91.23.11012.

[7] G. P. Mannaerts, L. J. Debeer, J. Thomas, and P. J. De Schepper, “Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats.,” *J. Biol. Chem.*, vol. 254, no. 11, pp. 4585–4595, Jun. 1979, doi: 10.1016/S0021-9258(17)30051-0.

[8] L. Madsen, A. C. Rustan, H. Vaagenes, K. Berge, E. Dyrøy, and R. K. Berge, "Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference," *Lipids*, vol. 34, no. 9, pp. 951–963, Sep. 1999, doi: 10.1007/s11745-999-0445-x.

[9] R. J. A. Wanders *et al.*, "Peroxisomal fatty acid α- and β-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases," *Biochem. Soc. Trans.*, vol. 29, no. 2, pp. 250–267, May 2001, doi: 10.1042/bst0290250.

[10] C. W. T. van Roermund*et al.*, "Identification of a Peroxisomal ATP Carrier Required for Medium-Chain Fatty Acid β-Oxidation and Normal Peroxisome Proliferation in Saccharomyces cerevisiae," *Mol. Cell. Biol.*, vol. 21, no. 13, pp. 4321–4329, Jul. 2001, doi: 10.1128/MCB.21.13.4321-4329.2001.

CHAPTER 11

BIOLOGICAL SIGNIFICANCE OF THE KETOGENSIS AND THE KETOLYSIS

Prof. Kapilesh Jadhav, Professor
Department of Biotechnology, Jaipur National University, Jaipur, India
Email Id-kapilesh@jnujaipur.ac.in

ABSTRACT:
When fatty acids are broken down by the liver, a substance that is water-soluble called ketone body is created. Oxidized ketone molecules are transported to the citric acid cycle to create ATP. During starvation, diabetes, etc., ketone bodies are crucial. The emphasis in this paper was on ketogenesis and ketolysis. This paper's second part addresses the regulation and biochemical significance of ketone bodies.

KEYWORDS:

Acetyl-Coa Carboxylase, Acid Cycle, Citric Acids, Fatty Acid, Ketone Bodies.

INTRODUCTION

The ketone groups that the liver creates from fatty acids are found in ketone bodies, which are water-soluble structures or substances (ketogenesis). Acetyl-CoA (acetyl-Coenzyme A) is produced when ketone bodies are transformed into these tissues outside the liver. Acetyl-CoA then joins the Krebs cycle and is oxidized for energy. Acetoacetic acid (acetoacetate), beta-hydroxybutyrate, and acetone, an acetoacetate spontaneous breakdown product, are among the ketone groups produced by the liver (Figure. 1). The liver produces ketone bodies under a variety of caloric-restrictive conditions, including starvation, carbohydrate-restrictive diets, extended vigorous exercise, alcoholism, and type 1 diabetes mellitus that is uncontrolled (or poorly managed). By breaking down fatty molecules, liver cells create ketone bodies. They are discharged into circulation after the liver's glycogen reserves are exhausted. (Glycogen stores typically are depleted within the first 24 hours of fasting[1]–[3].

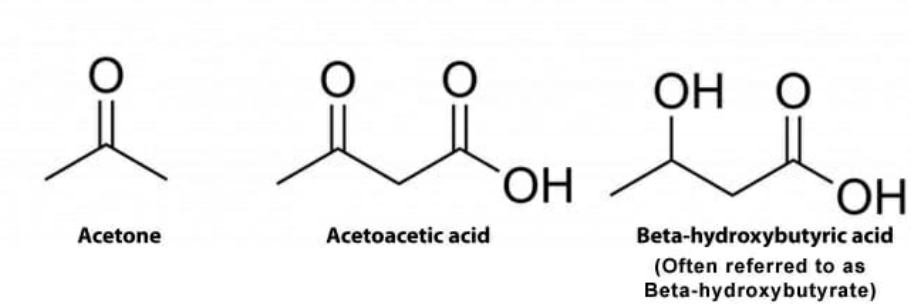

Figure 1: Ketone bodies: Diagram showing the structure of the ketone bodies (Biology Dictionary).

During times of dietary restriction, glial cells also create ketone bodies to support memory formation. Acetoacetate is a (covalent) dimer that can develop when two acetyl-CoA molecules shed their -CoAs (or coenzyme A groups). Acetoacetate is reduced to -hydroxybutyrate, where the ketone group is changed to an alcohol (or hydroxyl) group. Both are 4-carbon molecules that, with the noteworthy exception of the liver, can be easily converted back into acetyl-CoA by the majority of bodily cells. The decarboxylated version

of acetoacetate is acetone. It can only be transformed back into acetyl-CoA through liver detoxification, where it is changed into lactic acid, which can then be oxidized into pyruvic acid, and only then. People in ketosis and ketoacidosis can readily be identified by the distinctive scent of ketone bodies on their breath. It is frequently compared to fruity or nail paint stripper (which usually contains acetone or ethyl acetate). Other ketone bodies, such as -keto pentanoate and -hydroxy pentanoate, may be produced in addition to the three natural ketone bodies as a consequence of the metabolism of synthetic lipids, like triheptanoin.

When insulin levels are low and blood levels of glucagon and epinephrine are high, fats held in adipose tissue are released from the fat cells into the blood as free fatty acids and glycerol. When fasting, starving, or engaging in strenuous activity, blood glucose levels are likely to decline. All cells that are capable of metabolism and have mitochondria absorb fatty acids because they are extremely high energy sources. This is so because only the mitochondria can process fatty acids. Since red blood cells lack mitochondria, they must exclusively obtain their energy from anaerobic glycolysis. All other organs create acyl-CoA chains from the fatty acids that enter the metabolizing cells by combining them with coenzyme A. These are delivered into the mitochondria of the cells, where they undergo a series of processes known as -oxidation that convert them into acetyl-CoA molecules.

By joining with oxaloacetate to create citrate, the acetyl-CoA generated by -oxidation joins the citric acid cycle in the mitochondrion. The acetyl group of acetyl-CoA (see illustration above, on the right) completely burns away as a consequence, converting it to CO2 and water. Per the acetyl group (or acetic acid molecule) that is oxidized, the energy produced during this process is collected in the form of 1 GTP and 11 ATP molecules. Acetyl-CoA undergoes this destiny wherever fatty acid -oxidation takes place, except in the liver under specific conditions. Oxaloacetate is entirely or partly diverted into the gluconeogenic pathway in the liver when one fast when one is starved when one consumes a low carbohydrate diet when one engages in extended, arduous exercise, and when one has uncontrolled type 1 diabetes. In these conditions, oxaloacetate is changed to malate by hydrogenation, which is then expelled from the mitochondrion and transformed into glucose in the cytoplasm of the liver cells, where the glucose is released into the circulation. Therefore, when substantial gluconeogenesis has been induced by low (or nonexistent) insulin and high glucagon concentrations in the blood, oxaloacetate is not accessible in the liver for condensation with acetyl-CoA. Acetate and beta-hydroxybutyrate are produced instead of acetyl-CoA in these conditions. Ketone bodies include acetoacetate, beta-hydroxybutyrate, and their spontaneous decomposition byproduct, acetone. The liver releases the ketone molecules into circulation[4].

No other organ can divert its oxaloacetate into the gluconeogenic pathway in the manner that the liver does, so all cells with mitochondria can receive ketone bodies up from the blood and reconvert them into acetyl-CoA, which can then be used as fuel in their citric acid cycles. Ketone bodies, which can penetrate the blood-brain barrier unlike free fatty acids and can therefore be used as fuel for central nervous system cells instead of the glucose that these cells usually depend on to live, can cross the blood-brain barrier. Ketosis, and its extreme version, ketoacidosis, can result from the high amounts of ketone bodies that can build up in the blood during starvation, a low-carb diet, and protracted vigorous activity. For those who can scent it, acetoacetate, which is present in the air and urine during ketosis, has a very distinct odor. Contrarily, the majority of people can detect acetone, whose "sweet & fruity" flavor is also present in the breath of people who are in ketosis or, more specifically, ketoacidosis.

By dissolving fatty acids and ketogenic amino acids, organisms create ketone bodies through a metabolic process called ketogenesis. Under specified conditions like fasting, calorie limitation, sleep, or others, the mechanism provides energy to some organs, especially the brain, heart, and skeletal muscle. In rare metabolic diseases, insufficient gluconeogenesis can cause excessive ketogenesis and hypoglycemia, which may lead to a life-threatening condition known as non-diabetic ketoacidosis. Ketone bodies are not necessarily made from fatty acids; rather, a significant quantity of them is only made when there is a shortage of protein and carbohydrates, which leaves only fatty acids readily accessible as a source of energy for their synthesis.

When blood glucose levels are low, as they are when fasting, the liver cells' mitochondria, which are where ketone bodies are primarily made, can start to synthesize ketone bodies. Although other cells, such as human astrocytes, can carry out ketogenesis, their efficiency is lower. A fit person experiences continuous ketogenesis. The master regulatory protein AMPK, which is triggered in response to metabolic stressors like carbohydrate deficiency, eventually regulates ketogenesis in healthy people. The liver's activation prevents lipogenesis, encourages fatty acid oxidation, turns on malonyl-CoA decarboxylase instead of acetyl-CoA carboxylase, and subsequently triggers ketogenesis. Because ethanol is a strong AMPK inhibitor, it can significantly alter the liver's biochemical condition, stopping ketogenesis even when blood sugar levels are low. When blood glucose levels are low and other cellular carbohydrate reserves, such as glycogen, have been used up, ketogenesis occurs. When there is inadequate insulin, such as in type 1 (and less frequently type 2) diabetes, it can also happen, especially during times of "ketogenic stress" like concurrent sickness.

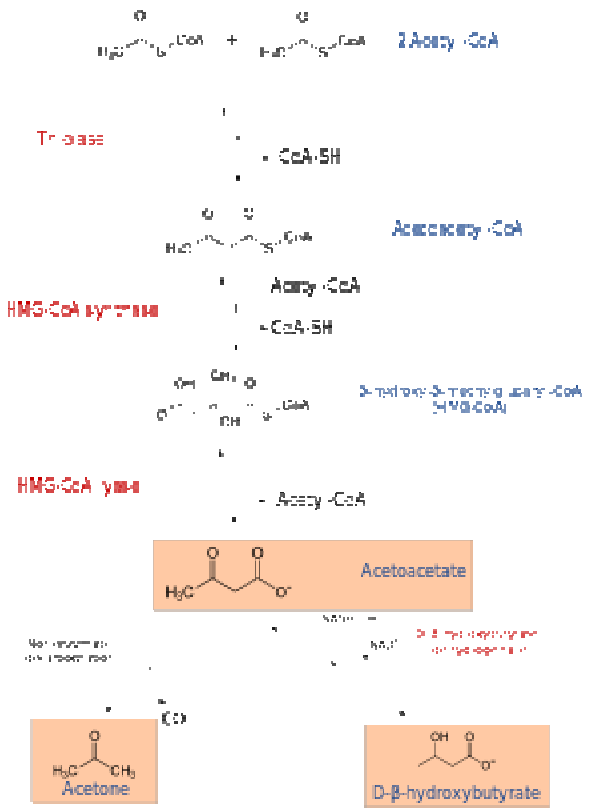

Figure 2: Ketone bodies formation: Diagram showing the reaction of the ketone bodies formation (Wikipedia).

Then, to release the energy that has been saved as fatty acids, the creation of ketone bodies is started. Acetyl-CoA is produced by the metabolic breakdown of fatty acids in -oxidation. Acetyl-CoA is oxidized further by the citric acid cycle (TCA/Krebs cycle) and then by the mitochondrial electron transport chain to liberate energy under typical circumstances. However, acetyl-CoA is used in the biosynthesis of ketone bodies via acetoacetyl-CoA and -hydroxy--methylglutaryl-CoA if the TCA cycle's processing capacity is challenged by the amounts of acetyl-CoA generated in fatty-acid -oxidation, i.e. if activity in the TCA cycle is low due to low amounts of intermediates like oxalic (HMG-CoA) (Figure .2). Additionally, because the liver only contains a small quantity of coenzyme A, the production of ketogenesis enables some of the coenzymes to be released so that fatty-acid -oxidation can proceed. Fasting, strenuous exercise, high-fat meals, and other medical conditions can all cause the depletion of glucose and oxaloacetate, which boosts ketone generation. Deaminated ketogenic amino acids, like leucine, fuel the TCA cycle by producing acetoacetate and ACoA, which in turn generate ketones. HMG-CoA plays a dual function in the production of ketone bodies and cholesterol, though the two processes are compartmentalized. Since cholesterol synthesis takes place in the cytosol rather than the mitochondria, ketogenesis, and cholesterol synthesis are separately controlled processes.

The process of catabolizing ketones is known as ketolysis, as opposed to the process of creating them, known as ketogenesis. Fatty acid oxidation uses more energy than ketolysis does for the production of ATP. Ketolysis occurs in non-liver cells, particularly in the heart, brain, and skeletal muscle, while ketogenesis occurs predominantly in the liver (Figure. 3). All human cells, except hepatocytes, have the SCOT enzyme in their mitochondria, which is necessary for ketolysis. Despite having monocarboxylate transporters to move beta-hydroxybutyrate intermediates into the cytoplasm, type II cells of the lung alveolus are unable to catabolize beta-hydroxybutyrate because they lack ketolytic enzymes. Elevated ketone levels and enhanced ketolysis have been linked to the cardioprotective benefits of SGLT2 inhibitors[5], [6].

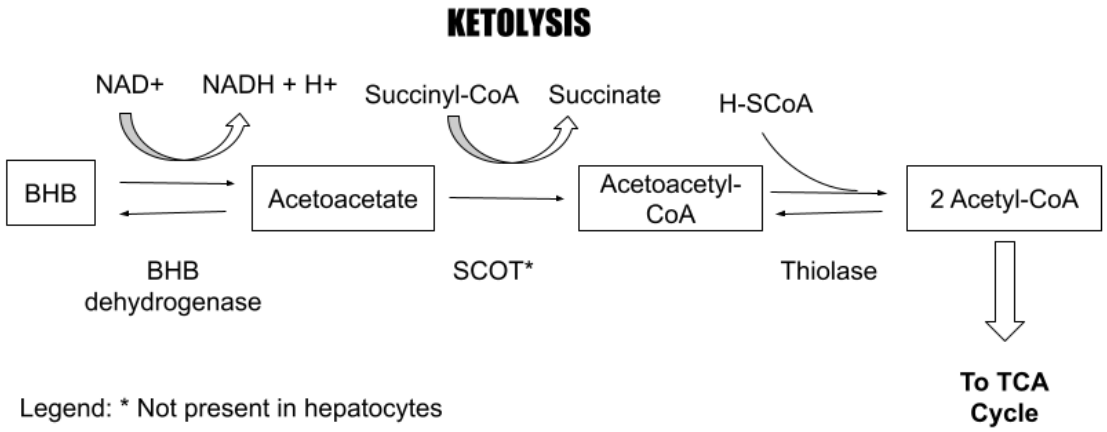

Figure 3: Ketone bodies breakdown: Diagram showing the reaction of the ketone bodies breakdown (Wikipedia).

Regulation of the ketogenesis: Depending on the number of accessible carbohydrates in the cell or organism, ketogenesis may or may not take place. This is directly linked to acetyl-CoA's pathways: When the body has a sufficient supply of carbs, glucose is fully oxidized to CO2; as an intermediate in this process, acetyl-CoA is created and first enters the citric acid cycle before being entirely converted to ATP through oxidative phosphorylation. When the body has more carbs than it needs, some of the extra glucose is completely metabolized, and the rest is either stored as fatty acids or glycogen in response to excess citrate. At this stage,

coenzyme A is regenerated. In the absence of unrestricted carbs, the body must convert fat into acetyl-CoA to produce energy.

Because the intermediates of the citric acid cycle, primarily oxaloacetate, have been exhausted to fuel the gluconeogenesis pathway, acetyl-CoA cannot be metabolized through this route. Acetyl-CoA builds up as a consequence, starting the ketogenesis process. Ketogenesis is controlled in part by the hormones insulin and glucagon, with insulin serving as the main modulator. Both hormones control acetyl-CoA carboxylase and hormone-sensitive lipase. Diglycerides are created from triglycerides by hormone-sensitive lipase, releasing a fatty acid molecule for decomposition. Malonyl-CoA is created from acetyl-CoA by the enzyme acetyl-CoA carboxylase. Carnitine palmitoyltransferase I, an enzyme that transports fatty acids into the mitochondria for -oxidation, is less active when malonyl-CoA is present. Insulin reduces the quantity of starting materials for fatty acid oxidation and limits their ability to reach the mitochondria by inhibiting hormone-sensitive lipase and activating acetyl-CoA carboxylase. Glucagon stimulates the synthesis of ketone bodies and facilitates entry into the mitochondria for -oxidation by activating hormone-sensitive lipase and inhibiting acetyl-CoA carboxylase.

In addition to blocking HMG-CoA lyase, insulin also prevents the synthesis of ketone bodies. Similar to cortisol, catecholamines, epinephrine, norepinephrine, and thyroid hormones, these substances can increase the production of ketone bodies by triggering lipolysis, which increases the quantity of fatty acids accessible for -oxidation. Contrary to glucagon, catecholamines have the ability to trigger lipolysis for use by peripheral tissues during severe stress, even in the presence of insulin. Since it has some influence over a number of the genes involved in ketogenesis, the peroxisome proliferator-activated receptor alpha (PPAR) can also upregulate ketogenesis. For instance, PPAR regulates the monocarboxylate transporter 1, which is involved in the transport of ketone bodies across membranes, including the blood-brain barrier, and as a result, affects the transfer of ketone bodies into the brain. Additionally, PPAR upregulates carnitine palmitoyltransferase, which may have an impact on how fatty acids are transported into the mitochondria[7]–[9].

Most extrahepatic organs, including the brain, undergo ketogenesis and use ketone bodies for energy generation. (excluding erythrocytes and most malignant cell types. The ability to endure times of hunger is significantly increased, which has significant genetic benefits. Brain cells would completely rely on liver and renal gluconeogenesis during prolonged deprivation in the absence of ketogenesis. There are only a few substances that can be used by the body to synthesize glucose; these substances include glucogenic amino acids, glycerol from lipids, regenerated lactate, and pyruvate via the Cori cycle (and ketone bodies). The brain of an adult could only operate for two to three weeks on gluconeogenesis alone, but if ketone bodies made from fat depots are used as an additional energy source, the brain can perform for at least two months. Even a much extended time of starvation wouldn't be enough to kill off an obese individual. Two-thirds of the energy required by the brain after several weeks of starvation is supplied by acetoacetate and OHB.

Early neonatal stages necessitate ketone bodies for the development of the human brain. The reduced lactose concentration of colostrum causes the ketotic metabolism of infants. The infant human brain receives almost half of its energy from OHB. After a few days of lactation, ketosis ends because the lactose level has risen. The heart is another component that is essential for life. It's interesting to note that myocardial cells heavily depend on the oxidation of fatty acids, which accounts for 60–85% of ATP generation, rather than much use of glucose for energy production. Amino acids, ketone bodies, and glucose/lactate are additional energy sources. Although free fatty acids continue to be the primary substrate for

ATP generation, the use of acetoacetate and OHB is proportionate to systemic amounts, leading to a rise in ketone body intake during ketosis. Acetoacetate and OHB each contribute differently to the skeletal muscle's ability to produce ATP. Ketone bodies add 10–20% of the energy supply after an overnight fast, and this percentage may increase to 50% after several days of fasting. Blood glucose accounts for more than half of the energy. Depending on the dietary state and exercise volume, the disposal of ketone bodies to the skeletal muscle during aerobic exercise may increase by up to five times, followed by post-exercise ketosis (0.3-2.0 mmol/l).

Together, these findings show that the liver constantly generates low amounts of ketone bodies during lipid catabolism, with a rapid rise in reaction to a drop in the availability of glucose/pyruvate from the diet for directing breakdown products of fatty acids into the TCA cycle for full oxidation. Acetoacetate and OHB's capacity to replace blood glucose in the generation of energy is crucial for life during extended starvation, particularly in terms of brain function.Ketogenic diets may be more protective of bodily processes than just reducing insulin production and supplying ketone bodies as a substitute auxiliary fuel. Increased blood levels of ketone bodies, such as those that occur during times of food scarcity, are a sign that fat is being broken down in the lack of enough carbohydrate supply. As a result, the body may use a rise in systemic levels of acetoacetate or OHB as a warning indication that hunger is imminent, followed by a suitable reaction to modify relevant physiological processes to improve survival during fasting. Such a situation will be covered in this volume.

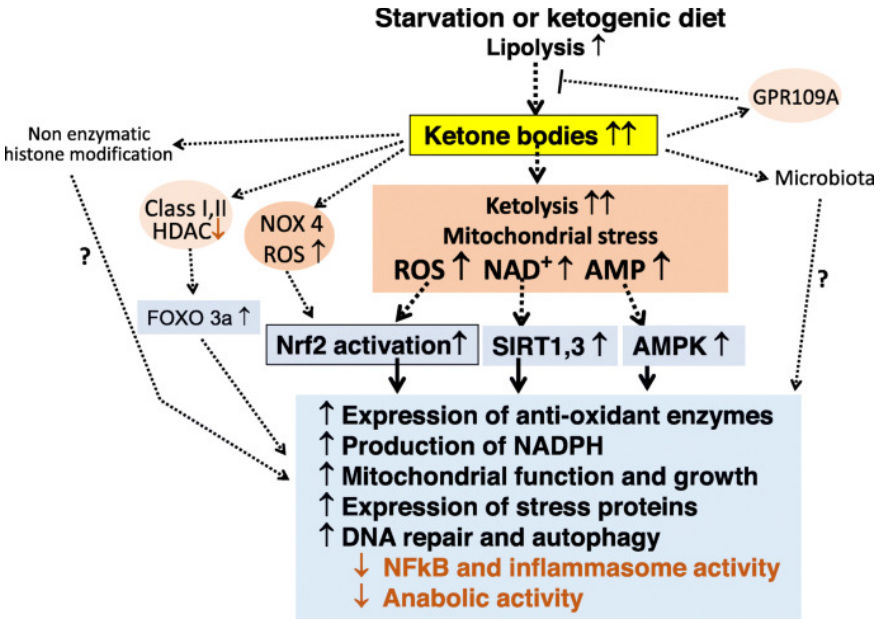

Figure 4: Ketone bodies significance: Diagram showing the significant effects of the ketone bodies (bmcmedicine).

The increased energy generation from ketone bodies is linked to higher radical oxygen species (ROS) release in the mitochondria, a concurrent drop in NADH levels in favor of NAD+ levels, and a decline in the AMP/ATP ratio when there is a food scarcity or starvation. Acetoacetate exposure of rodent hepatocytes or human endothelial cells also results in increased ROS generation. Bovine hepatocytes were also subjected to OHB or acetoacetate-induced markers of oxidative stress. NADPH oxidase 4 is triggered in human endothelial cells by elevated concentrations of acetoacetate (4 mmol/l) and OHB (12 mmol/l) in addition to higher ROS generation from the mitochondria. Oxidative stress frequently causes or is followed by the stimulation of inflammatory reactivity as well as lipid, protein, and DNA

damage at the cellular level. In reality, it was discovered that OHB stimulated the production of the chemokine CCL2 and the pro-inflammatory cytokines tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 in human capillary endothelial cells or calf hepatocytes.

Consideration of ketosis and ketone bodies as advantageous to the organism seems illogical in light of such undesirable effects on cell metabolism. Numerous studies, however, have found that following a ketogenic diet or being exposed to ketone bodies causes the activation of anti-oxidant and anti-inflammatory processes (Figure.4). When it is taken into account that the reaction to a ketogenic diet or exogenous ketone bodies has a temporal axis, these contentious results become clear. An adaptive cellular defense response that results in a sustained upregulation of cell-protective activities, such as enhanced anti-oxidative and anti-inflammatory activity, cell repair, and regenerative mechanisms, follows the initial rise of ROS and pro-inflammatory mediators. Numerous danger-responsive regulatory molecules, such as the nuclear factor erythroid 2-related factor 2 (Nrf2), histone deacetylases from the sirtuin (SIRT) family, and AMP-activated kinases, orchestrate these cellular reactions (Figure.4).

DISCUSSION

When glucose is not easily accessible, the liver produces ketone bodies, which are then used peripherally as an energy source. Acetone is the third and least prevalent ketone body. Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB) are the two major ketone bodies. Blood amounts of ketones are always present and rise during extended periods of fasting and activity. They can also be discovered in the blood of expectant mothers and newborns. The most frequent clinical reason for increased blood ketone levels is diabetes. High levels of ketones are generated in diabetic ketoacidosis (DKA) in reaction to low insulin levels and high levels of counterregulatory hormones. The ketone body ratio (3HB: AcAc) increases from the usual (1:1) to as high as 10:1 in severe DKA. Commonly, 3HB levels fall off in reaction to insulin treatment much earlier than AcAc levels. The commonly used nitroprusside test can only identify AcAc in pee and blood. This test is cumbersome, does not measure the best marker of ketone levels in the body (3HB), only gives a semiquantitative evaluation of ketone levels, and is linked to false-positive findings. Recently, it became possible to use tiny blood samples (5–25 l) with low-cost quantitative assays of 3HB levels. These studies provide fresh approaches to managing and treating diabetes and other conditions defined by atypical ketone body metabolism[10].

It has long been believed that the ketone body -hydroxybutyrate (OHB) transports energy from the liver to peripheral organs during fasting or exertion. However, OHB also communicates through external receptors and inhibits histone deacetylases naturally. (HDACs). These discoveries are consistent with a paradigm in which OHB mediates chromatin changes that connect the environment, in this case, food, and gene expression. We go over how ketone bodies are regulated and what they do, how they interact with calorie limitation, and how the ketone body OHB's HDAC inhibition affects aging and metabolic disorders.

Ketosis, defined as an increase in D-hydroxybutyrate (R-3-hydroxybutyrate) and acetoacetate, has been crucial to famished man's survival because it gives his genetically hypertrophied brain nonglucose substrate while protecting muscle from being destroyed for glucose synthesis. Unexpectedly, D-hydroxybutyrate, or OHB, may also be a more effective source of energy for the brain per unit of oxygen. This hypothesis is backed by a similar occurrence observed in the isolated working perfused rat heart and in sperm. In two human neural cultures, one a model for Alzheimer's disease and the other for Parkinson's disease, it

has also been demonstrated to reduce cell mortality. These findings suggest that various neurologic conditions, both inherited and acquired, may profit from ketosis. A rise in the energy of ATP hydrolysis (G') and its associated ionic gradients are among OHB's additional positive impacts. This may be important in anoxic and injury conditions as well as drug-resistant seizures. In reducing free radical damage, OHB's capacity to decompose coenzyme Q and decrease NADP + may also be significant. Synthetic esters or polymers of OHB given orally, presumably 100 to 150 g or more daily, may be necessary for clinical maneuvers to raise blood levels of OHB to 2-5 mmol. For animal research and potentially later clinical trials, this requires improvements in food science technology to provide at least enough synthetic material that can be consumed. The technology for analyzing numerous metabolic "phenotypes" must also be advanced to match the degree of complexity of the instruments used, for instance, in structural biology or genome science. By improving the information obtained from gene analysis and the following steps and changes of the protein products themselves, this technological approach will be essential to the classification of polygenic diseases.

Standard medication-resistant seizures continue to be a serious therapeutic issue. The high-fat, low-carb ketogenic diet is one underused choice for people with medication-resistant seizures. The name of the diet was inspired by the discovery that people following it generate ketone bodies, such as acetoacetate, -hydroxybutyrate, and acetone. Ketone bodies have been proposed as potential contributors to the diet's anticonvulsant and antiepileptic benefits, even though the precise mechanisms of action remain unclear. The ketogenic diet and the anticonvulsant characteristics of ketone bodies are addressed in this study. The effects of ketone bodies on developing neurons in vitro are also addressed due to the significance of ketone body metabolism in the early phases of development. Ketone bodies can be used more effectively to treat epilepsy and other neurological diseases if their impacts are understood.

Ketone bodies, along with free fatty acids, serve as an easily oxidizable fuel of oxidation in different tissues when the supply of glucose is limited. Ketone bodies are produced by special reactions and are not typical intermediates in the degradation of fatty acids. The moderate types of ketosis, such as those caused by starvation, low-carb diets, or mild diabetes, are physiological processes that are a component of "caloric homeostasis." There is evidence to support the working theory that the high rates of gluconeogenesis that take place in diabetic comas and lactating cows are related to their extreme types of ketosis.

Due to the elevated activity of the enzyme that transforms oxaloacetate to phosphopyruvate, gluconeogenesis is switched from the tricarboxylic acid cycle to gluconeogenesis. The liver increases the rate of metabolism outside of the tricarboxylic acid cycle to make up for the energy loss caused by the cycle's reduced rate. The conversion of fatty acids to ketone bodies is the primary process of this kind. These are produced as a byproduct of processes that provide the energy needed, far exceeding what is required. Therefore, the logical treatment for ketosis is to provide utilizable glucose.

The metabolism of ketone bodies is a crucial component of metabolic balance. In this overview, we go over the distinct metabolic roles that ketones play in optimizing organ and organism function in various nutrient conditions and safeguarding against inflammation and damage in various organ systems. Observations highlight the significance of ketone bodies as crucial metabolic and signaling factors when carbs are plentiful. They have traditionally been thought of as metabolic substrates used only in carbohydrate restriction. Prospective roles for ketone bodies in cancer have emerged, along with interesting protective roles in the heart and liver, offering therapeutic options in obesity-related and cardiovascular diseases. These findings complement a repertoire of known therapeutic options for diseases of the nervous

system. To balance traditional doctrine with modern findings, debates on ketone metabolism and signaling are addressed.

CONCLUSION

When there is an inadequate supply of glucose, the liver produces ketone bodies, which are then used as a supplementary energy source. Acetone is the third and least prevalent ketone body. Acetoacetate and 3-beta-hydroxybutyrate are the two major ketone bodies. Beyond the liver, ketone bodies are easily delivered to organs. It is transformed into acetyl-CoA in this location, which then participates in the Krebs cycle and is metabolized for energy. By expanding the quantity of free fatty acids accessible for use in the ketogenic pathway and causing a more substantial breakdown of them, hormones like glucagon in cortisol, thyroid hormones, and catecholamines can upregulate ketogenesis. The main endocrine driver of this process, though, is insulin. Ketone molecules Ketone bodies serve critical roles as signaling agents, promoters of amino acid post-translational modification, as well as regulators for inflammation and oxidative stress in addition to acting as energy fuels for extrahepatic organs like the brain, heart, and skeletal muscle.

REFERENCES:

[1] W. Wang *et al.*, "Characterization of the release and biological significance of cell-free DNA from breast cancer cell lines," *Oncotarget*, 2017, doi: 10.18632/oncotarget.17858.

[2] Y. Miura, "The biological significance of ω-oxidation of fatty acids," *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*. 2013. doi: 10.2183/pjab.89.370.

[3] N. E. Olson, "The Microarray Data Analysis Process: From Raw Data to Biological Significance," *NeuroRx*, 2006, doi: 10.1016/j.nurx.2006.05.005.

[4] J. St. John, K. Powell, M. Katie Conley-LaComb, and S. R. Chinni, "TMPRSS2-ERG fusion gene expression in prostate tumor cells and its clinical and biological significance in prostate cancer progression," *Journal of Cancer Science and Therapy*. 2012. doi: 10.4172/1948-5956.1000119.

[5] D. Chettri, M. Boro, L. Sarkar, and A. K. Verma, "Lectins: Biological significance to biotechnological application," *Carbohydr. Res.*, 2021, doi: 10.1016/j.carres.2021.108367.

[6] M. C. Reed, A. Lieb, and H. F. Nijhout, "The biological significance of substrate inhibition: A mechanism with diverse functions," *BioEssays*. 2010. doi: 10.1002/bies.200900167.

[7] K. S. Jain *et al.*, "Biological and medicinal significance of pyrimidines," *Current Science*. 2006.

[8] D. Gu, X. Ao, Y. Yang, Z. Chen, and X. Xu, "Soluble immune checkpoints in cancer: Production, function and biological significance," *Journal for ImmunoTherapy of Cancer*. 2018. doi: 10.1186/s40425-018-0449-0.

[9] R. J. Nevagi, S. N. Dighe, and S. N. Dighe, "Biological and medicinal significance of benzofuran," *European Journal of Medicinal Chemistry*. 2015. doi: 10.1016/j.ejmech.2014.10.085.

[10] L. Pessoa and R. Adolphs, "Emotion processing and the amygdala: From a 'low road' to 'many roads' of evaluating biological significance," *Nature Reviews Neuroscience*. 2010. doi: 10.1038/nrn2920.

CHAPTER 12

AMINO-ACID CATABOLISM AND THE UREA CYCLE

Mr. Vikas Bansal, Associate Professor
Department of Food Technology, Jaipur National University, Jaipur, India
Email Id-vikasbansal@jnujaipur.ac.in

ABSTRACT:

The catabolism of amino acids is the elimination of the amino group and disintegration of the ensuing carbon skeleton. The cellular system's ability to break down amino acids is maintained by several metabolic processes. This paper covered the processes involved in the metabolism of amino acids, such as transamination, deamination, and the urea cycle. The control of the urea cycle and the impact of urea cycle enzyme dysfunction on the human system were discussed in the second section of the paper.

KEYWORDS:

Amino-Acids, Acid Catabolism, Acetyl Coa, Carbamoyl Phosphate, Urea Cycle.

INTRODUCTION

Protein catabolism, which occurs when proteins are broken down into smaller peptides and then amino acids, is a concept in molecular biology. A crucial part of the digestive process is the breakdown of proteins. Pepsin, which transforms proteins into polypeptides, frequently initiates the breakdown of proteins.

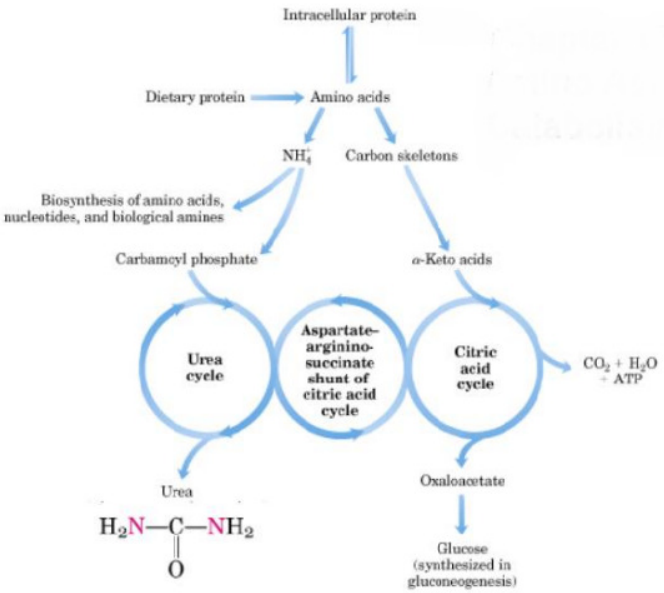

Figure 1: Amino acids catabolism: Diagram showing the overview of the amino-acids catabolism (Slide share).

Then these polypeptides undergo more degradation. The pancreas proteases found in people are composed of trypsin, chymotrypsin, and other enzymes. The small peptides in the gut are converted into amino acids that can enter the circulation. The amino acid breakdown process allows for the use of these ingested amino acids as an energy source or as building blocks for new proteins. There are several ways to use the amino acids created during catabolism,

including direct recycling to create new proteins, conversion into other amino acids, or Krebs cycle-mediated amino acid catabolism [1]. Amino acids produced by protein degradation can either be used to build bacterial proteins or metabolized to provide the energy that cells require. Deamination (removal of an amino group), transamination (transfer of an amino group), decarboxylation (removal of a carboxyl group), and dehydrogenation are a few of the mechanisms that degrade amino acids. The Krebs/Citric Acid (TCA) Cycle can be powered by the decayed amino acids that they can metabolize.

The amino acids must first undergo oxidative deamination to be broken down into carbohydrates (Figure. 2). The first step in the procedure is to remove the amino group from the amino acids. As the amino group is removed, it changes into ammonium, which then travels through the urea cycle in the liver to become urea. Following its discharge into the bloodstream, it travels to the kidneys, where it is then secreted as pee by the kidneys. The leftover amino acid undergoes oxidation and transforms into an alpha-keto acid. The TCA cycle will then be entered by the alpha-keto acid to begin the energy-producing process. The acid can also go through glycolysis and ultimately become pyruvate there. To join the TCA cycle and transform the initial pyruvate molecules into ATP, or useful energy for the organism, the pyruvate is then changed into acetyl-CoA.

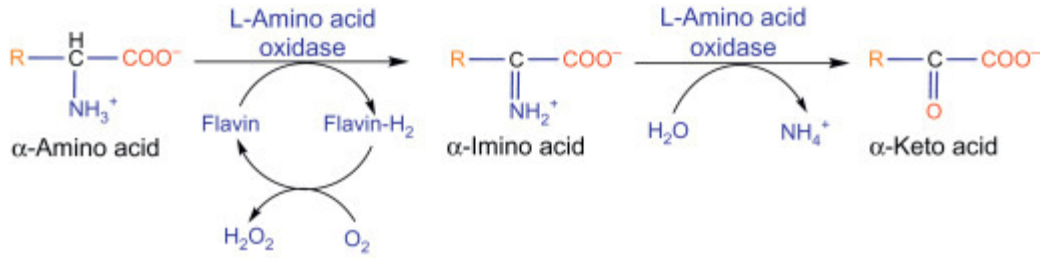

Figure 2: Oxidative deamination: Diagram showing the reaction of the oxidative deamination (Science direct.com).

The outcome of transamination is the same as that of deamination: the leftover acid will go through the TCA cycle or glycolysis to generate energy, which the organism's body will use for a variety of functions. Instead of losing the amino group to be converted into ammonium, this mechanism moves the amino group. To enable its conversion to glutamate, the amino group is moved to alpha-ketoglutarate (Figure. 3). The amino group is then transferred to oxaloacetate by glutamate.

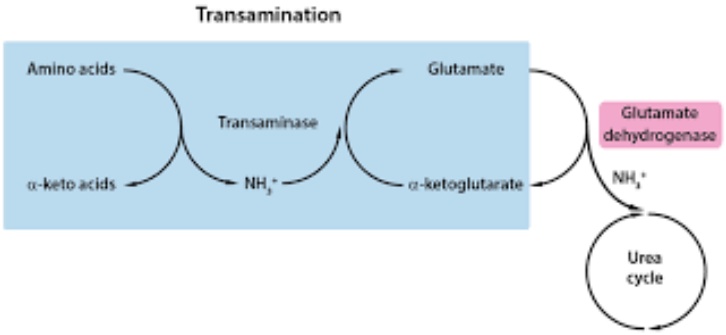

Figure 3: Transamination reaction: Diagram showing the Transamination reaction (Anita Popescu).

This shift is necessary for the conversion of oxaloacetate into aspartate or other amino acids. This product will ultimately also continue into oxidative deamination to generate ammonium, which will eventually go through the urea cycle, and alpha-ketoglutarate, an alpha-keto acid

that will go through the TCA cycle. Enzymes called transaminases aid in catalyzing the processes involved in transamination. When the amino group is transferred from the initial amino acid, such as glutamate to alpha-ketoglutarate, they assist in catalyzing the reaction and hang onto it to transfer it to another alpha-ketoacid.

Ammonium ions are produced in the cell by the glutaminase enzyme when it breaks down glutamine. Glutamate is an additional substance. Of course, glutamate can be transformed into alpha-ketoglutarate through a transamination process, which can then be metabolized in the citric acid cycle. Similar to how aspartate can be broken down into oxaloacetate for combustion in the citric acid cycle, aspartate can also be broken down into ammonium and aspartate by asparaginase. In a transamination process, alanine is transformed into pyruvate, rendering it glucogenic. The urea cycle hydrolyzes arginine to produce urea and ornithine. The catabolization of proline to glutamate reverses the biosynthetic route.

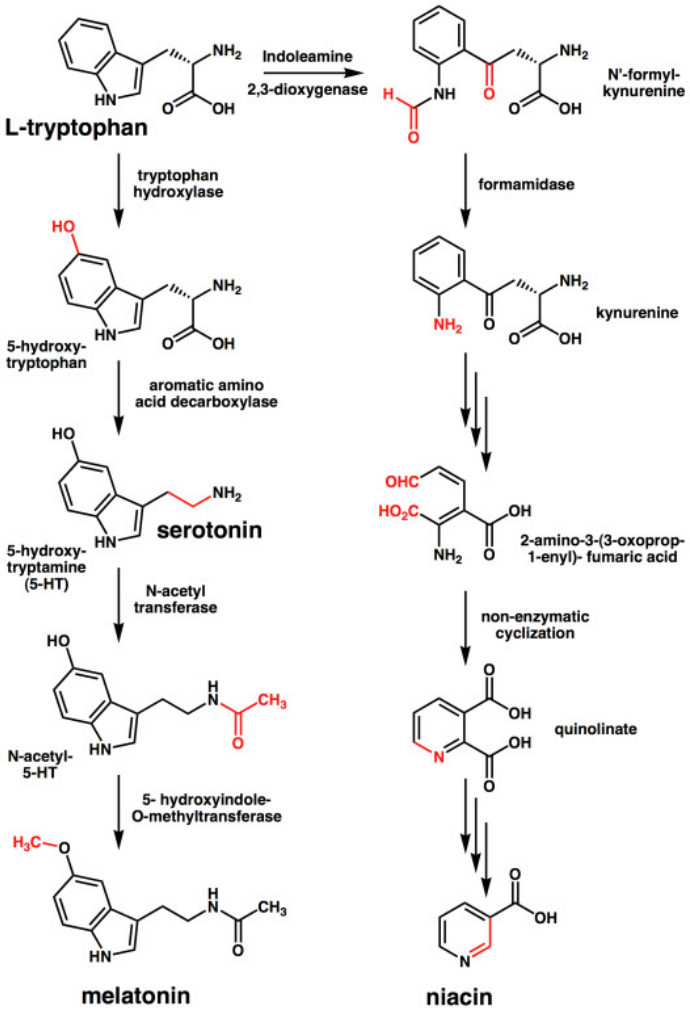

Figure 4: Amino-acids catabolism: Diagram showing the Conversion of L-Tryptophan into Serotonin, Melatonin, and Niacin (Bio. libertext)

Serine contributes carbon to create folate, and glycine, the other byproduct of the process, undergoes its oxidation to produce carbon dioxide and ammonia. Serine, which itself can be transformed back into 3-phosphoglycerate or pyruvate, can be produced from glycine (Figure .4). There are three possible routes for threonine degradation, but only two are applicable to people. One route results in glycine and acetyl-CoA. The opposite results in pyruvate. Cysteine can be degraded in several methods. The desulfurase in the liver can work on the

simplest to produce hydrogen sulfide and pyruvate. Cysteine can be created from methionine for use in additional processes. It is capable of becoming succinyl-CoA, which can then be oxidized in the citric acid cycle. It can also be changed into the carbon source S-Adenosyl-Methionine (SAM). After first being transformed to propionyl-CoA, isoleucine, and valine can also be changed to succinyl-CoA. Since vitamin B12 is necessary for the change of propionyl-CoA to succinyl-CoA, it is also necessary for the metabolism of these amino acids[2]. During catabolism, phenylalanine is transformed into tyrosine, which is eventually broken down into fumarate and acetoacetate. These two amino acids are therefore both glucogenic and ketogenic. Additionally, dopamine, norepinephrine, and adrenaline can be produced from tyrosine. It is possible to catabolize leucine and lysine into acetoacetate and acetyl-CoA. Another crucial component of carnitine is lysine. Bacteria in the gut can catabolize histidine to histamine, which when in overabundance, expands or dilates a variety of blood vessels. Although the breakdown of tryptophan is complicated, it can happen through alanine, acetoacetate, and acetyl-CoA. Altogether, the amino acids alanine, cysteine, glycine, serine, and threonine are converted to pyruvate in the body. Aspartate and asparagine are used to make oxaloacetate. It takes isoleucine, valine, and methionine to make succinyl-CoA. The amino acids arginine, glutamate, glutamine, histidine, and proline are used to make alpha-ketoglutarate. Tyrosine and phenylalanine are converted to fumarate and acetoacetate in the body. Acetoacetate and acetyl-CoA are produced from leucine and lysine. Alanine, acetoacetate, and acetyl-CoA are produced from tryptophan. Last but not least, amino acids function as precursors for several essential substances, such as serotonin (from tryptophan), porphyrin heme (from glycine), nitric oxide (from arginine), and nucleotides, in addition to being integrated into proteins. (from aspartate, glycine, and glutamine) [2].

The urea cycle, also referred to as the ornithine cycle, is a series of metabolic processes that converts ammonia into urea $(NH_2)_2CO$ (NH_3). Ureotelic animals are those that use this pattern, primarily mammals and frogs. Highly poisonous ammonia is transformed into urea by the urea cycle for elimination. Five years before the TCA cycle was found, this cycle was the first to be identified as a biochemical process (Hans Krebs and Kurt Henseleit, 1932). Later, Ratner and Cohen provided a more thorough explanation of this pattern. The liver and kidneys play a smaller role in the urea cycle than they do. Waste ammonia is produced during amino acid breakdown. All creatures require a method of excreting this substance. The majority of aquatic creatures, also known as ammonotelic organisms, simply discharge ammonia. Through the urea cycle, which primarily takes place in the liver, organisms that cannot quickly and securely eliminate nitrogen as ammonia change it to a less toxic substance, such as urea. The liver produces urea, which is then discharged into the circulation and carried to the kidneys where it is eventually eliminated as urine.

These creatures depend on the urea cycle because if nitrogen or ammonia is not removed from the body, it can have a negative impact. The ammonia is transformed into uric acid or its urate salt, which is excreted in solid form by animals such as birds and the majority of invertebrates. Additionally, the urea cycle uses the basic ammonia to combine with the acidic waste carbon dioxide and devour it, maintaining a neutral pH. Two amino groups one from aspartate and one from $NH+^4$ as well as a carbon atom from HCO are all converted during the process to the urea elimination product, which is comparatively harmless. This happens at the expense of four phosphate bonds with considerable energy (3 ATP hydrolyzed to 2 ADP and one AMP). There are five major stages in the process of turning ammonia into urea. The first is necessary for ammonia to start the cycle, and the subsequent four all take place during the cycle. Ammonia is changed into carbamoyl phosphate to join the cycle. One mitochondrial and three cytosolic enzyme processes make up the urea cycle. This employs six enzymes. Entering the urea cycle is the initial response. Ammonia is transformed into carbamoyl

phosphate before the start of the urea cycle. Carbamoyl phosphate synthetase I is responsible for catalyzing the process, which uses two ATP molecules. The urea cycle is then entered by the carbamoyl phosphate.

The urea cycle's steps: Citrulline is produced by converting carbamoyl phosphate. The carbamoyl phosphate group is given to ornithine and released along with a phosphate group through processing by ornithine transcarbamylase. To create argininosuccinate, a condensation process takes place between the amino group of aspartate and the carbonyl group of citrulline. Argininosuccinate synthase catalyzes this ATP-dependent process (Figure .4). The enzyme argininosuccinase breaks down argininosuccinate to produce arginine and fumarate. Urea and ornithine are produced when arginase cleaves arginine. After that, the ornithine is returned to the mitochondria to restart the urea cycle [3].

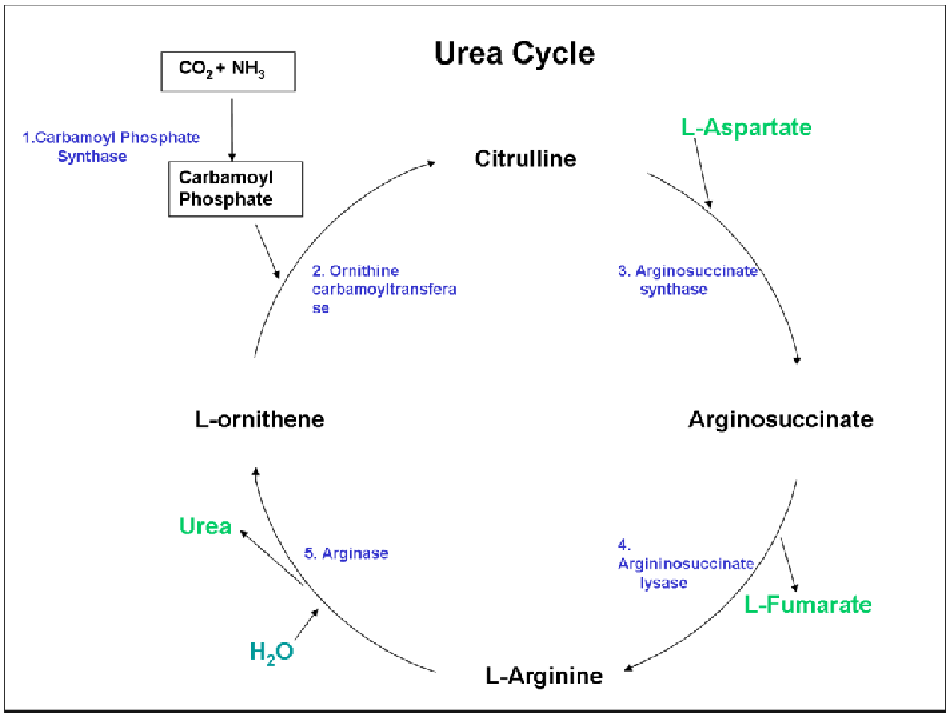

Figure 5: Urea cycle: Diagram showing the reaction of the urea cycle (Toppr).

NH^{+4} + HCO_3 in the initial process is identical to NH_3 + CO_2 + H_2O. The urea cycle's general formulation is as follows:

$$NH_3 + CO_2 + \text{aspartate} + 3\ ATP + 3\ H_2O \rightarrow \text{urea} + \text{fumarate} + 2\ ADP + 2\ Pi + AMP + PPi + H_2O$$

The equation can be simplified as follows because fumarate is produced by eliminating NH3 from aspartate (using processes 3 and 4), and PPi + H_2O 2 Pi.

$$2\ NH_3 + CO_2 + 3\ ATP + 3\ H_2O \rightarrow \text{urea} + 2\ ADP + 4\ Pi + AMP$$

Because the urea cycle reactions also result in the creation of 2 NADH, the total reaction produces a small amount more energy than it uses. There are two methods to make the NADH: When glutamate is transformed into ammonium and -ketoglutarate, the enzyme glutamate dehydrogenase generates one NADH molecule. The non-toxic transporter of amine groups is glutamate. This gives the ammonium ion needed to create carbamoyl phosphate in the first place.

Cytosolic fumarase converts the fumarate that has been produced into malate. The cytoplasmic malate dehydrogenase then converts this malate to oxaloacetate, resulting in a decreased NADH in the cytosol. Since oxaloacetate is one of the keto acids that transaminases favor, it will be converted back into aspartate, ensuring that nitrogen continues to enter the urea cycle.

By adding the responses, we can state this as follows:

$$CO_2 + \text{glutamate} + \text{aspartate} + 3\ ATP + 2\ NAD{+}{+} + 3\ H_2O \rightarrow \text{urea} + \alpha\text{-ketoglutarate} + \text{oxaloacetate} + 2\ ADP + 2\ Pi + AMP + PPi + 2\ NADH$$

A net production of two high-energy phosphate bonds for the urea cycle can be achieved from the two NADH generated (cytosolic NADH gives 2.5 ATP with the malate-aspartate shuttle in human liver cells). However, the latter reducing counterpart is used to cause the reversal of the GAPDH process rather than producing ATP if gluconeogenesis is active in the cytosol. Oxaloacetate either undergoes transamination to yield aspartate or is transformed into phosphoenolpyruvate, a precursor for gluconeogenesis.

Regulation of the urea cycle: N-acetyl glutamic acid (NAcGlu), which allosterically triggers CPS1, is necessary for the production of carbamoyl phosphate and the urea cycle. An essential regulator of carbamoyl phosphate synthase is NAcGlu. Both Arg, an allosteric stimulator of N-acetyl glutamate synthase (NAGS), and Glu, a byproduct of transamination processes and one of NAGS's substrates, which are both raised when free amino acids are elevated, induce the synthesis of NAcGlu by NAGS. As a result, Glu works as an initiator for the urea cycle in addition to being a substrate for NAGS. The amounts of their respective substrates regulate the activity of the cycle's surviving enzymes.

Therefore, the production of urea is not significantly affected by genetic defects in cycle enzymes other than ARG1 (if any cycle enzyme is entirely missing, death occurs shortly after birth). Instead, the substrate for the inadequate enzyme piles up, restoring the inadequate reaction's rate to normal. However, the unusual substrate accumulation comes at a price. Hyperammonemia results from the substrate amounts becoming raised back up the cycle to NH^{+4}. A high [$NH^{+}{}^{4}$] places a great deal of pressure on the $NH^{+}{}^{4}$-clearing system, particularly in the brain, even though the cause of NH+ 4 toxicity is not fully known (symptoms of urea cycle enzyme deficiencies include intellectual disability and lethargy). The 2-oxoglutarate (2OG) and Glu pools are reduced by the GLUD1 and GLUL clearance mechanisms. The loss of these reservoirs is most noticeable in the brain. While Glu is both a neurotransmitter and a precursor to GABA, another neurotransmitter, 2OG depletion lowers the incidence of TCAC.

Although separate processes, the citric acid cycle, and the urea cycle are connected. Oxaloacetate is transaminated to aspartate to produce one of the nitrogen atoms used in the urea cycle. The fumarate created in stage three is recycled back into the citric acid cycle where it serves as an intermediary. An uncommon condition, urea cycle disorders impact about one in 35,000 Americans. The cycle's enzymes can have genetic flaws, which typically become apparent a few days after delivery. The newborn usually goes through various episodes of vomiting and periods of lethargic behavior. In the end, the child might experience cerebral injury and fall into a coma. As a result of delayed screening procedures and incorrect diagnoses, newborns with UCD are much more likely to experience problems or pass away. Neonate peritonitis is the most typical incorrect diagnostic.

UCD symptoms may appear within the first two to three days of life, but the current way of confirming the diagnosis through test findings may take too long. Complications like

paralysis or mortality could result from this, among other things. Adults may also be identified with urea cycle diseases, and signs and symptoms may include delirium attacks, lethargy, and symptoms resembling a stroke. In addition to these signs, the patient may also acquire cirrhosis if the liver's urea cycle starts to malfunction. Sarcopenia can result from this as well (the loss of muscle mass). Mutations induce urea cycle diseases by depleting the different enzymes and transporters involved in the urea cycle. If people who lack any of the six cycle-dependent enzymes consume more amino acids than are required for their minimal daily needs, the ammonia that results will not be able to be transformed into urea. Hyperammonemia or a buildup of a cycle intermediary may occur in these people [4].

DISCUSSION

This part concentrates on the catabolism of amino acids. Within living tissue and cells, biological substances undergo catabolism, which is their disintegration and decay. The use of labeled substrates demonstrates that higher plants are capable of many of the catabolic processes found in other animals, even though they are autotrophs and not reliant on external organic substances for survival. All of the catabolic routes in heterotrophs lead to a substance that is either an intermediary in the tricarboxylic acid cycle or has the potential to become one. An amino acid's typical formula is R-CH (NH2)-COOH. Numerous transaminases are capable of easily converting glutamate to 2-oxoglutarate. The pyridine nucleotide-linked glutamate dehydrogenases are another pathway for the conversion of glutamate to 2-oxoglutarate. Leucine and valine share a lot of parallels with the degradative pathway that was discovered for isoleucine. Among the protein amino acids, glycine is distinctive in that it lacks a chiral C atom [5].

Important flavor molecules are produced by microbial degradation of amino acids and are used in foods like cheese, wine, and fermented meats. Since they have enzymatic systems for using amino acids in their digestion, lactic acid bacteria help give food its flavor. The breakdown of aromatic amino acids (Phe, Tyr, Trp) results in floral, chemical, and fecal flavors; the breakdown of aspartic acid (Asp) results in buttery flavors; and the breakdown of sulphuric amino acids (Met, Cys) results in flavors like boiled cabbage, meat, and garlic. Branched-chain amino acids (Leu, Ile, Val) are converted into compounds that contribute to the malty, fruity, and sweaty flavors [6].

The catabolism of food amino acids and arterial glutamine occurs in the small intestine, which is also in charge of final processing and nutritional intake. The small intestinal epithelium catabolizes the majority of the glutamine, glutamate, and aspartate in the food, with CO2 accounting for 56–64% of their metabolized carbons. As a result, between 30 and 50 percent of these dietary amino acids are not available to extraintestinal tissues. The small intestinal mucosa also plays a significant role in the breakdown of arginine, proline, and branched-chain amino acids as well as possibly methionine, lysine, phenylalanine, threonine, glycine, and serine in the diet. Dietary amino acids are a major source of energy for the small intestinal mucosa and are necessary building blocks for the production of glutathione, nitric oxide, polyamines, purine, and pyrimidine nucleotides, as well as amino acids (alanine, citrulline, and proline) and other essential compounds that are necessary for maintaining the mass and integrity of the intestinal mucosa. The efficacy with which dietary protein and amino acids are utilized by both animals and humans is significantly impacted by intestinal amino acid catabolism because it modulates the supply of dietary amino acids to extraintestinal tissues [7].

The control of amino acid metabolism in adipocytes is less well understood than the regulation of carbohydrate and lipid balance by adipocytes. Here, we used isotope tracking to

measure the contributions of various nutrients to tricarboxylic acid (TCA) metabolism and lipogenesis in pre-adipocytes and differentiated adipocytes. Differentiated adipocytes displayed increased branched-chain amino acid (BCAA) catabolic flux, such that leucine and isoleucine from medium and/or protein catabolism accounted for as much as 30% of lipogenic AcCoA pools. This is in contrast to proliferating cells, which use glucose and glutamine for acetyl-coenzyme A (AcCoA) synthesis. Lack of medium cobalamin led to the production of odd-chain fatty acids and the buildup of methylmalonic acid. These compounds were decreased by vitamin B12 intake, which also changed the ratio of substrates going into mitochondria. Finally, adipogenesis was hampered by BCAA catabolism suppression. These findings demonstrate numerically the importance of BCAAs for adipocyte metabolism and imply that BCAA degradation contributes to adipocyte differentiation [8].

Five enzymes make up the urea cycle, but it also needs other enzymes and mitochondrial amino acid carriers to work properly. The entire urea cycle can be seen in the liver and, to a lesser extent, in enterocytes. However, many other tissues also exhibit highly controlled expression of several urea cycle enzymes, which are also implicated in the synthesis of nitric oxide, polyamines, proline, and glutamate. Significant moderators of the production of urea cycle enzymes in the liver include glucagon, insulin, and glucocorticoids. In comparison, a variety of pro- and anti-inflammatory cytokines and other substances control the "urea cycle" enzymes in nonhepatic cells. In almost all cell types, transcription plays a major role in the regulation of these enzymes. The focus of this study is on new findings regarding the functions and control of the urea cycle and arginine metabolic enzymes in the liver and other cell types [9].

After a brief initial phase in which they look healthy, most patients with urea cycle disorders who arrive as newborns do so with worsening feeding, drowsiness, and tachypnoea. In such individuals, the plasma ammonia should be determined concurrently with the septic screen. Even if the patients pass away, a determination must be made for genetic counseling because ammonia levels above 200 mol/l are typically caused by inherited metabolic illnesses. The fastest potential reduction in ammonia amounts is the treatment's goal. Alternative nitrogen removal methods such as sodium benzoate, sodium phenylbutyrate, and arginine can be used, but hemodialysis or hemofiltration should be started if ammonia amounts are above 500 mol/l or if they do not drop quickly. Drugs, a protein-restricted diet, and the use of an emergency regimen during sickness are all part of long-term treatment. The possibility of stopping therapy should be addressed with the patient's family because severe hyperammonaemia is typically accompanied by permanent neurological damage, especially if levels have been above 800 mol/l for more than 24 hours [10].

The prevalence of urea cycle diseases is a crucial issue. Argininosuccinic synthetase and lyase deficiency are two UCDs that are presently picked up by neonatal screening in the United States. To ascertain the prevalence of these diseases, we analyzed data from the big US and European longitudinal databases and infant screening data collected from over 6 million newborns. According to estimates, there will be 113 new cases of urea cycle disorders presented annually across all age categories in the United States, with a rate of 1 patient for every 35,000 births [11].

The metabolic process known as the urea cycle is used to get rid of extra nitrogen, which is mainly produced as ammonia. Nitrogen is necessary for the development and life preservation, but too much ammonia can cause potentially fatal conditions. Urea cycle disorders (UCDs) are a group of illnesses that either manifest as hyperammonemia in the newborn phase (about 50% of cases) or later in life. The illness is brought on by urea cycle transporter or enzyme congenital abnormalities. This cycle uses five enzymes, three of which

are found in the cytoplasm (argininosuccinate synthase, argininosuccinate lyase, and arginase 1) and two of which are found in the mitochondrial matrix (carbamoylphosphate synthetase 1 and ornithine transcarbamylase). The urea cycle also depends on N-acetyl glutamate synthase and at least two carrier proteins. The severity and age of the stars are correlated with the corresponding gene mutations and rely on the remaining enzyme or transporter function. The goal of treatment is to stop the brain from becoming permanently toxic from excessive ammonia exposure. Due to the disease's rarity, the pathogenesis and natural course are inadequately known, necessitating the creation of an international registration system and cutting-edge clinical studies. The etiology, diagnostics, genetics, and therapy of UCDs are all currently held notions that we will cover here [12].

CONCLUSION

There are several ways that amino acids can degrade, including breakdown (the removal of an amino group), conversion (the transfer of an amino group), decarboxylation (the removal of a carboxyl group), and dehydrogenation. Except for amino acids with twisted chains, the metabolism of amino acids begins in the liver. Separated from the amine group, urea contains the amine group. The carbon compounds can be utilized in the production of glucose and the production of ketones or reduced to CO_2 and H_2O. End products of amino acid catabolism include glutamate, which produces pyruvate or acetyl CoA. The process of amino acid breakdown is controlled by hormones like insulin and cortisol. The main component of pee and the main method for eliminating nitrogen produced by the breakdown of amino acids is urea. The renal medulla expresses specific phloretin-inhibitable urea transporters, which are essential for the elimination of urea and water equilibrium.

REFERENCES:

[1] "Protein catabolism," *Wikipedia*. Nov. 09, 2021. Accessed: Mar. 24, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Protein_catabolism&oldid=1054268351

[2] "10.2: Amino Acid Catabolism," *Biology LibreTexts*, Aug. 23, 2021. https://bio.libretexts.org/Courses/Wheaton_College_Massachusetts/Principles_of_Biochemistry/10%3A_Nitrogen_metabolism_and_the_urea_cycle/10.02%3A_Amino_Acid_Catabolism (accessed Mar. 24, 2020).

[3] "Urea cycle," *Wikipedia*. Feb. 07, 2021. Accessed: Mar. 24, 2021. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Urea_cycle&oldid=1137950330

[4] L. D. Smith and U. Garg, "Paper 5 - Urea cycle and other disorders of hyperammonemia," in *Biomarkers in Inborn Errors of Metabolism*, U. Garg and L. D. Smith, Eds. San Diego: Elsevier, 2017, pp. 103–123. doi: 10.1016/B978-0-12-802896-4.00004-3.

[5] M. Mazelis, "15 - Amino Acid Catabolism," in *Amino Acids and Derivatives*, B. J. Miflin, Ed. Academic Press, 1980, pp. 541–567. doi: 10.1016/B978-0-12-675405-6.50021-8.

[6] Y. Ardö, "Flavour formation by amino acid catabolism," *Biotechnol. Adv.*, vol. 24, no. 2, pp. 238–242, Mar. 2006, doi: 10.1016/j.biotechadv.2005.11.005.

[7] G. Wu, "Intestinal Mucosal Amino Acid Catabolism," *J. Nutr.*, vol. 128, no. 8, pp. 1249–1252, Aug. 1998, doi: 10.1093/jn/128.8.1249.

[8] C. R. Green *et al.*, "Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis," *Nat. Chem. Biol.*, vol. 12, no. 1, Art. no. 1, Jan. 2016, doi: 10.1038/nchembio.1961.

[9] S. M. Morris, "Regulation of Enzymes of the Urea Cycle and Arginine Metabolism," *Annu. Rev. Nutr.*, vol. 22, no. 1, pp. 87–105, 2002, doi: 10.1146/annurev.nutr.22.110801.140547.

[10] J. V. Leonard and A. A. M. Morris, "Urea cycle disorders," *Semin. Neonatol.*, vol. 7, no. 1, pp. 27–35, Feb. 2002, doi: 10.1053/siny.2001.0085.

[11] M. L. Summar*et al.*, "The incidence of urea cycle disorders," *Mol. Genet. Metab.*, vol. 110, no. 1, pp. 179–180, Sep. 2013, doi: 10.1016/j.ymgme.2013.07.008.

[12] S. Matsumoto, J. Häberle, J. Kido, H. Mitsubuchi, F. Endo, and K. Nakamura, "Urea cycle disorders—update," *J. Hum. Genet.*, vol. 64, no. 9, Art. no. 9, Sep. 2019, doi: 10.1038/s10038-019-0614-4.

CHAPTER 13

AMINO ACID BIOSYNTHESIS PATHWAY

Dr Prithpal Singh Matreja, Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- drpsmatreja@yahoo.co.in

ABSTRACT:

Amino acids are classified as either essential or non-essential in the human body. The substance produced during glycolysis, the citric acid cycle, and the pentose phosphate route is used to formation of the amino acids. While the valine, leucine, and isoleucine are synthesized from pyruvate, the other aeromatic amino acids are synthesized from the shikimic acid route. The various biosynthetic pathways for both essential and non-essential amino acids are covered in this paper.

KEYWORDS:

Amino-Acids, Aromatic Amino-Acids, Acid Biosynthesis, Biosynthesis Pathway, Glutamate Proline.

INTRODUCTION

The collection of biochemical reactions (metabolic paths) that create amino acids is known as amino acid synthesis (Figure .1). The substrates for these processes are different substances found in the food or growing medium of the organism. All amino acids cannot be synthesized by every creature. For instance, 11 of the 20 essential amino acids can be produced by people. The non-essential amino acids are these eleven[1], [2].

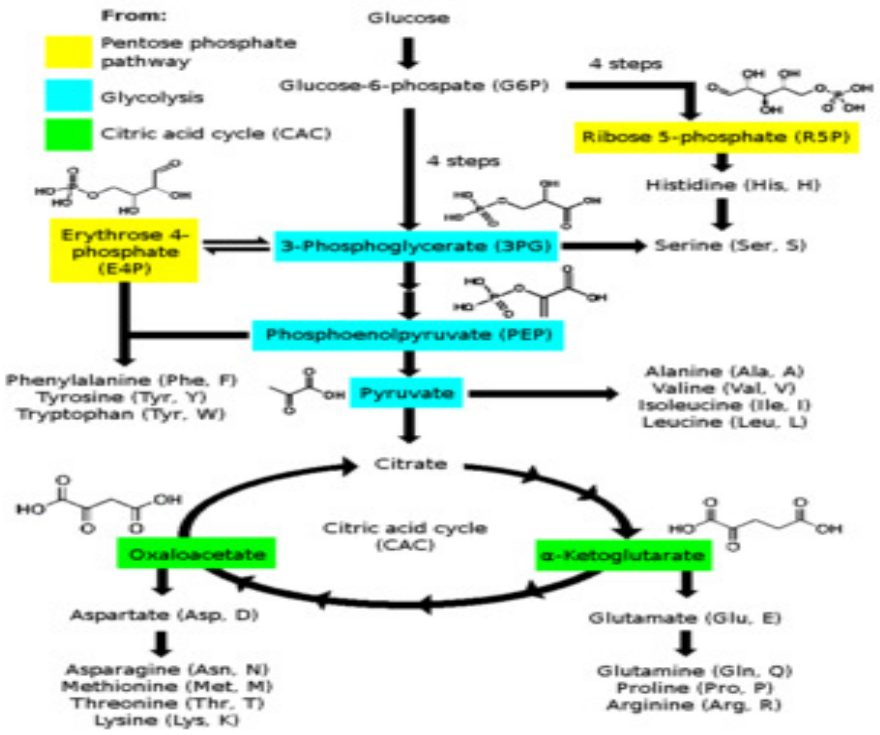

Figure 1:Amino acids biosynthesis. Diagrame showing the biosynthesis pathway of the different amino-acids (Wikipedia).

The majority of amino acids are created from -ketoacids and then transaminated, typically from glutamate, from another amino acid. An aminotransferase is the enzyme that is active in this process.

$$\alpha\text{-ketoacid} + \text{glutamate} \rightleftarrows \text{amino acid} + \alpha\text{-ketoglutarate}$$

Amination of -ketoglutarate results in the formation of glutamate itself.

$$\alpha\text{-ketoglutarate} + NH^{+}_{4} \rightleftarrows \text{glutamate}$$

Beginning with -ketoglutarate, an intermediary in the Citric Acid Cycle, is the -ketoglutarate family of amino acid synthesis (synthesis of glutamate, glutamine, proline, and arginine). The control of enzymatic activity, as well as cell function and metabolism, affect the quantity of -ketoglutarate. Citrate synthase, an enzyme in *E. coli* that participates in the condensation process that starts the Citric Acid Cycle, is severely suppressed by -ketoglutarate feedback inhibition and is also susceptible to DPNH and elevated ATP concentrations. This is one of the early rules governing the family of amino acids known as -ketoglutarates. Due to the reversible character of the transamination and glutamate dehydrogenase processes, the modulation of the synthesis of glutamate from -ketoglutarate is influenced by the citric acid cycle as well as mass action contingent on the concentrations of the reactants involved. A crucial stage in nitrogen biosynthesis is the transformation of glutamate into glutamine, which is controlled by glutamine synthetase. There are at least four distinct ways that this enzyme is controlled: The following factors affect the activity of an enzyme:

1. Repression and depression caused by nitrogen levels;
2. Activation and deactivation caused by enzymatic forms (taut and relaxed);
3. Cumulative feedback suppression caused by end product compounds; and
4. Alterations caused by adenylation and deadenylation (Figure .2).

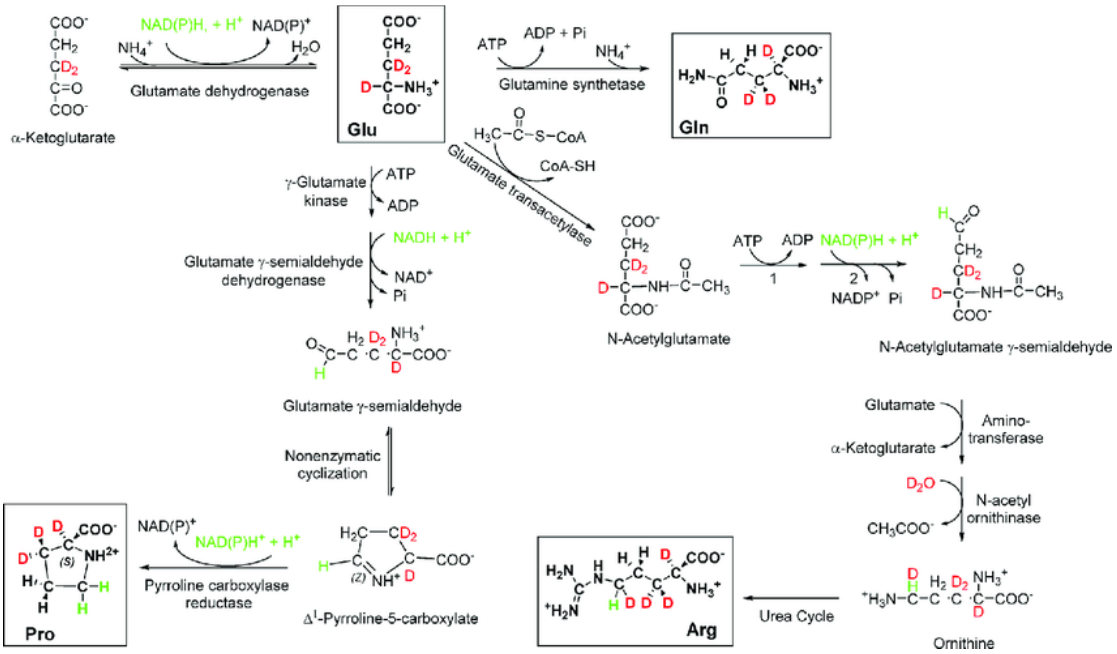

Figure 2: Biosynthesis of glutamate, glutamine, proline, and arginine from α-ketogulatarate and diagrame showing the biosynthesis pathway of the glutamate, glutamine, proline, and arginine (Research gate).

The amount of GS is low in abundant nitrogenous media or growth environments with high ammonia concentrations, whereas the specific activity of the enzyme is 20-fold greater in environments with low ammonia concentrations. Depending on whether GS is in the taut or

flexible shape, the confirmation of the enzyme affects control. The enzyme is in its completely active taut form when manganese is present; however, when manganese is absent, the enzyme becomes flexible. The specific structural state, which is also connected to adenylation, arises as a result of the attachment of particular divalent cations. A number of molecules, including L-tryptophan, L-histidine, AMP, CTP, glucosamine-6-phosphate and carbamyl phosphate, alanine, and glycine, contribute to the accumulated feedback that inhibits GS. An excess of any one product alone does not block the enzyme; however, the accumulation of all the end products has a potent inhibitory impact on the production of glutamine. Adenylation also inhibits the action of glutamine synthetase. The dual-function adenylyltransferase/adenylyl removal (AT/AR) enzyme catalyzes the adenylation action. The regulating protein PII and glutamine work together to promote adenylation. Through negative feedback inhibition, the original controlling process can affect how the proline biosynthesis is regulated. Proline allosterically blocks the activity of glutamate 5-kinase in E. coli, which catalyzes the conversion of L-glutamate to the unstable intermediary glutamyl phosphate. Negative feedback and repression are also used in the production of arginine by a regulator that is encoded by the gene argR. The locus of arginine production is influenced by the argR gene product, argR, an aporepressor, and arginine, a corepressor. The levels of the repressor protein and corepressor decide how much suppression is occurring[3], [4].

Figure 3: Biosynthesis of glutamate, glutamine, proline, and arginine from α-ketogulatarate: Diagrame showing the biosynthesis pathway of the glutamate, glutamine, proline, and arginine (Research gate).

Phenylalanine, tyrosine, and tryptophan, the aromatic amino acids, arise from chorismate (Figure .3). The first step, condensation of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) from PEP/E4P, uses three isoenzymes AroF, AroG, and AroH. Each one of these has its synthesis regulated from tyrosine, phenylalanine, and tryptophan, respectively. The rest of the enzymes in the common pathway (conversion of DAHP to chorismate) appear to be synthesized constitutively, except for shikimate kinase, which can be inhibited by shikimate through linear mixed-type inhibition. Tyrosine and phenylalanine are biosynthesized from prephenate, which is converted to an amino acid-specific intermediate.

This process is mediated by a phenylalanine (PheA) or tyrosine (TyrA) specific chorismate mutase-prephenate dehydrogenase. PheA uses a simple dehydrogenase to convert prephenate to phenylpyruvate, while TyrA uses a NAD-dependent dehydrogenase to make 4-hydroxylphenylpyruvate. Both PheA and TyrA are feedback inhibited by their respective amino acids. Tyrosine can also be inhibited at the transcriptional level by the TyrR repressor. TyrR binds to the TyrR boxes on the operon near the promoter of the gene that it wants to repress.

Tryptophan biosynthesis involves conversion of chorismate to anthranilate using anthranilate synthase. This enzyme requires either glutamine as the amino group donor or ammonia itself. Anthranilate synthase is regulated by the gene products of trpE and trpG. trpE encodes the first subunit, which binds to chorismate and moves the amino group from the donor to chorismate. trpG encodes the second subunit, which facilitates the transfer of the amino group from glutamine. Anthranilate synthase is also regulated by feedback inhibition: tryptophan is a co-repressor to the TrpR repressor.

Lysine, asparagine, methionine, threonine, and isoleucine are the members of the amino acid family that includes oxaloacetate and aspartate. Lysine, asparagine, methionine, and threonine are all products of the conversion of aspartate. Isoleucine is also produced from threonine. The related enzymes are susceptible to hereditary suppression and/or feedback inhibitory control. Additional control is present at each place where the metabolic route branches, as is usual for extremely convoluted metabolic pathways. The overall flow of the aspartate route as well as the total flux of the various amino acids can both be controlled by this kind of regulation structure. L-aspartic acid is used in the aspartate pathway as a precursor for the production of one-fourth of the amino acid building blocks.

Aspartate: Oxaloacetate is commonly transaminated during the production of aspartate. Three isozymes, AK-I, AK-II, and AK-III, make up the enzyme aspartokinase, which catalyzes the phosphorylation of aspartate and starts its change into other amino acids. Threonine inhibits the feed-back of AK-I, whereas lysine inhibits AK-II and AK-III. As a side aside, the critical stage in this biosynthesis route, the phosphorylation of aspartic acid, is catalyzed by AK-III. When threonine or lysine are present, aspartate kinase is suppressed.

Lysine: The diaminopimelate (DAP) route is used to convert aspartate into lysine. Aspartokinase and aspartate semialdehyde dehydrogenase catalyze the first two steps in the DAP pathway. Lysine, threonine, and methionine are biosynthesized in large part by these enzymes. Along with a monofunctional aspartokinase, LysC, there are two bifunctional aspartokinase/homoserine dehydrogenases, ThrA and MetL. Concentrations of the later generated amino acids, lysine, threonine, and methionine, control the transcription of aspartokinase genes. The gene is translated less frequently the greater these amino acid amounts are. Threonine and lysine also block the feed-back of ThrA and LysC. The final stage of lysine production is mediated by DAP decarboxylase LysA, which is present in all examined bacterial taxa. Both lysine and threonine suppress the synthesis of aspartate kinase (AK), which catalyzes the phosphorylation of aspartate and starts its translation into other amino acids, preventing the formation of the amino acids obtained from aspartate. Furthermore, dihydrodipicolinate synthase is inhibited by elevated lysine amounts. (DHPS). Therefore, lysine also suppresses the activity of the first enzyme following the branch point, i.e. the enzyme that is specialized for lysine's own synthesis, in addition to the first enzyme of the aspartate groups biosynthesis route.

Asparagine: Using the transaminase enzyme, aspartate is the starting point for the production of asparagine. Aspartate, glutamine, and ATP are converted by the enzyme

asparagine synthase into asparagine, AMP, glutamate, and pyrophosphate. ATP activates aspartate in the asparagine synthase process, resulting in the formation of -aspartyl-AMP. In order to create asparagine and unbound AMP, glutamine contributes an ammonium group, which interacts with -aspartyl-AMP. Bacteria have two asparagine synthetases. The AsnC protein covers both of them. The genes AsnA and AsnB encode for them. AsnC is autogenously controlled, meaning that the structural gene's offspring controls the operon's genes' expression. Asparagine inhibits the activating impact of AsnC on AsnA transcription. Asparagine has no impact on AsnC's autoregulation, though.

Methionine: Aspartic acid is the first step in the transsulfuration pathway's biosynthesis. Relevant enzymes include cystathionine-synthase, cystathionine-lyase, homoserine dehydrogenase, aspartate-semialdehyde dehydrogenase, and homoserine O-transsuccinylase. Homocysteine methyltransferase or betaine—homocysteine S-methyltransferase carries out this process in animals.

The production of methionine is strictly regulated. The suppression of methionine production is carried out by the repressor protein MetJ in association with the corepressor protein S-adenosyl-methionine. The regulator MetR serves as a transactivator of transcription for the MetE and MetH genes and is necessary for their translation. Homocystein, the biochemical source of methionine, controls the MetR regulatory activity. Furthermore, it is well known that vitamin B12 inhibits the MetE gene's ability to produce itself through the MetH holoenzyme.

Threonine: Threonine is produced from aspartic acid by plants and microbes using -aspartyl-semialdehyde and homoserine. When homoserine is O-phosphorylated, the phosphate ester is hydrolyzed and the OH group is moved at the same time. Aspartokinase, -aspartate semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, and threonine synthase are among the enzymes involved in a normal production of threonine[5]–[7].

Isoleucine: Alpha-ketoglutarate and pyruvic acid are used in the biosynthesis of isoleucine in both plants and microbes. Acetolactate synthase, also known as acetohydroxy acid synthase, acetohydroxy acid isomeroreductase, dihydroxyacid dehydratase, and valine aminotransferase are some of the enzymes involved in this process. End-product regulation governs the modulation of the enzymes threonine deaminase, dihydroxy acid dehydrase, and transaminase. I.e., the production of threonine will be downregulated in the presence of isoleucine. High levels of isoleucine also slow down the translation of aspartate into the intermediary aspartyl-phosphate, preventing further lysine, methionine, threonine, and isoleucine production.

Serine, glycine, and cysteine are synthesized from the 3-phosphoglycerates.

Serine: Phosphoglycerate dehydrogenase converts 3-phosphoglycerate, a product of glycolysis, to 3-phosphohydroxypyruvate and NADH to begin the production of serine. This ketone is reduced to 3-phosphoserine (O-phosphoserine) by phosphoserine transaminase, which is then converted to serine by phosphoserine phosphatase. These enzymes are encoded by the genes serA, serC and serB in microbes like E. coli. Biosynthesis of glycine: The bidirectional transformations of 5,6,7,8-tetrahydrofolate to 5,10-methylenetetrahydrofolate (mTHF) and L-serine to glycine (retro-aldol cleavage) are also carried out by serine hydroxymethyltransferase (SHMT = serine transhydroxymethylase) (hydrolysis). The enzyme SHMT is pyridoxal phosphate (PLP) reliant. NH+ and CO2 can also be used to create glycine. Glycine synthase catalyzes a process involving 4, and mTHF.

Glycine: Serine hydroxymethyltransferase catalyzes the biosynthesis of glycine from serine. (SHMT). A hydrogen atom is successfully substituted for a hydroxymethyl group by the enzyme. GlyA is the gene that codes for SHMT. It is well known that serine, glycine, methionine, purines, thymine, and folates are involved in the intricate control of glyA. The entire process is still not fully understood. Positive regulation of glyA has been linked to the methionine gene product MetR and the methionine metabolite homocysteine. As a coactivator of glyA, homocysteine needs to work with MetR. S-adenosylmethionine and PurR, a protein involved in purine production, are known to down regulate glyA, on the other hand. In order to prevent the bacteria from producing glycine, PurR attaches straight to the regulatory region of glyA. This successfully shuts down the gene.

Cysteine: The cys regulon encodes the genes necessary for the production of cysteine. CysB controls the favorable incorporation of sulfur. N-acetylserine (NAS) and incredibly tiny concentrations of reduced sulfur are efficient inducers of this regulon. By attaching to DNA half-sites on the cys regulon, CysB performs its role. Depending on the subject of interest, these half sites are different in terms of number and layout. One partial location, though, has been preserved. It is located immediately north of the promoter's -35 location. Additionally, there are numerous auxiliary locations, based on the organizer. CysB will attach to the DNA and encompass many of the auxiliary half sites in the absence of the inducer, NAS. The regulon cannot be translated and cysteine cannot be made without the auxiliary half sites. CysB is thought to experience a structural shift in the presence of NAS. CysB is now able to attach correctly to all of the half sites, which results in the induction of the RNA polymerase. The cys regulon will then be translated by the RNA polymerase, and cysteine will be created.

Glycolysis produces pyruvate, which can be used in both the TCA cycle and fermentation procedures. The production of alanine, valine, and leucine starts with either one or two units of pyruvate. The primary way of suppression is feedback inhibition of end products, and in E. coli, the ilvEDA locus also contributes to this control. One molecule of pyruvate is transaminated to create one molecule of alanine, and this is done in two different ways: first, glutamate-alanine transaminase converts glutamate to -ketoglutarate, and then Transaminase C converts valine to -ketoisovalerate. The control of alanine production is largely unknown. The bacterium's capacity to suppress Transaminase C activity by either valine or leucine is the only proven way. Other than that, there is no evidence of regulation of alanine biosynthesis.

Valine: There is a four-enzyme mechanism that produces valine. Beginning with the acetohydroxy acid synthase-catalyzed condensation of two units of pyruvate to produce -acetolactate. The second stage entails the movement of methyl groups and the $NADPH^{+}$-dependent reduction of -acetolactate to create, -dihydroxyisovalerate. Acetohydroxy isomeroreductase catalyzes this. The dehydration of, -dihydroxyisovalerate is carried out in the third stage and is facilitated by dihydroxy acid dehydrase. The resulting -ketoisovalerate then proceeds through transamination in the concluding stage, which is either mediated by an alanine-valine transaminase or a glutamate-valine transaminase. The generation of acetohydroxy acid synthase is susceptible to feedback suppression, which affects valine biosynthesis.

Leucine: Beginning with -ketoisovalerate, the valine pathway splits off to form the leucine synthesis route. This reaction with acetyl CoA is catalyzed by -isopropylmalate synthase to create -isopropylmalate. -isopropylmalate is changed into -isopropylmalate by an isomerase. The third stage involves a dehydrogenase catalyzing the NAD+-dependent oxidation of -isopropylmalate. The transamination of the -ketoisocaproate by a glutamate-leucine transaminase is the last stage.

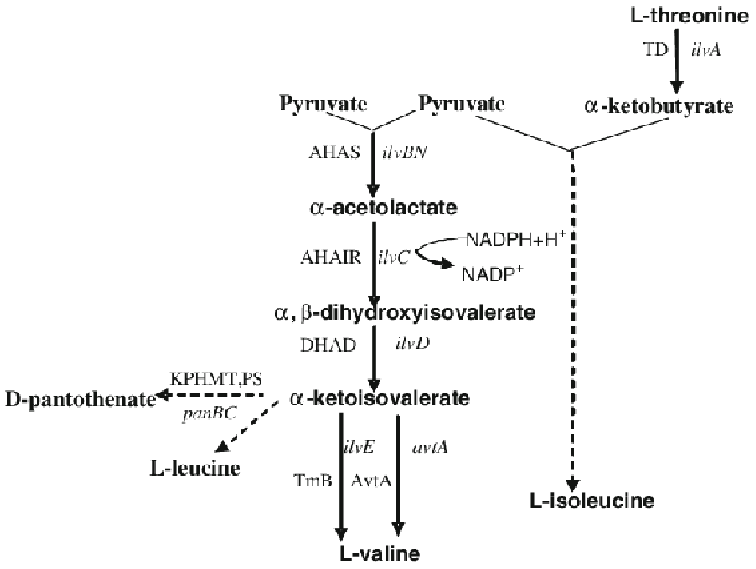

Figure 4: valine, leucine, and isoleucine from pyruvate and diagram showing the synthesis of the valine, leucine, and isoleucine from pyruvate (Research gate).

Similar to valine, leucine controls the first stage of its process by blocking the activity of -Isopropylmalate synthase. Because the synthesis of leucine diverts from the synthesis of valine, the feedback suppression of valine on its route can also prevent leucine from being synthesized. We will concentrate our attention on the other amino acids, the branched chain nonpolar amino acids Val, Leu, and Ile, since Ala can be readily made from the alpha-keto acid pyruvate by a transamination process (Figure. 4).

DISCUSSION

In addition to being necessary building blocks for protein production, the aromatic amino acids phenylalanine, tyrosine, and tryptophan in plants also act as sources for a variety of secondary compounds that are crucial for plant development as well as for human nourishment and health. The shikimate pathway, which is followed by the branched aromatic amino acids biosynthesis route, is used to create the aromatic amino acids, with chorismate acting as a key intermediary branch point molecule. However, it is still unclear how these amino acids are synthesized and how it is regulated and coordinated. Numerous different cross-regulated biosynthetic pathways with distinct genetic roots have been found by recent research on these pathways. Recent research suggests that plants can also manufacture phenylalanine via the intermediary metabolite phenylpyruvate (PPY), similarly to many microbes, although the primary pathway of Phe and Tyr production in plants happens via the intermediate molecule arogenate. Recent research has also uncovered a number of transcription factors that control the translation of genes in Arabidopsis and other plant species that code for the enzymes involved in the shikimate and aromatic amino acid pathways as well as a variety of secondary compounds generated from these processes.

The three aromatic amino acids tyrosine, phenylalanine, and tryptophan are biosynthesized and transported by Escherichia coli genes and proteins, which are thoroughly discussed in this paper. It offers a chronological view on the development of the different processes of the three final pathways that transform chorismate into phenylalanine, tyrosine, and tryptophan as well as those of the common pathway that converts erythrose-4-phosphate and phosphoenolpyruvate to chorismate. Also covered is how feedback suppression, reduction, restraint, and activation control critical responses. TrpR (108 amino acids) and TyrR (513 amino acids), two regulating proteins, are crucial for controlling transcription. When

tryptophan is present, the TrpR protein only acts as a monomer that represses the production of the trp locus and four additional genes the TrpR regulon.

The TyrR protein controls the regulation of nine genes that make up the TyrR regulon and can work as a dimer or a hexamer. TyrR can attach to all three aromatic amino acids as well as ATP, and depending on these interactions, it can either inhibit or activate gene translation. The different regions of this protein that are involved in transforming from a monomer to a dimer or a hexamer, binding the aromatic amino acids and ATP, identifying DNA binding sites, engaging with the alpha component of RNA polymerase, and binding DNA are all outlined. The numerous methods by which TyrR, in combination with particular amino acids, can differently influence the translation of specific TyrR regulon genes are also analyzed.

The aromatic amino acids (AAAs) tryptophan, phenylalanine, and tyrosine are necessary for the production of proteins and are also progenitors to a wide range of natural products in plants, including colors, toxins, hormones, and parts of cell walls. All three AAAs are derived from the shikimate pathway, to which ≥30% of photosynthetically fixed carbon is directed in vascular plants. The AAAs are important components of human foods due to the loss of their biosynthesis routes in animal ancestors, and pesticides have been developed to target the enzymes needed for their production. This overview outlines the structure of the pathway and the transcriptional/posttranscriptional control of the AAA biosynthesis network and emphasizes new molecular discovery of the pathway's enzymes. Additionally, it highlights our present limited understanding of the intracellular compartmentalization and metabolite transport processes connected to plant AAA pathways and talks about metabolic engineering initiatives targeted at enhancing the production of AAA-derived plant natural products[8]–[10].

Large groups of biosynthesis genes recently discovered on the chromosomes of Lactococcus lactis and, to a lesser degree, Lactobacillus, have shed light on how these organisms' genes are organized and how gene translation is regulated. An operon is made up of all the genes that function in a specific amino acid biosynthesis process and is located in a singular region of the chromosome. Some operons contain additional genes that are not necessary for metabolism. In general, genetic cues are comparable to those in other prokaryotes. There are numerous mechanisms that regulate gene translation, and transcription reduction appears to occur frequently. One of the reduction mechanisms discovered is similar to the one that prevents many microbes from synthesizing enough amino acids by causing ribosomes to stop at the codons matching to the limiting amino acid.

The others are distinct and could belong to a brand-new category of reduction mechanisms. Also examined is preliminary data supporting a novel regulation mechanism involving a metabolic switch.

Aroma volatiles are crucial to the biological health and fertility of plants. In order to entice pollinators and seed dispersers, deter animals and enlist their natural foes, as well as interact with other plants, plants create and use volatiles. The production of plant volatiles relies heavily on amino acids and the metabolic intermediates that they produce. To satisfy their unique biochemical requirements, different plants employ various biosynthesis routes and tactics. This study concentrates on the various biosynthesis pathways that plants use to create volatile amino acid-derived aromas, emphasizing both their shared and distinctive characteristics and highlighting the significance of the restricting enzymes found at the border between primary and specialized metabolism. We also provide a short overview of the role played by bioengineering in this interaction and suggest potential future paths for raising the standard of farming output and increasing the production of important volatiles.

Aroma volatiles are essential to a plant's biochemical well-being and reproduction. Plants produce and use volatiles to attract pollinators and pollen dispersers, fend off animals and engage their natural enemies, communicate with other plants, and all of these things. Amino acids and the biochemical products they generate are crucial for the synthesis of flammable compounds in plants. To satisfy their unique biochemical requirements, different plants employ various biosynthesis routes and tactics. This study focuses on the diverse biosynthesis pathways that plants use to produce volatile amino acid-derived aromas, highlighting both their shared and unique characteristics and emphasizing the importance of the restricting enzymes found at the boundary between primary and specialized metabolism. We also give a brief summary of the role that biotechnology has played in this interplay and offer some possible future directions for improving agricultural productivity and boosting the generation of significant volatiles.

In the past, amidated pectin was typically made by introducing ammonia gas while the pH was alkaline or by using an ethanol system, but these methods damage the galacturonic acid chain of the pectin and reduce the gel-forming benefits of amidated pectin. In order to broaden the use of pectin in food, common chemicals, medicine, and other areas, amidated pectin with ultra-high stiffness and low gelation limitation was created in this research by bonding with neutral aliphatic amino acids using an enzyme technique at an extremely low temperature. Leucine amidated pectin (L-PE), valine amidated pectin (A-PE), and alanine amidated pectin (V-PE) all had amino acid grafting ratios of 14.12%, 24.36%, and 18.94%, respectively. According to the dynamic viscosity findings, high methoxy pectin (HMP) dynamic viscosities rose from 186 to 39799 (V-PE), 20396 (A-PE), and 23781 mPa s (L-PE), respectively. The V-PE with the greatest amino acid grafting ratio had the maximum solution viscosity and gel toughness through ultra-low temperature enzyme. Additionally, the hydrogel has improved thermal stability, which enables it to maintain the gel's three-dimensional network structure without collapsing at higher temperatures. Amidated pectin can offer useful information in uses like food and medication due to its ultra-high viscosity and minimal gelation limitation.

All organisms capable of de novo synthesis share this pathway of aromatic amino acid biosynthesis. The production of shikimate 3-phosphate is catalyzed by the protein shikimate kinase. Tryptophan biosynthesis diverges from the common precursors of phenylalanine and tyrosine with the conversion of chorismate to anthranilate. In order to make anthranilate, the enolpyruvyl side chain of chorismate must be removed along with a glutamine-donated amidotransfer. The multibranched shikimic acid pathway is highly reliant on feedback mechanisms to regulate metabolism. This shikimate pathway enzymes occur in the chloroplast and also in the cytosol.

CONCLUSION

The citric acid cycle, the pentose phosphate pathway, or glycolysis intermediates are the sources of all amino acids.The two pathways by which nitrogen enters these networks are through glutamine and glutamate. Mammals can only synthesize about half of them, typically those with simple pathways. Biosynthesis of amino acids is controlled by feedback inhibition. The regulated biosynthetic pathway usually has the first committed step. Serine inhibits 3-phosphoglycerate dehydrogenase. More control thanks to cumulative feedback inhibition. Body protein as well as other important nitrogen-containing compounds like creatine, peptide hormones, and some chemicals cannot be made without amino acids. A biochemical requirement, amino acids are still allowed even though limits are stated in terms of protein.The food business, the medicine industry, and the chemistry industry all use amino acids in some capacity.

REFERENCES:

[1] V. Tzin and G. Galili, "New Insights into the shikimate and aromatic amino acids biosynthesis pathways in plants," *Molecular Plant*. 2010. doi: 10.1093/mp/ssq048.

[2] M. N. Price *et al.*, "Filling gaps in bacterial amino acid biosynthesis pathways with high-throughput genetics," *PLoS Genet.*, 2018, doi: 10.1371/journal.pgen.1007147.

[3] M. A. Bromke, "Amino acid biosynthesis pathways in diatoms," *Metabolites*. 2013. doi: 10.3390/metabo3020294.

[4] T. Parish and N. G. Stoker, "The common aromatic amino acid biosynthesis pathway is essential in Mycobacterium tuberculosis," *Microbiology*, 2002, doi: 10.1099/00221287-148-10-3069.

[5] M. Cao, M. Gao, M. Suástegui, Y. Mei, and Z. Shao, "Building microbial factories for the production of aromatic amino acid pathway derivatives: From commodity chemicals to plant-sourced natural products," *Metabolic Engineering*. 2020. doi: 10.1016/j.ymben.2019.08.008.

[6] A. Takénaka and D. Moras, "Correlation between equi-partition of aminoacyl-tRNA synthetases and amino-acid biosynthesis pathways," *Nucleic Acids Res.*, 2020, doi: 10.1093/nar/gkaa013.

[7] G. Caballero-Flores, J. M. Pickard, S. Fukuda, N. Inohara, and G. Núñez, "An Enteric Pathogen Subverts Colonization Resistance by Evading Competition for Amino Acids in the Gut," *Cell Host Microbe*, 2020, doi: 10.1016/j.chom.2020.06.018.

[8] H. Bono, H. Ogata, S. Goto, and M. Kanehisa, "Reconstruction of amino acid biosynthesis pathways from the complete genome sequence," *Genome Res.*, 1998, doi: 10.1101/gr.8.3.203.

[9] M. D. L. N. Peltzer, N. Roques, V. Poinsot, O. M. Aguilar, J. Batut, and D. Capela, "Auxotrophy accounts for nodulation defect of most Sinorhizobium meliloti mutants in the branched-chain amino acid biosynthesis pathway," *Mol. Plant-Microbe Interact.*, 2008, doi: 10.1094/MPMI-21-9-1232.

[10] J. A. Marchand, M. E. Neugebauer, M. C. Ing, C. I. Lin, J. G. Pelton, and M. C. Y. Chang, "Discovery of a pathway for terminal-alkyne amino acid biosynthesis," *Nature*, 2019, doi: 10.1038/s41586-019-1020-y.

CHAPTER 14

OXIDATIVE PHOSPHORYLATION

Dr Shilpa Patrick, Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- drshilpapatrick@gmail.com

ABSTRACT:

The majority of the ATP required for higher organisms and plants to sustain life is produced by oxidative phosphorylation, which also in role in establishing and preserving metabolic equilibrium. The main function of oxidative phosphorylation is to generate the ATP and the water molecule using the electron transport chain oxygen as the final electron acceptor. In this paper, we discussed the basic steps involved in oxidative phosphorylation, different complex, and the bioenergetics of Oxidative phosphorylation.

KEYWORDS:

ATP Synthase, Electron Transport Chain, Electrochemical Gradient, Inter-membrane Region, Mitochondrial Membrane.

INTRODUCTION

The biological mechanism known as oxidative phosphorylation uses oxygen reduction to produce high-energy phosphate molecules in the form of adenosine triphosphate (ATP). The electron transport chain in the mitochondria is a sequence of oxidation-reduction processes that involve the movement of electrons from NADH and $FADH_2$ to oxygen through several protein, metal, and lipid compounds. NADH and $FADH_2$, which are produced by several catabolic cellular processes, are used by the electron transport chain. Additionally, pure oxygen is used in oxidative phosphorylation as the ultimate reducing substance. Particularly when contrasted to anaerobic species, the electron transport chain, and mitochondrial function cast insight into the development and progress of aerobic cellular life. Numerous lifeforms need oxygen to live because it is characteristic of aerobic respiration [1].

In prokaryotes, these proteins are found in the cell's exterior membrane, whereas in eukaryotes, they are found in a sequence of protein structures within the interior membrane of the cell's mitochondria. The electron transport chain is made up of these connected protein groups. Five major protein complexes are involved in eukaryotes, whereas prokaryotes have many distinct enzymes that use a wide range of electron donors and acceptors. In a process known as electron transport, protons are transported across the interior mitochondrial membrane using the energy carried by the electrons moving through this electron transport chain. Through the creation of a pH difference and an electrochemical potential across this barrier, potential energy is produced. Through a big enzyme called ATP synthase, a process known as chemiosmosis, protons move back across the membrane and down the potential energy gradient to access this energy reserve.

In a process known as phosphorylation, the ATP synthase utilizes the energy to convert adenosine diphosphate (ADP) into adenosine triphosphate. The spinning of a portion of the enzyme is forced by the proton flow, which powers the process. The mechanical ATP synthase is a rotating generator. Although oxidative phosphorylation is an essential component of metabolism, it also generates reactive oxygen species like superoxide and hydrogen peroxide that damage cells, spread illness, and may even speed up the aging and senescence process. Many medications and toxins aim to block the activity of the enzymes

involved in this biochemical process. The interior membrane of the mitochondria contains several chemical compounds and proteins that make up the electron transfer chain. In a succession of redox processes, electrons are transferred from one component of the transport chain to another. In these processes, energy is released as a proton gradient, which is then used to produce ATP through a process known as chemiosmosis. Oxidative phosphorylation is a process that involves both chemiosmosis and the electron transport chain. The picture above highlights the main stages of this procedure in an abridged version, which includes: electron delivery by FADH and NADH. Near the start of the transport chain, reduced electron carriers (NADH and FADH) from other stages of cellular metabolism pass their electrons to molecules. They return to NAD+ and FAD as a result of the process, which can then be used in other stages of cellular metabolism.

Proton flow and electron transmission. Energy is released as electrons travel from a higher to a lower energy level along the circuit. H+ ions are pumped out of the matrix and into the intermembrane region using some of the energy. A difference in electrochemistry is created by this stimulation oxygen splitting creates water. Molecular oxygen and water receive electron transfers after the electron transport chain. ATP production is controlled by gradients. The H+ ions travel through an enzyme called ATP synthase as they run down their gradient and back into the matrix. This enzyme uses the passage of protons to create ATP [2]. Chemiosmosis is the passage of ions down their electrochemical gradient through a semipermeable membrane-linked structure. An essential illustration is the production of adenosine triphosphate (ATP) when hydrogen ions (H+) are transported across a barrier during photosynthesis or cellular metabolism.

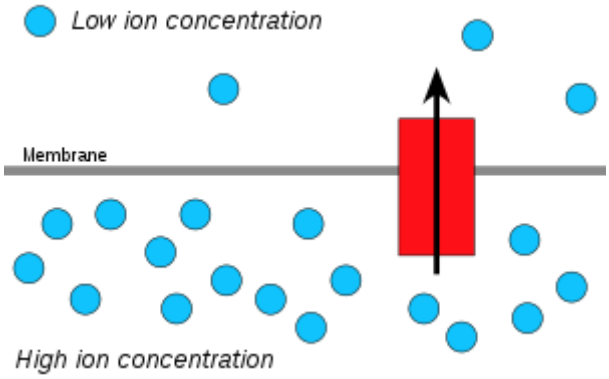

Figure 1: Ion gradient: Diagram showing the While ions move through the channel , a potential energy difference forms that can be used to fuel chemical processes (Wikipedia).

Protons, also known as hydrogen ppapers, will move from an area with a high proton concentration to an area with a low proton concentration. Using an electrochemical gradient of protons across a barrier, ATP can be produced. The term "chemiosmosis" refers to a process that is similar to osmosis in that it involves the passage of water across a selective barrier (Figure. 1). The protein that produces ATP via chemiosmosis is called ATP synthase. It permits protons to cross the membrane and generates ATP by phosphorylating adenosine diphosphate (ADP) with the free energy differential. In addition to mitochondria and chloroplasts, most bacteria and archaea also produce ATP through chemiosmosis. For instance, an electron transport chain transports H+ ions (protons) in the stroma (fluid) through the thylakoid membrane to the thylakoid gaps in chloroplasts during photosynthesis. As electrons pass through ATP synthase, the saved energy is used to photo-phosphorylate ADP, producing ATP. The chemiosmosis theory was put forth by Peter D. Mitchell in 1961. In a nutshell, the theory postulated that the majority of adenosine triphosphate (ATP)

production in respiring cells results from an electrochemical gradient across the mitochondrial inner membranes, using the energy of NADH and FADH2 produced during the oxidative decomposition of energy-rich molecules like glucose. When molecules like glucose are digested, an intermediary with a reasonable amount of energy is produced: acetyl CoA. The reduction of a transport molecule like nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide is linked to the oxidation of acetyl coenzyme A (acetyl-CoA) in the mitochondrial matrix. (FAD). The transporters transfer electrons to the inner mitochondrial membrane's electron transport chain (ETC), which then transfers them to other ETC proteins. Pumping protons out of the matrix and into the intermembrane space uses the energy of oxygen, the final acceptor in the ETC, which is then stored as a transmembrane electrochemical gradient. Through the ATP synthase enzyme, the electrons return across the interior membrane. ADP can join with calcium phosphate to create ATP with the help of the energy provided by the passage of protons back into the framework of the mitochondrion via ATP synthase.

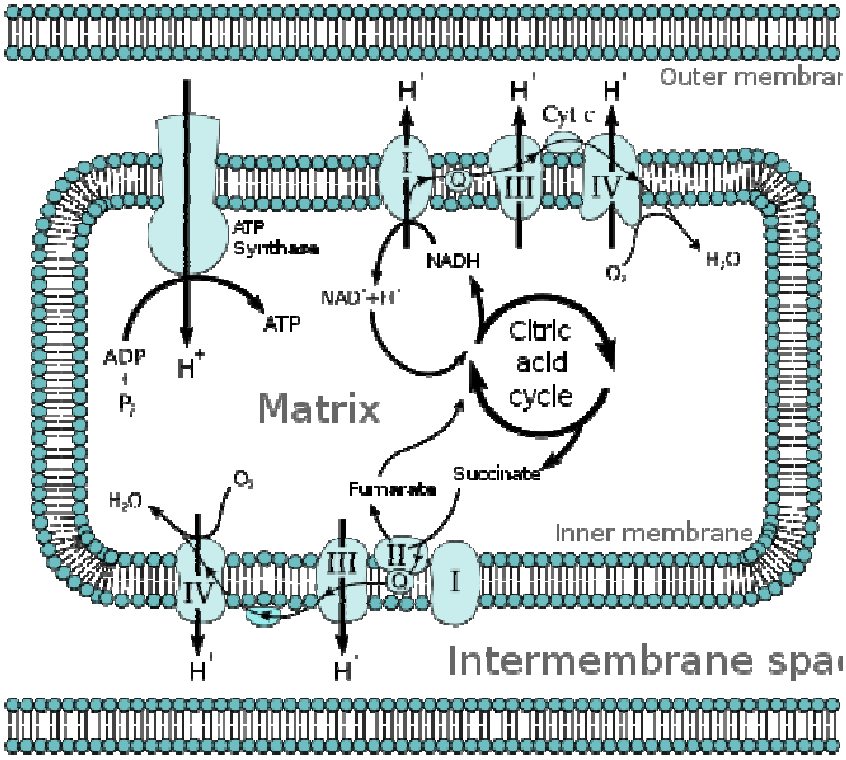

Figure 2: Electron transport chain: Diagram showing the organization of the different complexes used in the electron transport chain (Wikipedia).

At the time, this was a bold suggestion that was not well received. The dominant theory, which was biologically more cautious, was that the energy of electron transport was retained as a steady high potential intermediary. The earlier theory has a flaw in that no high-energy intermediary has ever been discovered, and the proof for proton pumping by complexes of the electron transfer chain got too strong to be disregarded. Eventually, the chemiosmosis theory started to receive more support from the data, and Peter Mitchell was given the 1978 Nobel Prize in Chemistry. ATP generation in mitochondria, chloroplasts, and many bacteria and archaea depends on chemiosmosis coupling [3].

The interaction of two variables determines how quickly ions travel across the membrane:

1. The power of concentration gradient-induced diffusion: All ppapers tend to spread from greater concentrations to lower concentrations.

2. Electrical potential gradient-induced electrostatic force - Cations, such as protons H+, tend to spread from the positive (P) side of the membrane to the negative (N) side. Anions naturally spread in the other way.

An electrochemical gradient can be described as the combination of these two gradients. However, the lipid bilayers in cellular membranes act as ion shields. This explains how a mixture of these two differences across the membrane can be used to store energy. Ions can occasionally only pass through the membrane when specific membrane proteins, such as ion channels, are present. A permeable ATP synthase is essential to the chemiosmosis theory because it transforms the energy of protons' natural passage through them into the molecular energy of ATP molecules. As a result, scientists invented the word proton-motive force (PMF), which they drew from the previously stated electrochemical gradient. It can be defined as the measurement of the potential energy held (chemiosmotic potential) as a result of a membrane's proton and voltage gradients. The charge difference across the membrane, which occurs when the protons H+ travel without a counterion like a chloride Cl, affects the electrochemical gradient.

Protons (hydrogen ions) are typically pumped across the membrane by an electron transport chain acting as a proton pump, which uses the Gibbs free energy of redox processes to separate the charge across the membrane. Protons are moved from the mitochondrial matrix (N side) to the intermembrane region in mitochondria using energy produced by the electron transport chain (P side). Because there are fewer positively charged protons inside the mitochondrion as a consequence of the protons being removed, the membrane's interior is overcharged with negative ions. Inside, the electrical potential difference is approximately -170 mV the combined electrochemical gradient produced by these gradients charge difference and proton concentration difference across the membrane is frequently denoted as the proton-motive force. (PMF). Since the passage of Cl and other anions neutralizes the charge of protons H+, the PMF in chloroplasts is primarily composed of the pH gradient rather than the electrical component that makes up the PMF in mitochondria. In either instance, the ATP synthase requires a PMF higher than approximately 460 mV (45 kJ/mol) to produce ATP.

The majority of the organic compounds and membrane-attached proteins that make up the electron transport chain are arranged into four sizable complexes with the letters I to IV. These compounds are present in large numbers in the interior mitochondrial membrane of animals. The components of the electron transport chain are located in the plasma membrane of prokaryotes. The electrons move from molecules that are less electron-hungry to molecules that are more electron-hungry as they move from a higher to a lower energy level along the chain. These "downhill" electron exchanges liberate energy, which is then used by several protein complexes to push protons out of the mitochondrial matrix and into the intermembrane region, creating a proton gradient. The NADH and FADH2 molecules created during the early phases of cellular respiration glycolysis, pyruvate oxidation, and the citric acid cycle provide all of the electrons that join the transport chain.

Since NADH's electrons have a high energy level and are excellent at giving electrons in redox processes, it can move its electrons to complex I without any intermediate steps, reverting to NAD+. Complex I utilizes the energy released as electrons travel through it in a sequence of redox processes to drive protons out of the matrix and into the intermembrane region.

FADH is unable to move its electrons to complex I because it is less effective at giving electrons than NADH is (that is, FADH's electrons are at a lower energy level). Instead of

pumping protons across the membrane, complex II sends them into the transport chain. Each FADH end subscript molecule pumps fewer protons than an NADH and adds less to the proton gradient as a result of this "bypass."

The path taken by the electrons from NADH and FADH end subscript after the first two compounds are identical. Ubiquinone (Q), which is reduced to produce QH2, and moves through the membrane, receives electrons from both complex I and complex II and transfers them to complex III. More H+ ions are pushed across the membrane as the electrons pass through complex III, and the electrons are eventually transferred to cytochrome C, another mobile transporter. A final load of H+ ions is pushed across the membrane by complex IV after Cyt C transports the electrons there. O2 separates into two oxygen atoms and takes up protons from the matrix to create water after receiving electrons from Complex IV. Each molecule requires the reduction of four electrons, resulting in the formation of two water molecules (Figure. 2).

Electron carrier regeneration. After receiving their electrons from the electron transport chain, NADH and FADH2 transform back into NAD+ and FAD. This is significant because glycolysis and the citric acid cycle both depend on the oxidized versions of these electron transporters and thus require their availability. Creates a stream of the proton. With a greater quantity of H+ in the intermembrane region and a lower concentration in the matrix, the transport chain creates a proton gradient across the inner mitochondrial membrane. As we'll see, this difference reflects a type of energy that is saved and can be used to create ATP. The majority of contemporary sources, however, predict that a glucose molecule can only produce up to 32 ATP at its highest rate. Because it takes into consideration the required transfer of ADP into and ATP out of the mitochondrion, this range is smaller than earlier predictions. Glycolysis generates two net ATP, and the citric acid cycle generates an additional two ATP (or chemically similar GTP). The final four ATP molecules all originate from oxidative phosphorylation. Numerous experiments have led to the conclusion that to fuel the production of one ATP molecule, four H+ must return to the matrix via ATP synthase.

Each NADH produces about 2.5 ATP when its electrons pass through the transport chain, which pumps about 10 H+ ions from the matrix into the intermembrane region. Only 6 H+ are pumped by later-arriving electrons from FADH2, which results in the generation of about 1.5 ATP. Your body's cells have a transit mechanism that uses FADH2 to transfer electrons to the transport chain. Only three ATP are generated in this instance for the two NADH involved in glycolysis. Your body's other cells have a transit mechanism that transports the electrons via NADH, leading to the creation of 5 ATP. Since glycolysis and the citric acid cycle take place in the cytoplasm of bacteria, there is no transport required for the production of 5 ATP. An optimistic estimate of the output from the decomposition of one glucose molecule is 30-32 ATP; the actual yield might be smaller. For instance, the cell may take some products from cellular metabolism and use them in other metabolic processes, which lowers the amount of ATP generated. The network of metabolic pathways that are connected by cellular respiration is much bigger than the network formed by the glucose breakdown pathways alone.

DISCUSSION

Without influencing the respiratory chain or ATP synthase (H(+)-ATPase), uncouplers of oxidative phosphorylation in mitochondria prevent the link between the electron transport and phosphorylation processes. This inhibits ATP production. Numerous substances are uncouplers, but slightly acidic uncouplers stand out because of their strong actions. The restricted phenol SF 6847 and the hydrophobic salicylanilide S-13, which are active in vitro

at quantities in the 10 nM range, are the most powerful uncouplers so far identified. Acid-dissociable groups, large hydrophobic moieties, and powerful electron-withdrawing groups are necessary for the onset of uncoupling. Weakly acidic uncouplers are thought to cause uncoupling through the H(+)-impermeable mitochondrial membrane through their protonophoric activity. The persistence of the corresponding uncoupler anions in the hydrophobic membrane is crucial for achieving these results. Delocalization of the polar ionic charge through uncoupler-specific (chemical) processes results in high stability. Such a mildly acidic uncoupler action is typical of a non-site-specific form of bioactive compound's extremely effective membrane targeting action [4].

The last metabolic process in the synthesis of ATP is the mitochondrial oxidative phosphorylation (OXPHOS) system. The mitochondrial or nuclear genomes each encode one component of the five multiprotein complexes that make up the OXPHOS system. Devastating, typically multisystemic illnesses are caused by defects in the OXPHOS system, and recent years have seen the discovery of the underlying genomic abnormalities in nuclear and mitochondrial genes. This field has benefited from developments in numerous genome studies as well as advancements in our capacity to produce useful animal models [5].

We review the basics of photophosphorylation and oxidative phosphorylation. We present new laboratory evidence on the role of succinate and malate anions in oxidative and photophosphorylation, respectively. The energy coupling and ATP production that takes place in mitochondria and chloroplast thylakoids are explained by these new findings in a unique molecular mechanical manner. Since Mitchell's chemiosmotic theory's initial publication 50 years ago, when it was praised as a ground-breaking mechanical account of what is arguably the most significant process in cellular energetics, many studies have pointed out its flaws. The mechanism does not suffer from these flaws. The novel results are in line with Nath's twisting process of energy transfer and ATP production forecasts very well. It is claimed that this mechanism, which Sunil Nath has developed over at least 15 years of theoretical and practical research, represents a completely new theory of the energy conversion process that resolves all the problems with Mitchell's chemiosmotic theory that have been brought up by other writers.

It is inferred that rather than just being electrogenic proton translocators, the energy-transducing complexes in oxidative phosphorylation and photosynthesis are proton-dicarboxylic acid anion cotransporters. These findings call for a rethink of earlier ideas regarding ATP production, coupling, and cellular energy transfer. The innovative molecular mechanism is expanded to include ATP production in prokaryotes, specifically in alkaliphilic and haloalkaliphilic bacteria. This effectively turns the theory into a full one that addresses mechanical, kinetic, and thermal aspects. Finally, quantifiable values for the P/O ratio—the quantity of ATP produced per redox packet of the reduced substrates—are computed based on the novel understanding of oxidative phosphorylation and contrasted with experimental values for fermentation on various substrates. We anticipate that the description of oxidative phosphorylation and photophosphorylation from a completely new angle will reignite scientific debate about a crucial bioenergetics process and open up new research directions in a genuinely multidisciplinary area [6].

This study focuses on the information that shows diminished mitochondrial oxidative phosphorylation occurs during aging and contributes to compromised cellular metabolism. Aging reduces cellular energy production, inhibits substrate oxidation, and raises the production of harmful reactive intermediates. First, a quick recap of the fundamental concepts of mitochondrial oxidative metabolism is provided. The "rate of living" and "uncoupling to survive" theories of aging, which emphasize the connection between changed mitochondrial

metabolism and the increased generation of reactive oxygen species, are then addressed. Third, a summary of the organ-based method used in animal systems to examine the decline of respiratory function with aging is presented. Fourth, the present status of understanding regarding changes in the makeup and operation of important mitochondrial components brought on by aging is discussed. Where appropriate, model animals like *C. elegans* and D. melanogaster are used. Fifth, an understanding of the generation of reactive oxygen species from particular locations of the electron transport pathway is linked to these flaws [7].

To function properly, the majority of body cells need the ATP created by oxidative phosphorylation. The primary causes of a large and diverse range of multisystem illnesses are incomplete abnormalities in this system. Because both mitochondrial and nuclear genes contain structural components of the enzyme complexes involved in oxidative phosphorylation, illnesses can be inherited in a Mendelian, maternal, or random way. In the last 12 years, more than 100 mtDNA variations have been discovered, the majority of which are connected to adult-onset illnesses. Recently, there has been a lot of interest in the research of anomalies in the nucleus oxidative phosphorylation gene. The majority of these are inherited hereditary traits that produce severe, commonly fatal illnesses in infants. Mendelian oxidative phosphorylation diseases with adult development have a weakened pattern, and the bulk of them are associated with multiple mtDNA losses. These diseases have the potential to be inherited dominant or recessive traits. In genes that code for the complexes' structural components, assembly/maintenance components, and components crucial for preserving the integrity of the mtDNA, approximately 20 unique nuclear gene anomalies have now been identified. Some unique genotype-phenotype associations have emerged, implicating mitochondria as oxygen sensors in the hypoxia response, and there is an unexpected link between some structural gene alterations and rare tumors[8].

The process of oxidative phosphorylation in oxidative human skeletal muscle was created as a dynamic computer model. The cytosolic proton production/consumption system (proton production/consumption by the creatine kinase-catalyzed reaction, efflux/influx of protons), physiological size of the adenine nucleotide pool, and some other minor changes were added to the previously published model of oxidative phosphorylation in isolated skeletal muscle mitochondria. Since inorganic phosphate only slightly alters the relationship between the respiration rate and [ADP], theoretical studies using the extended model showed that the CK system, which allows for large changes in Pi in comparison to the isolated mitochondria system, has no significant influence on the kinetic properties of oxidative phosphorylation. Additionally, it was determined through computer models that the second-order reliance of oxidative phosphorylation on [ADP] advocated in the literature only applies to the flow of ATP production and not the flux of oxygen intake the difference between these two fluxes is due to the proton leak). Then, time sequences of flow and metabolite concentration variations as a result of transitioning between various steady-states were modeled. According to previous theoretical predictions, the model suggests that at low work intensities, activation of oxidative phosphorylation by an increase in [ADP] can (roughly) explain the behavior of the system, whereas, at high work intensities, parallel activation of various steps of oxidative phosphorylation is involved [9].

A newly recognized characteristic of tumor biology, metabolic reprogramming is a hotly sought chance in the search for new cancer medicines. Numerous attempts have been made to therapeutically target glycolysis, but little has been done to treat mitochondrial oxidative phosphorylation (OXPHOS), in part because we don't fully comprehend the circumstances in which OXPHOS is required in tumors. Here, we announce the identification of IACS-010759, a small molecular inhibitor of complex I of the mitochondrial electron transport

chain that is clinical-grade. In models of brain cancer and acute myeloid leukemia (AML) dependent on OXPHOS, treatment with IACS-010759 strongly suppressed growth and caused death. This effect is most likely attributable to a mix of energy loss and decreased aspartate production, which impairs nucleotide biosynthesis. IACS-010759 therapy at well-tolerated dosages significantly suppressed tumor development in vivo in models of AML and brain cancer. Phase 1 clinical studies are presently being conducted to assess IACS-010759 in patients with relapsed/refractory AML and solid malignancies [10].

The assembly of the oxidative phosphorylation (OXPHOS) system in the interior mitochondrial membrane is a complex procedure involving numerous interdependent variables. The OXPHOS system is made up of four respiratory chain complexes that are in charge of producing the proton gradient in the mitochondrial intermembrane space and transporting electrons, as well as the ATP synthase that utilizes this gradient to create ATP. Human mitochondrial diseases are brought on by OXPHOS system malfunction, and many of them are correlated with changed OXPHOS system component assembly. It has been helpful to fully grasp the mechanisms underlying the formation of these big multimeric groups by studying assembly errors in human cases. Based on research into the related diseases, we summarize here the present understanding of the formation of OXPHOS complexes [11].

One tenet of Mitchell's chemiosmotic hypothesis of oxidative phosphorylation is the presence of an electrical potential differential at the mitochondrial membrane1, 2. The uncoupling of oxidative phosphorylation is caused by shunting of the mitochondrial membrane. Uncoupling substances have been shown to enhance the proton permeability of synthetic bimolecular membranes4–7 and phospholipid micelle membranes3. Uncouplers can function as proton transporters in mitochondrial membranes, as demonstrated by Mitchell and Moyle8 [12].

Nitric oxide (NO) and potassium cyanide (KCN) have been investigated for their effects on the effectiveness of oxidative phosphorylation. Concentrations of NO or KCN that improve oxidative phosphorylation efficacy in mitochondria oxidizing succinate or palmitoyl-L-carnitine but not in mitochondria oxidizing malate + glutamate decreased stationary oxygen utilization by 10–20%. Succinate or palmitoyl-L-carnitine decreased the redox state of cytochrome oxidase in comparison to malate + glutamate. At various stages of ATP production, the link between membrane potential and oxygen utilization rates was assessed. The association was impacted by the substitution of malate plus glutamate for succinate, which altered the H+/2e stoichiometry of the respiratory chain but was unaffected by alterations to membrane permeability. The association was also impacted by NO or KCN, indicating that they altered the respiratory chain's H+/2e stoichiometry. We suggest that NO may be a redox-sensitive short-term modulator of mitochondrial metabolism that lowers proton pump slippage and boosts oxidative phosphorylation efficacy [13].

For quantified investigations of the modulation of mitochondrial oxidative phosphorylations, metabolic control analysis has frequently been used. (OXPHOS). This research's primary addition has been to demonstrate how different oxidative phosphorylation stages can share control of mitochondrial metabolic flows, and how the allocation of control varies depending on the tissue and stable state. The results of these studies do not, however, indicate whether the experimental setup or the makeup of the mitochondria is to blame for the reported variance in the OXPHOS regulation. We calculated the control coefficients of seven OXPHOS complexes on the oxygen-consumption flux in rat mitochondria isolated from five different tissues under identical experimental conditions to ascertain whether there is a tissue variation in the distribution of OXPHOS control coefficients. Therefore, in this study, any differences in control coefficient values between organs can only be explained by the

structure of the mitochondria. Two tissue groups can be identified based on the analysis of control coefficient distribution:

(i) The liver, the kidney, and the brain, which are primarily controlled at the phosphorylation level by ATP synthase and the phosphate carrier.

(ii) The muscle and the heart, which are primarily controlled at the level of the respiratory chain. We suggest that part of the tissue specialization seen in mitochondrial cytopathies can be explained by this difference in control coefficient according to the tissue origin of the mitochondria [14].

CONCLUSION

Cellular metabolism is a set of metabolic procedures in which biological energy is extracted from organic materials (such as glucose) and saved in energy carriers (such as ATP) for use in the cell's energy-consuming processes. The proton gradient helps the mitochondrion's ability to produce ATP from ADP and Pi. Because the conversion of ADP to ATP depends on the oxygen processes taking place in the mitochondria, the procedure is known as oxidative phosphorylation. The majority of the ATP required for higher creatures and plants to sustain life is produced by oxidative phosphorylation, which is also in charge of establishing and preserving metabolic equilibrium.

REFERENCES:

[1] O. A. Deshpande and S. S. Mohiuddin, *Biochemistry, Oxidative Phosphorylation*. StatPearls Publishing, 2020. Accessed: Mar. 24, 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK553192/

[2] "Oxidative phosphorylation | Biology (paper) | Khan Academy." https://www.khanacademy.org/_render (accessed Mar. 24, 2021).

[3] "Chemiosmosis," *Wikipedia*. Feb. 12, 2021. Accessed: Mar. 24, 2021. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Chemiosmosis&oldid=1138930229

[4] H. Terada, "Uncouplers of oxidative phosphorylation.," *Environ. Health Perspect.*, vol. 87, pp. 213–218, Jul. 1990, doi: 10.1289/ehp.9087213.

[5] J. Smeitink, L. van den Heuvel, and S. DiMauro, "The genetics and pathology of oxidative phosphorylation," *Nat. Rev. Genet.*, vol. 2, no. 5, Art. no. 5, May 2001, doi: 10.1038/35072063.

[6] S. Nath and J. Villadsen, "Oxidative phosphorylation revisited," *Biotechnol. Bioeng.*, vol. 112, no. 3, pp. 429–437, 2015, doi: 10.1002/bit.25492.

[7] E. J. Lesnefsky and C. L. Hoppel, "Oxidative phosphorylation and aging," *Ageing Res. Rev.*, vol. 5, no. 4, pp. 402–433, Nov. 2006, doi: 10.1016/j.arr.2006.04.001.

[8] E. A. Shoubridge, "Nuclear genetic defects of oxidative phosphorylation," *Hum. Mol. Genet.*, vol. 10, no. 20, pp. 2277–2284, Oct. 2001, doi: 10.1093/hmg/10.20.2277.

[9] B. Korzeniewski and J. A. Zoladz, "A model of oxidative phosphorylation in mammalian skeletal muscle," *Biophys. Chem.*, vol. 92, no. 1, pp. 17–34, Sep. 2001, doi: 10.1016/S0301-4622(01)00184-3.

[10] J. R. Molina *et al.*, "An inhibitor of oxidative phosphorylation exploits cancer vulnerability," *Nat. Med.*, vol. 24, no. 7, Art. no. 7, Jul. 2018, doi: 10.1038/s41591-018-0052-4.

[11] E. Fernández-Vizarra, V. Tiranti, and M. Zeviani, "Assembly of the oxidative phosphorylation system in humans: What we have learned by studying its defects," *Biochim. Biophys. Acta BBA - Mol. Cell Res.*, vol. 1793, no. 1, pp. 200–211, Jan. 2009, doi: 10.1016/j.bbamcr.2008.05.028.

[12] E. A. Liberman, V. P. Topaly, L. M. Tsofina, A. A. Jasaitis, and V. P. Skulachev, "Mechanism of Coupling of Oxidative Phosphorylation and the Membrane Potential of Mitochondria," *Nature*, vol. 222, no. 5198, Art. no. 5198, Jun. 1969, doi: 10.1038/2221076a0.

[13] P. Clerc, M. Rigoulet, X. Leverve, and E. Fontaine, "Nitric oxide increases oxidative phosphorylation efficiency," *J. Bioenerg. Biomembr.*, vol. 39, no. 2, pp. 158–166, Apr. 2007, doi: 10.1007/s10863-007-9074-1.

[14] R. ROSSIGNOL, T. LETELLIER, M. MALGAT, C. ROCHER, and J.-P. MAZAT, "Tissue variation in the control of oxidative phosphorylation: implication for mitochondrial diseases," *Biochem. J.*, vol. 347, no. 1, pp. 45–53, Mar. 2000, doi: 10.1042/bj3470045.

CHAPTER 15

BIOSYNTHESIS AND DEGRADATION PATHWAY OF NUCLEOTIDES

Dr Preeti Singh, Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- drpreetisinghtmu@gmail.com

ABSTRACT:

Purine and pyrimidine serve as the basic unit for the Genetic material, DNA. Nucleotide synthesis and degradation are known as nucleotide anabolism and catabolism. The Denovo pathway and the salvage pathway are the two pathways used in the biosynthesis of nucleotides. In this paper, we cover the metabolic pathway of purine and pyrimidine synthesis. We also cover the degradation pathway of the nucleotides in this paper.

KEYWORDS:

Denovo Pathway, Nucleic Acids, Purine Synthesis, Pyrimidine Synthesis, Salvage Pathway.

INTRODUCTION

The phrase "nucleic acid metabolism" refers to the range of molecular processes that are used to either produce or break down nucleic acids (DNA and/or RNA). Nucleic acids are compounds, or so-called "biopolymers," made up of numerous nucleotide molecules. A nitrogenous base, phosphate, and pentose sugar undergo a molecular process known as nucleotide synthesis, which is a common anabolic mechanism. Nucleic acid degradation is a catabolic process, and the leftover nucleotides or nucleobases can be used to make new nucleotides. For both production and breakdown processes, several enzymes are needed. These enzymes can have flaws or defects that cause several illnesses. The molecules that polymerize into nucleic acids are called nucleotides.

All nucleotides comprise a nitrogenous base, a phosphate, and a sugar. Either purines or pyrimidines serve as the bases in nucleic acids. They are both mainly made in the liver of more complicated multiple organisms, but the two distinct categories are created in various ways. However, phosphoribosyl pyrophosphate (PRPP), which provides the phosphate and ribose needed to make a nucleotide, is required for all nucleotide production. Purine synthesis: There are two purine synthesis routes in mammals: a de novo pathway and a recovery pathway that uses recycled nucleotide bases. Since purine bases are produced by the degradation of nucleic acids, the recovery route is usually adequate. Adenine phosphoribosyltransferase (APRT) or hypoxanthine-guanine phosphoribosyltransferase (HGRT) are enzymes that link the free bases adenine, guanine, and hypoxanthine to phosphoribosyl pyrophosphate (PRPP) to create nucleoside monophosphates (NMP), which are then converted into IMP and GMP (Figure .1) [1].

In both the salvage and de novo routes, PRPP serves as a scaffold. Figure.1 gives a comprehensive description of the general de novo and rescue routes for purine production.In humans, 6 gene products initiate 10 molecular stages in the conserved de novo biosynthesis process that produces IMP. They include the trifunctional enzyme TGART, which is made up of the GAR synthetase (GARS), GAR transformylase (GARTfase), and AIR synthetase (AIRS) domains; the bifunctional enzymes PAICS and ATIC, which are made up of the AICAR transformylase (AICART) and the IMP cyclohydrolase (IMPCH); and three monofunctional enzymes, phosphoribosyl (ADSL). Through a series of sequential processes

involving IMP dehydrogenase (IMPDH), GMP synthase (GMPS), and ADSL, downstream IMP is transformed into (1) GMP and (2) AMP. The hypoxanthine phosphoribosyltransferase (HPRT)-mediated one-step processes PRPP uses to produce IMP and GMP using hypoxanthine and guanine bases are necessary for the rescue pathway. Adenine phosphoribosyltransferase (APRT) uses adenine base and PRPP as substrates to produce AMP. Glycine, N10-formyl THF, and aspartic acid are among the substrates that mitochondria provide for purine de novo production through their one-carbon cycle (1C cycle) and tricarboxylic acid cycle (TCA).

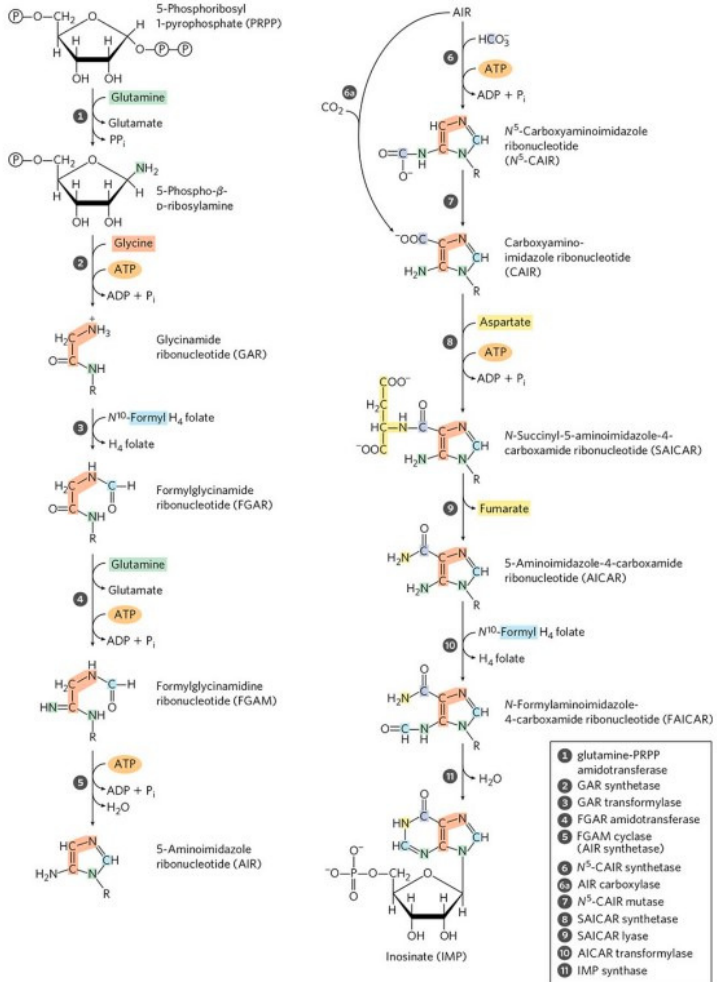

Figure 1: De novo pathway of the purine synthesis: Diagramed showing the reaction of the de novo pathway of the purine synthesis (Zoologytalks)

When there is a large demand for purines, the de novo routes begin to function. The 10-stage process requires six enzymes. These include the two bifunctional enzymes phosphoribosylaminoimidazole carboxylase (PAICS) and AICAR transformylase/inosine monophosphate cyclohydrolase (ATIC), as well as the trifunctional enzyme glycinamide ribonucleotide transformylase, which catalyzes multiple steps in the pathway (TGART). When the pathway is active, it is constrained by the supply of the precursor as well as the rate at which the enzyme phosphoribosylpyrophosphateamidotransferase converts PRPP to phosphoribosylamine (PRA) (PPAT) (Figure. 2). A precursor for the creation of both AMP and GMP, IMP is the end result of the de novo biosynthesis route. 1 IMP is created from PRPP using 6 ATP. In the recovery route, none are necessary

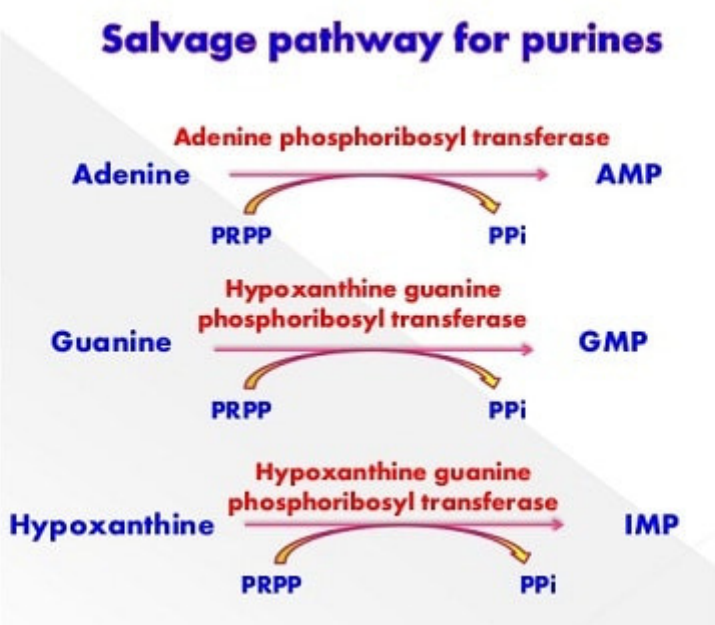

Figure 2: Salvage pathway of the purine synthesis: Diagramed showing the reaction of the salvage pathway of the purine synthesis (medicoapps.org).

Pyrimidine production Cytidine-triphosphate (CTP), at right, is created when uridine-triphosphate (UTP), at left, interacts with glutamine and other substances. Cytidine, uridine, and thymidine are examples of pyrimidine nucleosides. Any pyrimidine nucleotide synthesis starts with the production of uridine. Aspartate, glutamine, bicarbonate, two ATP molecules (to provide energy), and PRPP, which supplies ribose-monophosphate, are all needed for this process. The sugar/phosphate molecule from PRPP is introduced to the nitrogenous base later in the process than it is in purine synthesis. Uridine-monophosphate can be created and then combine with two ATP molecules to create uridine-triphosphate or UTP. CTP synthase can facilitate the conversion of UTP to CTP (cytidine-triphosphate) (Figure.3) [2].

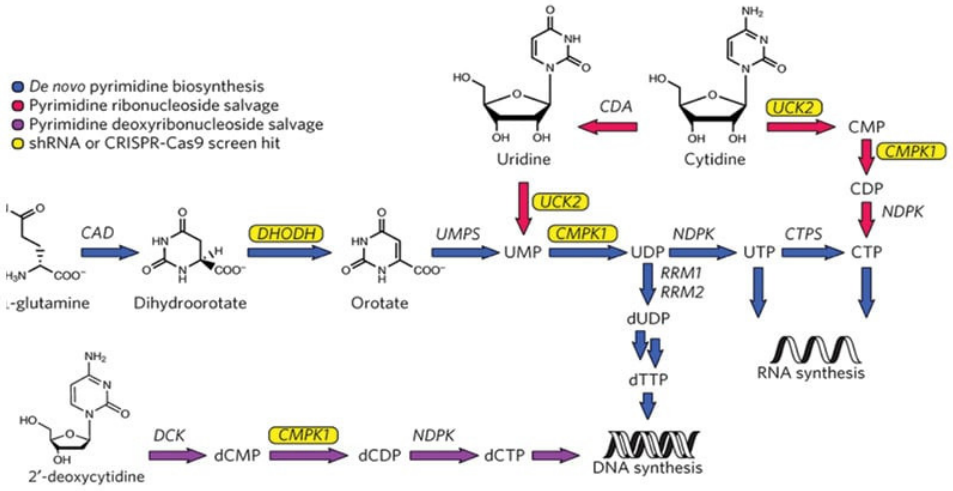

Figure 3: De novo and the salvage pathway of the pyrimidine synthesis: Diagramed showing the reaction of the de novo and the salvage pathway of the pyrimidine synthesis (Microbe notes)

Before the base being modified to create thymidine, the uridine must first be reduced to deoxyuridine. The production of pyrimidines is activated by the purine nucleotide ATP and

inhibited by the pyrimidine nucleotide CTP. Because equivalent quantities of purines and pyrimidines are needed for DNA production, this control serves to maintain the purine/pyrimidine ratios in a comparable range. Genetic illness can result from deficiencies of enzymes involved in pyrimidine production. Orotic acid production in the urine is high in people with orotic aciduria. Ribose, a characteristic of RNA, is used as the sugar component in the early synthesis of nucleotides. However, deoxyribose, which lacks the 2'-hydroxyl (-OH group) on the ribose, is what DNA needs. Ribonucleotide reductase is the enzyme that catalyzes the process to eliminate this -OH. The NDPs (nucleoside-diphosphate) are changed into dNDPs by this enzyme. For the process to take place, the nucleotides must be in the diphosphate form. Uridine is changed to deoxyuridine (by ribonucleotide reductase) and then modified by thymidylate synthase to produce thymidine, a component of DNA that only appears in the deoxy state.

Purine nucleotides, which are broken down into adenosine (Ado) and deoxyadenosine (dAdo), as well as guanosine (Guo) and deoxyguanosine, accumulate as a result of normal nucleic acid breakdown (dGuo). All cells contain ADA, which transforms the compounds Ado and 2′-dAdo into inosine (Ino) and 2′-deoxyinosine (dIno), respectively. PNP turns Guo and dGuo into guanine and Ino and 2′-dIno into hypoxanthine (Figure. 4). The purine salvage pathway is depicted in green. When these molecules reach this pathway, they are transformed back into ATP and GTP, which can then be repurposed into new purines to prepare cells for cell division. Therefore, ADA and PNP are essential for converting components of old purines into new purines, especially in regions where cell division happens quickly (such as the bone marrow, thymus, and lymph nodes).

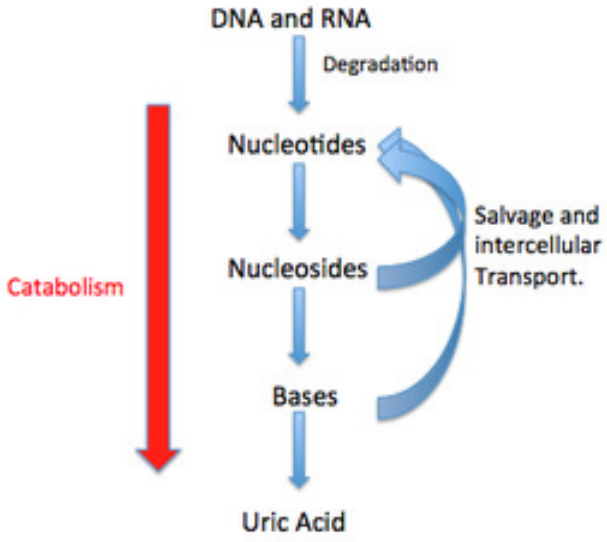

Figure 4: Degradation of the nucleotides: Diagramed showing the reaction of the degradation of the nucleotides (Wikipedia)

High amounts of Ado, dAdo, Guo, and dGuo build up in the absence of ADA/PNP activity (the red route in the image), where they are then converted to dATP and dGTP. These harmful molecules cause DNA breakage, prevent normal DNA methylation, and obstruct de novo DNA synthesis, all of which lead to the induction of cell death. Since dATP and dGTP buildup most severely affects rapidly multiplying cells like T and B lymphocytes and NK cells, ADA and PNP defects cause a varying loss of these cell types as well as clinical signs of SCID [3].

DISCUSSION

DNA and RNA are composed of pyrimidines and purine (deoxy) nucleotides as their building elements. Nearly all glycosidic links between sugars are created through the production of nucleoside diphosphate sugars, such as UDP-glucose. In animals, UMP de novo synthesis and, to a greater or smaller degree, recovery of liberated nucleosides, are used to meet the demand for pyrimidines. One emphasis of the current study is the unusual compartmentation of the de novo synthesis concerning mitochondrially bound dihydroorotate dehydrogenase. The dihydroorotate-dependent oxygen utilization or the UV absorbance of the orotate product with mitochondria separated from mouse and pig tissues were used to measure DHODH activity. In contrast, the oxygen utilization of mitochondria from various organs was evaluated using cytochrome c and choline. The liver 2.3 10-3 mol/min mg protein was the organ with the greatest specific activity of the rat DHODH, followed by the kidney and the heart. The precision of the used activity assays was confirmed by using sodium cyanide for cytochrome c oxidase and the known enzyme inhibitors Brequinar Sodium and Leflunomide for DHODH. The liver mitochondria had the greatest rates and the heart mitochondria had the lowest when comparing DHODH activity to cytochrome c oxidase activity. It is advised to include improving the pyrimidine nucleotide state in treatment procedures because abnormalities in mitochondrial energy metabolism may result in significant disruption of pyrimidine biosynthesis via respiratory-chain-linked DHODH [4].

Analogs of pathway intermediate that are produced synthetically or naturally, or, more recently, inhibitors that were logically designed using knowledge of the catalytic mechanism, are effective inhibitors of enzymes that catalyze processes in the de novo pathways for the production of purine and pyrimidine nucleotides. Such inhibitors could be useful medications for treating infections, inflammatory diseases, or tumors. The purine pathway may be a more effective target for suppression in human cancer than the pyrimidine pathway, where harmful side effects are more noticeable. Drugs with numerous areas of action, like 6-mercaptopurine and methotrexate, make it challenging to forecast their impacts on cells numerically. The possibility of creating medicines with just one site of action in human cells exists through the rational creation of inhibitors based on the X-ray structure of the target enzyme. A powerful suppressor of the purine enzyme IMP dehydrogenase is VX-497 [5].

Since the release of prostaglandins from different separate perfused tissues is typically influenced by the stimulus, some triggers caused prostaglandin release while others did not. Adenosine monophosphate and adenosine were inert while adenosine triphosphate and adenosine diphosphate were effective stimulators of prostaglandin production in the kidney, spleen, splenic adipose pad, heart, liver, and lung. While angiotensin was agonistic in the kidney, spleen, splenic fat pad, and liver, epinephrine caused prostaglandin production from the kidney, spleen, and liver. Each agonist's production (biosynthesis) of prostaglandins from all tissues was stopped by indomethacin [6].

Under different nutrient-limiting circumstances, Alcaligenes eutrophus poly-hydroxybutyrate biosynthesis was investigated. Both the amounts of NAD(P)H and the ratios of NAD(P)H/NAD(P) were higher in the cells grown in nitrogen-limited media than in nitrogen-sufficient media. It was discovered that the particular poly-hydroxybutyrate production rate increased with the values of both NADH/NAD and NADPH/NADP, proving that the ratios of nicotinamide nucleotides directly control poly-hydroxybutyrate synthesis. Regarding enzyme rates, the impacts of nicotinamide nucleotides on poly--hydroxybutyrate biosynthesis were examined. Citrate synthase activity was substantially decreased by NADH and NADPH, suggesting that increasing the metabolic flow of acetyl-CoA to the poly-hydroxybutyrate synthase pathway could improve poly-hydroxybutyrate buildup. It was also discovered that

the total biosynthesis activity of poly-3-hydroxybutyrate in this strain was regulated by the cellular NADPH, which served as a limiting substrate for NADPH-linked reductase [7].

In this paper, we'll talk about how nucleotides are made. The basic pyrimidine ring is formed using carbon dioxide, aspartate, and the glutamine amide group, with ATP serving as the energy source. Orotic acid is then combined with the phosphoribosyl moiety of 5-phosphoribosyl 1-pyrophosphate (PRPP), producing orotidine 5-phosphate. (OMP). The carboxyl group of OMP is then taken out, producing UMP, which is then sequentially modified to produce UDP and UTP. The amide group of another glutamine is added to UTP in order to replace the amino group linked to cytosine at position 6 in CTP. Several bacterial species, some euglenoid flagellates, including *Euglena gracilis*, and 10 species of freshwater blue-green algae use a second method of deoxynucleotide production. Nucleoside triphosphates are converted to deoxynucleoside triphosphates, and a cofactor called 5'-deoxy adenosylcobalamin, which contains vitamin B12, provides the hydrogen ion that takes the place of deoxyribose's 2'-hydroxyl group. For electron transfer to occur from NADPH to thioredoxin to 5'-deoxy adenosylcobalamin to the nucleoside triphosphate, NADPH and thioredoxin are still required. ATP, GTP, UTP, and CTP are all reduced by the same enzyme [8].

Trypanosoma brucei, a human harmful parasite, has both de novo and rescue pathways for pyrimidine nucleotide biosynthesis. As a result, they can develop without recoverable pyrimidines. Thymidine kinase (TK), one of several rescue enzymes that seem superfluous to the de novo route, catalyzes the creation of dTMP and dUMP. Surprisingly, we demonstrate that TK is needed for growth and infectivity in a rodent model and that its activity requires a catalytically active enzyme by analyzing TK conditional null and RNAi cells. *T. brucei* and all other kinetoplastids lack the enzyme dCMP deaminase (DCTD), which offers an additional pathway for dUMP production. The RNAi growth trait was completely rescued by ectopic translation of human DCTD, which made it possible to choose healthy TK null cells. In TK-deficient cells, LC-MS/MS metabolite analysis showed an accumulation of polypyrimidine nucleosides. Thymidine/deoxyuridineauxotrophy was caused by cytidine deaminase (CDA) knockout, which transforms deoxycytidine into deoxyuridine. These surprising outcomes indicated that *T. brucei* genes an unknown 5'-nucleotidase that transforms deoxy pyrimidine nucleotides to their respective nucleosides, causing their dead-end accumulation in TK deficient cells at the cost of dTTP pools. An HD-domain protein that we demonstrate catalyzes the dephosphorylation of deoxyribonucleotide 5'-monophosphates was among several possible candidate genes that could contain 5'-nucleotidase activity that was discovered through bioinformatics analysis. We conclude that regardless of whether the nucleoside intermediates come from the de novo route or through salvage, TK is necessary for the production of thymine nucleotides. A chance to specifically target a variety of harmful single-celled organisms with a single medication may exist due to trypanosomatids' dependence on TK in the lack of DCTD [9].

Nucleotides are continuously produced *denovo* in all cells and are necessary for a broad range of cellular functions. For DNA replication and RNA synthesis to enable protein synthesis at various phases of the cell cycle, during which these processes are controlled at numerous levels, greater nucleotide synthesis is required when cells multiply. As a result, there are numerous stages at which the production of the constituent nucleotides is tightly controlled. Several biochemical routes from various cell divisions are used in the energy-intensive process of nucleotide synthesis, which also utilizes a variety of carbon and nitrogen sources. A group of master transcription factors, as well as allosteric regulation and feedback suppression at the level of the enzymes, control the processes at the transcriptional level. The

requirements for nucleotide production at the cellular level, as well as their biochemical routes and control mechanisms during the cell cycle, are discussed here. Stable isotope tracers are used to identify the biosynthesis pathways of the various overlapping pathways, and it is emphasized how these are numerically regulated under various circumstances. Additionally, the significance of nucleotide synthesis for maintaining cell survival is addressed, along with how this might suggest fresh ideas for developing drugs to treat conditions like cancer [10].

CONCLUSION

The bases of nitrogen, phosphate, and pentose sugar undergo a molecular process known as nucleotide synthesis, which is a common anabolic mechanism. The deamination process, the elimination of phosphate from the nucleoside monophosphates, phosphorolytic removal of the ribose to produce ribose-1-phosphate, and ultimately oxidation of the nucleobases to uric acid are all steps in the metabolism of purine nucleotides. The three amino acid donor processes are involved in nucleotide metabolic processes: glutamine to glutamate for amine donation, aspartate to fumarate for methyl donation, and serine to glycine for methyl donation. Nucleic acid degradation is a catabolic process, and the leftover nucleotides or nucleobases can be used to make new nucleotides. The bases and the nucleoside created during the breakdown of RNA and DNA are recovered using nucleotide salvage mechanisms. Because some cells in some systems cannot experience de novo production, this is significant. The products that can be recovered can then be transformed back into RNA.

REFERENCES:

[1] "22.4: Biosynthesis and Degradation of Nucleotides," *Biology LibreTexts*, Dec. 03, 2018. https://bio.libretexts.org/Bookshelves/Biochemistry/Fundamentals_of_Biochemistry_(Jakubowski_and_Flatt)/02%3A_Unit_II-_Bioenergetics_and_Metabolism/22%3A_Biosynthesis_of_Amino_Acids_Nucleotides_and_Related_Molecules/22.04%3A_Biosynthesis_and_Degradation_of_Nucleotides (accessed Mar. 24, 2020).

[2] "Nucleic acid metabolism," *Wikipedia*. Mar. 22, 2020. Accessed: Mar. 24, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Nucleic_acid_metabolism&oldid=1146040907

[3] "Nucleic Acid Degradation - an overview | ScienceDirect Topics." https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/nucleic-acid-degradation (accessed Mar. 24, 2020).

[4] M. Löffler, J. Jöckel, and G. Schuster, "Dihydroorotat-ubiquinone oxidoreductase links mitochondria in the biosynthesis of pyrimidine nucleotides," *Mol. Cell. Biochem.*, vol. 174, no. 1, pp. 125–129, Sep. 1997, doi: 10.1023/A:1006859115450.

[5] R. I. Christopherson, S. D. Lyons, and P. K. Wilson, "Inhibitors of de Novo Nucleotide Biosynthesis as Drugs," *Acc. Chem. Res.*, vol. 35, no. 11, pp. 961–971, Nov. 2002, doi: 10.1021/ar0000509.

[6] P. Needleman, M. S. Minkes, and J. R. Douglas, "Stimulation of Prostaglandin Biosynthesis by Adenine Nucleotides," *Circ. Res.*, vol. 34, no. 4, pp. 455–460, Apr. 1974, doi: 10.1161/01.RES.34.4.455.

[7] I. Y. Lee, M. K. Kim, H. N. Chang, and Y. H. Park, "Regulation of poly-β-hydroxybutyrate biosynthesis by nicotinamide nucleotide in Alcaligenes eutrophus," *FEMS Microbiol. Lett.*, vol. 131, no. 1, pp. 35–39, Aug. 1995, doi: 10.1111/j.1574-6968.1995.tb07750.x.

[8] C. W. Ross, "4 - Biosynthesis of Nucleotides," in *Proteins and Nucleic Acids*, A. Marcus, Ed. Academic Press, 1981, pp. 169–205. doi: 10.1016/B978-0-12-675406-3.50010-4.

[9] C. Leija*et al.*, "Pyrimidine Salvage Enzymes Are Essential for De Novo Biosynthesis of Deoxypyrimidine Nucleotides in Trypanosoma brucei," *PLOS Pathog.*, vol. 12, no. 11, p. e1006010, Nov. 2016, doi: 10.1371/journal.ppat.1006010.

[10] A. N. Lane and T. W.-M. Fan, "Regulation of mammalian nucleotide metabolism and biosynthesis," *Nucleic Acids Res.*, vol. 43, no. 4, pp. 2466–2485, Feb. 2015, doi: 10.1093/nar/gkv047.

CHAPTER 16

BIOSYNTHESIS OF THE TRIGLYCERLYDHYDE

Dr Ruchi Choudhary, Associate Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- ruchi.upmanyu@gmail.com

ABSTRACT:

All living forms, including animals, have the ability to produce and retain fatty acids. Triacylglycerols are produced in the cytoplasm and the adipose tissue of mammals via the Kennedy route, the dihydroxyacetone-phosphate pathway, and the glycerol phosphate pathway, among other biochemical pathways. In this paper, we discussed the various metabolic routes that lead to the creation of triacylglycerols.

KEYWORDS:

Biosynthesis Pathway, Endoplasmic Reticulum, Fatty Acids, Glycerol Phosphate, Lipid Droplets.

INTRODUCTION

In mammals, many cell types and tissues can make triacylglycerols, but the liver, gut, and fatty tissue are the most active, with the last of these housing the majority of the body's reserves. Triacylglycerols are kept in the cytosol of all cell types, including those in the brain, and are encased in monolayers of phospholipids and hydrophobic proteins, such as perilipins in fat tissue or oleosins in seeds. These lipid droplets are no longer considered to be uninteresting clumps of fat but rather separate structures with unique biochemical pathways and related enzymes. However, since Mycobacteria and yeasts also have fatty inserts, they are not only found in mammals and vegetation. The lipid acts as a reservoir of fatty acids for structural reasons or as eicosanoids' constituents, as well as a storage of fatty acids for energy that can be quickly released on demand. Furthermore, lipid droplets function as a defensive mechanism in cells, securing any surpluses of physiologically active and possibly hazardous lipids like free fatty acids, oxylipins, diacylglycerols, cholesterol (as cholesterol esters), retinol esters, and coenzyme A esters. Even though triacylglycerols are necessary for regular metabolism, an abnormal buildup in human fatty tissue and other tissues causes obesity and other health issues, such as insulin resistance, steatohepatitis, and cardiomyopathy. As a result, pharmaceutical companies are very interested in medicines that impact the production and breakdown of triacylglycerol [1].

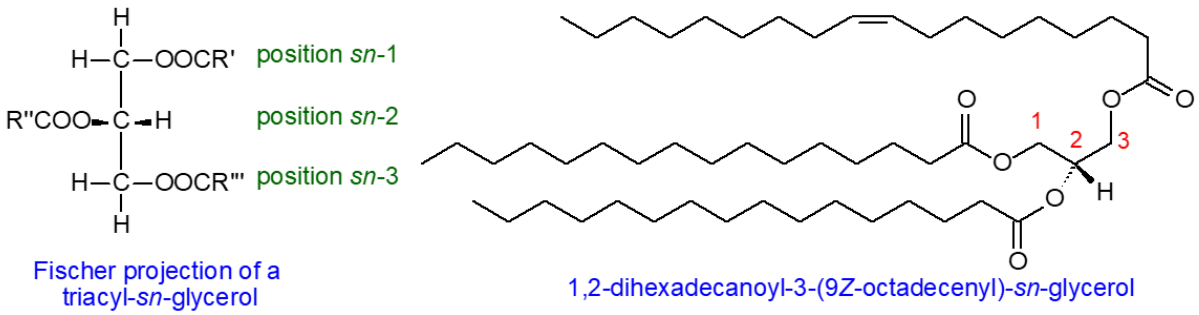

Figure 1: Triacylglycerols structure: Diagram showing the structure of the triacylglycerols (lipids maps).

The lipid acts as a reservoir of fatty acids for structural reasons or as eicosanoids' constituents, as well as a storage of fatty acids for energy that can be quickly released on demand. Free fatty acids, oxylipins, diacylglycerols, cholesterol (as cholesterol esters), retinol esters, and coenzyme A esters are just a few examples of physiologically active and possibly damaging lipids that are sequestered by lipid droplets as a defensive mechanism in cells (Figure. 1). Even though triacylglycerols are necessary for regular metabolism, an abnormal buildup in human fatty tissue and other tissues causes obesity and other health issues, such as insulin resistance, steatohepatitis, and cardiomyopathy. As a result, pharmaceutical companies are very interested in medicines that impact the production and breakdown of triacylglycerol [2].

The sn-glycerol-3-phosphate and dihydroxyacetone phosphate pathways, which prevail in the liver and fatty tissue, as well as a monoacylglycerol pathway in the bowels, are the three major routes for triacylglycerol production that are known. A fourth route involving diacylglycerol transferase has been identified in developing plant seeds and some mammal tissues. Kennedy process: More than 90% of liver triacylglycerols are generated through the sn-glycerol-3-phosphate, or Kennedy pathway, which was first characterized by Professor Eugene Kennedy and coworkers in the 1950s. The primary source of the glycerol backbone in this pathway was previously thought to be the sn-glycerol-3-phosphate produced by the catabolism of glucose (glycolysis) (Figure. 1). However, it is now known that a sizeable portion of the glyceroneogenesis, which may be the primary source in adipose tissue, is produced de novo by a process known as glyceroneogenesis via pyruvate.

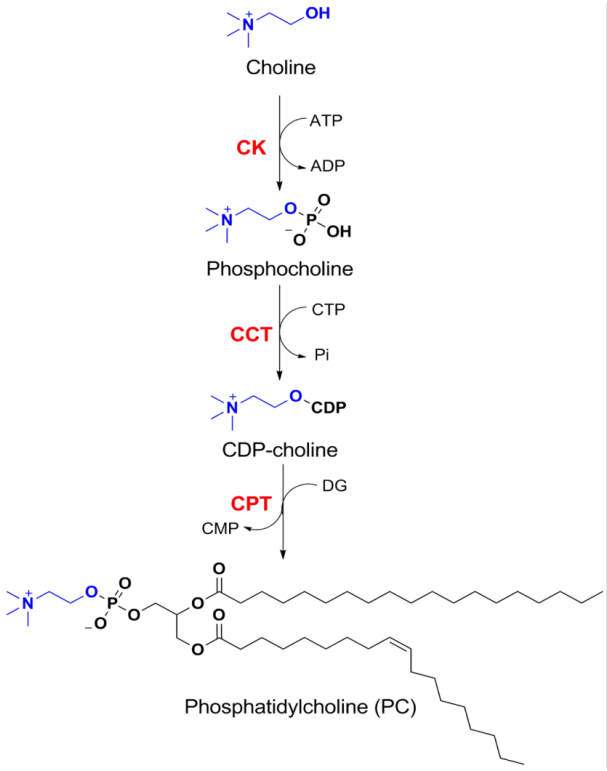

Figure 1: Kennedypathway: Diagram showing the Kennedy pathway for the synthesis of the triacylglycerols (Research gate).

The following processes mostly take place in or near the endoplasmic reticulum. A fatty acid cofactor first esterifies the precursor sn-glycerol-3-phosphate. An ester in a reaction catalyzed by a glycerol-3-phosphate acyltransferase (GPAT) at position sn-1 to form lysophosphatidic acid, and this is in turn acylated by an acylglycerophosphate acyltransferase (AGPAT) in

position sn-2 to form a key intermediate in the biosynthesis of all glycerolipids - phosphatidic acid, reactions described in greater detail in our web page on this lipid. These enzymes have a large number of known variants; they are produced with distinct tissue and membrane patterns; and they are controlled in various ways. Then, a group of enzymes known as phosphatidic acid phosphohydrolases (PAPs, also known as phosphatidate phosphatases, lipid phosphate phosphatases, or "lipids") removes the phosphate group, resulting in the production of sn-1,2-diacylglycerols, which are crucial intermediates in the biosynthesis of triacylglycerols and the phospholipids phosphatidyl and of mono galactosyl diacylglycerols in plants). As it may control the flux of lipids for storing or membrane formation, this is a crucial branch point in lipid biosynthesis. Three related intracellular proteins, originally referred to as lipids, namely lipin-1, lipin-2, and lipid-3, which have tissue-specific functions in glycerolipid synthesis, are responsible for a large portion of this phosphatase activity resulting in triacylglycerol formation in mammals. Unusually, these were designated and given descriptive names prior to the discovery of their enzyme functions.

While the expression and roles of each lipin appear to vary, lipin-1 (PAP1), which exists in three variants (named 1, 1, and 1), is responsible for the majority of PAP action in fatty tissue and skeletal muscle in people. While lipin-3's activity combines with that of lipin-1 and lipin-2's and is located in the GI system and liver, lipin-2 is the most prevalent lipin in the liver and is also significantly expressed in the small intestine, macrophages, and some areas of the brain. Although lipins lack trans-membrane domains, they are translocated to the endoplasmic reticulum in reaction to increased amounts of fatty acids in cells. Lipins are cytoplasmic enzymes that momentarily interact with membranes to access their substrate. N-ethylmaleimide inhibits the lipin-1 activity, which needs Mg2+ ions to function. In contrast, the membrane-bound activity that produces diacylglycerols as a phospholipid intermediary is not reliant on Mg2+ content and is not affected by the inhibitor.

Highland rose unexpectedly, lipin-1 plays a dual role in modulating lipid metabolism by acting as a transcriptional coactivator with known nuclear receptors. Lipin 1 is also linked to the induction of lipogenic genes like fatty acid synthase, stearoyl-CoA desaturase, and DGAT. They may have significant impacts on various cell kinds' signaling. It is known that some human illness conditions that can result in metabolic syndrome and inflammation diseases also involve abnormalities in lipin-1 production. A related phosphatidate phosphohydrolase called Lipin-2, which is actively controlled by hunger and adiposity, (in mice).

The 1,2-diacyl-sn-glycerol intermediary is acylated by diacylglycerol acyltransferases (DGAT) in the pathway's concluding stage to create triacyl-sn-glycerol from a variety of fatty acyl-CoA esters. Animals have two DGAT enzymes that differ in both structure and function. DGAT1 is primarily found in the endoplasmic reticulum, is expressed in skeletal muscle, epidermis, and gut, and is expressed in lesser amounts in the liver and fatty tissue. Surprisingly, however, it is the only one to be found in the mammary gland's epithelium cells that synthesize milk fat. It is thought to have a dual structure that contributes to the production of triacylglycerol on both sides of the endoplasmic reticulum membrane, but it only esterifies fatty acids that have already been produced outside the cell. A broader variety of substrates, such as monoacylglycerols, long-chain alcohols (used in the production of wax), and retinol, can be used by DGAT1. It is also thought to play a significant part in defending the endoplasmic reticulum from the lipotoxic impacts of high-fat meals. Other than yeasts, most organisms have orthologues of this enzyme, and plants in particular rely heavily on them.

Although it is produced much more broadly in tissues, the predominant type of enzyme in hepatocytes and adipocytes (lipid droplets) is DGAT2. It esterifies fatty acids of both native and external origin and is connected to various parts of the endoplasmic reticulum, the surface of lipid droplets, and mitochondria. Triacylglycerols are believed to be channeled from the endoplasmic reticulum's synthesis site to the developing lipid droplet, where they accumulate and cause the latter to grow, thanks to DGAT2's targeting domain, which enables it to tether between the two structures. Although DGAT2 appears to be particularly significant in regulating the balance of triacylglycerols in vivo, both enzymes are significant regulators of energy metabolism. This phase might be the rate-limiting one because the glycerol-3-phosphate acyltransferase (GPAT) has the lowest specific activity of these enzymes. However, because DGATs are specifically designed to produce triacylglycerol, they are thought to be the best drug targets for treating obesity and its associated diseases. Clinical research on DGAT1 inhibitors is still in its early stages. Additional DGAT-active enzymes have been discovered, one of which is a soluble DGAT3 in Arabidopsis.

Dihydroxyacetone-phosphate pathway: In a second pathway for the synthesis of triacylglycerols, dihydroxyacetone-phosphate in peroxisomes or the endoplasmic reticulum can be acylated with fatty acid CoA esters to produce 1-acyl dihydroxyacetone-phosphate, which is then reduced to lysophosphatidic acid by the dihydroxyacetone-phosphate oxidoreductase (Figure. 2). The biosynthesis pathway that leads to neutral plasmalogens, which can be major components of cytoplasmic droplets in many mammalian cell types but not in fat tissue, includes the precursor dihydroxyacetone-phosphate.

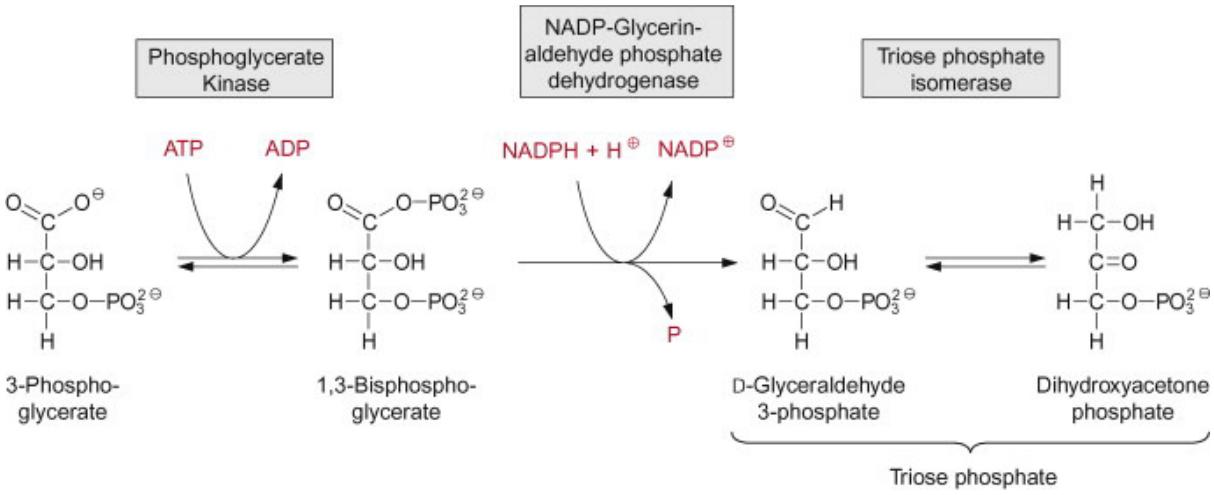

Figure 2:Dihydroxyacetone-phosphate pathway: Diagram showing the Dihydroxyacetone-phosphate pathway for the synthesis of the triacylglycerols (Sciecne direct.com).

The activity of diacylglycerol lipases causes microbes, fungi, plants, and mammals to make monoacylglycerols from diacylglycerols but not from triacylglycerols. In particular, 2-arachidonoylglycerol is produced by the release of 1,2-diacyl-sn-glycerols by the activity of phospholipase C on membrane phospholipids in the brain and nerve tissue. To create cutin chains in plants like Arabidopsis, specialized glycerol-3-phosphate acyltransferases create an intermediary 2-lysophosphatidic acid. (GPATs). The corresponding human enzyme has little monoacylglycerol lipase activity, whereas bacterial diacylglycerol lipases can hydrolyze diacylglycerol to glycerol and free fatty acids via monoacylglycerol intermediates.It is now recognized that 2-monoacylglycerols and 2-oleoyl glycerol in particular have a signaling function in the intestines by activating a specific G-protein coupled receptor GPR119, sometimes termed the 'fat sensor', which is believed to be the only receptor responsible for

the fat-induced release of the gut hormones glucagon-like peptide-1 (GLP-1), peptide tyrosine (PYY) and neurotensin. When activated, this prevents rodents from gaining weight and reduces food consumption while controlling insulin release that is triggered by glucose. Initially believed to be at the apical membrane confronting the digestive tract, the receptor may be at the basolateral membrane, according to new research. The actions of 1-oleoyl-lysophosphatidylcholine and oleoyl ethanolamide are identical, but 2-oleoyl glycerol is the most prevalent of the possible ligands. It is unknown if these 2-monoacylglycerols act as signaling molecules in other organs.

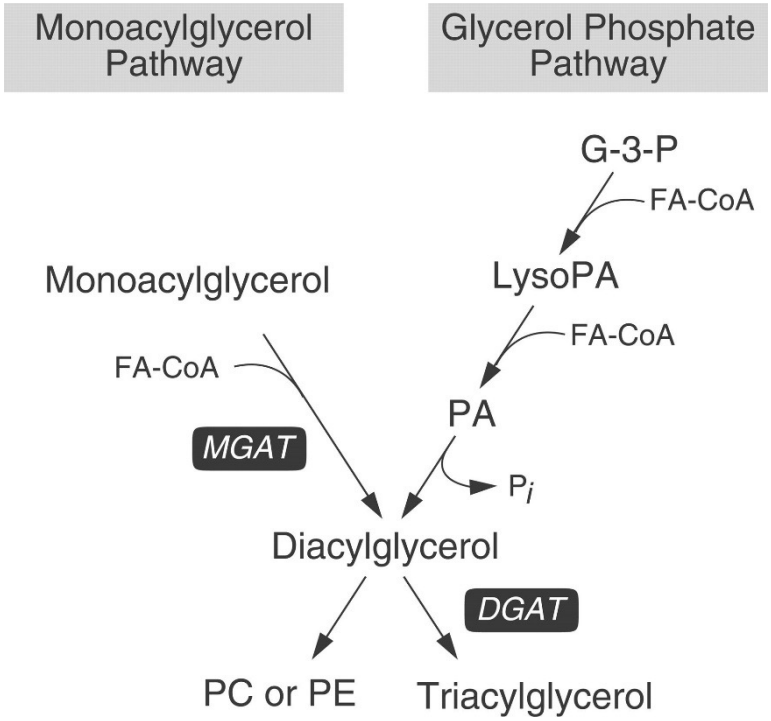

Figure 3:Monoglycerol pathway: Diagram showing the Monoglycerol pathway for the synthesis of the triacylglycerols (Sciecne direct.com).

Similar to endocannabinoids, 2-linoleoyl-glycerol has to signal properties in the fruit fly Drosophila melanogaster. Adipocytes that are developing produce the angiogenesis component 1-butyryl-glycerol (aminobutyric), which promotes the development of blood vessels from the established capillaries. Human milk contains glycerol monolaurate, which is sometimes added to meals for its claimed antibacterial and anti-inflammatory effects on a variety of Bacillus and Staphylococcus species. In poplar leaves and blossoms (propolis), monoacylglycerols with 3,8-dihydroxy fatty acids (C18 to C24) have been found. These monoacylglycerols have been shown to have potent antiproliferative action against human GI tumors in vitro. A protein called Munc13-1's C1-domain binds to Scottish thistle1-Monoacylglycerol species, which contain long-chain saturated fatty acids. Munc13-1 then works with SNARE proteins to help prime insulin granules, which in turn stimulates insulin release in pancreas beta-cells. Additionally, by engaging PPAR and PPAR, 1-monoacylglycerol is thought to play a significant part in brown fat stimulation and energy utilization.

From brain and other tissues, acylglycerol kinases that can phosphorylate both 1- and 2-monoacyl-sn-glycerols to produce the crucial signaling chemical lysophosphatidic acid have been identified. A monoacylglycerol lipase primarily catabolizes monoacylglycerols in animal cells, releasing free fatty acids and glycerol in the process. However, a ubiquitously

expressed serine hydrolase with the hydrolase domain number 6 (ABHD6) is thought to play a key role in controlling monoacylglycerol signaling. Due to the monoacylglycerol lipase's high expression in initial tumors and invasive human cancer cells, it is presently of significant biochemical interest. The high lipolytic activity appears to lead to an increase in free fatty acid levels in cancer cells, which feed into a variety of pro-tumorigenic signaling lipids that support malignancy by promoting motility, survival, and tumor development in vivo. As a result, a lot of work is being put into developing the enzyme's particular antagonists. Monoacylglycerol lipase suppression has the potential to be beneficial for several other disease conditions. For instance, blockage of 2-arachidonoylglycerol breakdown may lessen the amount of arachidonic acid available for the production of pro-inflammatory prostaglandins [3].

DISCUSSION

Numerous cellular processes are controlled by N-3 polyunsaturated fatty acids (PUFA) and the numerous groups of lipid molecules generated from them. comprehending the n-3 biosynthesis route, which starts with alpha-linolenic acid (18:3n-3), and is generally believed to conclude with the creation of docosahexaenoic acid, is the first step in comprehending the methods by which n-3 PUFA control cellular processes. (DHA, 22:6n-3). However, we still know less about this route than was once thought. In the current study, we give an overview of the data supporting the pathway as it is presently known and provide reports on new research that question three n-3 PUFA metabolism dogmas. Recent evidence is presented that focuses on in vivo kinetic modeling and compound-specific isotope abundance studies in rodents and humans, which have been crucial in advancing our understanding of the pathway. This evidence builds on nearly three decades of research, primarily in cell culture and oral dosing studies. We specifically point out three significant changes to the route for n-3 PUFA biosynthesis: Tetracosahexaenoic acid (24:6n-3) is both a product and a precursor to DHA, which means that (1) DHA production rates cannot be as low as originally thought, (2) DHA is both a product and a precursor, and (3) rises in EPA in reaction to DHA intake are not the consequence of greater retro conversion [4].

Nearly all polyunsaturated fatty acids (PUFA) come from main providers, but as they move up the food chain (a process known as trophic upgrading), they can be altered by bioconversions. Therefore, higher trophic levels have biochemical pathways that can create new and distinctive PUFA, despite the fact that phytoplankton is the major primary providers of PUFA in the aquatic ecosystem. The mechanisms of PUFA production and metabolism in the stages between primary producers and fish, which are predominately occupied by crustaceans, are, however, poorly understood. It is becoming more and clearer that some lesser animals can synthesize PUFA from scratch in addition to trophic boosting. The existence of other species within many crustaceans makes it difficult to identify the PUFA biosynthesis routes. These organisms vary from gut flora to commensal partnerships that can entail PUFA transfer to host organisms, and they include bacteria and phytoplankton with PUFA biosynthetic pathways. This highlights the significance of researching biosynthesis networks at the molecular level, and developments in molecular analysis and genetic tools are making this easier. In this paper, new findings on the molecular and metabolic processes of PUFA production in marine crustaceans, with a special emphasis on mollusk mollusks, are reviewed [5].

Arachidonic acid (AA) and adrenic acid (AdA), which are phospholipididylethanolamine (PE)-linked, are well-known lipid peroxidation intermediates and essential for ferroptosis, controlled apoptosis that is iron-dependent. It is not entirely clear how cells control the intracellular concentrations of AA and AdA differently. Here, the differential expression of

fatty acid desaturase 1 (FADS1) and elongation of very long-chain fatty acid protein 5 (ELOVL5) in stomach cancer cells identify the cellular vulnerability to ferroptosis. The idea that ELOVL5 and FADS1 are necessary to preserve intracellular amounts of AA and AdA and encourage ferroptosis is supported by biochemical and lipidomics studies. Our research emphasizes the importance of ELOVL5 and FADS1 in the production of AA and AdA as a crucial step in the ferroptosis pathway [6].

In reaction to greater energy accumulation in adipose tissue, the protein leptin, which is expressed by the obese (ob) gene, is produced and released. The exact mechanism by which inbound energy is detected and converted into elevated production of the ob gene is still unknown. In addition to acting as a cellular "sensor" of energy availability, the hexosamine biosynthesis pathway also regulates the impacts of glucose on the production of a number of gene products. Here, we present proof that glucosamine quickly activates the ob gene in skeletal muscle. Leptin messenger RNA and protein levels rise quickly and noticeably as tissue amounts of UDP-N-acetylglucosamine (UDP-GlcNAc), the pathway's final output, rise. although these levels were much lower than those in fat. Adipose tissue's amounts of leptin protein, mRNA, and plasma levels all rise as well. Most importantly, hyperlipidemia or hyperglycemia, which also raises tissue levels of UDP-N-acetylglucosamine in cognizant rodents7, replicates the activation of leptin production. Finally, glucosamine quickly triggers the production of the ob gene in L6 myocytes and 3T3-L1 pre-adipocytes. Our results reveal an essential molecular connection between higher food supply and leptin expression and provide the first proof of inducible leptin production in skeletal muscle [7].

Advanced biodiesel generation primarily centers on non-agricultural energy sources. Among them, microalgae are viewed by many as the best option for the production of different biofuels, including biodiesel, due to their distinctive properties, including a natural resistance to sewage and salty water, sustainable biomass production, and high lipid content (LC). In the current research, lipid content was increased and biofuel quality was improved by manipulating the carbon flow into the fatty acid biosynthesis pathway in Dunaliella salina using pGH plasmid carrying the AccD and ME genes. After several passes, PCR was used to validate the durability of the change. With no read-through transcription by native regulators, Southern hybridization of the AccD probe with genomic DNA showed steady incorporation of the cassette in the designated locations in the chloroplast genome. The over-expression of the ME/AccD genes in the transformants led to a 12% increase in total LC and significant improvements in biodiesel properties, particularly by increasing algal oil oxidation stability, according to a comparison of the LC and fatty acid profile of the transformed algal cell line and the control. The entire procedure effectively used here for changing algal cells by genes implicated in the route that produces lipids may be useful for producing biofuel on a big scale from microalgae [8].

PtdCho buildup is a regular, S phase-specific occurrence that is influenced in part by variations in CTP: phosphocholine cytidylyltransferase (CCT) activity that is contingent on the cell cycle. To produce the diacylglycerol (DG) components for phosphatidylcholine (PtdCho) production, a supply of fatty acids is necessary, but it is unknown if the DG supply is also connected to the cell cycle. The growth factor CSF-1 significantly increased the rate of fatty acid production in a macrophage cell type, but the cell cycle had no control over this process. Elevated steady-state mRNA levels for acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) were associated with increased fatty acid production. The creation of membrane PL required cellular fatty acid production. Cerulenin's suppression of native fatty acid synthesis hindered PtdCho synthesis as well, and external fatty acids had no effect to reverse this inhibition. The freshly produced DG was directed to the TG pool where it was

collected when CCT activity was inhibited by the presence of lysophosphatidylcholine (lysoPtdCho) or temperature-shifting a conditionally faulty CCT. PtdCho biosynthesis was promoted and TG synthesis was decreased by forced translation of CCT. As a result, neither PtdCho nor TG biosynthesis was regulated by the cellular DG supply, and CCT activity controls how DG is divided between the PL and TG pools [9].

The main mechanism for producing membrane phospholipid acyl chains in microbes and plants is the type II fatty acid synthesis pathway. The process is orchestrated by a collection of distinct soluble proteins, each of which is expressed by a separate genome. The intermediates of the pathway are transported between the enzymes as thioesters of an acyl carrier protein. High-resolution X-ray and/or NMR structures of typical members of every enzyme in the type II pathway are now accessible. The Escherichia coli system is the model for the research of this system. The three-dimensional characteristics of the enzymes that explain substrate identification, chain length selectivity, and the catalytic processes that determine their functions in creating the wide range of products produced by the type II system are revealed by the structural biology of these proteins. The development of antimicrobial drugs can be aided by these shapes [10].

CONCLUSION

The process of esterifying fatty acids to glycerol produces triglycerides. Through biochemical pathways, the acyl groups in fatty acyl-CoAs are moved to the hydroxyl groups of glycerol-3-phosphate and diacylglycerol, causing fatty acid esterification to occur in the endoplasmic reticulum of cells. Triglycerides are hydrolyzed by the majority of body cells using comparable routes, frequently to produce fatty acids to meet energy needs. The liver and fatty tissue are the two primary locations for natural lipid production. Hepatic triglyceride is normally released in very low-density lipoproteins (VLDL). One of the main elements of the glycerolipid family is triacylglycerols (TAGs). They primarily exist in lipid droplets and serve the purpose of storing extra fatty acids in cells. Triacylglycerols have acyl chains with a range of lengths and levels of unsaturation, giving rise to hundreds of biologically unique species.

REFERENCES:

[1] "21.2: Biosynthesis of Triacylglycerols," *Biology LibreTexts*, Dec. 03, 2018. https://bio.libretexts.org/Bookshelves/Biochemistry/Fundamentals_of_Biochemistry_(Jakubowski_and_Flatt)/02%3A_Unit_II-_Bioenergetics_and_Metabolism/21%3A_Lipid_Biosynthesis/21.02%3A_Biosynthesis_of_Triacylglycerols (accessed Mar. 24, 2020).

[2] H. M. Alvarez, "Triacylglycerol and wax ester-accumulating machinery in prokaryotes," *Biochimie*, vol. 120, pp. 28–39, Jan. 2016, doi: 10.1016/j.biochi.2015.08.016.

[3] P. Poursharifi, S. R. M. Madiraju, and M. Prentki, "Monoacylglycerol signalling and ABHD6 in health and disease: POURSHARIFI ET AL .," *Diabetes Obes. Metab.*, vol. 19, pp. 76–89, Sep. 2017, doi: 10.1111/dom.13008.

[4] A. H. Metherel and R. P. Bazinet, "Updates to the n-3 polyunsaturated fatty acid biosynthesis pathway: DHA synthesis rates, tetracosahexaenoic acid and (minimal) retroconversion," *Prog. Lipid Res.*, vol. 76, p. 101008, Oct. 2019, doi: 10.1016/j.plipres.2019.101008.

[5] Ó. Monroig, D. R. Tocher, and J. C. Navarro, "Biosynthesis of Polyunsaturated Fatty Acids in Marine Invertebrates: Recent Advances in Molecular Mechanisms," *Mar. Drugs*, vol. 11, no. 10, Art. no. 10, Oct. 2013, doi: 10.3390/md11103998.

[6] J.-Y. Lee *et al.*, "Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer," *Proc. Natl. Acad. Sci.*, vol. 117, no. 51, pp. 32433–32442, Dec. 2020, doi: 10.1073/pnas.2006828117.

[7] J. Wang, R. Liu, M. Hawkins, N. Barzilai, and L. Rossetti, "A nutrient-sensing pathway regulates leptin gene expression in muscle and fat," *Nature*, vol. 393, no. 6686, Art. no. 6686, Jun. 1998, doi: 10.1038/31474.

[8] A. F. Talebi, M. Tohidfar, A. Bagheri, S. R. Lyon, K. Salehi-Ashtiani, and M. Tabatabaei, "Manipulation of carbon flux into fatty acid biosynthesis pathway in Dunaliella salina using AccD and ME genes to enhance lipid content and to improve produced biodiesel quality," *Biofuel Res. J.*, vol. 1, no. 3, pp. 91–97, Sep. 2014, doi: 10.18331/BRJ2015.1.3.6.

[9] S. Jackowski, J. Wang, and I. Baburina, "Activity of the phosphatidylcholine biosynthetic pathway modulates the distribution of fatty acids into glycerolipids in proliferating cells," *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids*, vol. 1483, no. 3, pp. 301–315, Jan. 2000, doi: 10.1016/S1388-1981(99)00203-6.

[10] S. W. White, J. Zheng, Y.-M. Zhang, and C. O. Rock, "The Structural Biology of Type Ii Fatty Acid Biosynthesis," *Annu. Rev. Biochem.*, vol. 74, no. 1, pp. 791–831, 2005, doi: 10.1146/annurev.biochem.74.082803.133524.

CHAPTER 17

BIOSYNTHESIS OF THE FATTY ACIDS

Mr. Lalendra Yadav, Assistant Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- yadavlalendra@gmail.com

ABSTRACT:

Fatty acid chains are components of membranes and a source of energy. They engage in biochemical processes that affect the metabolism, mitochondrial functionality, and receptivity of cells and tissues to endocrine and additional cues.The cellular system produces a variety of fatty acid molecules, including saturated, unsaturated, and polyunsaturated kinds. This paper covered the process by which various fatty acid molecules are formed.

KEYWORDS:

Acetyl Coa, Acid Synthase, Biosynthesis Fatty Acids, Malonyl Coa, Polyunstuatraed Fatty Acids.

INTRODUCTION

The entire production of palmitate from acetyl-CoA in the cytoplasm is accomplished by an extramitochondrial mechanism, which produces fatty acids. Most animals use glucose as their major feedstock for lipogenesis, but ruminants use acetate as their main dietary fuel source. Human reports of critical illnesses on the route are lacking. However, type 1 (insulin-dependent) diabetes mellitus inhibits lipogenesis, and changes in the process's activity have an impact on the type and degree of fat. The phospholipids in the cell membrane's phospholipids play a crucial role in keeping the flexibility of the membrane. It is thought that a meal with a high polyunsaturated fatty acid to the saturated fatty acid ratio (P:S ratio) will help avoid ischemic heart disease. Animal cells can only desaturate a certain amount of fatty acids, so they need specific nutritional polyunsaturated fatty acids from vegetation.

These important fatty acids are converted into eicosanoic (C20) fatty acids, from which prostaglandins, thromboxanes, leukotrienes, and lipoxins are derived. In addition to mediating pain, and inflammation, and inducing slumber, prostaglandins also control blood clotting and fertility. Aspirin and ibuprofen are examples of nonsteroidal anti-inflammatory medications (NSAIDs) that work by preventing the production of prostaglandins. Leukotrienes play a crucial role in allergy responses and inflammation because they have chemotactic and muscle-contracting characteristics[1], [2].

Based on the structure of the yeast fatty acid synthase (FAS) complex, whose complete structure is known, we show the complete fatty acid synthase pathway. We will then go into greater depth about each stage. We will list the key distinctions between the human FAS complex and the animal FAS complex next. A short synopsis of the bacterial route will conclude this section. Let's examine the net response first, and from there, work our way backward to structure and process. The overall response is shown in Equation 1 below.

$$8\ \text{AcetylCoA (2C)} + 14\text{NADPH} + 14\text{H}^+ + 7\text{ATP} \rightarrow 1\ \text{Palmitate} + 8\text{CoA} + 6\text{H}_2\text{O} + 14\text{NADP}^+ + 7\text{ADP} + 7\text{Pi}$$

The 16:0 fatty acid palmitate is produced by the condensation of eight 2C-acetyl-CoAs. The fact that most fatty acids have an even number of carbon atoms provides an instant answer for how acetyl-CoA is used to make and lengthen fatty acids.

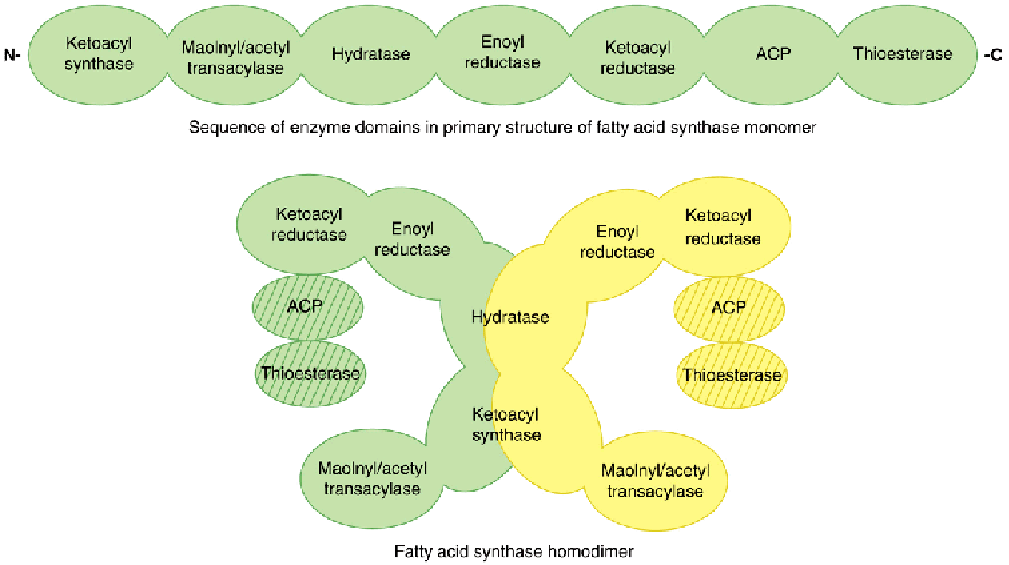

Figure 1: Fatty acids synthase complex: Diagram showing the organization of the Fatty acids synthase complex (basicmedical. key).

The overall process is a reductive biosynthesis where several short molecules are combined to form one long molecule. Therefore, a supply of energy (7 ATPs) and a reducing substance are required to fuel the process. (typically NADPH). This cytosolic process utilizes the nicotinamide-based redox couple NADPH/NADP+ to take place. These elements distinguish beta-oxidation, which takes place in the mitochondria and utilizes the redox couple NAD+/NADH, from fatty acid production. Chemically speaking, acetyl-CoAs, which have high energy molecules concerning the breakdown products they produce, need to be triggered by ATP in some manner to promote C-C bond formation. The enzyme acetyl-CoA carboxylase, which utilizes the ATP already stated and an attached coenzyme called biotin to add CO_2 to acetyl-CoA and create malonyl-CoA, catalyzes that process. Since they are present on both sides of the net reaction and cancel out in the final balanced equation, neither malonyl-CoA nor CO_2 is shown in the equation above. The increasing acyl group is added to the 3C malonyl to create a C-C bond, and the true addition process is fueled by CO_2 emission. Malonyl-CoA generated by acetyl-CoA carboxylase is now incorporated into the cycle of fatty acid production.

The fatty acid synthase (FAS) complex is the sole additional enzyme complex needed for the complete process (Figure. 1). But don't be fooled into believing the process is easy because of that! Fatty acid synthase complexes come in two different varieties, I and II. The majority of microbes, plants, and organelles all contain Type II FAS. Since fatty acid metabolism takes place in the mitochondria, its existence there might seem strange. The production of fatty acids is catalyzed by numerous different enzymes in Type II FAS systems. We won't talk about those. The Type I FAS complex, which is present in some bacteria, fungi like yeast, and higher organisms, will be the subject of our attention. The Type I FAS is a massively complicated system. It has two strands with a combined molecular weight of 540,000 in animals. It is a 6x6 hetero dodecamer with a 2.6 million molecular weight found in yeast. The alpha and beta strands in both complexes contain a variety of distinct enzyme active regions. Bound intermediates usually move through "channels" to the following active site in large enzyme complexes, allowing bound substrates to progress from substrate to product without breakup. In the instance of Type I FAS, an acyl-carrier protein tethers the developing intermediate, which ultimately reaches the end product dimension 16:0. To enable the repetitive sequential chemical processes to finish before adding another 2C acetyl CA to the

expanding chain, the attached intermediate can "swing" from one active site to another. The 3C malonyl-CoA produced by the extra cyclic (outside of the route) enzyme acetyl-CoA carboxylase and the rapid emission of CO2 is what contribute to the extra 2Cs that aid the process progress. The Process of Making Fatty Acids: The creation of malonyl-CoA is the first and most important regulating stage in the formation of fatty acids. Acetyl-CoA is carboxylated to malonyl-CoA in the early process in the presence of ATP and acetyl-CoA carboxylase, which requires bicarbonate as a source of CO2. Controlling the production of fatty acids depends on this enzyme.

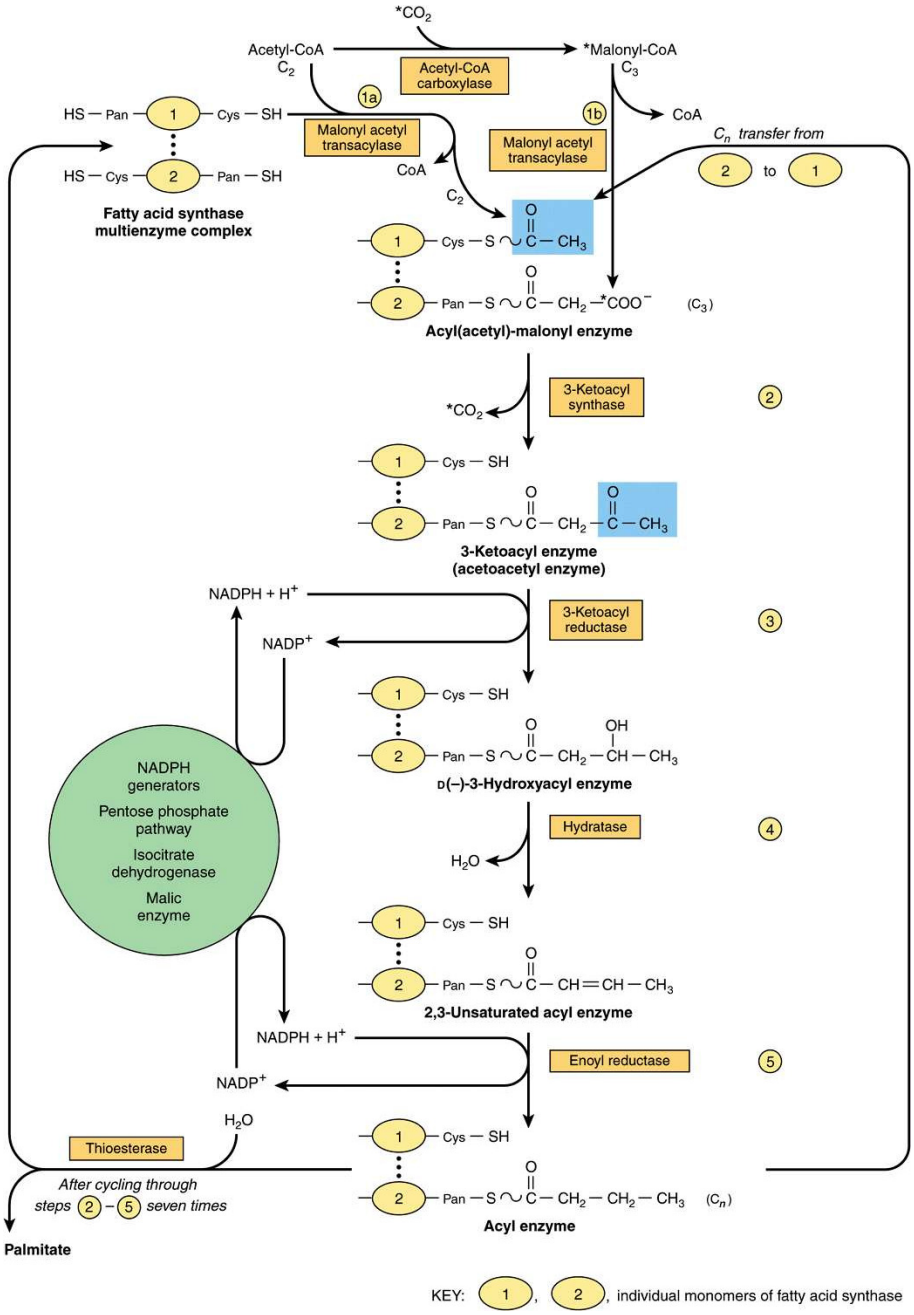

Figure 2: Biosynthesis of the long chain fatty acids: Diagram showing the synthesis of the long chain fatty acids (basic medical. key).

Following the production of malonyl-CoA, the fatty acid synthase (FAS) enzyme complex creates fatty acids. The acyl carrier protein (ACP) is a multienzyme polypeptide construct that connects the various enzymes required for fatty acid production. The pantothenic acid

vitamin 4′-phosphopantetheine is a component of the multienzyme complex. An acetyl-CoA primer molecule and a cysteine (-SH group) first unite, and a malonyl-CoA molecule and the -SH group on the 4′-phosphopantetheine of the other monomer then join. These processes are catalyzed by malonyl acetyl transacylase, which results in the production of the acetyl (acyl)-malonyl enzyme. The acetyl group assaults the methylene group of the malonyl residue with the aid of 3-ketoacyl synthase, which then creates the 3-ketoacyl enzyme and unleashes the cysteine -SH group. The reaction can continue until it is finished thanks to decarboxylation, which advances the complete series of processes. The 3-ketoacyl group is first reduced, then desiccated, and finally reduced once more to produce the matching saturated acyl-enzyme. When a fresh malonyl-CoA molecule combines with the -SH of 4′-phosphopantetheine, the saturated acyl residue is moved to the liberated cysteine -SH group. The sequence of events is continued a further six times, up until the synthesis of a concentrated 16-carbon acyl radical (palmitoyl). The unbound palmitate first needs to be converted to acyl-CoA before it can proceed along any other biochemical route[3], [4].

Glucose is converted to acetyl-CoA in the mitochondria by pyruvate metabolism. The primary location of fatty acid production, the extramitochondrial cytoplasm, is where it does not spread easily. Citrate is produced in mitochondria when acetyl-CoA and oxaloacetate condense to form the citric acid cycle. Citrate is then transported into the extramitochondrial compartment via the tricarboxylate transporter, where it is converted to acetyl-CoA and oxaloacetate by the ATP-citrate lyase, whose activity rises in the well-fed state. The acetyl-CoA is then available for the production of malonyl-CoA into palmitate. Following the formation of malate from the ensuing oxaloacetate by NADH-linked malate dehydrogenase, NADPH is produced by the malic enzyme. After being transported into the mitochondrion, the pyruvate can be used to replenish acetyl-CoA and the NADPH becomes accessible for lipogenesis. Through this route, reducing equivalents can be moved from extramitochondrial NADH to NADP. As an alternative, malate itself can enter the mitochondrion and recombine with oxaloacetate there. Be aware that malate is necessary for the citrate (tricarboxylate) transporter in the mitochondrial membrane to swap with citrate. Ruminants don't have a lot of ATP-citrate lyase or malic enzyme, which is presumably because the primary source of acetyl-CoA in these species is acetate, which comes from food breakdown in the rumen and is converted to acetyl-CoA extra mitochondrially.

Enzymes referred to as elongates facilitate the elongation process in the endoplasmic reticulum, which results in fatty acids with more than 16 carbons. Fatty acids can also be extended by mitochondria, but their beginning components are typically less than 16 carbons long. Both habitats use comparable processes to the cytoplasm (a malonyl group, for example, is used to add two carbons), but CoA, not ACP, is affixed to the intermediates. Furthermore, the enzymes in these compartments are separate and not a component of a complex, in contrast to cytosolic synthesis, which uses the fatty acid synthase complex (Figure. 3)

An extra mitochondrial mechanism completes the production of palmitate from acetyl-CoA in the cytoplasm. A mechanism outside of the mitochondria makes fatty acids. Ruminants use acetate as their primary nutritional source of fuel, while the majority of animals use glucose as their primary feedstock for lipogenesis. Lipogenesis is inhibited by type 1 (insulin-dependent) diabetes mellitus, and variations in the activity of the process affect the type and degree of fat. The flexibility of the cell membrane is significantly influenced by the phospholipids present in the membrane. A high polyunsaturated to saturated fatty acid ratio (P:S ratio) diet is believed to reduce the risk of ischemic heart disease.

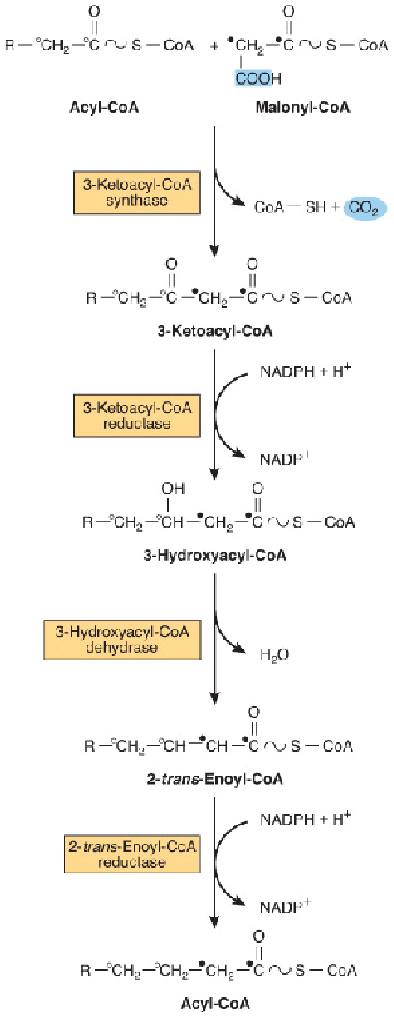

Figure 3: Elongation of the fatty acids in the ER: Diagram showing the elongation of the fatty acids in the endoplasmic reticulum (basic medical. key).

DISCUSSION

Carbon chain extension is used to transform a significant portion of the palmitic acid (C16:0) produced by fatty acid synthase into stearic acid (C18:0). Here, we discuss the cloning and production of the rat liver enzyme rELO2, which is thought to be involved in the condensing process and C16:0 extension. The elongation activity of rELO2 on C16:0 and, to a lesser extent, C18:0 and fatty acids with low desaturation degree, was shown by heterologous translation studies in the yeast Saccharomyces cerevisiae. Contrary to rELO1, a rat counterpart of HELO1, which primarily promoted the extension of mono- and polyunsaturated fatty acids of C16-C20, this was different. The Northern analysis demonstrated that the periods of starving and refeeding rodents on a fat-free diet triggered the expression of rELO2, but not rELO1, in hepatocytes. The rELO1 was persistently expressed in different organs under these circumstances, but the rELO2 RNAs were primarily found in the liver.

Super enhancers fuel tumors' aberrant gene expression and encourage cancer. Unknown is the connection between aberrant metabolism and long noncoding RNA (lncRNA) linked with super-enhancers. The fatty acid synthesis-related lncRNA (FASRL), whose translation is regulated by an upstream stimulatory factor 1 (USF1) through its super enhancer, is discovered in this research. Hepatocellular carcinoma (HCC) cell growth is stimulated by

FASRL both in vitro and in vivo. Additionally, FASRL interacts with the fatty acid biosynthesis pathway to increase fatty acid production by binding to the rate-limiting enzyme acetyl-CoA carboxylase 1 (ACACA). Additionally, FASRL, USF1, and ACACA expression are elevated, and their high expression predicts a poorer prognosis in HCC patients. In conclusion, USF1 activates a super-enhancer to promote FASRL transcription. To mechanistically worsen HCC, FASRL binding to ACACA causes a rise in fatty acid production and lipid buildup. A new predictive predictor and therapeutic focus for HCC may be FASRL[5]–[7].

Uncertainty surrounds the biochemical mechanisms by which the powerful tumor inhibitor p53 works. Here, we report that p53 activates many genes that produce peroxisomal fatty acid -oxidation enzymes by interacting with the Vitamin D receptor (VDR). (FAO). The enzyme 5-Aminoimidazole-4-Carboxamide Ribonucleotide Formyltransferase/IMP Cyclohydrolase (ATIC), which catalyzes the final two stages in the purine biosynthesis pathway, is acetylated as a result, raising the amount of acetyl-CoA in the cytoplasm. In vitro and in vivo colorectal cancer (CRC) tumor growth is inversely correlated with this acetylation step, which is mediated by lysine acetyltransferase 2B (KAT2B), and acetylation of ATIC is downregulated in human CRC samples. High amounts of ATIC in p53-deficient CRCs make them more sensitive to ATIC suppression. Collectively, these results demonstrate that the amounts of ATIC acetylation control the relationships between p53 and peroxisomal FAO, purine biosynthesis, and CRC development.

Conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA), two of the most studied conjugated fatty acid derivatives, are among the most intriguing classes of fatty acid derivates and are linked to a variety of positive health effects. Some bug species, such as the tobacco hornworm (Manduca sexta), produce rare C16 long conjugated fatty acids with two and three-conjugated double bonds as compared to C18 long CLA and CLNA in their sex scent combinations. The *M. sexta* desaturases MsexD2 and MsexD3 were expressed in numerous Y. lipolytica strains of various alleles in this research. The medium was supplemented with fatty acid methyl esters during experiments, and this led to the creation of new fatty acids. Twenty novel fatty acids with two or three double bonds were discovered using GCxGC-MS. In small quantities, fatty acids with solitary, conjugated, or both double bonds as well as a mixture of both were generated. The findings of this research demonstrate that *Y. lipolytica* can produce C16-conjugated fatty acids. Increased output of new fatty acid compounds with biotechnologically intriguing characteristics could result from further genetic optimization of the *Y. lipolytica* DNA and improvement of the fermentation process.

a rabbitfish The ability to synthesize long-chain polyunsaturated fatty acids (LC-PUFAs) was first discovered in the marine teleost *Siganuscanaliculatus*. Although its regulatory mechanisms have been studied at the transcriptional and posttranscriptional levels, little is known about how it is regulated at the cellular signaling level. The G-protein-coupled receptor 120 (GPR120) signaling pathway's regulating function in rabbitfish LC-PUFA production was examined in the current research. Docosahexaenoic acid (DHA) concentration and mRNA levels of critical genes implicated in LC-PUFA biosynthesis, encoding 6/5 Fads2, Elovl5, and regulatory component Srebp1c, were substantially reduced in *S. canaliculatus* hepatocyte line (SCHL) cells treated with GRP120 agonists (TUG891 and GW9508). The extracellular signal-regulated kinase 1 (ERK1), AMP-activated protein kinase 2 (AMPK2), the target of rapamycin (TORC2), and Srebp1c genes showed significantly lower mRNA levels in the treated SCHL cells, indicating that these proteins may be involved in the GRP120 signaling pathway.

The role of the ERK1-Srebp1c signaling pathway in the control of LC-PUFA production was further supported by the treatment of SCHL cells with signaling molecules of ERK1, AMPK, TORC2, and Srebp1c. Srebp1c, 6/5 fads2, and elovl5 mRNA levels were significantly higher in cells treated with ERK1 inhibitors compared to those treated with PUFAs (linoleic, -linolenic, arachidonic, eicosapentaenoic, and DHA). (U0126 and CI-1040). The DHA concentration of the other treatment groups (aside from PD98059) was considerably lower than that of the cells treated with CI-1040. These findings suggest that a new regulating mechanism in vertebrates, the GPR120-ERK1-Srebp1c signaling pathway, controls LC-PUFA production in rabbitfish[8]–[10].

Mycobacterium smegmatis and other non-tuberculous mycobacteria (NTM) have large surface concentrations of glycopeptidolipids (GPLs). Critically connected to the biofilm development by NTM, which also contains opportunistic bacteria like *Mycobacterium abscessus*, is the production of GPLs. The production of GPLs using external fatty acids in M. smegmatis exposed to stressors faced by mycobacteria during infection of the human body has not been examined, even though GPLs have been probed in numerous previous works. As a result, we exposed *M. smegmatis* to various combinations of the three stresses—hypoxia, an acidic pH, and nutrient deprivation—and can now report that these stresses significantly increased the metabolic incorporation of radiolabeled long-chain fatty acids into alkali-stable GPLs. *M. smegmatis* exposed to the triple stress mixture did not favor endogenously produced fatty acids for GPL production. Our findings suggest that GPLs may be crucial in the cell surface alterations connected to *M. smegmatis'* non-replicating condition. Our experimental model, which is described in this paper, will be helpful in the future investigation of GPL biosynthesis from exogenous fatty acid sources in *M. smegmatis* under hypoxic, nutrient-starved, and acidic stress conditions. It will also aid in the identification of potential therapeutics that target this biochemical pathway in pathogenic NTM.

CONCLUSION

All living things use highly preserved processes to produce fatty acids. Fatty acid biosynthesis generally begins with acetyl-CoA, followed by carboxylation to create the building elements of malonyl-CoA, which are then compressed and reduced repeatedly as long as the fatty acid chain is ready for cellular use. Acetyl-CoA is the starting point for the production of fatty acids using NADPH, a glycolytic pathway intermediary. The process is catalyzed by the protein fatty acid synthases. Since the body is unable to produce linoleic acid or linolenic acid, they are considered important fatty acids. Up until palmitoyl ACP is produced, the process of transfer, lengthening, reduction, drying, and reduction is repeated. Polyunsaturated fatty acids, such as omega-3 and omega-6, appear to be the most significant fatty acids because of their numerous biochemical functions, including affecting the inflammation cascade, lowering reactive stress, providing neuroprotection, and providing cardiovascular protection.

REFERENCES:

[1] J. S. Meng, Y. H. Tang, J. Sun, D. Q. Zhao, K. L. Zhang, and J. Tao, “Identification of genes associated with the biosynthesis of unsaturated fatty acid and oil accumulation in herbaceous peony ‘Hangshao’ (Paeonia lactiflora ‘Hangshao’) seeds based on transcriptome analysis,” *BMC Genomics*, 2021, doi: 10.1186/s12864-020-07339-7.

[2] Y. Su *et al.*, “Enhanced Biosynthesis of Fatty Acids Is Associated with the Acquisition of Ciprofloxacin Resistance in Edwardsiella tarda,” *mSystems*, 2021, doi: 10.1128/msystems.00694-21.

[3] Ó. Monroig, D. R. Tocher, and J. C. Navarro, "Biosynthesis of polyunsaturated fatty acids in marine invertebrates: Recent advances in molecular mechanisms," *Marine Drugs*. 2013. doi: 10.3390/md11103998.

[4] Ó. Monroig *et al.*, "Biosynthesis of polyunsaturated fatty acids in Octopus vulgaris: Molecular cloning and functional characterisation of a stearoyl-CoA desaturase and an elongation of very long-chain fatty acid 4 protein," *Mar. Drugs*, 2017, doi: 10.3390/md15030082.

[5] A. Tomčala *et al.*, "Fatty acid biosynthesis in chromerids," *Biomolecules*, 2020, doi: 10.3390/biom10081102.

[6] K. Liu and S. Li, "Biosynthesis of fatty acid-derived hydrocarbons: perspectives on enzymology and enzyme engineering," *Current Opinion in Biotechnology*. 2020. doi: 10.1016/j.copbio.2019.07.005.

[7] A. Demidenko, I. R. Akberdin, M. Allemann, E. E. Allen, and M. G. Kalyuzhnaya, "Fatty acid biosynthesis pathways in Methylomicrobium buryatense 5G(B1)," *Front. Microbiol.*, 2017, doi: 10.3389/fmicb.2016.02167.

[8] M. He, C. X. Qin, X. Wang, and N. Z. Ding, "Plant Unsaturated Fatty Acids: Biosynthesis and Regulation," *Frontiers in Plant Science*. 2020. doi: 10.3389/fpls.2020.00390.

[9] C. S. Heil, S. S. Wehrheim, K. S. Paithankar, and M. Grininger, "Fatty Acid Biosynthesis: Chain-Length Regulation and Control," *ChemBioChem*. 2019. doi: 10.1002/cbic.201800809.

[10] M. Leesong, B. S. Henderson, J. R. Gillig, J. M. Schwab, and J. L. Smith, "Structure of a dehydratase-isomerase from the bacterial pathway for biosynthesis of unsaturated fatty acids: Two catalytic activities in one active site," *Structure*, 1996, doi: 10.1016/S0969-2126(96)00030-5.

CHAPTER 18

BIOSYNTHESIS PATHWAY OF THE ECOSINOIDES

Dr.Dhyuti Gupta, Demonstrator
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- dhyuti92@rediffmail.com

ABSTRACT:

Eicosanoids, also known as prostaglandins, thromboxanes, leukotrienes, and lipoxins, serve as autocrine or paracrine hormones. The polyunsaturated fatty acids, which are produced by several biochemical pathways, including cyclooxygenase, cytochrome P450, and lipoxygenase, are the precursors to eicosanoids. In this paper, we summarised the biosynthesis pathway of the eicosanoids and their biological significance in the human system.

KEYWORDS:

Arachidonic Acids, Anti-Inflammatory, Blood Pressure, Eicosanoid Synthesis, Prostaglandins Leukotrienes.

INTRODUCTION

Arachidonic acid or other polyunsaturated fatty acids (PUFAs) that are comparable to arachidonic acid and are approximately 20 carbon units in length are oxidized either enzymatically or non-enzymatically to produce eicosanoids, which are signaling molecules. Eicosanoids are a subclass of oxylipins, which are oxidized fatty acids with a variety of carbon unit lengths. They differ from other oxylipins by having a disproportionately large role as cell signaling molecules. Eicosanoids play a role in several physiological and pathological processes, including mounting or inhibiting inflammatory, allergic, inflammatory, fever, and other immune responses; controlling pregnancy termination and normal childbirth; regulating cell growth; regulating blood pressure; and modulating regional blood flow to tissues. Eicosanoids typically play these roles by acting as autocrine signaling agents to influence their cells of origin or as paracrine signaling agents to influence cells nearby their cells of origin. Eicosanoids may also serve as hormonal molecules to regulate the activity of cells miles away.

Eicosanoids are classified into several subfamilies, the most notable of which are prostaglandins, thromboxanes, leukotrienes, lipoxins, resolvins, and toxins. Each subgroup can have at least four different sequences of compounds, two of which are generated from PUFAs (arachidonic and dihomo-gamma-linolenic acids)-6, one from PUFAs (eicosapentaenoic acid)-3, and one from PUFAs. This difference between subfamilies is crucial. Humans and other mammals cannot change omega-6 PUFA into omega-3 PUFA. As a result, the ratio of nutritional -6 to -3 PUFAs ingested directly correlates with organ amounts of -6 and -3 PUFAs and their associated eicosanoid compounds. It has frequently been proposed that the negative effects of consuming a diet high in omega-6 PUFAs reflect the excessive production and activities of omega-6 PUFA-derived eicosanoids, whereas the positive effects of consuming a diet high in omega-3 PUFAs reflect the excessive production and activities of omega-3 PUF. This is because certain omega-6 and omega-3 PUFA series of metabolites have almost opposite physiological and pathological activities. According to this theory, the adverse and advantageous effects of -6 and -3 PUFA-rich diets on inflammation and allergy reactions, atherosclerosis, hypertension, cancer growth, and a host of other

processes are due to the opposing effects of -6 PUFA-derived and -3 PUFA-derived eicosanoids on important target cells [1].

To create the eicosanoids, fatty acid oxidation is catalyzed by two groups of enzymes: The prostanoids are produced by cyclooxygenase or COX. LOX comes in a variety of types. The leukotrienes are produced by 5-lipoxygenase (5-LO). Eicosanoids are produced as needed and are not retained by cells. They come from the fatty acids found in the nucleus and cell membranes. When a cell is triggered by physical damage, cytokines, growth factors, or other triggers, eicosanoid production starts (Figure. 1). The stimulus may even be an eicosanoid from a neighboring cell; the pathways are complex. This causes a phospholipase to be released at the cell membrane. The nucleus barrier is reached by the phospholipase. There, the phospholipase catalyzes diacylglycerol or phospholipid ester degradation (by A2) (by phospholipase C). This liberates an important fatty acid with 20 carbons. The rate-determining stage for eicosanoid production appears to be this breakdown.

Any of the phospholipases can liberate fatty acids. Since cells missing type IV cytoplasmic phospholipase A2 (cPLA2) are typically bereft of eicosanoid production, cPLA2 is the main player in these processes. Phospholipids with AA, EPA, or GPLA at the SN2 location are specifically recognized by the phospholipase cPLA2. It's interesting to note that lysophospholipid that develops into a platelet-activating factor may also be released by cPLA2.

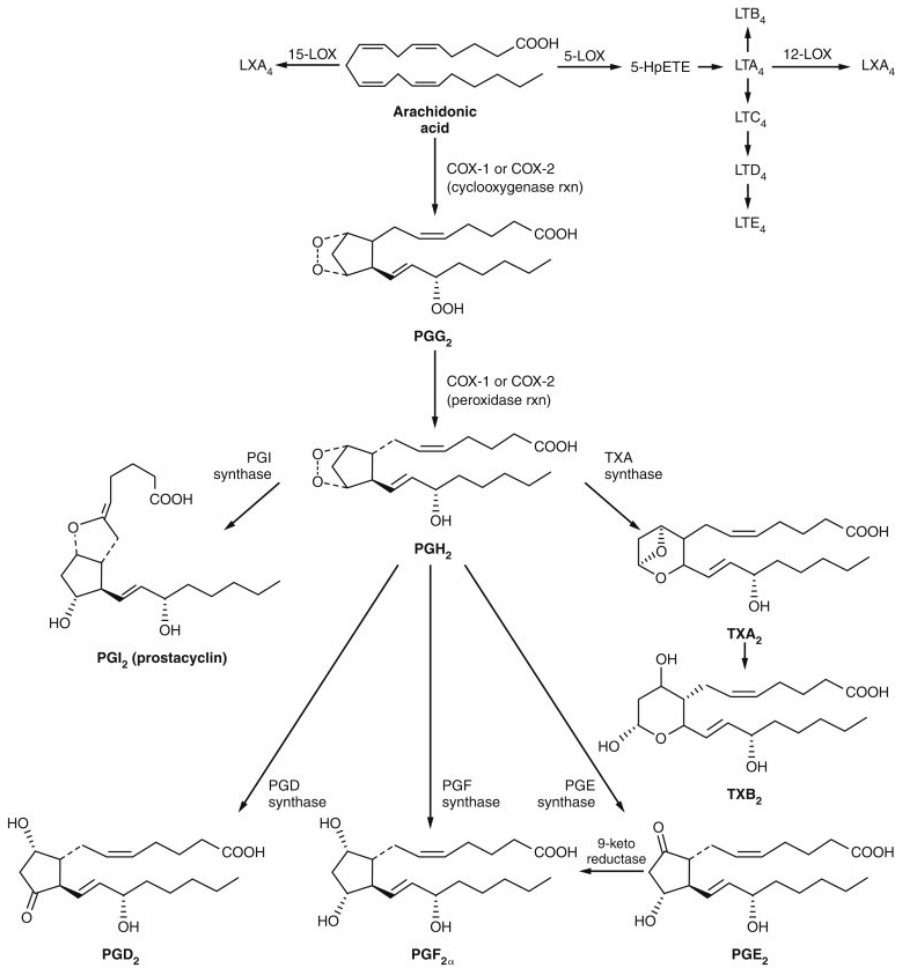

Figure 1: Eicosanoids synthesis pathway: Diagram showing the synthesis pathway of the eicosanoids (Science direct.com).

Von Euler first used the word "prostaglandin" in the 1930s to describe a potent substance from the prostate and seminal receptacles. In particular, this substance aided in the tightening of the uterus and digestive muscles and reduced blood pressure. Prostanoids and thromboxanes, byproducts of the COX branch of the pathway, have since been discovered as numerous closely related substances with various metabolic impacts (Figure .2). The hypothesized prostanoid acid has a C8–C12 cyclopentane structure and prostanoids are compounds of that acid. Structurally, The A, E, and F families are the three main groups of prostanoids. All have a five-membered ring and are linked to prostanoic acid (Figure .2). They differ based on where the functional groups are located. For instance, the 1,3-diols in the F series, the a, b-unsaturated ketones in the A series, and the b-hydroxy ketone in the E variety. The amount of double bonds in the side chain is indicated by the notation 2, which is 2. For instance, prostaglandin F2a (PGF2a), which is also a vasoconstrictor, activates the uterus and lung smooth muscle. By promoting blood flow, pH output, and mucous formation in the stomach mucosa, PGE2 helps to keep the healthy function and structure of the gastric mucosa. PGE2 also prevents gut mucosal mast cells from releasing inflammatory molecules like platelet-activating factor (PAF) and tumor necrosis factor (TNFa).

Additionally, PGE2 is a significant prostanoid that interacts with at least four nephron receptors, three of which are strongly expressed in various kidney areas. The production of prostaglandin D2 (PGD2, 9,15-dihydroxy-11-oxoprosta-5,13-dien-1-oic acid) occurs in response to mast cell and alveolar macrophage stimulation. Inhibition of PAF, bronchoconstriction, and deleterious impacts are some of its primary biochemical processes. Prostaglandins typically only remain in tissues for less than 5 minutes before being absorbed by cells and becoming inactive. Circulating prostanoid is effectively deactivated by the lungs. Prostaglandin seems to be released and cleared by particular carriers. Isoprostanes, a novel family of physiologically active compounds produced from AA, have recently come under consideration as having potential significance for human arterial disease. They appear to be produced in vivo by a nonenzymatic process of lipid peroxidation, though it has been demonstrated that a tiny percentage can be produced enzymatically in some circumstances.

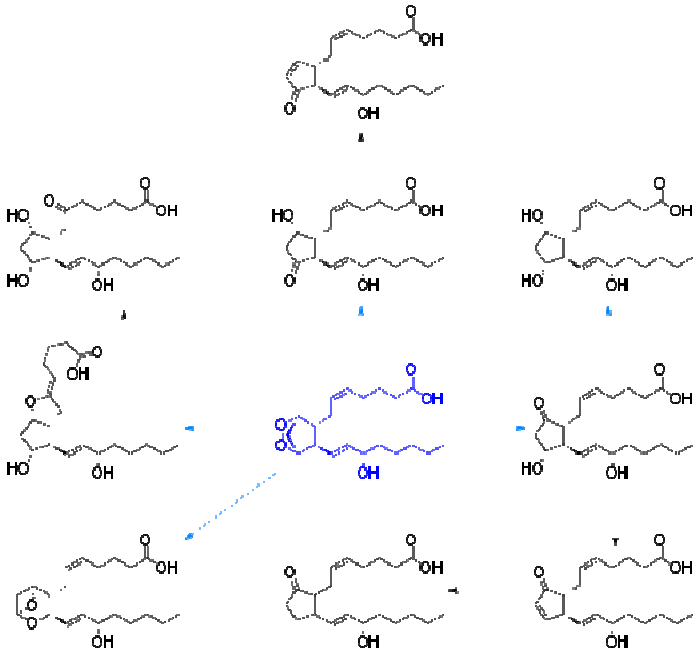

Figure 2:Strucutre of theprostanoids: Diagram showing the structure of the prostanoids (Wikipedia).

Arachidonic acid is converted by the enzyme 5-lipoxygenase (5-LO) into 5-hydroperoxy eicosatetraenoic acid (5-HPETE), which then naturally decreases to 5-hydroxyeicosatetraenoic acid. (5-HETE). 5-HPETE is changed into leukotriene A4 (LTA4) by the enzyme LTA synthase, which can then be changed into LTB4 by the enzyme leukotriene A4 epoxide hydrolase. Leukotriene C4 synthase is an enzyme that is used by eosinophils, mast cells, and lung macrophages to combine glutathione with LTA4 to create LTC4, which is then transferred outside the cell and modified to create LTD4. Dipeptidases then break the leukotriene LTD4 to create LTE4. The cysteinyl leukotrienes, which include the leukotrienes LTC4, LTD4, and LTE4, all contain cysteine. Eicosanoids function as mediators in the central nervous system and complicated regulators of many physiological systems, especially those related to inflammation and defense. The majority of organic organisms contain them. Eicosanoids are limited in people. Most cells produce hormones that operate on their own or neighboring cells (i.e., autocrine and paracrine messengers before being quickly deactivated. Eicosanoids have a brief half-life that can be anywhere between seconds and minutes. Dietary antioxidants, such as trans-resveratrol against thromboxane and some leukotrienes, prevent the production of some inflammatory eicosanoids. See the Receptors chart or the page eicosanoid receptors for more information. The majority of eicosanoid receptors belong to the G protein-coupled receptor class.

Leukotriene levels are higher in conditions like asthma that are inflammatory and hypersensitive. Such adverse consequences are an unavoidable result of anti-inflammatory drugs inhibiting the COX pathway. These LOX pathway products (Figure .3) are produced when LOXs react with arachidonate. For instance, AA is transformed into 12-hydroperoxy-eicosatetraenoic acid by 12-LOX action. (12-HPETE). Polymorphonuclear leukocytes' 5-LOX generates 5- HPETE, which is later changed into leukotrienes. Although leukocytes are a significant source, the word "leukotriene" was initially used to describe these compounds, which are tetraenes (conjugated trienes). Leukotrienes LTC4, LTD4, and LTE4 are parts of the anaphylaxis-causing slow-reacting compound (SRS-A) as a result of an immune assault, the lung releases. Their primary biochemical impact is the tightening of smooth muscles in the breathing system. beginning with research.

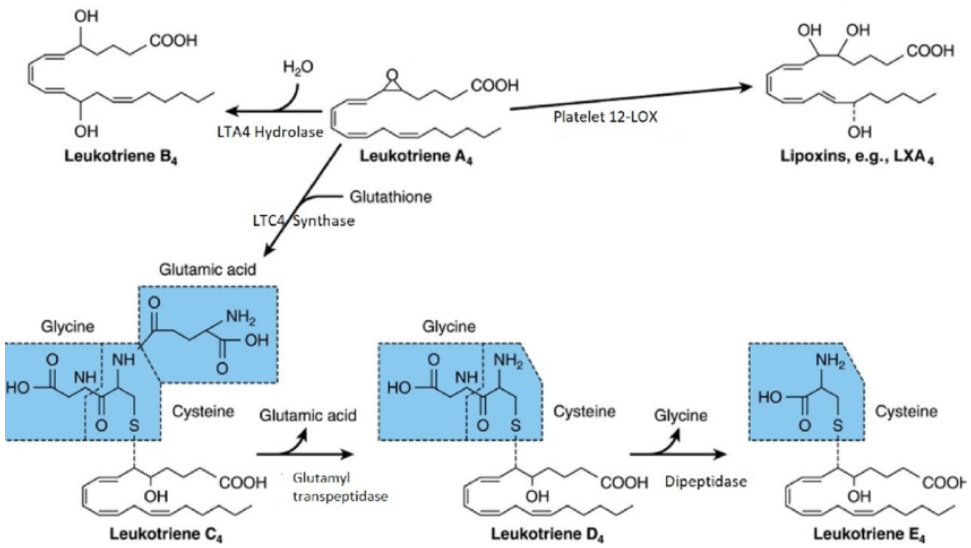

Figure 3:Biosyntheis of the Leukotriene: Daigrame showing the biosynthesis pathway of the Leukotriene (Research gate).

Leukotrienes are agents of bronchoconstriction connected to acute allergic responses, such as asthma, on SRS-A. Leukotrienes make the skin's blood vessels more permeable and narrow the heart, pulmonary, and epidermal arteries. Polymorphonuclear leukocytes, monocytes, and macrophages, which are cells engaged in inflammatory processes and defense mechanisms, produce leukotrienes. PAF and chemotactic peptides are examples of inflammatory triggers that cause LTB4 production in phagocytes. LTB4 is a strong chemoattractant for both human and rat neutrophils and is the first biological action of this substance that has been directly linked to the activation of neutrophil movement. Leukotrienes may play a hormonal function because they are produced in the central nervous system and induce the production of luteinizing hormone in rat pituitary cells. Leukotrienes can stay in the bloodstream for up to 4 hours, but little is known about the process that breaks them down or gets rid of them. The end product of the 5- and 15-LOXs' activity on arachidonate is lipoxins (Figure 3).

They have three alcohol groups as well as a twisted tetraene structure. Lipoxin A (LXA) and Lipoxin B (LXB), two subtypes, have been identified. Lipoxin B4 (LXB4) is its structural isomer, which is 5S,14R,15S-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid, and lipoxin A4 (LXA4) is 5S,6R,15S-trihydroxy-7,9,13-trans-11-ciseicosatetraenoic acid. LXA is implicated in chemotaxis, superoxide anion production, and spasmogenic action in neutrophils. In rodent and human endothelium cells, LXA4 has been shown to counteract the impacts of LTD4 and promote PGI production. LXA4 and LXB4 both prevent the killing of natural killer cells. LXA4 has been discovered to have PPAR ligand properties. In general, lipoxins have contributed to the pro-resolution of inflammation.

These AA compounds have impacts that are regulated by both internal and external receptor interaction. promote pulmonary endothelium cell growth and result in ERK1/2 and Akt activation. These lipidic intracellular molecules control the intracellular PI3K and MAPK pathways and have been associated with the process of inflammatory resolve. The endothelium produces EETs, which then trigger large-conductance Ca2+-activated K1 channels (BKCa), hyperpolarizing arterial smooth muscle and relaxing blood vessels. To lower blood pressure, EETs work as an endothelium-derived hyperpolarizing factor (EDHF) in several arterial pathways, including the heart and kidney circulations. Most EET has generally been linked to positive and preventive benefits [2].

The CYP membrane-bound proteins known as epoxygenase enzymes catalyze the conversion of AA into four regioisomeric EETs: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. At least 16 gene families and about 35 subfamilies make up the human P450 gene group. In several organ tissues of animals, several members of the CYP2C P450 gene subgroup have been found as the main AA epoxygenases. (Capdevila and Falck, 2002). In rodent and human tissues, CYP2C23, 2C11, 2C8, and 2C9 are classified as kidney and arterial epoxygenases.

The synthesis of EET is regioselectively mediated by each enzyme. EETs are either esterified to lysophospholipids by lysolipid acyltransferases or enzymatically hydrated to their corresponding inert dihydroxyeicsatrienoic acids (DHET) by epoxide hydrolases. A different method of eicosanoid production includes cell- contact between cells and the handling of biochemical products produced in one cell by a vicinal cell to produce a potent substance that neither cell can produce on its own. Growing evidence points to an eicosanoid transcellular metabolism in a complex environment that involves the creation of eicosanoids through the transmission of reactive intermediates as a specialized method of cell communication, especially among neutrophils, endothelium cells, and platelets. Recent data indicated that LT was produced in vivo by transcellular means in a model of in vivo inflammation. The four classic indicators of inflammation have been called calor (warmth), dolor (pain), tumor

(swelling), and rubor (redness) since ancient times (redness). Each of these symptoms involves eicosanoids.

Redness

The traditional inflammation reaction is brought on by a bug bite. TXA2, which has a short half-life, is rapidly produced after damage. The area might briefly become paler. The production of the vasodilators PGE2 and LTB4 is then mediated by TXA2. The laceration turns redder as the blood vessels swell.

LTB4 causes swelling, which increases blood artery permeability. In the fibrous tissues, plasma escapes, causing swelling. During the process, anti-inflammatory peptides are also lost.

Pain:

The cytokines make COX-2 more active. PGE2 levels rise as a result, making pain receptors more sensitive.PGE2 is a strong pyretic substance that can cause heat. Aspirin and NSAIDs, inhibit the COX pathways and prevent the production of prostanoids, reducing temperature or the intensity of localized inflammation [3].

DISCUSSION

Complex molecular signaling networks with synchronized and frequently conflicting actions are required for controlled immune reactions to infection and damage. A significant bioactive lipid network made up of eicosanoids and associated bioactive lipid molecules generated from polyunsaturated fatty acids is one of the most intricate and difficult routes to characterize in a physiological setting. Eicosanoid signaling has traditionally been thought of as a pro-inflammatory aspect of the innate immune response, similar to cytokine signaling and the formation of inflammasomes. However, recent developments in lipidomics have helped to elucidate specific eicosanoids and related docosanoids with anti-inflammatory and pro-resolution functions. Our knowledge of the inflammation reaction and its treatment consequences has improved as a result. This paper reviews how the stimulation of inflammatory receptors by pathogenic pathogens causes a pro-inflammatory and anti-inflammatory eicosanoid maelstrom[4].

Potent eicosanoid lipid mediators called prostaglandins and leukotrienes, which are produced from arachidonic acid released by phospholipase, are engaged in a variety of cellular processes that are homeostasis and inflammatory. They are produced by the enzymes cyclooxygenase isozymes and 5-lipoxygenase, respectively, and the more recent line of coxibs (specific inhibitors of cyclooxygenase-2) and leukotriene modifiers prevent their production and effects. The primary mechanism of action of prostaglandins and leukotrienes is via particular G protein-coupled receptors, many of which have only recently been identified, allowing the creation of specific receptor agonists and blockers. Our present knowledge of eicosanoid biology has provided significant new information about the processes underlying inflammation reactions, discomfort, and temperature [5].

Arachidonic acid (AA), which is released by the rate-determining hydrolytic action of heart phospholipase A2, is primarily stored in myocardial phospholipids. (PLA2s). A common PLA2 in the heart is calcium-independent phospholipase A2 (iPLA2), which is controlled by changes in local cellular Ca2+ amounts and ventricular bioenergetic state through its calmodulin (CaM) and ATP-binding regions, respectively. It is significant to note that iPLA2 is triggered by ischemia through an increase in cardiac fatty acyl-CoA content, which eliminates Ca2+/CaM-mediated suppression of iPLA2. As a precursor for eicosanoids

produced by processes reliant on cyclooxygenases (COX), lipoxygenases (LOX), and cytochromes P450, AA is released by PLA2-catalyzed breakdown of phospholipids. (CYP). Eicosanoids engage with cellular receptors and ion channels to start and spread a variety of signaling pathways. Eicosanoids, on the other hand, contribute to several maladaptive changes during pathologic states like ischemia or congestive heart failure, including inflammation, changes to cellular growth programs, and activation of numerous transcriptional events that result in the harmful aftereffects of these pathologic states. The main COX, LOX, and CYP pathways of eicosanoid generation in the myocardium, as well as the effects of significant eicosanoids on receptor-, ion channel-, and transcription-mediated processes that promote cardiac hypertrophy, mediate ischaemic preconditioning, and precipitate arrhythmogenesis in response to pathologic stimuli, are summarized in this review [6].

Eicosanoids, which include prostaglandins, thromboxanes, leukotrienes, and lipoxins, are a class of oxidized products of arachidonic acid. These lipid messengers are crucial for maintaining both healthy cellular balance and a variety of illness conditions. This overview will emphasize particular findings and accomplishments while concentrating on current developments in the area of eicosanoids. Structure and receptor biology, which have considerable pharmaceutical and therapeutic implications, will be the focus of the study [7].

Prostaglandins, leukotrienes, and lipoxins are examples of eicosanoids, which are signaling lipids produced from the breakdown of arachidonic acid and play significant roles in both normal and disease processes. The control of eicosanoid synthesis and activities has recently been shown to be significantly influenced by the intracellular compartmentalization of the eicosanoid-synthetic apparatus. Significant headway has been made in recent years showing that lipid bodies, also known as lipid droplets, are separate sites for eicosanoid synthesis and that precursors and enzymes implicated in eicosanoid synthesis locate there. Here, we'll go over what is currently known about how lipid bodies work as specialized intracellular locations for compartmentalizing signaling that play key roles in the production of eicosanoids in cells involved in inflammatory, contagious, and malignant processes [8].

Studies in animal tumor models have shown that blocking eicosanoids' synthesis can prevent colon cancer, and epidemiological studies have shown that long-term aspirin and other nonsteroidal anti-inflammatory drug users have a lower risk of developing colon cancer. These findings suggest that eicosanoids may play a role in colon carcinogenesis. (NSAIDs). The amounts of the cyclooxygenase and 5-lipoxygenase pathway markers prostaglandin E2 (PGE2), PGF2, PGI2, thromboxane A2 (TXA2), and leukotriene B4 (LTB4) were measured in 21 pairs of surgically removed human colon cancer and histologically normal mucosa samples that were 5 to 10 cm away from the tumor. In contrast to histologically normal mucosa samples far from cancer, the levels of PGE2 were higher in colorectal cancer samples (p 0.01) while prostacyclin (PGI2) levels were lower (p 0.05). PGF2, TXA2, and LTB4 amounts between healthy and cancerous tissue did not vary in a statistically meaningful way. There was no statistically significant correlation between Dukes' colon cancer stage and the levels of any of the measured eicosanoids. These results, which support and build upon previous research from tumors and cell culture, indicate that the beneficial impact of aspirin and other NSAIDs in the formation of human colon cancer may be mediated, at least in part, through their suppression of cyclooxygenase catabolism of arachidonic acid [9].

A model for the use of oxygen in the transfer of information and communication by arachidonic acid is outlined in the opening to this Perspective series (1). Arachidonic acid communication, however, can also aid in the spread of cellular harm. The signaling cascade that (a) stops 5-lipoxygenase (5-LO) from activating in dormant cells and (b) causes the

creation and release of leukotrienes (LTs) is a good example of this dichotomy. This cascade needs the successive activation and interaction of at least eight distinct proteins. In actuality, membrane transfer is necessary for all lipoxygenases to function. Humans have evolved two sets of biosynthetic enzymes that differ not only in their subcellular localization and requirement for reduced glutathione, a cellular defense against oxidative damage but also in the formation of COX products, particularly prostaglandin E2 (PGE2) and PGD2. Three facets of the biochemistry of arachidonic acid will be the emphasis of this study. First, we'll talk about how eicosanoid synthesis is compartmentalized and organized, with a focus on LTs and PGs. It will also demonstrate that enzymes like glutathione-S-transferases, epoxide hydrolases, and carrier proteins that are typically thought of as biosynthetic also belong to families that are typically considered to play a role in detoxification. These complex mechanisms that prevent unwanted lip-oxygenation will be demonstrated. Second, it is investigated whether the use of oxygen and fatty fuels can result in cellular reactive harm. The methods by which a nucleus of LTs and PGs is used to increase signaling variety are then described [10].

CONCLUSION

Eicosanoids are usually not retained by cells; instead, they are created as needed. It comes via fatty acids found in the nucleus and cell membranes. Eicosanoids come in a variety of forms, but prostaglandins, thromboxanes, and leukotrienes are the three that have been the subject of the most study. Arachidonic acid (AA), eicosapentaenoic acid (EPA), and dihomo-linolenic acid are converted into eicosanoids when phospholipase A2 releases them from tissue phospholipids (PL). Arachidonic acid is the source of the local proteins known as eicosanoids. They perform a wide range of tasks, such as triggering asthma episodes, blood coagulation, labor, and edema and inflammation at the locations of damage. In the process of reproduction, eicosanoids play several functions, which are related to ovulation, corpus luteum function, luteolysis, implantation, and decidualization. Eicosanoids, which include prostaglandins, leukotrienes, thromboxanes, and lipoxins, are physiologically active lipid mediators implicated in several pathological processes related to asthma, allergens, and related illnesses.

REFERENCES:

[1] "Eicosanoid," *Wikipedia*. Jan. 07, 2020. Accessed: Mar. 24, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Eicosanoid&oldid=1132069259

[2] L. R. Engelking, "Paper 68 - Eicosanoids I," in *Textbook of Veterinary Physiological Chemistry (Third Edition)*, L. R. Engelking, Ed. Boston: Academic Press, 2015, pp. 434–438. doi: 10.1016/B978-0-12-391909-0.50068-2.

[3] "Arachidonic Acid - ScienceDirect." https://www.sciencedirect.com/science/paper/pii/B0123868602000435 (accessed Mar. 24, 2020).

[4] E. A. Dennis and P. C. Norris, "Eicosanoid storm in infection and inflammation," *Nat. Rev. Immunol.*, vol. 15, no. 8, Art. no. 8, Aug. 2015, doi: 10.1038/nri3859.

[5] C. D. Funk, "Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology," *Science*, vol. 294, no. 5548, pp. 1871–1875, Nov. 2001, doi: 10.1126/science.294.5548.1871.

[6] C. M. Jenkins, A. Cedars, and R. W. Gross, "Eicosanoid signalling pathways in the heart," *Cardiovasc. Res.*, vol. 82, no. 2, pp. 240–249, May 2009, doi: 10.1093/cvr/cvn346.

[7] J. Z. Haeggström, A. Rinaldo-Matthis, C. E. Wheelock, and A. Wetterholm, "Advances in eicosanoid research, novel therapeutic implications," *Biochem. Biophys. Res. Commun.*, vol. 396, no. 1, pp. 135–139, May 2010, doi: 10.1016/j.bbrc.2010.03.140.

[8] P. T. Bozza, I. Bakker-Abreu, R. A. Navarro-Xavier, and C. Bandeira-Melo, "Lipid body function in eicosanoid synthesis: An update," *Prostaglandins Leukot. Essent. Fat. Acids PLEFA*, vol. 85, no. 5, pp. 205–213, Nov. 2011, doi: 10.1016/j.plefa.2011.04.020.

[9] B. Rigas, I. S. Goldman, and L. Levine, "Altered eicosanoid levels in human colon cancer," *J. Lab. Clin. Med.*, vol. 122, no. 5, pp. 518–523, Nov. 1993, doi: 10.5555/uri:pii:002221439390010V.

[10] R. J. Soberman and P. Christmas, "The organization and consequences of eicosanoid signaling," *J. Clin. Invest.*, vol. 111, no. 8, pp. 1107–1113, Apr. 2003, doi: 10.1172/JCI18338.

CHAPTER 19

BIOSYNTHESIS OF THE MEMBRANE PHOSPHOLIPIDS

Dr Pooja Agarwal, Assistant Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- poojaagr.sgrrr@gmail.com

ABSTRACT:

The components of the cell membrane are proteins, carbohydrates, and lipids. Lipids are the major component of the cell membrane and play an essential role in cell signaling and maintaining the fluidity of the cells. Phospholipids serve a significant part in the transfer and elimination of cholesterol from cells with the help of proteins, phospholipids create the basic elements of the cell membrane. In this paper, we discussed the biosynthesis pathway of the phospholipids and their importance in the cells.

KEYWORDS:

CDP Choline, Endoplasmic Reticulum, Fatty Acids, Mitochondrial Membrane, Phospholipids Biosynthesis.

INTRODUCTION

A hydrophilic "head" made up of a phosphate group and two hydrophobic "tails" made up of fatty acids are connected by an alcohol residue that makes up the structure of phospholipids (usually a glycerol molecule). Omega-3 fatty acids EPA and DHA are frequently incorporated into the phospholipid structure of marine phospholipids. Simple organic compounds like serine, ethanolamine, or choline can modify the phosphate group. All cell walls contain phospholipids, which are essential elements. Due to their amphiphilic nature, they can create lipid bilayers. Along with phospholipids, the cell walls of eukaryotes also contain sterols, a different type of lipid. The combo offers mechanical power against tearing along with the two-dimensional flow[1]–[3]. Commercially available, purified phospholipids are used in materials science and nanotechnology. Lecithin, or phosphatidylcholine, in poultry egg yolks, was the first phospholipid discovered in living tissues in 1847 by French scientist and physician Theodore Nicolas Gobley. Amphiphilic phospholipids are present. The hydrophobic end typically consists of two "tails" that are lengthy fatty acid residues, while the hydrophilic end typically includes a negatively charged phosphate group. The hydrophobic interactions that drive phospholipids in watery environments cause the fatty acid ends to aggregate to reduce contact with the water molecules (Figure. 1).

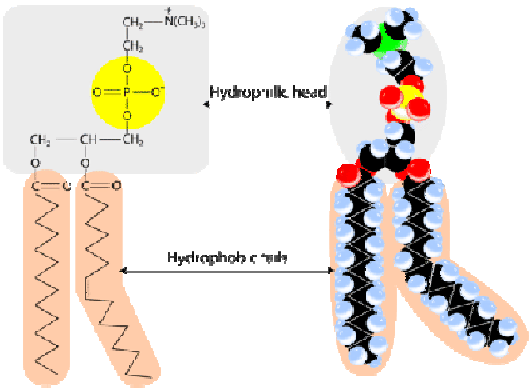

Figure 1: Phospholipids: Diagram showing the structure of the Phospholipids (Chemistry libera text).

The end product is frequently a membrane made of two layers of phospholipid molecules that are oppositely oriented, with their heads guided into the membrane and their tails exposed to the liquid on both sides. The membranes of all cells and some other biological structures, like vesicles or viral coverings, share this main structural pattern. The primary structural element of cell membranes is composed of phospholipid bilayers. In biological membranes, phospholipids frequently co-occur in a bilayer, like a cell membrane, with other compounds (such as proteins, glycolipids, and sterols). When hydrophobic tails align with one another to create a membrane with hydrophilic heads on either side confronting the water, lipid bilayers are created.Phospholipids can play a significant role in the cell membrane thanks to these particular characteristics. The fluid mosaic model, which sees the membrane as a mosaic of lipid molecules that serves as a solvent for all the chemicals and proteins inside of it, can be used to explain their movement. In this model, proteins and lipid molecules are free to diffuse laterally through the lipid matrix and migrate over the membrane. Sterols prevent phospholipids from clumping together, which promotes membrane mobility. This paradigm has since been replaced, though, as research on lipid variation has revealed that lipid behavior under metabolic (and other) circumstances is not straightforward.

Acid Phosphatidic biosynthesis: Although phosphatidic acid makes up a small percentage of membrane phospholipids, it serves as the structural basis for the production of triacylglycerol and other phospholipid species. Lysophosphatidic acid is created by adding a fatty acyl-CoA, typically saturated, to glycerol 3-phosphate at the sn-1 location. This is the first step in the production of phosphatidic acid. The rate-limiting step in the production of phosphatidic acid is this process, which is mediated by glycerol 3-phosphate acyltransferase (Figure. 2). This enzyme exists in two different forms, one of which is located in the exterior mitochondrial membrane and the other in the endoplasmic reticulum. Arylglycerol-3-acyltransferase converts lysophosphatidic acid to phosphatidic acid at the sn-2 location by adding a second fatty acyl-CoA, frequently an unsaturated one. The endoplasmic reticulum is where this mainly happens.

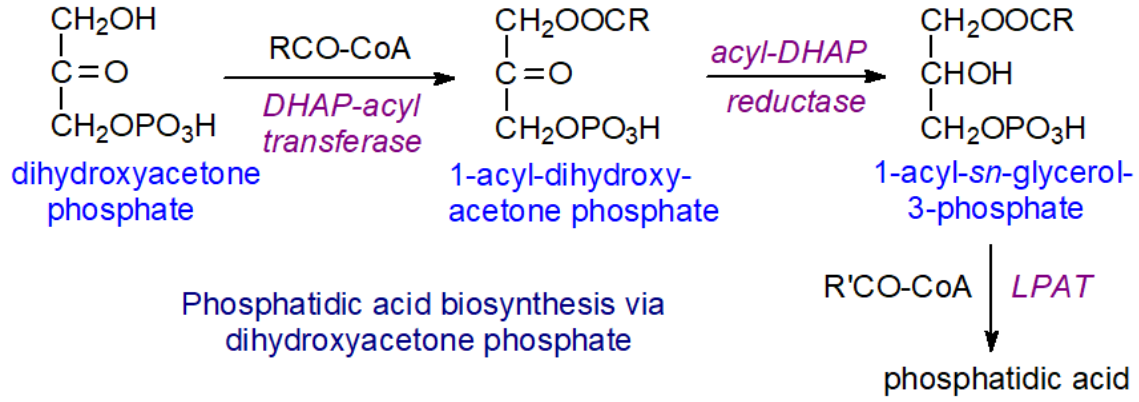

Figure 2: Phosphatidic acid biosynthesis: Diagram showing the biosynthesis pathways of the Phosphatidic acids (Chemistry libera text).

There are two distinct ways that phospholipidic acid can be used in the production of various phospholipids. The phosphate molecule in phosphatidic acid is hydrolyzed in the first process, resulting in diacylglycerol. This is accomplished by the endoplasmic reticulum membrane's phosphatidic acid and the cytoplasmic phosphatidic acid phosphatase, also known as lipids. The succeeding biosynthesis routes for phosphatidylcholine and phosphatidylethanolamine, which are covered in the following parts, both use diacylglycerol. Triacylglycerol, the primary type of energy storage, is also a forerunner to diacylglycerol.

The second way to make more phospholipids from phosphatidic acid uses cytidine triphosphate (CTP) as an energy source to produce a CDP-diacylglycerol molecule. Overall, this process enables the formation of phosphatidylinositol, phosphatidylglycerol, or cardiolipin by substituting other phosphate functional groups for the phosphate group of phosphatidic acid. (also known as diphosphatidylglycerol). This summary will not go over how these glycerophospholipids are made.

The Kennedy pathway, also known as the CDP-choline route, produces PC in all nucleated mammalian cells (Figure. 3). Extracellular choline is first brought into the cell and quickly modified by the cytoplasmic enzyme choline kinase to become phosphocholine. Two different genes encode this kinase function. CTP: phosphocholine cytidylyltransferase catalyzes the second step of this PC biosynthetic route, which results in CDP-choline. The rate-limiting process for PC biosynthesis occurs during the generation of CDP-choline under the majority of biochemical circumstances. There are two types of CTP: phosphocholine cytidylyltransferase, and, both of which become active when they attach to membranes. The isoform has a nucleus localization gene, but not the isoform. The isoform is extranuclear, whereas the isoform is predominantly found in the nucleus and is also present in the cytoplasm. Because CDP-choline catalyzes the concluding process of the CDP-choline route for PC formation, the molecular reason for why CDP-choline is produced in the nucleus is unclear[4], [5]:

ER-specific 1,2-diacylglycerol choline phosphotransferase is an essential membrane protein that converts diacylglycerol to CDP-choline to produce PC (Figure. 3). PE N-methyltransferase, an enzyme that is anchored in ER membranes, catalyzes three consecutive methylation processes that transform PE to PC in a different route for PC production. Only hepatocytes (30% of the total) are a form of rodent cell in which this process generates appreciable quantities of PC. Although yeast uses parallel routes for PC synthesis, the majority of PC in this organism is produced by PE methylation under the majority of growth circumstances. Two different methyltransferases are needed in yeast for the conversion of PE to PC, whereas in mammalian cells, a single methyltransferase carries out all three methylation processes. This is a significant distinction between the methylation pathways in yeast and mammalian cells.

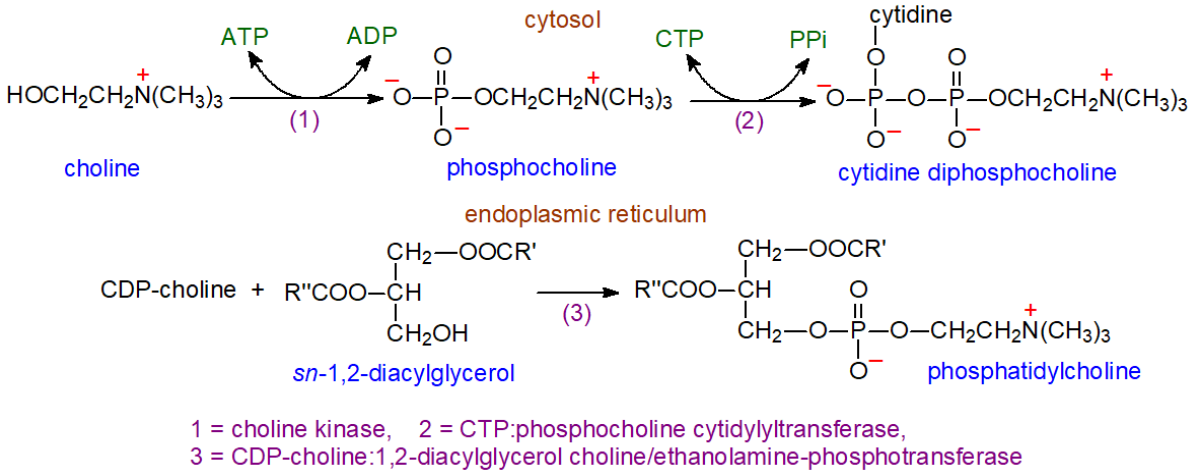

Figure 3: Phosphatidylcholine biosynthesis: Diagram showing the biosynthesis pathway of the Phosphatidylcholine (lipid maps).

When compared to other organelle membranes of human cells, the plasma membrane is greatly abundant in the phosphosphingolipid sphingomyelin, another choline-containing lipid.

Ceramide and PC, two components that are produced in the ER and transferred to the Golgi for SM synthesis, are used to create SM, with SM synthase-1 in the Golgi apparatus producing the bulk of the SM. The plasma membrane contains SM synthase-2, a second enzyme that produces small amounts of SM. Additionally, the ER contains an enzyme that is closely linked to SM synthase-R, which creates tiny quantities of the ethanolamine analog of SM, ceramide phosphoethanolamine.

In human membranes, PC is the most prevalent phospholipid and makes up about 50% of all cellular phospholipids. The exterior layer of the plasma membrane contains the majority of the PC. Due to its cylinder form, PC is a crucial structural element that supports the stability and functionality of membranes. The liver produces and secretes very low-density lipoproteins that transport viscous material (such as cholesterol and energy in the form of fat) to other tissues. PC is necessary for this process. This phospholipid also contributes to the creation of micelles in the digestive tract, which helps people absorb lipid-soluble minerals from food.

Phosphatidylethanolamine is produced through the cytidine diphosphate-ethanolamine and phosphatidylserine decarboxylation routes. The enzyme used to decarboxylate phosphatidylserine in the first route is phosphatidylserine decarboxylase. The primary route for the production of phosphatidylethanolamine in the mitochondrial membranes is phosphatidylserine decarboxylation (Figure. 4).

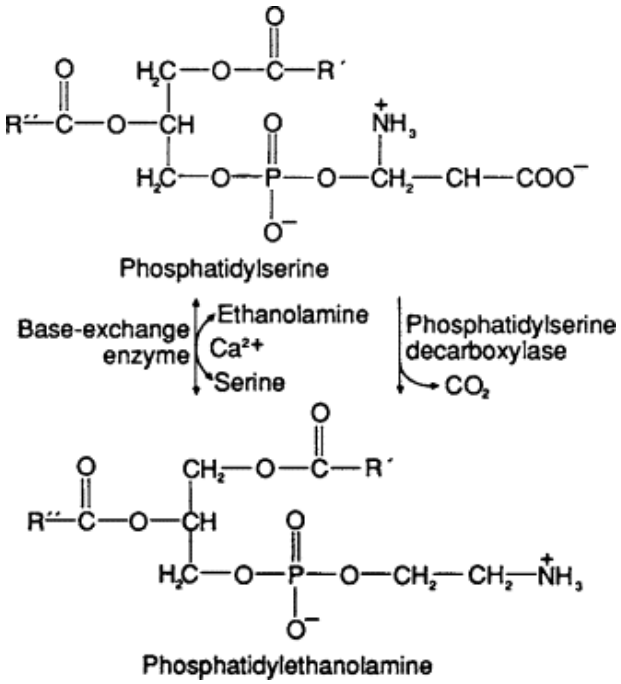

Figure 4: phosphatidylethanolamine biosynthesis: Diagram showing the biosynthesis pathway of the phosphatidylethanolamine (Science direct.com).

The cell transports phosphatidylethanolamine, which is generated in the mitochondrial membrane, to other membranes for use. Phosphatidylethanolamine is also produced via the cytidine diphosphate-ethanolamine pathway, using ethanolamine as the precursor, in a procedure that is similar to the production of phosphatidylcholine. The manufacturing route results in the final product of phosphatidylethanolamine after several stages that take place in both the cytoplasm and endoplasmic reticulum. Additionally widely present in soy or egg lecithin, phosphatidylethanolamine is synthesized economically through chromatography separation.

In microorganisms (including *E. coli*), phosphatidylserine is created when the hydroxyl functional group of serine engages in a nucleophilic assault on cytidine monophosphate (CMP). PS synthase converts CDP-diacylglycerol into CMP. The enzyme PS decarboxylase can ultimately convert phosphatidylserine to phosphatidylethanolamine (forming carbon dioxide as a byproduct) (Figure. 5). Using the same route as bacteria, yeast can produce phosphatidylserine.

In animals, phosphatidylserine is instead produced by one of two Ca2+-dependent head-group exchange processes in the endoplasmic reticulum from phosphatidylethanolamine or phosphatidylcholine. Both processes result in ethanolamine or choline, but they both need serine as a starting material. Phosphatidylserine synthase 1 (PSS1) or PSS2 promotes these (PSS2). On the other hand, phosphatidylserine can also result in phosphatidylethanolamine and phosphatidylcholine, though in mammals, the liver is the only organ where the route to produce phosphatidylcholine from phosphatidylserine is active.

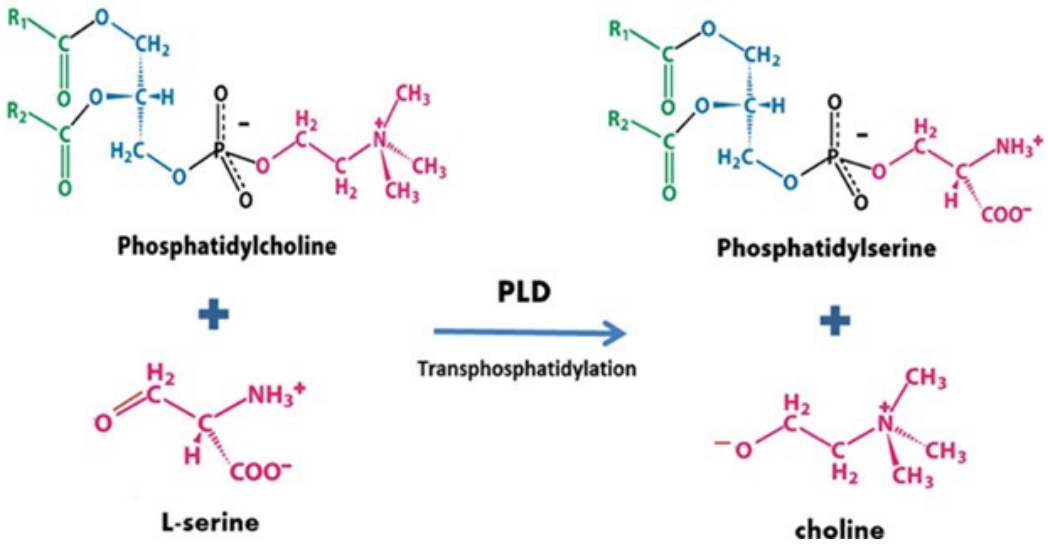

Figure 5: phosphatidylserine biosynthesis: Diagram showing the biosynthesis pathway of the phosphatidylserine (Wiley online. library).

PS makes up 5–10% of the phospholipids in cells. The interior aspect of the plasma membrane is where it is most prevalent. An essential signaling process for blood coagulation, the externalization of PS to the exterior layer of the membrane causes the absorption of dead cells. In the mitochondrion, freshly made PS serves as an essential forerunner to PE. The anionic nature of the serine head group is one intriguing PS feature. As a result, positively charged proteins can interact with PS in the inner membrane and may make it easier for proteins to engage with their membrane-bound receptors. Protein kinase C, which is in charge of many biological reactions to cues, is stimulated by PS.

The supply of substrate is necessary for PC synthesis via the CDP pathway; sufficient choline and CTP must be provided. A supply of diacylglycerol is also necessary. The CDP-choline pathway's rate-limiting enzyme is CT. This enzyme is present in a liquid state that serves as a dormant reserve. The enzyme develops a stronger preference for its precursor, CTP, when it establishes a connection with lipids in the membrane. Phosphatidylglycerol, oleic acid, and diacylglycerol are lipids that increase CT action. Through the CDP-choline route, the bidirectional transfer of CT controls PC synthase activity. Enzyme phosphorylation, which favors the soluble dormant arrangement, and dephosphorylation, which favors the active-membrane-related version, control translocation.

The PEMT pathway and the CDP-choline pathway may be controlled in tandem. When the amount of choline is insufficient to support PC production via the CDP-choline route, PEMT activity is enhanced. S-adenosylmethionine and PE are just two of the compounds that are necessary for PC synthesis by PEMT. S-adenosylhomocysteine buildup prevents PE from being methylated[6], [7].

The interior mitochondrial membrane quickly produces PE through the decarboxylation of PS. However, the endoplasmic reticulum is where PS is produced. The transport of freshly synthesized PS from the endoplasmic reticulum domain known as the mitochondrial-associated membrane to the mitochondrial membrane and its subsequent transport to the inner mitochondrial membrane, where phosphatidylserine decarboxylase resides, is thus proposed as the rate-limiting steps in the phosphatidylserine decarboxylation pathway. We presently don't know the exact processes at play in this transit process. In the CDP-ethanolamine pathway, ET is the rate-limiting enzyme. The availability of diacylglycerol and CDP-ethanolamine are both necessary for this process. ET action also depends on the cytoplasmic protein's proximity to the endoplasmic reticulum, which houses the membrane-bound enzymes of this pathway.

DISCUSSION

Unique membrane phospholipids found in Archaea are typically isoprenoid ethers constructed from sn-glycerol-1-phosphate. (G1P). In comparison, the phospholipids found in bacterial and eukaryal membranes are fatty acid compounds connected to sn-glycerol-3-phosphate. (G3P). G1PDH and G3PDH, the two main dehydrogenase enzymes that result in G1P and G3P, respectively, are not identical. There is disagreement over the character of lipid membranes in the last universal common ancestor (cenancestor), including whether or not they even existed. Various theories suggest that these enzymes evolved during the separation of the two bacterial groups. The fact that G1PDH and G3PDH are members of two distinct superfamilies that are equally dispersed suggests that both superfamilies had members in the common progenitor. Additionally, archaea produce fatty acid phospholipids and have homologs of known bacterial genes implicated in fatty acid biosynthesis. It appears probable that the common progenitor had access to membrane lipids, whose production was likely catalytic but not stereospecific.

The plasma membrane, nuclear envelope, mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and endosomes are all made of bilayer membranes made of phospholipids and cholesterol in human cells. According to their chemical makeup, phospholipids can be categorized into different groups, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, cardiolipin, and sphingomyelin. Phospholipids are crucial for a variety of cellular processes, including membrane protein control, membrane transport, cell development, death, and intracellular communication, in addition to their structural functions. So, aberrant phospholipid biosynthesis is linked to several illnesses. Phospholipid classes are produced by several biochemical processes in human cells, primarily in the endoplasmic reticulum, mitochondria, and Golgi apparatus. Enzymes involved in the production of different phospholipid groups have been discovered in recent years. The regulating processes underpinning the production of various phospholipid groups are, however, poorly understood. We have shown variations in phospholipid makeup in intracellular compartments during cell development using our newly designed enzyme fluorometric tests for all main phospholipid classes. In this overview, we address the regulating processes that affect phospholipid biosynthesis enzymes as well as our present knowledge of their characteristics and roles.

The dynamic changes in nuclear morphology at various cell cycle phases and during cell development depend on the remodeling of the nuclear membrane. It is unknown what chemical process controls nuclear membrane formation. Here, we demonstrate how the nucleus membrane development during the cell cycle is significantly regulated by Smp2, the yeast equivalent of mammalian lipin. Smp2 is phosphorylated by Cdc28/Cdk1 and dephosphorylated by a Nem1 and Spo7-containing CPD phosphatase complex that is confined to the nuclear/endoplasmic reticulum (ER) membrane. Loss of SMP2 or its dephosphorylated version results in a huge enlargement of the nucleus and transcriptional activation of important enzymes involved in lipid production. On the other hand, Smp2's inherent dephosphorylation prevents cell division. We demonstrate that Smp2 interacts with phospholipid biosynthesis enzyme regulators in a Nem1-Spo7-dependent way. Our findings imply that Smp2 plays a crucial role in synchronizing nuclear/ER membrane phospholipid production with nuclear development during the cell cycle.

Maintaining the flexibility and porosity barrier of the cell membrane is essential for all living things. When compared to bacteria and eukarya, the phospholipid membrane makeup of archaea is different. Isoprenoid hydrocarbon side chains in archaea are joined to the sn-glycerol-1-phosphate backbone by an ether bond. On the other hand, in bacteria and eukarya, fatty acid side chains are joined to the sn-glycerol-3-phosphate backbone by an ester bond. The three areas of existence all share the same polar head groups worldwide. The distinctive membrane lipids of archaea have been linked to the last common ancestor's membrane makeup as well as the survival and adaptation of the organisms to harsh habitats. (LUCA). The diether (or archaeol) and tetraether (or caldarchaeol) lipids, which create a bilayer, are the most prevalent archaeal lipids in nature. These lipids are diverse due to variations in chain length, cyclization, and other changes. Although development over the past ten years has led to a thorough knowledge of the biosynthesis of archaeol, the biosynthesis of these lipids is still not fully known. The present state of understanding regarding the biosynthesis process for archaeal ether lipids, discoveries regarding the durability and resilience of archaeal lipid membranes, and evolutionary implications of the lipid split and the LUCA are all covered in this study. It looks at new developments in the study of bacterial pathway rebuilding. An summary of human phospholipid production and the cellular sites of the metabolic processes that result in membrane lipid molecule species are provided in this study. Examples of the developing connections between changes in lipid makeup, control of membrane lipid biosynthesis, and cellular secretion function are addressed. The generalized endoplasmic reticulum compartment is a key location for membrane lipid biogenesis[8]–[10].

This study examines the most recent data on the interactions between cell biology and phospholipid metabolism.

The most prevalent phospholipid is phosphatidylcholine, and its production has received a great deal of attention. Recent developments in our knowledge of the processes governing choline cytidylyltransferase include the finding of numerous transcripts and a more thorough grasp of the lipid control of enzyme activity.

Choline cytidylyltransferase governs phosphatidylcholine synthesis. Along with current knowledge of the controlling processes, similarities between the creation of phosphatidylcholine and the biosynthesis of phosphatidylethanolamine and phosphatidylinositol are addressed. The cyclical production and breakdown of membrane phospholipids is responsible for their tripling during the development of the cell cycle. Insufficient phosphatidylcholine causes cells to undergo apoptosis, whereas excessive phospholipid is broken down by the enzyme phosphalipase A to ensure membrane equilibrium.

CONCLUSION

Phospholipids, especially phosphatidylcholine (PC), are made in the ER where they play important roles in maintaining cholesterol balance, supplying walls for protein production and export, and storing and secreting triacylglycerol. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine are the four most prevalent phospholipids. Complex compounds known as phospholipids in the membrane, such as proteins, contain functional groups which are known to organize copper ions. Suggested binding locations include the phosphate, carboxyl, and amine moiety of the hydrophilic head that is exposed to the surface of phospholipids. Mechanisms that influence the production of enzymes and the regulation of their functions control how phospholipids are synthesized. Numerous variables, such as the supply of nutrients, the stage of development, pH, and heat, regulates the activation of the genes involved in phospholipid production.

REFERENCES:

[1] W. Raphael and L. M. Sordillo, "Dietary polyunsaturated fatty acids and inflammation: The role of phospholipid biosynthesis," *International Journal of Molecular Sciences*. 2013. doi: 10.3390/ijms141021167.

[2] H. Santos-Rosa, J. Leung, N. Grimsey, S. Peak-Chew, and S. Siniossoglou, "The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth," *EMBO J.*, 2005, doi: 10.1038/sj.emboj.7600672.

[3] W. Dowhan, "Understanding phospholipid function: Why are there so many lipids?," *J. Biol. Chem.*, 2017, doi: 10.1074/jbc.X117.794891.

[4] J. Royes, V. Biou, N. Dautin, C. Tribet, and B. Miroux, "Inducible intracellular membranes: Molecular aspects and emerging applications," *Microbial Cell Factories*. 2020. doi: 10.1186/s12934-020-01433-x.

[5] M. Exterkate, A. Caforio, M. C. A. Stuart, and A. J. M. Driessen, "Growing Membranes in Vitro by Continuous Phospholipid Biosynthesis from Free Fatty Acids," *ACS Synth. Biol.*, 2018, doi: 10.1021/acssynbio.7b00265.

[6] D. E. Sastre *et al.*, "Membrane fluidity adjusts the insertion of the transacylase PlsX to regulate phospholipid biosynthesis in Gram-positive bacteria," *J. Biol. Chem.*, 2020, doi: 10.1074/jbc.RA119.011122.

[7] G. A. Coleman, R. D. Pancost, T. A. Williams, and T. Dagan, "Investigating the Origins of Membrane Phospholipid Biosynthesis Genes Using Outgroup-Free Rooting," *Genome Biol. Evol.*, 2019, doi: 10.1093/gbe/evz034.

[8] P. Fagone *et al.*, "Phospholipid biosynthesis program underlying membrane expansion during B-lymphocyte differentiation," *J. Biol. Chem.*, 2007, doi: 10.1074/jbc.M608175200.

[9] Y. Murata *et al.*, "Dimethyl sulfoxide exposure facilitates phospholipid biosynthesis and cellular membrane proliferation in yeast cells," *J. Biol. Chem.*, 2003, doi: 10.1074/jbc.M300450200.

[10] M. Lehmann, "Diverse roles of phosphatidate phosphatases in insect development and metabolism," *Insect Biochem. Mol. Biol.*, 2021, doi: 10.1016/j.ibmb.2020.103469.

CHAPTER 20

SYNTHESIS AND REGULATION OF THE STEROIDS

Dr Shilpa Patrick, Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- drshilpapatrick@gmail.com

ABSTRACT:

The endoplasmic reticulum and the mitochondria are where steroid compounds, which are biologically active chemicals, are made. The cholesterol, mevalonate, and non-mevalonate pathways are used to synthesize steroids. The growth, development, metabolism, and maintenance of the cell's homeostasis are all impacted by steroids. In this paper, we covered the metabolic process that produces steroids and the hormones that control them.

KEYWORDS:

Acute Regulatory, Hormone Biosynthesis, MEP Pathway, Star Protein, Steroid Hormones.

INTRODUCTION

A steroid is a physiologically active organic substance with four rings organized in a particular molecular arrangement. The word "steroid" is derived from the steroid cholesterol, which was first identified in gallstones. The two main biological roles of steroids are as signaling molecules and as critical elements of cell membranes that affect membrane flexibility. Numerous steroid species can be found in fungus, mammals, and plants. Lanosterol (opisthokonts) or cycloartenol are the two sterols used to make all steroid compounds in cells (plants). Squalene, a triterpene, is cyclized to produce lanosterol and cycloartenol.

The normal steroid core structure consists of seventeen carbon atoms bound in four "fused" rings: three cyclohexane rings with six members and one cyclopentane ring with five members. (the D ring). The functional groups that are joined to this four-ring center and the redox state of the rings determine how different steroids are. With a third hydroxy group and a structure formed from cholestane, sterols are a type of steroid. Additionally, steroids can undergo more drastic modifications, such as ring structure alterations, such as the removal of one of the rings. Vitamin D3 is one of the secosteroids produced by cutting Ring B. Examples include the reproductive hormones estrogen and testosterone, fat cholesterol, bulking drugs, and the anti-inflammatory medication dexamethasone.Lanosterol (found in animals and fungi; see samples above) or cycloartenol are the building blocks for the hundreds of steroids found in animals, fungi, and plants (in other eukaryotes). The triterpenoid squalene undergoes cyclization to produce lanosterol and cycloartenol. Because they are the building blocks for all other steroids, lanosterol, and cycloartenol are sometimes referred to as phytosterols[1]–[3].

The anabolic process called steroid biosynthesis creates steroids from uncomplicated starting materials. Animals (compared to many other species) follow a distinct biosynthesis route, which makes the pathway a popular target for antibiotics and other anti-infection medications. Human steroid biosynthesis is also a focus of cholesterol-lowering medications like statins. The mevalonate pathway, which utilizes acetyl-CoA as a building component for dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IDP), is used in the production of hormones in humans and other mammals (IPP).

Following their conjugation, DMAPP and IPP create farnesyl diphosphate (FPP), which then combines with itself to create the linear triterpenoid squalene. Squalene synthase, a member of the squalene/phytoene synthase family, catalyzes the production of squalene. Lanosterol is produced by the subsequent epoxidation and cyclization of squalene, and it serves as the precursor for further changes that result in the production of other steroids (steroidogenesis). Cycloartenol is the byproduct of the cyclization of epoxidized squalene (oxidosqualene) in other organisms. A crucial biochemical process found in humans, archaea, and some bacteria is the mevalonate pathway, also referred to as the isoprenoid pathway or the HMG-CoA reductase pathway. Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), two five-carbon building blocks produced by the process, are used to create isoprenoids, a varied family of over 30,000 proteins that include cholesterol, vitamin K, coenzyme Q10, and all steroid hormones. Acetyl-CoA is the starting point of the mevalonate pathway, which results in the synthesis of IPP and DMAPP. It is best recognized for being the focus of the statin medication family, which lowers cholesterol. The mevalonate pathway's HMG-CoA reductase is inhibited by statins. Eubacteria, archaea, and humans all start on the same mevalonate route. Acetyl-CoA serves as the pathway's primary carbon feedstock. Acetoacetyl-CoA is produced in the first stage by condensing two acetyl-CoA molecules. (Figure. 1).

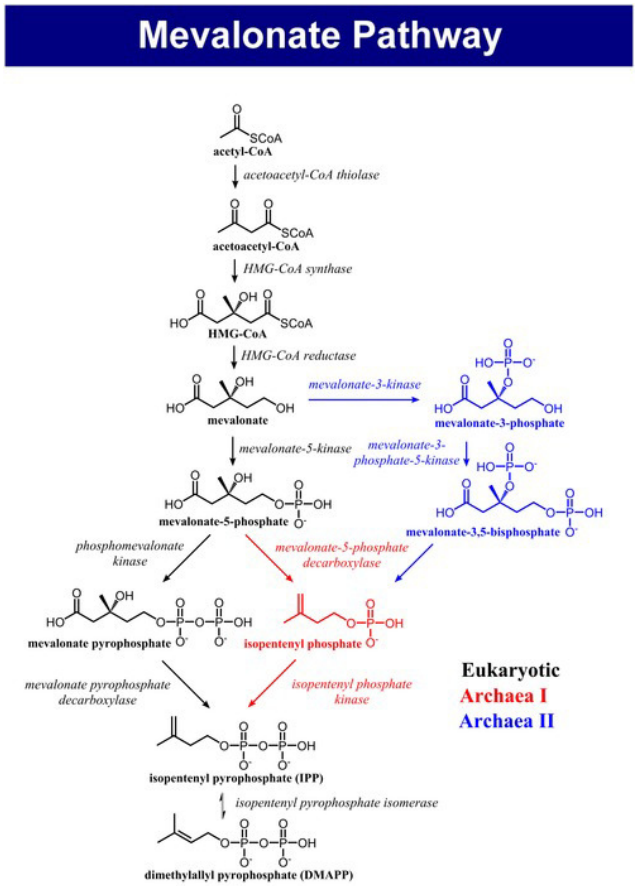

Figure 1: Mevalonate pathway: Diagram showing the steps involved in the mevalonate pathway(Wikipedia).

A second condensation occurs after that to create HMG-CoA.(3-hydroxy-3- methyl-glutaryl-CoA). HMG-CoA is reduced to produce (R)-mevalonate. The higher mevalonate route refers

to the first three enzyme stages. There are three variations of the lower mevalonate pathway, which turns (R)-mevalonate into IPP and DMAPP. Mevalonate is phosphorylated twice in the 5-OH location in eukaryotes, followed by decarboxylation to produce IPP. Mevalonate is phosphorylated once in the 5-OH position in some archaea, like *Haloferaxvolcanii*, decarboxylated to produce isopentenyl phosphate (IP), and then phosphorylated again to produce IPP (Archaeal Mevalonate Pathway I). *Thermoplasmaacidophilum* has a third mevalonate pathway variation that phosphorylates mevalonate at the 3-OH position, followed by phosphorylation at the 5-OH position. Mevalonate-3,5-bisphosphate, the ensuing molecule, is decarboxylated to produce IP and then phosphorylated to produce IPP. (Archaeal Mevalonate Pathway II). Activation of SREBP via DNA transcriptional control can trigger a number of important enzymes (sterol regulatory element-binding protein-1 and -2). When the intracellular sensor senses inadequate cholesterol levels, the HMG-CoA reductase pathway is stimulated, and the LDL receptor is upregulated to increase lipoprotein absorption. Controlling the rate of mRNA translation, reductase breakdown, and phosphorylation are additional methods for regulating this process.

An alternative metabolic pathway for the biosynthesis of the isoprenoid precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate is the non-mevalonate pathway, also known as the mevalonate-independent pathway and the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway (DMAPP). Since MEP is the first committed molecule on the road to IPP, the MEP pathway is the term that is presently most frequently used to refer to this pathway. The majority of microbes, plants, and apicomplexan protozoa, including malaria parasites, can make isoprenoid intermediates by using the MEP route, a different non-mevalonate mechanism (Figure 2). Both the MVA and MEP pathways are still present in plants and many other green organisms. The MEP pathway is used for the biosynthesis of IPP/DMAPP, whereas the MVA pathway is used for the biosynthesis of IPP/DMAPP in the cytosol.MEP and MVA pathway co-expression has been designed for bacteria like *Escherichia coli*. Using 13C-glucose isotopomers, it is possible to analyze how metabolic flows between the MEP and MVA pathways are distributed *Mycobacterium TB* and other significant diseases are among the bacteria that use the MEP route[4], [5].

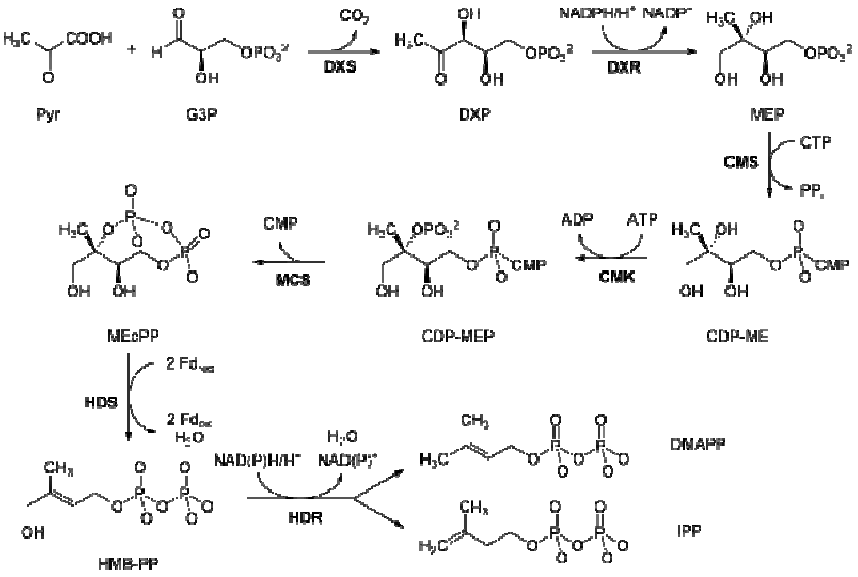

Figure 2: Non-Mevalonate pathway: Diagram showing the steps involved in the Non-mevalonate pathway (Wikipedia).

Several labs have investigated the connection between steroidogenesis and the cytoskeleton to show that steroid hormone synthesis is compromised. These studies have used microtubule depolymerization agents. Before ovulation, the quantity of polymerized tubulin declines, followed by rises in microtubule production as progesterone biosynthesis increases, according to immunostaining of sheep ovaries for microtubules. Colchicine also stops these ovarian cells from producing more progesterone, demonstrating the significance of dynamic changes in microtubule organization in reaction to shifting requirements for steroid hormone synthesis. This microtubule polymerization inhibitor has been demonstrated to promote progesterone biosynthesis in pig luteal cells, in contradiction to colchicine's inhibiting impact on progesterone production in sheep. Taxol inhibits both baseline and human choriogonadotropin-stimulated progesterone and 17-estradiol in pig granulosa cells by stabilizing microtubules. Preventing microtubule polymerization has been shown to lessen ACTH-stimulated cortisol production in human adrenocortical sections. Benis and Mattson also discovered that strengthening microtubules suppresses the steroidogenesis induced by ACTH in cultivated mouse adrenocortical cells. Even though the studies discussed here collectively show that cytoskeletal proteins play a crucial role in regulating hormone biosynthesis, using chemical inhibitors to manipulate the formation of cytoskeletal protein polymers has led to conflicting results regarding the precise function of these proteins. Although many of these discrepancies can probably be ascribed to variations between species and cells, more research is required to clarify these contradictory results. To find out if the cytoskeleton also controls hormone production processes that take place after cholesterol absorption and transfer, a more thorough examination of each of the stages in steroidogenesis is also necessary.

DISCUSSION

Pituitary trophic hormones and other steroidogenic cues rapidly control the production of steroid hormones. The rate-limiting stage in the production of steroid hormones, cholesterol translocation from the exterior to the inner mitochondrial membrane in steroidogenic cells, is necessary for this control. The protein known as steroidogenic acute regulation (StAR) plays a crucial part in this process and is the best option to act as the potential regulator. Congenital lipoid adrenal hyperplasia, a condition in which steroid hormone biosynthesis is seriously impaired, is caused by defects in the StAR gene. StAR is produced in steroidogenic tissues in reaction to substances that trigger the production of steroids. A nearly similar pattern to the human illness exists in the StAR-deficient rodent. StAR is responsive to substances that promote positive and negative steroid production, respectively. StAR facilitates cholesterol transport in the mitochondria, but the exact method by which it does so has not been completely defined. The START domain of a StAR ortholog has, however, had its tertiary structure determined, and the discovery of a cholesterol-binding hydrophobic tunnel within this domain raises the prospect that StAR functions as a cholesterol-shuttling protein.

Steroid hormones like glucocorticoids and mineralocorticoids were once solely believed to be released by the adrenal glands. Corticosteroids can also be locally produced in several other tissues, including main lymphatic organs, the gut, the epidermis, the brain, and potentially the heart, according to new research. Finding steroidogenic enzymes and elevated local corticosteroid levels, even after adrenalectomy, are examples of evidence for local synthesis. High corticosteroid amounts, sometimes significantly higher than systemic levels, are produced by local production in extra-adrenal tissues. It's interesting to note that locally produced regulators of the hypothalamic-pituitary-adrenal (HPA) axis or the renin-angiotensin system can control local cortisol production (RAS). These local regulatory pathways may function as tiny analogs of the pathways that govern the generation of adrenal

corticosteroids in some tissues (such as the epidermis). While locally produced mineralocorticoids control blood volume and pressure, locally produced glucocorticoids control immune cell activation. Because local synthesis suppression has significant impacts even in individuals with healthy adrenals, the metabolic significance of extra-adrenal glucocorticoids and mineralocorticoids has been demonstrated. In conclusion, local production of corticosteroids leads to high geographic precision of steroid action, whereas adrenal release of glucocorticoids and mineralocorticoids into the circulation integrates numerous organ systems. When considered collectively, research on these five significant organ systems casts doubt on accepted theories about the production and use of corticosteroids.

The regulating stage for steroid synthesis is believed to be the cyclic AMP-dependent translation of the steroidogenic acute regulatory (StAR) protein, but it is unclear how exterior cues convert into enhanced StAR gene transcription. We show that the phosphorylation and activation of ERKs are necessary for the cyclic AMP-induced induction of steroid synthesis. ERK activation also increases the phosphorylation of SF-1 and the generation of steroid hormones by increasing StAR gene transcription. Forskolin (FSK)-induced adenylate cyclase stimulation increased ERK activity and transfer from the cytoplasm to nucleus in a time-dependent manner, which was accompanied by an uptick in StAR mRNA levels, StAR protein buildup, and steroidogenesis. Similar to this, ERK suppression decreased the amounts of StAR mRNA, StAR protein, and steroid release that were induced by FSK. The discovery that ERK activity is necessary for the phosphorylation of SF-1, a transcription factor necessary for the control of StAR gene transcription, was ascribed to these effects. Our evidence showing an ERK-dependent rise in the binding of SF-1 from FSK-treated Y1 nuclei to three consensus double-stranded DNA segments from the StARpromoter region helped to corroborate this finding. These findings imply that cAMP-induced ERK2/1 activation is a necessary and controlled step in the induction of steroid production by cyclic AMP-producing triggers.

According to recent research, locally generated hormones known as neurosteroids regulate the activity of neurons. Changes in the steroidogenic acute regulatory protein (StAR) regulate gonadal and adrenal steroidogenesis; however, little is known about the modulation of neurosteroid synthesis. We have now established beyond a shadow of a doubt that StAR mRNA and protein are expressed within glia and neurons in specific areas of the rodent brain, and that glial StAR expression is induced. StAR colocalizes with the cholesterol side-chain cleaving enzyme P450scc in both rat and human brains, which is consistent with a function in de novoneurosteroidogenesis[6]–[8].

These findings pinpoint possible locations of active de novo steroid synthesis in the brain and suggest a function for StAR in the synthesis of neurosteroids. Breast cancer formation and spread appear to be significantly influenced by endogenous ovarian estrogens and progestins. Local collagenolytic enzyme production and activation may be similarly important for local intrusive processes, and local growth factor production and activation likely also add to the development of cancerous cells. Characterization of the growth factor-receptor systems active in healthy and cancerous breast epithelial is the main goal of the current study. The influences on local infiltration are also discussed, including adhesion, mode, and protease release. Finally, information on how hormones and antihormonal drugs control infiltration and growth in hormone-dependent breast cancer is addressed. The findings point to novel drug targets that should be investigated to slow the development and spread of breast cancer.

Androgens and the androgen receptor are crucial for the development of prostate cancer. (AR). Hormonal treatments either prevent the creation of extragonadal androgens or gonadal

testosterone, or they directly interfere with AR. Castration-resistant prostate cancer (CRPC), which results from resistance to surgical castration, is fueled by the revival of the androgen-AR axis. The rate-limiting stage for powerful testosterone production from extragonadal substrates is 3-hydroxysteroid dehydrogenase-1 (3HSD1), which activates CRPC. A man's genetic makeup supports the function of 3HSD1 in causing CRPC. 3HSD1 is necessary for the production of aromatase substrates in postmenopausal women and is crucial in the development of breast cancer. As a result, 3HSD1 is located at a crucial juncture for the production of androgens and estrogens, and this biochemical flow is controlled by processes that are passed from the mother. We demonstrate that, including in patient tissues, tyrosine 344 (Y344) phosphorylation happens and is necessary for 3HSD1 cellular activity and production of 4, 3-keto-substrates of 5'-reductase, and aromatase. The direct interaction between BMX and 3HSD1 is required for the activation of enzymes and testosterone production. CRPC is inhibited in vivo when 3HSD1 Y344 phosphorylation is blocked. These results point to novel hormonal treatment pharmaceutical weaknesses for sex-steroid-dependent tumors in our opinion.

Retinoids (vitamin A and its compounds) are essential for a variety of processes, including neurotoxicity and balance that are affected by steroid hormones. The steroidogenic acute regulating (StAR) protein mediates the rate-limiting stage in steroid production. In the current research, we show that retinoids increased StAR expression and pregnenolone production, and that stimulation of the PKA pathway in rodent hippocampal neural HT22 cells significantly increased these parameters. The significance of a retinoic acid receptor (RAR)/retinoid X receptor (RXR)-liver X receptor (LXR) heterodimeric pattern at 200/185 bp region in retinoid reactivity was discovered through deletion and mutational studies of the 5′-flanking sections of the StAR gene. The RAR/RXR-LXR sequence motif can bind both RAR and RXR, and it was discovered that the LXR pathway influences retinoid-regulated transcription of the StAR gene, illustrating signaling cross-talk in hippocampus neurosteroid biosynthesis. Steroidogenesis diminishes during senescence as a consequence of changes to the central neural and endocrine systems, causing hormone deficits, suggesting that hormonal equilibrium is necessary for good aging.

Alzheimer's disease is characterized by the loss of neural cells and the buildup of amyloid beta (A) and/or modified tau in the brain. (AD). StAR and pregnenolone levels were reduced in HT22 cells that overexpressed either mutant APP (mAPP) or mutant Tau (mTau), which created circumstances mimicking AD and increased toxins. StAR's beneficial function in AD is suggested by the fact that co-expression of StAR with either mAPP or mTau reduced neurotoxicity and concurrently increased neurosteroid production. These findings shed light on the molecular processes by which retinoid signaling increases StAR and steroid levels in hippocampal neuronal cells. StAR then modulates neuro steroidogenesis and restores hormonal balance by rescuing mAPP and/or mTau-induced toxicities, which may have significant implications for preventing AD and age-related diseases and complications[9], [10].

As ovarian follicles grow, granulosa cells (GCs) undergo steroidogenesis, which depends on miRNAs. As a result, in this study, the effects of miR-202-5p on lipid metabolism and steroidogenesis in goose hierarchical follicular GCs (hGCs), as well as its mechanisms of action, were assessed. Previous research has shown that miR-202-5p exhibits a stage-dependent expression pattern in GCs from goose follicles of different sizes, suggesting that this miRNA could be involved in the regulation of the functions of goose GCs. The increase of miR-202-5p significantly suppressed fat accumulation in hGCs, as evidenced by Oil Red O staining and studies of intracellular cholesterol and triglyceride levels. In addition, miR-202-

5p significantly inhibited progesterone release in hGCs. Acyl-CoA synthetase long-chain family member 3 (ACSL3), which triggers long-chain fatty acids for the production of cellular lipids, has been identified as a possible target of miR-202-5p through computational analysis and luciferase reporter experiment. Silencing of ACSL3 prevented hGCs from secreting hormone and accumulating fat. These findings imply that miR-202-5p targets the ACSL3 gene to have negative effects on lipid accumulation and steroidogenesis in gander hGCs.

Fluoride is a frequent natural pollutant that, in high amounts, is detrimental to human health. When individuals drink fluoride-contaminated groundwater, fluoride is taken by the gastric tract and then reaches circulation. By assessing sperm quality, sex hormones, testicular antioxidant state, histology, and StAR gene expression, the current research sought to ascertain whether polyphenol-rich nano *Moringa oleifera* (NMO) could safeguard rat testicles from sodium fluoride (NaF) injury. Twenty-eight mature Wistar rats were split into four groups, each receiving a different treatment: group one received purified water, group two received NMO at a dosage of 250 mg/kg/body weight, group three received NaF at a dosage of 10 mg/kg/body weight, and group four got both NaF and NMO. For eight weeks, the rodents received daily oral administration. The results of the study showed that, in comparison to rats exposed to NaF alone, co-administration of NMO and NaF improved testicular histology, increased the Johnson score, decreased sperm morphological changes, increased sperm motility and viability, restored the balance between oxidant and antioxidant status, improved testosterone and dehydroepiandrosterone levels, and improved testosterone and dehydroepiandrosterone status. These results demonstrate the potential of NMO as a preventative treatment for sperm injury caused by sodium fluoride because NMO delivery had no side effects and improved sexual health.

CONCLUSION

The placenta, the gonads, and the adrenal medulla all produce steroid hormones that are all derived from cholesterol and have a variety of clinically significant functions. The smooth endoplasmic reticulum and mitochondria are where steroid hormones are made. In terms of growth, development, reproductive division, and fertility, steroids are crucial. The impacts of the four groups of steroids androgens, estrogens, progestogens, and glucocorticoids on the brain differ. Negative feedback is mainly responsible for controlling hormone release and synthesis. In systems with negative feedback, a stimulation results in the release of a substance, the consequences of which prevent further release. The hormone levels in the blood are kept within a specific limit in this manner. A wide range of metabolic processes, such as the preservation of glucose metabolism, salt and fluid balance, fertility, and the emergence of secondary sex traits, are all significantly regulated by steroid hormones.

REFERENCES:

[1] H. T. Jacobs, J. George, and E. Kemppainen, “Regulation of growth in Drosophila melanogaster: The roles of mitochondrial metabolism,” *Journal of Biochemistry*. 2020. doi: 10.1093/jb/mvaa002.

[2] P. Duarte-Guterman, L. Navarro-Martín, and V. L. Trudeau, “Mechanisms of crosstalk between endocrine systems: Regulation of sex steroid hormone synthesis and action by thyroid hormones,” *General and Comparative Endocrinology*. 2014. doi: 10.1016/j.ygcen.2014.03.015.

[3] C. Paz *et al.*, “Role of Protein Phosphorylation and Tyrosine Phosphatases in the Adrenal Regulation of Steroid Synthesis and Mitochondrial Function,” *Frontiers in Endocrinology*. 2016. doi: 10.3389/fendo.2016.00060.

[4] S. Mao, Z. Song, M. Wu, X. Wang, F. Lu, and H. M. Qin, "Expression, Purification, Refolding, and Characterization of a Neverland Protein From Caenorhabditis elegans," *Front. Bioeng. Biotechnol.*, 2020, doi: 10.3389/fbioe.2020.593041.

[5] P. F. Hall, "The role of endozepine in the regulation of steroid synthesis," *Mol. Neurobiol.*, 1995, doi: 10.1007/BF02740835.

[6] P. T. T. Nguyen, A. J. Conley, T. K. Soboleva, and R. S. F. Lee, "Multilevel regulation of steroid synthesis and metabolism in the bovine placenta," *Molecular Reproduction and Development*. 2012. doi: 10.1002/mrd.22021.

[7] Y. Kobayashi, Y. Yamamoto, S. Kageyama, H. Hirayama, K. Kimura, and K. Okuda, "Regulation of bovine oviductal NO synthesis by follicular steroids and prostaglandins," *Reproduction*, 2016, doi: 10.1530/REP-15-0254.

[8] K. L. Schmidt, D. S. Pradhan, A. H. Shah, T. D. Charlier, E. H. Chin, and K. K. Soma, "Neurosteroids, immunosteroids, and the Balkanization of endocrinology," *General and Comparative Endocrinology*. 2008. doi: 10.1016/j.ygcen.2008.03.025.

[9] D. M. Stocco, X. J. Wang, Y. Jo, and P. R. Manna, "Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: More complicated than we thought," *Molecular Endocrinology*. 2005. doi: 10.1210/me.2004-0532.

[10] C. Zhang *et al.*, "Interleukins 4 and 13 drive lipid abnormalities in skin cells through regulation of sex steroid hormone synthesis," *Proc. Natl. Acad. Sci. U. S. A.*, 2021, doi: 10.1073/pnas.2100749118.

CHAPTER 21

BIOSYNTHESIS OF STARCH, SUCROSE, AND CELLULOSE

Mayur Porwal, Associate Professor
Department of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- mayur.porwal1@gmail.com

ABSTRACT:

Plants synthesize the carbohydrates starch and cellulose from the glucose molecule. While sucrose is produced by the photosynthesis pathway. Enzymes involved in the synthesis of this starch and sucrose are present in the chloroplast. This paper is a brief discussion of the biosynthesis pathway of starch, sucrose, and cellulose.

KEYWORDS:

Cellulose Synthesis, Fructose Bisphosphate, Glucose Phosphate, Plasma Membrane, Sucrose Phosphate.

INTRODUCTION

A complex carbohydrate known as starch or amylum is made up of multiple glucose molecules connected by α-(1→4)-D glycosidic links. Most verdant plants make this carbohydrate as a means of storing energy. It is the most prevalent carbohydrate in human meals worldwide and is present in significant quantities in popular foods like wheat, potatoes, maize (corn), rice, and tapioca (manioc). A whitish, flavorless, and colorless substance known as pure starch is impervious in both frigid water and alcohol. It is made up of two different kinds of molecules: branched amylopectin and straight helix amylose. Depending on the plant, starch typically has a weight ratio of 75 to 80% amylopectin and 20 to 25% amylose. Animals' energy store, glycogen, is a more complexly branched form of amylopectin. Starch is frequently transformed into carbohydrates in industry, such as through malting.

These carbohydrates can be turned into ethanol through fermentation, which is used to make alcohol, whiskey, and energy. In addition, a lot of prepared meals use carbohydrates made from starches that have been treated. Most carbohydrates can be made into pastes by combining them with tepid water. One such paste is a wheat paste, which can be used as a hardening, strengthening, or binding substance. The main non-food commercial application of starch is as a glue in the production of paper. Certain textile products can be stiffened by applying garment starch, a comparable substance, before pressing. Using the enzyme glucose-1-phosphate adenylyltransferase, plants first transform glucose 1-phosphate to ADP-glucose to create starch. Energy in the shape of ATP is needed for this process. ADP is then released and amylose is produced when the enzyme starch synthase joins the ADP-glucose to an expanding chain of glucose residues via a 1, 4-alpha glycosidic link. Similar to how UDP-glucose is added to the non-reducing end of glycogen during glycogen formation, ADP-glucose is almost definitely added to the non-reducing end of the amylose chain[1]–[3].

The branched amylopectin is produced by the starch branching enzyme, which adds 1,6-alpha glycosidic links between the amylose strands. Some of these stems are cut off by the enzyme isoamylase, which breaks down starch. Due to the existence of multiple versions of these enzymes, the manufacturing process is extremely complicated (Figure. 1). Glycogen and amylopectin both have comparable structures, but the former has about one branch point for every ten 1,4-alpha links while the latter has one for every thirty. While humans and plants

produce glycogen from UDP-glucose, microbes typically produce glycogen from ADP-glucose. Amylopectin is produced from ADP-glucose (analogous to starch). In addition to being produced by plants, starch can also be produced from non-food starch using a combination of enzymes.Beta-1,4-glycosidic bond-linked cellulose is partly converted to cellobiose in this cell-free biosystem. The other enzyme, potato alpha-glucan phosphorylase, can add a glucose molecule from glucose 1-phosphorylase to the non-reducing extremities of starch. Cellobiose phosphorylase cleaves to glucose and glucose 1-phosphate. Internal phosphorus recycling occurs in it. A yeast can take up glucose, the other substance. This cell-free bioprocessing can be carried out in a liquid solution, requires no expensive chemical or energy input, and does not result in sucrose depletion.

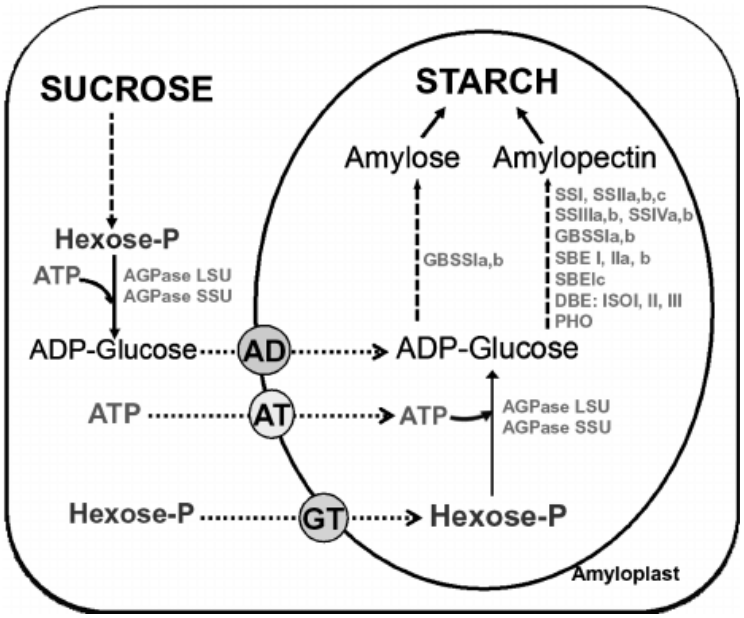

Figure 1: Synthesis of the starch: diagram showing the starch synthesis in the plant (Research gate).

Plant leaves create starch during the day, which is then kept as crystals and used as a source of energy at night. To make the intractable, highly twisted starch molecules available to the enzymes that break them down, phosphorylation is required. The glucose molecule's C-6 location, which is near the chain's 1,6-alpha branching links, is phosphorylated by the enzyme glucan, water dikinase (GWD). The glucose molecule is phosphorylated at the C-3 location by phosphoglucose, water dikinase (PWD), a second enzyme. A starch surplus (sex) trait is caused by the loss of these enzymes, such as the loss of the GWD, and because starch cannot be activated, it builds up in the plastids. The first disintegrating enzyme, beta-amylase (BAM), can target the glucose molecule at its non-reducing end following phosphorylation. The primary byproduct of starch breakdown is maltose. BAM cannot produce maltose if the glucose chain has three molecules or fewer.

Disproportionating enzyme-1 (DPE1), a second enzyme, joins two maltotriose molecules. A glucose molecule is discharged from this sequence. The last maltose molecule in the chain can now be released by BAM. The loop keeps going until all of the starch is broken down. BAM can no longer produce maltose if it gets too near to the modified branching point of the glucose chain. The enzyme isoamylase (ISA) is needed to break down the modified strand. The main byproducts of starch breakdown are maltose and, to a lesser extent, glucose. Maltose is transported from the plastid to the cytoplasm by the maltose transporter, whose mutation (MEX1-mutant) causes a buildup of maltose in the plastid. The plastidic glucose

translocator (pGlcT) is used to transport glucose. These two sugars serve as building blocks for the production of sucrose. The mitochondria's oxidative pentose phosphate pathway can then use sucrose to make ATP at night. A connection between the anomeric carbons in sucrose, a disaccharide of glucose and fructose, creates the nonreducing sugar O-D-glucopyranosyl-(12)-D-fructofuranoside. Similar to how ketone bodies are transport forms of fatty acids, sucrose can be thought of as a transport form of carbon. As mentioned above, the anomeric C-1 link of glucose and the anomeric C-2 link of fructose form the connection between the carbohydrates.

As a result, it is not broken down by normal enzymes that break down carbohydrates, such as amylases. Additionally, unlike other sugars with unbound cyclic hemiacetals that can open and produce volatile aldehydes, it doesn't combine with proteins (Figure. 2). The circular monosaccharide glucose, for instance, can make covalent links to amine groups in proteins like hemoglobin, which results in the glycosylated version of hemoglobin. A Schiff base is formed, and then the reaction moves on to a rearrangement. A diabetes measure is HbA1c. These characteristics of sucrose may help to explain why plants use it as a primary source of glucose synthesis.

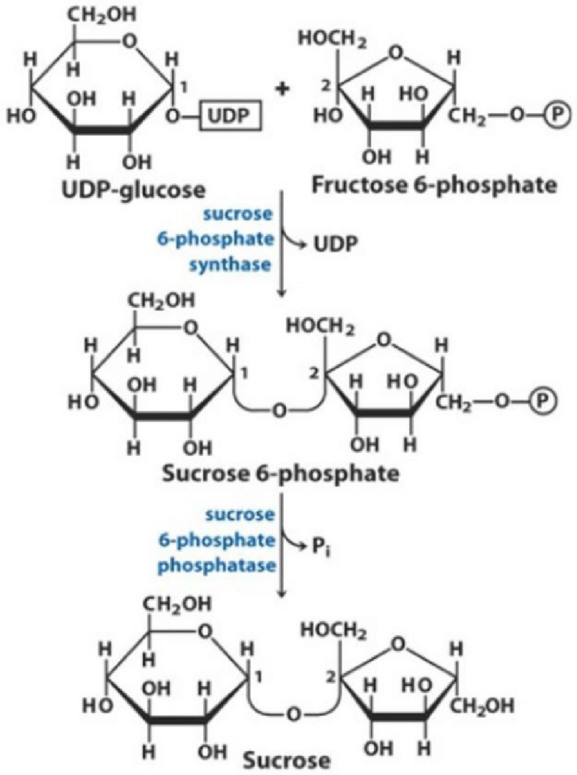

Figure 2: Synthesis of the sucrose: diagram showing the sucrose synthesis in the plant (Research gate).

By isolating and separating the compartments from one another during cell separation, the location of sucrose production has been investigated. According to enzyme studies, the cytosolic synthesis of sucrose from triose phosphates follows a comparable route to that of starch, i.e., via fructose-1,6-bisphosphate and glucose-1-phosphate. A particular UDP glucose pyrophosphorylase, similar to the ADP glucose pyrophosphorylase of chloroplasts, is used in the production of sucrose to transform glucose-1-phosphate to UDP glucose. At this point, the production of sugar is finished by two related processes. First, fructose-6 phosphate and

UDP-glucose react with the help of the enzyme sucrose-6-phosphate synthase to produce sucrose-6 phosphate and UDP. Second, the phosphate from sucrose-6-phosphate is broken down by the enzyme sucrose-6-phosphate phosphatase (phosphohydrolase), producing sucrose. The second process pushes the first in the path of sucrose production and is irrevocable. The pyrophosphate produced in the process mediated by UDP-glucose pyrophosphorylase is digested, just like in the production of starch, but not right away like in chloroplasts. The pyrophosphate can be used by other enzymes in transphosphorylation processes since there is no exogenous pyrophosphatase present. One illustration is the enzyme fructose-6-phosphate phosphotransferase, which catalyzes a similar reaction to phosphofructokinase but uses pyrophosphate instead of ATP as the phosphoryl source. The change of triose phosphates to glucose-1-phosphate in the routes leading to the production of starch and sucrose share several stages, according to a study of the processes.

However, these routes use isozymes, which are specific to the plastid or cytoplasm and are distinct versions of the same enzyme that catalyze the same process. The characteristics of the isozymes vary noticeably. For instance, fructose-2,6-bisphosphate and AMP do not affect how the chloroplastic fructose-1,6-bisphosphatase is controlled by the thioredoxin system. In contrast, the cytoplasmic version of the enzyme is untouched by thioredoxin and is controlled by fructose-2,6-bisphosphate (see the next section). It is also responsive to AMP in the presence of fructose-2,6-bisphosphate. In addition to the intracellular fructose-1,6 bisphosphatase, the allosteric enzyme sucrose phosphate synthase, which is triggered by glucose-6-phosphate and blocked by orthophosphate, controls the production of sucrose. A protein kinase-mediated phosphorylation of a particular serine site renders the enzyme inactive in the dark, and a protein phosphatase-mediated dephosphorylation renders the enzyme activity in the presence of light. The kinase is inhibited by glucose-6-phosphate, and the phosphatase is inhibited by pi.

The structural and functional characteristics of this enzyme are now better-understood thanks to the recent isolation and cloning of sucrose-6-phosphate phosphatase from rice leaves. According to these investigations, sucrose-6-phosphate synthase and sucrose-6-phosphatase are present as a supramolecular complex that exhibits greater catalytic activity than the individual component enzymes. The two enzymes implicated in the final two stages of sucrose production engage noncovalently, suggesting a new regulation mechanism for plant glucose metabolism. Major determinants of whether photosynthetically fixed carbon is partitioned as starch in the plastid or as sucrose in the cytoplasm are the respective amounts of orthophosphate and triose phosphate. The phosphate/ triose phosphate translocator, also known as the phosphate translocator, a stringent stoichiometric antiporter, connects the two sections. Between the chloroplast and the cytoplasm, the phosphate translocator catalyzes the passage of orthophosphate and triose phosphate in oppositional directions. The export of triose phosphate from the chloroplast through the translocator is constrained by a low level of orthophosphate in the cytoplasm, which encourages the production of starch[4], [5].

On the other hand, an excess of orthophosphate in the cytosol prevents starch from being made in the chloroplast and encourages the release of triose phosphate, which is then transformed to sucrose, in the cytoplasm. Numerous regulating enzymes in the biosynthesis pathways for sugar and starch are controlled by the orthophosphate and triose phosphate metabolites. The primary enzyme that controls the production of starch from glucose-1-phosphate is the plastid enzyme ADP-glucose pyrophosphorylase. Orthophosphate inhibits this enzyme while 3-phosphoglycerate stimulates it. Lit chloroplasts that are constantly making starch usually have a high ratio of 3-phosphoglycerate to orthophosphate. In the gloom, reciprocal circumstances rule. A crucial regulatory component that enables greater

sucrose synthesis in the light and reduced production in the nighttime is fructose-2,6-bisphosphate. It is present in the cytoplasm in extremely low quantities and regulates the interconversion of fructose-1,6-bisphosphate and fructose-6-phosphate in the cytosol: Because fructose-2,6-bisphosphate is a potent regulator of cytoplasmic fructose-1,6-bisphosphatase and a stimulator of the pyrophosphate dependent (PPi-linked) phosphofructokinase, it is related to reduced rates of sucrose production. (reaction 4b). What, then, regulates the level of fructose-2,6-bisphosphate in the cytosol? A unique fructose-6-phosphate 2- kinase converts fructose-6-phosphate into fructose-2,6-bisphosphate. According to recent research, both plant functions are found on a single protein chain, just like in animal cells. Orthophosphate and triose phosphate regulate the kinase and phosphatase functions. Triose phosphate suppresses the 2-kinase while orthophosphate activates fructose-6-phosphate 2-kinase and inhibits fructose-2,6-bisphosphatase. Due to the creation of fructose-2,6-bisphosphate being encouraged by a low cytoplasmic ratio of triose phosphate to orthophosphate, the breakdown of cytosolic fructose-1,6-bisphosphate is inhibited, which delays the rate of sucrose synthesis.

The reverse is true when there is a large triose phosphate to orthophosphate ratio in the cytoplasm. Through photosynthesis-related processes, light affects the quantity of these activators and inhibitors, which in turn governs the amount of fructose-2,6-bisphosphate in the cytoplasm. In plants, fructose-2,6-bisphosphate has little to no impact on the glycolytic enzyme phosphofructokinase, which is also involved in the reduction of fructose-6-phosphate to fructose-1,6-bisphosphate. The relative amounts of ATP, ADP, and AMP appear to control the action of phosphofructokinase in plants. Recent gene excision studies with modified tobacco plants provided new evidence of the extraordinary flexibility of plants. This experiment demonstrates that the altered plants can continue to develop in the absence of a working fructose-6-phosphate kinase enzyme. In this instance, phosphofructokinase appears to be the only enzyme capable of catalyzing the change of fructose-6-phosphate to fructose-1,6-bisphosphate.

In addition to being tasteless and colorless, cellulose is also chiral, compostable, hydrophilic with a contact angle of 20 to 30 degrees, and impermeable in water and the majority of organic liquids. It can be chemically converted into glucose molecules by being heated and treated with strong mineral acids. D-glucose molecules that consolidate through (1-4)-glycosidic links are the source of cellulose. In opposition to this linking pattern, starch, and glycogen contain (14)-glycosidic links. A polysaccharide with a linear strand is cellulose. Because of the equatorial shape of the glucose molecules, unlike starch, the molecule assumes an elongated and relatively rigid rod-like structure (Figure. 3). To keep the chains tightly together and create microfibrils with high compressive strength, the numerous hydroxyl groups on the glucose from one chain make hydrogen bonds with oxygen atoms on the same or a neighboring chain.

In cell walls where cellulose microfibrils are woven into a polysaccharide framework, this gives compressive strength. The organization of cellulose fibers that are evenly dispersed throughout the lignin matrix is another factor contributing to the high compressive strength of plant stalks and tree timber. The mechanical function of cellulose fibers in the wood matrix, which is responsible for its high structural resilience, is comparable to that of the reinforcing bars in concrete, with lignin serving as the "glue" that holds the cellulose fibers together. The development and enlargement of plant cells are linked with the mechanical characteristics of the cellulose in the main cell wall. Rosette terminal complexes at the plasma membrane of plants make cellulose. (RTCs). The hexameric protein structures are known as RTCs, which have a width of about 25 nm, and house the cellulose synthase enzymes responsible for

producing the individual cellulose strands. Each RTC "spins" a microfibril into the cell wall as it drifts in the plasma membrane of the cell.

At least three distinct cellulose synthases are present in RTCs and are expressed by the CesA (cellulose synthase) gene in an undetermined stoichiometry. The production of the main and secondary cell walls is regulated by different groups of CesA genes. The plant CesA superfamily is known to contain about seven subfamilies, some of which contain the more enigmatic, provisionally called Csl (cellulose synthase-like) enzymes. These cellulose processes create the (14)-linked cellulose using UDP-glucose. Although the gene is frequently referred to as CelA for "cellulose" instead of BcsA for "bacterial cellulose synthase," bacterial cellulose is made using the same family of proteins. In actuality, the endosymbiosis process that gave rise to the chloroplast is where plants obtained CesA. The glucosyltransferase family 2 includes all known cellulose synthases (GT2). Chain start and extension are required steps in the production of cellulose, but they are carried out independently. Utilizing a steroid precursor, sitosterol-beta-glucoside, and UDP-glucose, cellulose polymerization is started by the enzyme cellulose synthase (CesA). The lengthening of the cellulose strand is then accomplished using UDP-D-glucose intermediates. The precursor may be separated from the final chain by a cellulase.

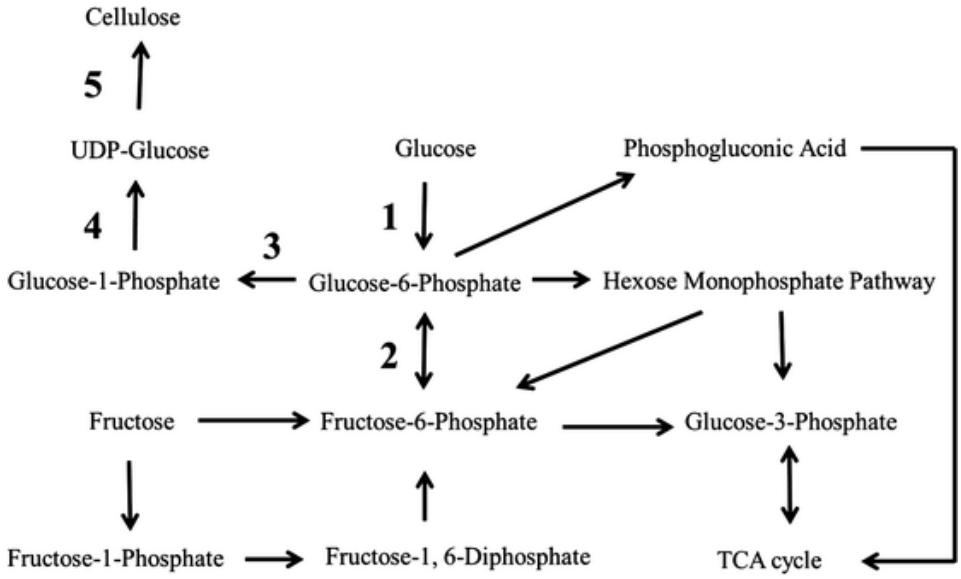

Figure 3: Synthesis of the cellulose: diagram showing the cellulose synthesis in the plant (Springer link).

Animals classified as tunicates can also synthesize cellulose, especially in the tests of ascidians, where the substance was formerly known as "tunicine." Cellulolysis is a chemical mechanism that converts cellulose into cellodextrins, which are smaller carbohydrates, or entirely into glucose molecules. Comparatively challenging to the decomposition of other polysaccharides, cellulolysis is made more challenging by the powerful interactions between cellulose molecules. However, in the right fluid, such as an ionic liquid, this process can be greatly accelerated. The capacity of most animals to process food fiber like cellulose is generally restricted. The rumen ecology of some ruminants, like cows and sheep, contains specific commensal anaerobic bacteria (like Cellulomonas and Ruminococcus spp.), which make cellulases, which hydrolyze cellulose. The microbes then use the degradation byproducts for growth. The ruminant's eating system (stomach and small intestine) eventually breaks down the bacterium bulk. Through fermentation in their rumen, horses use cellulose in their food. Some termites have specific flagellate protozoa in their hindguts that make these

enzymes, while others have bacteria or may manufacture cellulase. Glycoside hydrolases, such as endo-acting cellulases and exo-acting glucosidases, are the enzymes responsible for cleaving the glycosidic bond in cellulose. These enzymes are typically released as a component of multienzyme complexes that may also contain dockerins and modules that bond carbohydrates. Cellulose experiences thermolysis, also known as "pyrolysis," at temps above 350 °C, breaking down into solid char, fumes, ppapers, and gases like carbon dioxide. At 500 °C, the highest output of fumes that coalesce into bio-oil is produced. It has been demonstrated that semi-crystalline cellulose polymers react at pyrolysis temperatures (350–600 °C) in a matter of seconds, with the liquid (referred to as intermediate liquid cellulose or molten cellulose) existing for only a brief period. The liquid is made up of two to seven-molecule-long small cellulose strands as a result of glycosidic bond breakage. Aerosols are created by the vapor bursting of intermediary liquid cellulose and contain short-chain hydro-oligomers that are obtained from the dissolution. Levoglucosan, furans, pyrans, light oxygenates, and fumes are among the flammable substances that are produced as liquid cellulose continues to decompose through primary reactions. Levoglucosan undergoes "secondary reactions" to flammable products like pyrans and light oxygenates like glycolaldehyde in samples of dense cellulose.

DISCUSSION

In plant cells, sucrose synthase (SuSy; EC 2.4.1.13; sucrose + UDP reversible UDP-glucose + fructose) degrades sucrose and supplies carbon for metabolism as well as the production of cell wall carbohydrates and starch. Here, we find that the plasma membrane is closely linked with at least half of the total SuSy of growing cotton strands (Gossypium hirsutum). As a result, this particular SuSy could act as a straight conduit for carbon from sucrose to the plasma membrane's cellulose and/or callose synthases. We demonstrate that carbon from sugar can be changed at high rates to both cellulose and callose using disconnected and permeable cotton strands. The presence of EGTA, calcium, or cellobiose favors the synthesis of cellulose or callose, respectively. These results go against the conventional wisdom that callose is primarily produced in vitro when UDP-glucose is used as a source. SuSy can locate at the fiber surface in ways that are compatible with the accumulation of cellulose or callose, according to immunolocalization studies. These findings, therefore, support the hypothesis that SuSy functions as a carbon transfer enzyme between sucrose and glucan by forming a complex with the beta-glucan synthases[6]–[8].

A granular type of sucrose (Suc) synthase was suggested to promote secondary wall cellulose production by reducing Suc to fructose and UDP-glucose based on research with cotton fibers. According to the hypothesis, UDP-glucose was then transported to cellulose synthase in the plasma membrane, and it follows that the abundance of Suc in cellulose sink cells would have an impact on the rate of cellulose synthesis. Therefore, cellulose synthesis might be promoted if cellulose sink cells could make Suc and/or return the fructose produced by Suc synthase back to Suc. By examining the Suc phosphate synthase (SPS) activity of three heterotrophic systems with cellulose-rich secondary walls, the ability of cellulose sink cells to produce Suc was evaluated. Although SPS is a key modulator of the rate of Suc synthesis in leaves and some heterotrophic tissues that store Suc, cellulose synthesis has not previously been linked to SPS activity. Etiolated kidney bean (*Phaseolus vulgaris*) hypocotyls and cultivated mesophyll cells of *Zinnia elegans* L. var. Envy both contained developing tracheary elements. Fibers from cotton (*Gossypium hirsutum L. cv. Acala SJ-1*) that were synthesized during the primary and secondary walls were also examined. In all three systems, SPS activity increased when cellulose accumulation in secondary walls was at its highest. The Z. elegans culture method was altered to demonstrate that SPS activity was independent

of the supply of starch for breakdown and that there was a close correlation between the onset of tracheary element division and increasing SPS activity. It will be addressed how important these results are for steering metabolic flow toward cellulose.

According to earlier research, increased availability of sucrose, a key byproduct of photosynthesis in source leaves and the carbon source for the production of secondary wall cellulose in fiber sinks, may enhance the quality of fiber under abiotic stress circumstances. Due to its function in controlling the production of sucrose in photosynthesis and heterotrophic tissues, spinach sucrose-phosphate synthase (SPS) was overexpressed in a family of transgenic cotton plants (*Gossypium hirsutum cv. Coker 312 elite*) to verify this theory. The translation of spinach SPS, synthesis of spinach SPS protein, and improvement of extractable V max SPS activity in leaf and fiber were all examined in a family of 12 separate transgenic lines. In comparison to wild-type and transgenic null controls, lines with the greatest V max SPS activity were further examined for carbon segregation and fiber quality. The ratio of sugar to starch in the leaves of transgenic SPS over-expressing lines was greater, and 14C was partitioned to sucrose rather than starch. The transgenic line with the greatest SPS activity in leaves and fiber had higher fiber micronaire and development ratios correlated with increased thickness of the cellulosic secondary wall in two growth chamber tests with mild nights, atmospheric CO2 content, and little light below the canopy.

It was assessed how different carbon and nitrogen sources affected Acetobacter xylinum's ability to produce cellulose membranes. Sucrose, glucose, and mannitol were discovered to be the carbon sources best suited for optimal cellulose synthesis. For the production of cellulose, the strain was able to use a variety of nitrogen and protein sources, including peptone, soybean powder, glycine, casein hydrolysate, and glutamic acid. Pellicle proteins (PP) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which identified electrophoretic bands with molecular masses between 116 and 20 kDa. The strain can also help remove different nitrogenous and carbon compounds found in sewage fluid.

The significance and consequences of controlling carbon allocation to cellulose synthesis, the features of cells that function as primary sources for cellulose accumulation, and enzymes involved in the conversion of given carbon to cellulose are all covered in this paper. A main model system is cotton filaments, which secrete almost pure cellulose into their secondary cell walls. Concerning sucrose synthase, we go over its gene family, its modification in transgenic plants, and potential processes that control its affiliation with polysaccharide synthesis sites. It is thought that this enzyme may route UDP-Glc to cellulose synthase during the formation of secondary walls. We go over the gene family structure of cellulose synthase and how protein variation might affect how carbon is distributed during cellulose production. UDP-Glcpyrophosphorylase and sucrose phosphate synthase are two additional enzymes that are highlighted. New information is presented on the phosphorylation of sucrose synthase in cotton fibers, the potential regulation of sucrose synthase localization by Ca2+, the immunolocalization of sucrose synthase in cotton fibers using electron microscopy, and the phylogenetic relationships between the cellulose synthase proteins, including three new ones discovered in *Zinnia elegans'* differentiating tracheary elements. We create a model for cellulose synthesis-related metabolism that includes the shifting intracellular location of sucrose synthase as a molecular switch between growth and differentiation processes involving cellulose synthesis and survival metabolism[9], [10].

Incoming sucrose (Suc) is partitioned into three main sources in the developing cotton (*Gossypium hirsutum L.*) seed, which are the filaments, seed coat, and cotyledons, which produce cellulose, starch, and storing proteins or lipids, respectively. In this research, we

looked into how Suc synthase (SuSy) helps Suc get mobilized into these sites. In opposition to what was discovered for other plants, analyses of SuSy gene expression at different levels came to the unexpected result that SuSy does not appear to be involved in starch production in the cotton seed. SuSy, however, appears to play a significant role in allocating carbon to fiber cellulose production as evidenced by our showing of functional symplastic links between the phloem-unloading region and the fiber cells as well as the SuSy expression pattern in fibers. Additionally, transfer cells of the seed sheath confronting the cotyledons exhibit significant levels of SuSy mRNA. Suc outflow to the seed apoplast and the production of energy for it could both be facilitated by such high amounts of SuSy. SuSy appears to play a part in the production of lipids and proteins in cotyledons. In conclusion, the growing cotton seed offers a superb illustration of the variety of functions SuSy performs in carbon metabolism.

The byproduct of photosynthesis, sucrose is the main sugar carried by most plants' phloem. Sucrose synthase (SuSy) is a glycosyl transferase enzyme that is mainly found in sink tissues and is essential for sugar metabolism. The reversible breakdown of sucrose into fructose and either uridine diphosphate glucose (UDP-G) or adenosine diphosphate glucose is catalyzed by the enzyme SuSy. (ADP-G). SuSy can cleave sugar into molecules that can be used in a variety of biochemical processes, including those that produce energy, main metabolites, and complicated carbs. Typically homotetramers, SuSy proteins have an average monomeric molecular weight of about 90 kD (about 800 amino acids long). Although some SuSy proteins are found in the cell wall, vacuoles, and mitochondria, plant SuSy isozymes are primarily found in the cytoplasm or close to the plasma membrane. Typically, plant SUS gene groups are tiny, with four to seven genes, and have unique exon-intron architectures. There are three distinct clades of plant SUS genes, which are found in both monocots and dicots.

According to a thorough phylogenetic analysis, there may have been a first SUS duplication event before gymnosperms and angiosperms diverged, and a second duplication event most likely took place in a shared angiosperm ancestor, resulting in the existence of all three clades in both monocots and dicots. There is evidence that plants with lower SuSy activity develop slower, synthesize less starch, cellulose, or callose, are less tolerant to anaerobic stress, and have changed shoot apical meristem function and leaf shape. SUS is a high-potential candidate gene for the enhancement of farming characteristics in food plants due to increased development, increased xylem area and xylem cell-wall breadth, and increased cellulose and glucose amounts in plants overexpressing SUS. The information that is currently known about plant SuSy is compiled in this overview, along with recently identified potential embryonic functions for SuSy in meristem activity that involve sucrose and endocrine communication.

It is thought that the plasma membrane-associated rosette structure, which can be seen by electron imaging, is responsible for cellulose production in plants. It has not been proven that the rosette is the location of cellulose synthesis's enzymatic activity, despite ten years of conjecture. We were able to effectively separate detergent-insoluble rosettes from the plasma membrane of bean epicotyls to investigate the connection between this structure and the production of cellulose. The pure rosettes lacked in vitro cellulose manufacturing ability, though. On the other hand, detergent-soluble grainy ppapers of about 9.5–10 μ in diameter were also separated, showed UDP-glucose binding activity, and could synthesize 1,4-glucan (cellulose) in a test tube. The ppaper, known as the active unit of cellulose synthesis, was loaded with a 78 kDa protein that was confirmed by mass spectrometry and immunoblotting to be similar to sucrose synthase. When added with magnesium, the catalytic units were still

able to attach to the rosettes and maintain their cellulose synthesis activity in the presence of UDP-glucose or sucrose + UDP. Under an electron microscope, immunogold staining with anti-sucrose synthase antibodies verified that the catalytic unit had been integrated into the rosette structure. Our findings imply that the catalytic unit of cellulose synthesis is anchored by the plasma membrane-associated rosette to create the functional cellulose synthesis machinery.

CONCLUSION

The triose phosphate produced by the Calvin cycle is used to create both glucose and sugar. The picture below illustrates the starch and sugar production routes. The chloroplast synthesizes starch. Research on the location of enzymes as well as the predominant starch residues. Three essential enzymes ADP-glucose pyrophosphorylase, starch synthases, and starch branching enzymes contribute to the ADP-glucose route for the production of starch (glucans and amylopectins). Then, a debranching enzyme selectively debranches the haphazardly branched glucan molecules to create amylopectins. The enzyme cellulose synthase, a membrane protein, catalyzes the direct synthesis of glucose from the precursor UDP-glucose into a cellulose product, which is how cellulose is produced. Numerous microbes, *Dictyosteliumdiscoideum*, and higher plants have had cellulose synthase genes discovered in their genomes. The Synthesis and Control of Cellulose. The most prevalent biopolymer produced on land, cellulose, is composed of straight strands of ß (1-4) connected D-glucose. Cellulose plays a crucial role in the cell wall's structural integrity and is crucial for both commercial applications and the growth and development of plants. Plants and cyanobacteria produce it to store energy in a solid, osmotically inactive state. Starch has important uses for people because it is a continuous base material for industry and the primary source of carbohydrates in a balanced diet.

REFERENCES:

[1] E. Baroja-Fernández *et al.*, “Sucrose synthase activity in the sus1/sus2/sus3/sus4 Arabidopsis mutant is sufficient to support normal cellulose and starch production,” *Proc. Natl. Acad. Sci. U. S. A.*, 2012, doi: 10.1073/pnas.1117099109.

[2] C. M. Figueroa, J. E. Lunn, and A. A. Iglesias, “Nucleotide-sugar metabolism in plants: The legacy of Luis F. Leloir,” *Journal of Experimental Botany*. 2021. doi: 10.1093/jxb/erab109.

[3] P. S. Chourey, E. W. Taliercio, S. J. Carlson, and Y. L. Ruan, “Genetic evidence that the two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis,” *Mol. Gen. Genet.*, 1998, doi: 10.1007/s004380050792.

[4] Y. Zheng, S. Anderson, Y. Zhang, and R. M. Garavito, “The structure of sucrose synthase-1 from arabidopsis thaliana and its functional implications,” *J. Biol. Chem.*, 2011, doi: 10.1074/jbc.M111.275974.

[5] C. Fan *et al.*, “Sucrose synthase enhances hull size and grain weight by regulating cell division and starch accumulation in transgenic rice,” *Int. J. Mol. Sci.*, 2019, doi: 10.3390/ijms20204971.

[6] C. Li *et al.*, “Transcriptome profiling helps to elucidate the mechanisms of ripening and epidermal senescence in passion fruit (Passiflora edulia Sims),” *PLoS One*, 2020, doi: 10.1371/journal.pone.0236535.

[7] E. Baroja-Fernández, F. J. Muñoz, A. Bahaji, G. Almagro, and J. Pozueta-Romero, "Reply to Smith et al.: No evidence to challenge the current paradigm on starch and cellulose biosynthesis involving sucrose synthase activity," *Proceedings of the National Academy of Sciences of the United States of America*. 2012. doi: 10.1073/pnas.1201538109.

[8] T. H. Yeats and C. R. Somerville, "A dual mechanism of cellulose deficiency in shv3svl1," *Plant Signal. Behav.*, 2016, doi: 10.1080/15592324.2016.1218108.

[9] D. M. Braun, L. Wang, and Y. L. Ruan, "Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security," *Journal of Experimental Botany*. 2014. doi: 10.1093/jxb/ert416.

[10] J. Kreuzwieser *et al.*, "Differential response of gray poplar leaves and roots underpins stress adaptation during hypoxia," *Plant Physiol.*, 2009, doi: 10.1104/pp.108.125989.

CHAPTER 22

CARBON ASSIMILATION, CALVIN CYCLE; A DARK REACTION

Dr Preeti Singh, Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- drpreetisinghtmu@gmail.com

ABSTRACT:

Plants and algae used the Calvin cycle for the assimilation of the carbon molecule, and the generation of energy. The Calvin cycle is the dark reaction complete in three steps; Carbon fixation, reduction, and regeneration. The main function of the Calvin cycle is to generate the glucose molecule by using the chemical energy NADPH and the ATP molecules. In this paper, we discussed a brief overview of the Calvin cycle, its steps, and the light-dependent regulation of the Calvin cycle.

KEYWORDS:

Carbon Fixation, Enzyme Cycle, Glyceraldehyde Phosphate, Penotse Phosphate, Riulose Bisphosphate.

INTRODUCTION

A set of molecular processes known as the Calvin cycle, also known as the nighttime reactions, biosynthetic phase, light-independent reactions, or photosynthetic carbon reduction (PCR) cycle of photosynthesis, transform carbon dioxide and hydrogen-carrier molecules into glucose. All photosynthetic organisms as well as many photosynthetic microbes contain the Calvin cycle. These processes take place in the stroma, the fluid-filled area of a chloroplast that is not covered by the thylakoid membranes, in plants. These reactions carry out additional molecular processes on the byproducts (ATP and NADPH) of light-dependent reactions. The Calvin cycle generates carbohydrates for use by the plant using the molecular energy of ATP and the reduction power of NADPH from light-dependent processes.

There is no direct reaction that changes several molecules of CO2 into sugar; instead, these compounds are used in a sequence of reduction-oxidation processes to create carbohydrates in a step-by-step manner. The three stages of the Calvin cycle, also known as the light-independent processes, are carboxylation, reduction reactions, and ribulose 1,5-bisphosphate (RuBP) renewal. The Calvin cycle does not truly take place in the dark or at night, despite the name "dark reaction." This is because the procedure needs NADPH, which is transient and obtained from light-dependent processes. Instead, plants discharge sugar from their carbohydrate stores into the phloem to give the plant energy at night. So, regardless of the type of photosynthesis (C3 carbon fixation, the Calvin cycle occurs when light is accessible[1], [2].

CAM plants need to hold malic acid in their vacuoles every night and discharge it during the day to make this process function. A set of metabolic redox processes known as the Calvin cycle, Calvin-Benson-Bassham cycle, reductive pentose phosphate cycle (RPP cycle), or C3 cycle occur in the stroma of chloroplasts in photosynthesis animals. At the University of California, Berkeley, Melvin Calvin, James Bassham, and Andrew Bensonused the unstable element carbon-14 to find the cycle in 1950. An organism goes through two phases of photosynthesis. The energy of light is first captured by light-dependent processes, which then use it to produce the energy-storing molecule ATP and the moderate-energy hydrogen transport NADPH. These substances are used by the Calvin cycle to transform carbon dioxide

and water into organic molecules that the creature can utilize (and by animals that feed on it). Carbon fixation is another name for this series of processes. RuBisCO is the name of the cycle's important protein. The chemical species (phosphates and carboxylic acids) in the accompanying metabolic formulae coexist in stable charged states that are controlled by pH. Although the majority of the enzymes used in other metabolic pathways, such as gluconeogenesis and the pentose phosphate pathway, are essentially identical to those in the Calvin cycle, the Calvin cycle enzymes are located in the chloroplast stroma rather than the cell cytoplasm, dividing the processes. The term "dark reaction" is deceptive because they are triggered by both the byproducts of the light-dependent reaction and light itself. These control mechanisms stop carbon dioxide from being breathed out during the Calvin cycle. These processes would be carried out with no net output, wasting energy (in the form of ATP).

The Calvin cycle's cumulative responses are as follows:

$$3\ CO_2 + 6\ NADPH + 6\ H^+ + 9\ ATP + 5\ H_2O \rightarrow \text{glyceraldehyde-3-phosphate (G3P)} + 6\ NADP^+ + 9\ ADP + 8\ P_i \quad (P_i = \text{inorganic phosphate})$$

The Calvin cycle does not produce hexose (six-carbon) carbohydrates. Even though C6H12O6 is frequently listed as a photosynthetic byproduct in textbooks, this is primarily done for ease to fit the equation for aerobic respiration, in which six-carbon carbohydrates are metabolized in mitochondria. Three-carbon sugar phosphate compounds, or "triose phosphates," such as glyceraldehyde-3-phosphate, are the carbohydrates produced by the Calvin cycle (G3P). Two molecules of ATP and two molecules of NADPH, which were created in the light-dependent stage, are used up as a CO2 molecule is integrated into one of two three-carbon molecules (glyceraldehyde 3-phosphate, or G3P), in the first stage of the Calvin cycle. The three stages are as follows:

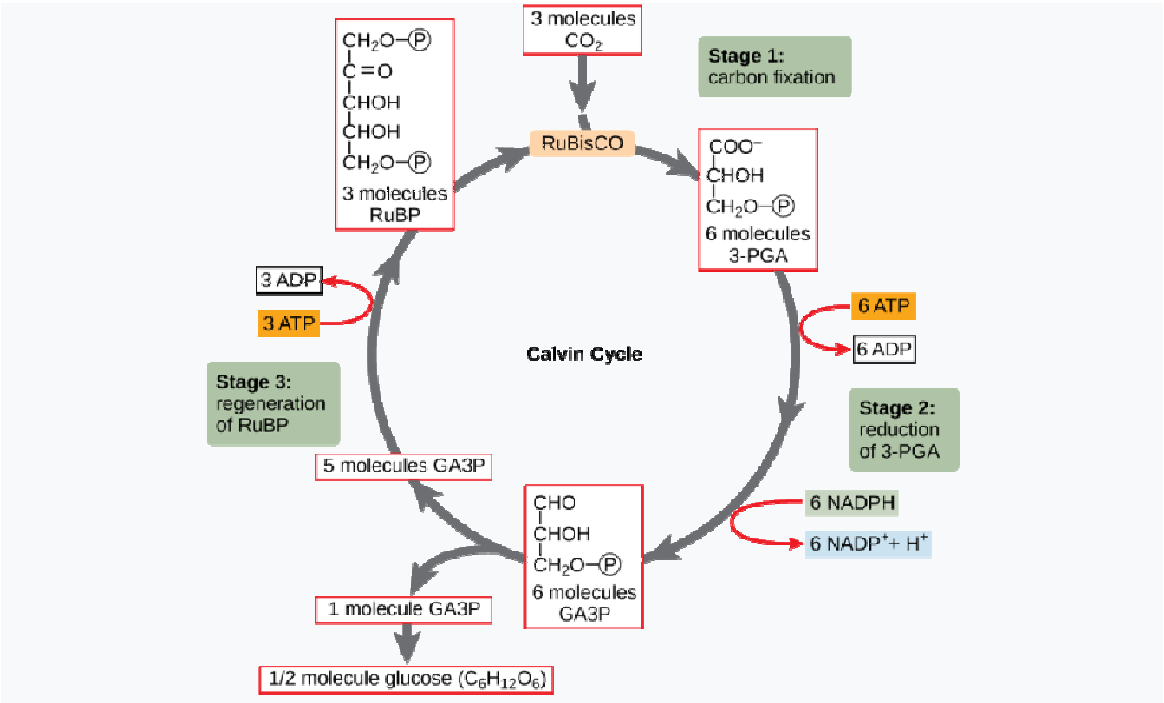

Figure 1: Calvin cycle: Diagram showing the steps involved in the Calvin cycle (Khan Academy).

In a two-step process, the enzyme RuBisCOcatalyzes the carboxylation of the 6-carbon molecule ribulose-1, 5-bisphosphate, or RuBP, a 5-carbon compound. The first stage results in the creation of an enzyme-enediol compound that can absorb CO or O. The actual

carboxylase/oxygenase is therefore an enediol-enzyme complex. When CO is captured by enediol in the second step, an unstable six-carbon compound known as 2-carboxy 3-keto 1, 5-biphosphoribotol (CKABP) or 3-keto-2-carboxyarabinitol 1,5-bisphosphate is produced. This unstable compound immediately splits into two molecules of 3-phosphoglycerate, also known as 3-phosphoglyceric acid, PGA, 3PGA, or 3-PGA, a three-carbon compound (Figure.1). The phosphorylation of 3-PGA by ATP (which was generated in the light-dependent step) is carried out by the enzyme phosphoglycerate kinase. The end products are ADP and 1, 3-bisphosphoglycerate (glycerate-1,3-bisphosphate). The reduction of 1,3BPGA by NADPH, another by-product of the light-dependent step, is catalyzed by the enzyme glyceraldehyde 3-phosphate dehydrogenase. In addition to the creation of glyceraldehyde 3-phosphate (also known as G3P, GP, TP, PGAL, or GAP), NADPH undergoes oxidation and changes into NADP+. Again, each COfixed is fixed with two NADPH.

Calvin cycle step of regeneration: Regeneration of RuBP is the subsequent phase of the Calvin cycle. Three ATP molecules are consumed in the production of three RuBP molecules by five G3P molecules. Three CO2 molecules produce six G3P molecules because each CO2 molecule generates two G3P molecules, and since five of those G3P molecules are used to replenish RuBP, there is a net increase of one G3P molecule for every three CO2 molecules. (as would be expected from the number of carbon atoms involved). Several stages make up the healing period. All of the G3P is reversibly transformed into the 3-carbon compound dihydroxyacetone phosphate (DHAP) by triose phosphate isomerase. A G3P and a DHAP are converted into fructose 6-phosphate by aldolase and fructose-1,6-bisphosphatase (6C). The fluid loses a phosphate molecule. Then, two more G3P are produced by the binding of a second CO_2. Transketolase removes two carbons from F6P to produce erythrose-4-phosphate.(E4P).

Transketolase's two carbons are combined with a G3P to produce ketose xylulose-5-phosphate (Xu5P). Aldolase enzyme transforms E4P and a DHAP (created from one of the G3P from the second CO2 fixation) into sedoheptulose-1,7-bisphosphate (7C). One of the three Calvin cycle enzymes that are specific to plants, sedoheptulose-1,7-bisphosphatase, breaks down sedoheptulose-1,7-bisphosphate into sedoheptulose-7-phosphate and releases an inorganic phosphate ion into solution. Two more G3P are produced by the fixation of a third CO2. Transketolase removes two carbons from the ketose S7P, producing ribose-5-phosphate (R5P), and transfers the two carbons that are still on transketolase to one of the G3P, producing another Xu5P. The remaining product of the fixing of three CO2 is one G3P, which results in the production of three pentoses that can be transformed to Ru5P. Phosphopentose isomerase changes R5P into ribulose-5-phosphate (Ru5P, RuP). Phosphopentose epimerase transforms Xu5P into RuP. The Calvin cycle is then finished by the plant-specific enzyme phosphoribulokinase, which phosphorylates ribulose-1,5-bisphosphate (RuP) into RuBP. One ATP needs to be supplied for this. Thus, of the six G3P generated, five are used to create three RuBP (5C) molecules, each of which contains three carbons[3]–[5].

Only one G3P is left over for later translation to a hexose. For every three CO2 molecules, nine ATP and six NADPH molecules are needed. At elevated temps, photorespiration occurs more quickly. RuBP is transformed by photorespiration into 3-PGA and 2-phosphoglycolate, a 2-carbon compound that can be changed into glycine via glycolate and glyoxalate. Two glycines are broken down into serine and CO by the glycine cleavage system and tetrahydrofolate. 3-phosphoglycerate can be produced once more from serine. To return to 3-PGA, only 3 of the 4 carbons from two phosphoglycolates can be transformed. Photorespiration has detrimental effects on plants because, instead of repairing carbon

dioxide (CO2), this mechanism results in CO2 loss. To avoid photorespiration, C4 carbon fixation developed, but it can only happen in specific plants that are endemic to extremely humid or equatorial regions, like maize. Additionally, to reduce the oxygenation reaction, RuBisCOs that catalyze the light-independent processes of photosynthesis typically show an enhanced selectivity for CO2 compared to O2. After RuBisCO added a novel protein component, the selectivity improved. Two glyceraldehyde-3-phosphate (G3P) molecules, three ADP molecules, and two NADP+ molecules are the direct byproducts of one revolution of the Calvin cycle. (ADP and NADP+ aren't considered "products. They are renewed and subsequently utilized once more in the light-dependent processes. A G3P atom has three carbons in it. RuBP (ribulose 1,5-bisphosphate) must be renewed for the Calvin cycle to proceed. Therefore, 5 of the 6 carbons in the 2 G3P molecules are utilized in this process. As a result, each turn's available total carbon production is limited to one. It takes 3 carbons and 3 cycles of the Calvin cycle to produce 1 excess G3P. It would take 6 cycles of the Calvin cycle to produce one glucose molecule (which is produced from 2 G3P molecules). Depending on the requirements of the plant, extra G3P can also be used to create other carbs like glucose, sugar, and cellulose.

DISCUSSION

The Calvin cycle's reaction order as well as the molecular properties of the participating enzymes have long been understood. However, it has long been unclear to what degree any particular enzyme regulates the rate of carbon fixation. Antisense mutant plants have been used as instruments to solve this over the past ten years and have discovered some surprising facts about the Calvin cycle. It was demonstrated that the amount of Rubisco protein had little effect on the regulation of carbon fixation under a variety of climatic circumstances. Additionally, the cycle was barely controlled by the three thioredoxin-regulated enzymes FBPase, PRKase, and GAPDH. Unexpectedly, aldolase and transketolase, two unregulated enzymes that catalyze reversible reactions, both exercised substantial control over carbon flow. Furthermore, it was demonstrated that SBPase significantly controls the Calvin cycle under a variety of development circumstances. These findings supported the theory that elevating the levels of these enzymes could boost photosynthesis carbon uptake. Surprisingly, tobacco plants that expressed a bifunctional SBPase/FBPase enzyme showed greater photosynthesis capability and development. Future research is addressed to improve our comprehension of this intricate and crucial process, especially about the mechanisms that control and coordinate enzyme activity.

The Calvin cycle is where larger organisms begin to metabolize carbon. Comparative biology of all of its enzymes from prokaryotes and eukaryotes has shown that it is a usually eubacterial route. Reviewing the structural underpinnings of the Calvin cycle function, we make an effort to take both biochemical and molecular results into account. Given that plants have all received the genes for the process from prokaryotes through endosymbiosis, the molecular variety of bacterial enzymes is highlighted. Curiously, the enzymes that make up the pathway vary among species and are physically unrelated; therefore, only the collection of substrate transformations has been preserved throughout evolution, not the enzymes that activate them. Some of the molecular and regulating characteristics of the enzymes were inherited from their prokaryotic ancestors, while others were only recently gained. Numerous variables influence how Calvin cycle genes are expressed, though the precise biochemical mechanisms underlying this control are still unknown. The results that point to the possibility of multienzyme Calvin cycle compounds are outlined. The thioredoxin system serves as the molecular foundation for redox-modulated light regulation, and its significance for adaptable control of the pathway under various circumstances is demonstrated. Recent discoveries from

the expression of antisense designs of Calvin cycle enzymes in transgenic plants are quickly discussed, along with the expression of Calvin cycle enzymes in reaction to internal or exterior stimuli.

Autotrophic organisms convert raw carbon to organic carbon through assimilation. They are a crucial element of the world's carbon cycle and contribute significantly to ecological health by supplying other creatures with a type of carbon that would otherwise be inaccessible. The Calvin-Benson-Bassham (CBB) cycle was long believed to be the only important metabolic autotrophic CO2 fixing process in the water. However, research in the last ten years in ecology, biochemistry, and genomics has not only revealed novel pathways but also demonstrated that autotrophic carbon fixation via routes other than the CBB cycle can be important. This has implications for how we perceive the ocean's energy flux and the carbon cycle. Here, we discuss the most recent findings in the area of autotrophic carbon fixation, including the biology and evolutionary history of the various processes and their biological applicability in diverse marine ecosystems.

Numerous scholars have developed and examined mutant plants with altered levels of the enzyme activity involved in the Calvin cycle, sucrose production, and starch metabolism to investigate the control of photosynthesis carbon flux in higher plants. By adding specific Calvin cycle enzymes, such as fructose-1,6-bisphosphatase and/or sedoheptulose-1,7-bisphosphatase, we were able to increase the photosynthesis carbon fixation capability. In this overview, we talk about how certain enzymes and mechanisms help regulate metabolism, glucose storage, and plant development in modified plants. These findings prompt a reexamination of theories regarding the control of carbon metabolism and have implications for the development of transgenic tactics to boost plant food output.

The most significant carbon absorption process in the environment is the Calvin cycle. Rubisco, its carboxylation enzyme, creates 2-phosphoglycolate by accepting oxygen as well. The Calvin cycle has been used to describe phosphoglycolate recovery mechanisms in photoautotrophs but not in chemolithoautotrophs. In this paper, we investigate the recovery of phosphoglycolate in the chemolithoautotrophic model bacteria *Cupriavidusnecator H16*. We show that this bacteria primarily uses the glycerate route to reassimilate 2-phosphoglycolate. When this pathway is blocked, a different mechanism that we refer to as the malate cycle promotes photorespiration by fully reducing 2-phosphoglycolate to CO2. Although 2-phosphoglycolate was not previously known to be metabolized by the malate cycle in nature, a bioinformatic study indicates that phosphoglycolate recovery may be supported by the malate cycle in a variety of chemoautotrophic bacteria[6]–[8].

The ability of seedlings to efficiently store carbon is essential for plant health and farming output. The majority of seeds1 contain a significant amount of oil, and this oil is the biggest natural supply of reusable decreased carbon compounds. However, one-third of the carbon is lost as CO2 as a consequence of the fermentation process, which transforms carbohydrates into the oil. Here, we demonstrate that Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) works in growing *Brassica napus* L. (oilseed rape) embryos outside of the Calvin cycle2 and in a previously unrecognized biochemical setting to improve the efficacy of carbon use during the production of oil. The biochemical change we describe results in 40% less carbon loss as CO2 and 20% more acetyl-CoA for fatty-acid production than glycolysis. Our findings are supported by analyses of basic flow patterns, observations of mass balance, enzyme activity, and stable isotope labeling.

The 12 enzymes that initiate the Calvin cycle processes in higher-plant chloroplasts have brief evolutionary paths. It has been determined that a combination of nucleus genes with

cyanobacterial and proteobacterial ancestry encodes them. Furthermore, the genes that almost always encode the cytoplasmic versions of these enzymes are of obviously endosymbiont origin. Invading eubacterial genes frequently replaced pre-existing nucleus equivalents to which they were either functionally or physically identical, indicating that endosymbiosis produced functional duplication that was removed through differential gene loss. Our results do not support the "product-specificity corollary," which asserts that nuclear-encoded gene products will be redirected to the organelle whose genome they originally came from. Instead, it would seem that regardless of where they came from, the enzymes of central glucose metabolism have developed new targeting options. Based on the empirical theory that some gene products carried by organelles might be harmful when present in the cytoplasm or other unsuitable cellular compartments, our results propose a novel hypothesis to explain the survival of the organelle genome.

The degree of Photosystem II photoinactivation in photosynthesis species is increased when the Calvin cycle enzymes' functions are compromised. (PSII). We looked into the chemical process causing this occurrence in the *Chlamydomonas reinhardtii*, a single-celled green alga. The degree of photoinactivation of PSII was increased when glycolaldehyde, which is known to block phosphoribulokinase, disrupted the Calvin cycle. Glycolaldehyde's impact was very comparable to chloramphenicol's, which prevents protein production in chloroplasts from beginning from scratch. The major component of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) gene was introduced with a nonsense mutant, which disrupted the Calvin cycle and increased the degree of PSII photoinactivation. Glycolaldehyde and chloramphenicol have no extra effects on photoinactivation in such mutated 10-6C cells. After photodamage to PSII, when wild-type cells were cultured under dim light, PSII activity progressively restored and returned to a level that was nearly equal to the original level. Glycolaldehyde, however, prevented healing in 10-6C cells as well as in wild-type cells. While the amounts of psbA and psbD mRNA were unaffected by the Calvin cycle disruption, radioactive labeling and Northern blotting showed that it reduced the production of chloroplast proteins such as the D1 and D2 proteins. According to our findings, the photoinactivation of PSII that causes the Calvin cycle to stop is mainly caused by the suppression of PSII's protein synthesis-dependent healing at the level of translation in chloroplasts.

In separated undamaged chloroplasts, hydrogen peroxide (6x10-4 M) results in a 90% suppression of CO_2 fixation. With the addition of DTT or catalase (2500 U/ml), the blockage is reversed. (10 mM). The incorporation of carbon into hexose- and heptulose bisphosphates as well as pentose monophosphates is significantly increased when hydrogen peroxide is added to a suspension of intact chloroplasts in the light, whereas the incorporation of carbon into hexose monophosphates and ribulose 1,5-bisphosphate is decreased. The production of 6-phosphogluconate is also significantly boosted at the same time that ATP levels are raised. By adding catalase or DTT, all these changes brought on by hydrogen peroxide are reverted. Additionally, it has been investigated how [14C]glucose-6-phosphate is transformed by disrupted chloroplasts in the dark into various compounds. Hydrogen oxygen inhibits the synthesis of ribulose-1,5-bisphosphate while promoting the synthesis of other bisphosphates, triose phosphates, and pentose monophosphates. DTT again has the reverse impact. DTT (0.5 mM) inhibits and hydrogen peroxide at similar amounts reactivates the oxidative pentose phosphate cycle processes that allow the liquid portion of disrupted chloroplasts to liberate 14CO2 from added [14C]glucose-6-phosphate. These findings show that hydrogen peroxide interacts with reduced sulfhydryl groups that are necessary for the light activation of Calvin cycle enzymes at the sites of fructose- and sedoheptulose bisphosphate and

phosphoribulokinase, as well as the light inactivation of the oxidative pentose phosphate cycle at the site of glucose-6-phosphate dehydrogenase[9], [10].

Although nitrogen addition to the understory (UAN) is frequently used in field studies of forest ecosystems to mimic nitrogen deposition, it overlooks the impacts of air nitrogen precipitation on the top. In a subtropical evergreen broadleaved forest in South China, we investigated the impacts of nitrogen precipitation replicated by UAN and by canopy addition of nitrogen (CAN) on leaf structure, molecular characteristics, Calvin cycle, and photosynthate dispersal strategy of typical woody plant species. According to the findings, at the same percentage of N addition, the maximal photosynthesis rate (Amax) of the plant species *Blastuscochinchinensis* and *Ardisia quinquegona* under CAN treatments was considerably greater than that under UAN treatments. In contrast to UAN therapies, CAN substantially boosted the amounts of intermediates (PGK, DPGA, and G3P) in the Calvin cycle of *B. cochinchinensis*, *A. quinquegona*, and *Castanea henryi*. *Lasianthus chinensis* and *B. cochinchinensis* shrub species had significantly higher starch concentrations after exposure to CAN25, whereas *A. quinquegona* shrub species and *C. henryi* tree species had considerably lower sucrose concentrations. Correlation studies revealed that the quantity of nitrogen applied under various conditions contributed to the explanation of changes in the intermediates of the Amax and Calvin cycles. In conclusion, nitrogen deposition may encourage the Amax and Calvin cycle in shrub species, and shrub species are better able to adjust to nitrogen deposition than tree species, which may help to explain why subtropical evergreen broad-leaved forests are deteriorating Microbial development depends on the movement of materials in the carbon cycle. Microorganisms contain a variety of CO_2-fixation processes. Another interesting topic covered in the paper is the breakdown of natural compounds like carbs, proteins, and lipids. Additionally, microbes can break down natural compounds like cellulose, glucose, and lignin that are found in plants. A significant factor in climate change is the contribution of bacteria activity to the methane cycle.

This paper gives a thorough summary of the present state of our understanding of the Calvin-Benson cycle (CBC), which supplies the cell with fixed carbon, as well as the generation and dispersal of reducing power within the chloroplast of Chlamydomonas. Following the absorption of light energy, photosynthesis continues with its transformation into reduction power (NADPH) and high-energy molecules (ATP), both of which are primarily directed to the CBC to drive carbon fixation and are also used for other metabolic processes. We explain how ferredoxins (FDXs) control electron transfer after Photosystem I to produce NADPH and reduce thioredoxins. (TRXs). Chlamydomonas has been widely researched for its FDX-TRX system, which supplies electrons for antioxidant and signaling functions. This system's function in the light-dependent stimulation of the CBC has received the most attention. We describe the Chlamydomonas CBC's role in depth, draw attention to how it differs from that of other photosynthetic species, and go over what is known about its control and molecular layout. We also focus on the different pathways that help to maintain optimum NADPH/NADP+ concentrations in the light, as well as the energetics and dual functions of NADPH cycling in supplying the assimilatory power needed to power the CBC and keeping straight electron flow. Finally, we address the potential for designing and enhancing carbon fixation using synthetic biology methods using Chlamydomonas as a substrate.

CONCLUSION

The Calvin cycle, also known as a light-dependent process, is used to convert carbon dioxide into glucose. It takes place in the chloroplast's stroma. As a result of the reaction's independence from light, it is known as a "dark reaction." Calvin cycle is another name for a dark response. Glucose is generated and carbon dioxide is assimilated during the night-time

process. Six molecules of 3-PGA are transformed into six molecules of glyceraldehyde 3-phosphate using ATP and NADPH. Due to the electron increase caused by 3-PGA, this process is a reduction. Remember that a ppaper or molecule gains an electron during a reduction. Adenosine triphosphate (ATP), a substance involved in energy transmission, and reduced nicotinamide adenine dinucleotide phosphate (NADPH), a supply of hydrogen atoms for reduction processes, are needed for some Calvin cycle reactions. During photosynthesis's light-energized processes, ATP and NADPH are produced. The Calvin cycle's main job is to convert carbon dioxide and water into basic carbohydrates through a process called carbon fixation. Producing three-carbon carbohydrates that can be used to create other sugar compounds, like starch, glucose, and cellulose, is the primary goal of the Calvin Cycle. Plants use these carbohydrates to build other molecules. Carbon is immediately absorbed from the air and transformed into plant matter in the Calvin cycle.

REFERENCES:

[1] A. Piotrowska and R. Czerpak, "Cellular response of light/dark-grown green alga Chlorella vulgaris Beijerinck (Chlorophyceae) to exogenous adenine- and phenylurea-type cytokinins," *Acta Physiol. Plant.*, 2009, doi: 10.1007/s11738-008-0267-y.

[2] L. Guidi, C. Nali, G. Lorenzini, F. Filippi, and G. F. Soldatini, "Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity," *Environ. Pollut.*, 2001, doi: 10.1016/S0269-7491(00)00194-9.

[3] A. M. Nonomura, D. Shevela, S. S. Komath, K. Y. Biel, and G. Govindjee, "The carbon reactions of photosynthesis: Role of lectins and glycoregulation," *Photosynthetica*. 2020. doi: 10.32615/ps.2020.064.

[4] A. Mortain-Bertrand, "Photosynthetic metabolism of an antarctic diatom and its physiological responses to fluctuations in light," *Polar Biol.*, 1988, doi: 10.1007/BF00441764.

[5] U. Heber, R. Bligny, P. Streb, and R. Douce, "Photorespiration is essential for the protection of the photosynthetic apparatus of C3 plants against photoinactivation under sunlight," *Bot. Acta*, 1996, doi: 10.1111/j.1438-8677.1996.tb00578.x.

[6] L. Zhang, W. Zhang, and X. Chen, "The effects and mechanism of climate change on vegetables quality: a review," *Chinese Journal of Eco-Agriculture*. 2021. doi: 10.12357/cjea.20210327.

[7] A. S. Denning, *Relating Surface Energy Budgets to the Biochemistry of Photosynthesis: A Review for Non-Biologists*. 2002.

[8] R. L. Burnap, R. Nambudiri, and S. Holland, "Regulation of the carbon-concentrating mechanism in the cyanobacterium Synechocystis sp. PCC6803 in response to changing light intensity and inorganic carbon availability," *Photosynth. Res.*, 2013, doi: 10.1007/s11120-013-9912-4.

[9] Y. H. Zhou and J. Q. Yu, "Allelochemicals and photosynthesis," in *Allelopathy: A Physiological Process with Ecological Implications*, 2006. doi: 10.1007/1-4020-4280-9_6.

[10] F. Xiong, Y. Gao, and P. Song, "A long-lasting photorespiration in CO2-free air, measured as the postirradiation CO2 burst, indicates mobilization of storage photosynthates," *Photosynthetica*, 1998, doi: 10.1023/A:1006882101535.

CHAPTER 23

AN OVERVIEW OF THE PHOTOSYNTHESIS; LIGHT REACTION

Mukesh Singh Sikarwar, Professor
Department of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- MUKESHSIKARWAR@GMAIL.COM

ABSTRACT:

The plant and another photosynthetic organism can utilize the quantum energy and convert it to chemical energy for the production of ATP and NADPH. This process is the light dependents involved in photosystem I, photosystem II, and the water splitting complex. In this paper, we summarised the light reaction of photosynthesis that occurs in the plant for the production of energy and food.

KEYWORDS:

Cytochrome B6f, Electron Acceptor, Electron Transport, Light Dependent, Photosystem I.

INTRODUCTION

Plants obtain energy, which are referred to as "light-dependent reactions" in scientific language. There are two light-dependent reactions; the first takes place at photosystem II (PSII), and the second at photosystem I (PSI). PSII captures a photon to create a so-called high energy electron, which then travels through an electron transport chain to cytochrome b6f and then to PSI via a second light-dependent reaction. The second photon that is absorbed by the then-reduced PSI produces an even more strongly reducing electron, which changes NADP+ into NADPH. Water serves as the first electron source in oxygenic photosynthesis, producing oxygen (O2) as a byproduct. (Figure. 1). Various electron sources are used in anoxygenic photosynthesis. In two different methods, ATP synthase and cytochrome b6f collaborate to generate ATP (photophosphorylation). In non-cyclic photophosphorylation, cytochrome b6f pumps protons from the stroma to the lumen using electrons from PSII and energy from PSI. A proton-motive force is produced by the ensuing proton gradient across the thylakoid membrane, which is used by ATP synthase to produce ATP. Cytochrome b6f utilizes electrons and energy from PSI to increase ATP generation and decrease NADPH synthesis during cycle photophosphorylation. To produce ATP and keep NADPH in the proper ratio for the light-independent processes, cyclic phosphorylation is crucial[1]–[3].

All light-dependent processes in oxygenic photosynthesis result in the following net reaction:

$$2H_2O + 2NADP^+ + 3ADP + 3P_i \rightarrow O_2 + 2\,H^+ + 2NADPH + 3ATP$$

Light-harvesting complexes PSI and PSII. In a photosynthesis reaction center, if a specific pigment molecule receives a photon, an electron in this pigment enters the energized state and is then passed to another molecule. The electron movement begins with this process, known as photoinduced charge separation, which converts light energy into molecular compounds.

The thylakoid membrane contains the response core. A group of chlorophyll pigment molecules close to the periplasmic (or thylakoid lumen) side of the membrane receive the received light energy. The primary function of this dimer in photosynthesis is the reason it is referred to as a unique pair. The PSI and PSII response centers have subtly distinct versions of this unique combination. It is known as P680 because it captures electrons with a spectrum

of 680 nm in PSII. It is known as P700 in PSI and captures light at 700 nm. The unique combination in microbes is referred to as P760, P840, P870, or P960. Here, "P" stands for pigment, and the number that follows it denotes the light frequency that was absorbed. At certain energies, electrons in color compounds can exist. They are in the ground condition, which has the lowest energy level when things are routine. However, they can be raised to a greater energy level by absorbing light with the proper ppaper energy. Any light that is either too weak or too strong to be received is deflected. The electron is unstable at the higher energy level and will soon fall to the lower energy level where it belongs. It must let go of the ingested energy to accomplish this. This may occur in several ways. The excess energy can be used to move molecules, which releases heat, or it can be reemitted by the electron as light. (fluorescence). Resonance energy transfer describes the process by which the energy, but not the electron, can be transferred to another ppaper.

Resonance energy transfer cannot be used to move an energized electron of the unique pair in the reaction center to another color. Since an appropriate electron acceptor is close by in the reaction center, the energized electron is absorbed by the acceptor instead of returning to the ground state as it would normally do. The unique couple acquires a positive charge as a result of the loss of an electron, which also increases its energy through the process of ionization. Photoinduced charge separation is the creation of a positive charge on the unique pair and a negative charge on the recipient. Another atom may receive the electron. The oxygen-developing complex splits water into electrons, protons, and molecule oxygen as the charged pigment returns to its ground state by absorbing an electron and releasing energy (after receiving energy from the pigment four times). The last two of these processes are typically used by plant compounds to transform solar energy into their own. This early charge separation takes place in 10–11 picoseconds or less than that. The electron on the acceptor may travel back to cancel the positive charge on the special pair in their high-energy states, causing charge recombination between the special pigment and the acceptor. Its return to the unique couple would merely transform the ingested light energy into heat while wasting a priceless high-energy electron.

This overflow of electrons in the instance of PSII can result in the production of reactive oxygen species and photoinhibition. Charge exchange is almost entirely suppressed by the reaction center's structural mix of three elements: The distance between the second electron acceptor and the first acceptor is less than 1 micron, which allows the electron to move swiftly farther from the unique couple. The unique pair is less than 1 nanometer from an electron source, so an additional electron is transferred to balance the positive charge. Particularly slow is the electron transmission from the electron receiver to the positively charged special pair. Up until a certain point, the rate of an electron transfer process rises with its thermal favorability before falling. Because of how advantageous the reverse transfer is, it occurs in the inversion area, where electron-transfer rates decelerate down. As a result, electron transfer moves successfully from one electron acceptor to the next, forming a chain of electron transport that concludes NADPH. The first protein complex in the light-dependent processes of oxygenic photosynthesis is known as photosystem II (or water-plastoquinone oxidoreductase). It can be found in plants, algae, and cyanobacteria's thylakoid membranes. Enzymes in the photosystem absorb light ppapers to energize electrons, which are then moved through a range of coenzymes and cofactors to convert plastoquinone to plastoquinol. By reducing water to produce hydrogen ions and molecular oxygen, the charged electrons are swapped out for new ones.

Photosystem II supplies the electrons necessary for the entire photosynthetic process by replacing wasted electrons with electrons obtained from the fission of water. The hydrogen

molecules (protons) produced when water is oxidized contribute to the formation of a proton gradient that ATP synthase uses to produce ATP. The charged electrons that were added to the plastoquinone are subsequently used in non-cyclic electron flow or to decrease NADP+ to NADPH. A substance called DCMU is frequently used in experimental environments to prevent photosynthesis. DCMU prevents electrons from moving from photosystem II to plastoquinone when it is present. Two identical proteins, D1 and D2, form a pseudo-symmetric pair at the center of PSII. The positive charge on the chlorophyll dimer that experiences the early photoinduced charge separation is shared evenly by the two molecules in the reaction centers of all other photosystems, but in undamaged PSII the charge is primarily localized on one chlorophyll center (70–80%).P680+ can participate in the breaking of water because it is a strong oxidizer. Depending on the organism, photosystem II (of cyanobacteria and green plants) is made up of up to 20 components as well as additional, auxiliary proteins that capture light. The Mn4CaO5 cluster, which includes two chloride ions, one non-heme Fe2+, two hypothetical Ca2+ ions per monomer, 20 lipids, 35 chlorophyll a, 12 beta-carotene, 2 pheophytins, 2 plastoquinone, 2 heme, 1 bicarbonate, and 99 cofactors are all present in at least one photosystem II.

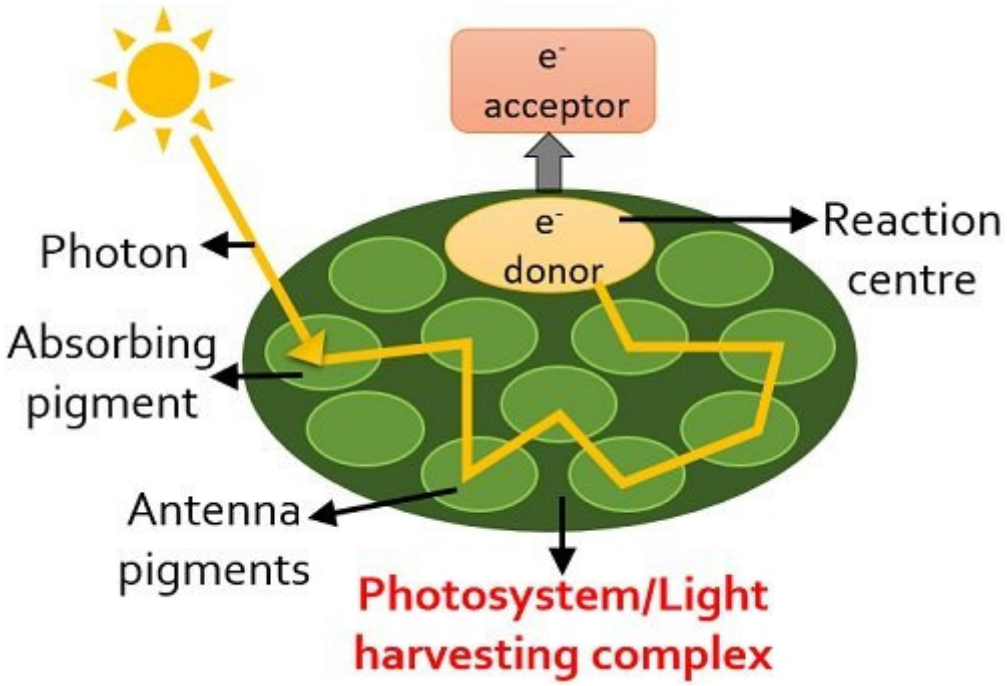

Figure.1: Photosynthesis: Diagram showing the overview of photosynthesis (Biology reader).

Since it is the source of almost all of the oxygen in the atmosphere, photosynthetic water splitting, also known as oxygen evolution, is one of the most significant processes on the globe. Additionally, manufactured photosynthesis water splitting might improve the efficiency of using solar as a substitute energy source. A great deal of information is known about the process of water decomposition. Four electrons and four protons from two water molecules must be removed to oxidize water to molecular oxygen. Pierre Joliot et al. gave direct proof that oxygen is produced via the periodic reactivity of an oxygen-evolving complex (OEC) within one PSII.They have demonstrated that when dark-adapted photosynthetic material, such as higher plants, algae, and cyanobacteria, is exposed to a series of single turnover flashes, oxygen evolution can be detected with a typical period-four damped oscillation, with maxima on the third and seventh flash and minima on the first and fifth flash. This experiment prompted Bessel Kok and colleagues to propose a cycle of five

flash-induced changes of the so-called S-states, which described the four redox states of OEC: OEC returns to its fundamental S0-state after storing four oxidizing equivalents (at the S4-state). The OEC will "relax" to the S1 state in the lack of light; the S1 state is frequently referred to as being "dark-stable." Most scientists believe that manganese ions in the S1 state have the oxidation states Mn3+, Mn3+, Mn4+, and Mn4+. Last but not least, Jablonsky and Lazar suggested the intermediary S-states as a regulating mechanism and connection between S-states and tyrosine Z.Renger first proposed the concept of interior water molecular modifications into normal oxides in various S-states during water splitting in 2012. Both PSI and PSII are intricate, highly structured transmembrane structures that include phylloquinone, a reaction center (P700), antenna chlorophylls, and various iron-sulfur proteins that function as intermediary redox transporters. Multiple versions of the transmembrane proteins are used by PSII in its light-harvesting mechanism (Figure. 2). In the reaction center, where specific chlorophyll molecules (P700, with maximal light absorption at 700 nm) are excited to a higher energy level, the energy of ingested light (in the form of delocalized, high-energy electrons) is channeled.

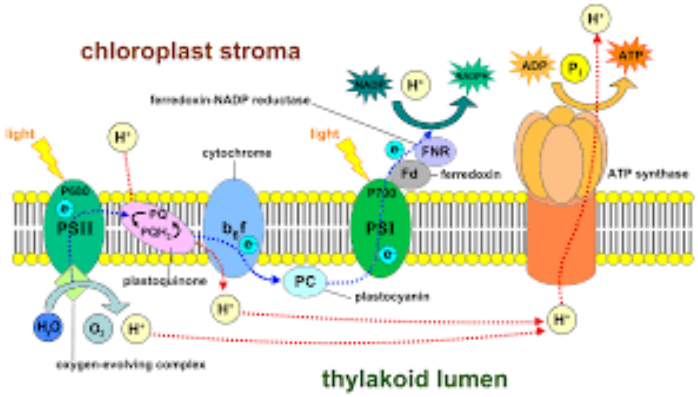

Figure.2: Components involved in photosynthesis: Diagram showing the organization of Photosystem I and Photosystem II involved in photosynthesis (Wikipedia).

The effectiveness of the procedure is astoundingly great. Excited chlorophyll molecules release their electrons, which are then transmitted to ferredoxin, a water-soluble electron carrier, via a sequence of intermediary carriers. This is a 100% efficient solid-state method, similar to PSII. In PSI, there are two distinct electron transport routes.

Ferredoxin transports the electron in noncyclic electron transport to the enzyme ferredoxin NADP+ reductase (FNR), which converts NADP+ to NADPH. Electrons from ferredoxin are moved (via plastoquinol) to a proton pump, cytochrome b6f, in cycle electron transport. Then, they are transferred back to P700 (using plastocyanin). To create biological compounds from CO_2, NADPH, and ATP are needed. By changing the equilibrium between circular and noncyclic electron transfer, the ratio of NADPH to ATP generation can be changed. Chloroplasts contain cytochrome b6f, PSII, and PSI. Chloroplasts are found in all plants and green phytoplankton, and they use the aforementioned processes to create ATP and NADPH. Cyanobacteria contain essentially the same transmembrane proteins.

Cyanobacteria, in contrast to plants and phytoplankton, are prokaryotes. They resemble chloroplasts but do not contain them, despite their remarkable similarity. This indicates that the genetic ancestors of chloroplasts were creatures that were similar to cyanobacteria. One can envision early eukaryotic cells acquiring cyanobacteria as internal symbionts through an endosymbiotic process. Notably, PSI shares many similarities with the photosynthesis structures of green sulfur bacteria, just as PSII does with those of purple bacteria[4], [5].

DISCUSSION

Plants accomplish the amazing task of turning light energy into useful chemical forms during photosynthesis, which includes controlling highly volatile intermediates without damaging plant cells. This necessitates a device that is not only effective and reliable but also adaptable to shifting weather circumstances. It also necessitates matching the metabolic needs with the production of the energy-storing processes. The processes by which this adaptability is attained for momentary external shifts are covered in this essay. The output ratio of ATP: NADPH, which is controlled by circular electron flows around photosystem I, and the regulating sensitivity of the light-harvesting antenna to electron (and proton) flow, are the two kinds of adaptable processes that we contend chloroplasts require.

In this study, we focus on new findings and emerging concepts for enhancing the productivity of light processes for photosynthesis in plants. We observe that the efficacy of photosynthesis is a compromise between the amount of energy used for development and the amount of energy lost or used to prevent photodamage to the photosynthetic apparatus. There are good reasons to be hopeful about improving photosynthesis efficacy, but there are still a lot of interesting concepts in the works. The cereals of the future are expected to undergo intensive genetic engineering to be tailored to particular natural or fabricated climatic circumstances. Some of the biology's most intensely energetic intermediates are channeled by plants during photosynthesis in a manner that harnesses a significant amount of their energy to power the plant. A functional photosynthesis device must be well incorporated into the metabolic and physiological networks of the plant in addition to being an effective and durable piece of equipment. This necessitates adaptability in how it responds to metabolic requirements as well as the drastically shifting external circumstances. First, the energy-storing light processes' product needs to correspond to what the plant system requires. Second, the control of the antenna needs to be adaptable to accommodate reactions to various problems that might lead to excessive light capture and subsequent photoinhibition. Evidence is provided for the interaction of two different kinds of molecular plasticity, one of which modifies the antenna down-regulation's susceptibility to electron flow and the other of which mainly modifies the ATP/NADPH output ratio but also influences down-regulation**.**

The fundamental processes of photosynthesis light reactions are exceptionally quick and efficient. They are found in highly ordered, biologically and physiologically well-defined protein-pigment clusters. Our comprehension of the underlying physical principles is aided by the new structural analysis at the atomic level of some significant elements of the main photosynthesis processes in bacteria[6]–[8].

By observing the varying chlorophyll-a fluorescence transients induced by single-turnover saturating bursts, rate-limiting stages in the dark-to-light transition of Photosystem II (PSII) were found. (STSFs). It was demonstrated that in diuron-treated samples: (i) the first STSF only produced an F1(Fm) fluorescence level, despite completely reducing the QA quinone acceptor molecule; (ii) additional excitations were necessary to produce the maximum (Fm) level; however, (iii) these excitations were only successful with sufficiently long waiting times between consecutive STSFs. In-depth investigations demonstrated the PSIIL, a light-adapted charge-separated state, gradually forming. The information provided here supports this assignment: *Thermostichusvulcanus* and spinach thylakoid membranes' isolated PSII core complexes (CCs) showed similar temperature dependences between 5 and -80 °C, with significantly higher values at low temperatures. (i) The 1/2 values in PSII CC were essentially invariant on the Fkto-Fk+1 (k = 1-4) increments both at 5 and at 80 °C, indicating the involvement of the These findings are consistent with the previous hypothesis that

dielectric relaxation processes were involved in the development of the light-adapted charge-separated state and the varying chlorophyll-a luminescence of PSII.

It has been widely suggested that a significant route of sulfate generation during smog occurrences in China is the mixed decomposition of SO2 by NO2. However, little is known about the rates and process of SO2 oxidation by NO2 on the surface of complicated ppapers. Here, we thoroughly investigate the process and dynamics of the SO2 and NO2 reaction on diesel black carbon (DBC) when exposed to light. The laboratory findings demonstrate that DBC photochemistry can implicitly support the creation of OH radicals in addition to substantially promoting the mixed reduction of NO2 to generate HONO through the transmission of photoinduced electrons. The mixed oxidation of SO2 on DBC is significantly aided by these NO2 reduction products as well as by NO2 itself. Three primary surface oxidation routes of SO2 have been identified: HONO oxidation, surface photooxidation, and NO2 oxidation. The timing findings show that the surface photooxidation route (63%), HONO oxidation pathway (27%), and direct oxidation by NO2 (10%) account for the bulk of the total SO2 absorption. This study emphasizes the important joint contributions of DBC, NO2, and light exposure in boosting air oxidation capacity and encouraging mixed sulfate production**.**

This study looked into the process by which light oil is produced during the decomposition of bituminous coal from Shenfu. The light tar reaction process was revealed using reactive force field models (ReaxFF), TGA, Py-GC/MS, TGA-FTIR, and other techniques. The decomposition of the suggested coal structure model, C3198H3261N51O513S33, was modeled using the ReaxFF technique. The decomposition process was split into ignition, pyrolysis, and condensation, according to simulation results, which were in line with the TGA findings. It was found that coal pyrolysis was started by the splitting of oxygen-containing groups based on the evolutions of gases in the activation stage. Results from simulations using the coal monomolecular structure demonstrated that the reaction process of light tar was a free radical addition reaction, with free radicals primarily produced by the breaking of the C-O chemical bond. Additionally, it was discovered that the crossover point for the predominant bond breakage from the C-O bond to the C-C bond occurred at a temperature of 2400 K. Chemical bond variance showed good agreement with TGA-FTIR and Py-GC/MS data, demonstrating the validity and veracity of the generated results. Therefore, it was determined that the reaction mechanism of light tar was the free radical addition reaction, which was primarily governed by the breaking of the C-O bond.

Synthetic chemists have lately become interested in photochemistry to perform photolysis processes of diazoalkanes. In this feature paper, we give a succinct overview of this field, beginning with advancements in physical organic chemistry and moving on to examples in the organic synthesis of singlet carbene intermediates. These examples range from traditional reactivity to cascade reactions and novel protonation reactions occurring under photochemical conditions. After that, we talk about the developments in the use of faintly colored diazoalkanes in dye-sensitized processes to reach radical or triplet carbene intermediates. Finally, we talk about the electrical management of singlet and triplet carbene intermediates[9], [10].

From straightforward aromatic chemicals, dearomatization processes quickly produce three-dimensional structures with complicated molecular architectures. Numerous studies have in recent years shown their usefulness in the production of natural products, pharmaceutical chemistry, and materials science. Recently, photocatalysis facilitated by visible light has become a potent instrument for fostering a variety of changes. Over the past few years, major advancements have also been made in the dearomatization responses brought on by visible

light. This study gives a summary of reactions that are caused by visible light and categorized according to how aromaticity is disturbed.

CONCLUSION

Four significant steps make up a light-dependent reaction: the absorption of light energy, the breaking of water molecules, the release of oxygen, and the creation of the energy-carrying molecules ATP and NADPH. Photosystems are useful structures made up of auxiliary pigments and chlorophyll-a (the reaction core). Producing organic energy molecules like ATP and NADPH, which are required for the following dark reaction, is the primary goal of the light reaction. The red and blue portions of white light are absorbed by chlorophyll, and it is at these frequencies that photosynthesis is most effective.

REFERENCES:

[1] M. Messant, A. Krieger-Liszkay, and G. Shimakawa, "Dynamic changes in protein-membrane association for regulating photosynthetic electron transport," *Cells*. 2021. doi: 10.3390/cells10051216.

[2] J. J. Fuhrmann, "Microbial metabolism," in *Principles and Applications of Soil Microbiology, Third Edition*, 2021. doi: 10.1016/B978-0-12-820202-9.00003-4.

[3] D. Pavlovic, B. Nikolic, S. Djurovic, H. Waisi, A. Andjelkovic, and D. Marisavljevic, "Chlorophyll as a measure of plant health: Agroecological aspects," *Pestic. i fitomedicina*, 2014, doi: 10.2298/pif1401021p.

[4] M. O. Senge, A. A. Ryan, K. A. Letchford, S. A. MacGowan, and T. Mielke, "Chlorophylls, symmetry, chirality, and photosynthesis," *Symmetry*. 2014. doi: 10.3390/sym6030781.

[5] G. Wang and K. Castiglione, "Light-driven biocatalysis in liposomes and polymersomes: Where are we now?," *Catalysts*. 2019. doi: 10.3390/catal9010012.

[6] A. Pannwitz *et al.*, "Roadmap towards solar fuel synthesis at the water interface of liposome membranes," *Chemical Society Reviews*. 2021. doi: 10.1039/d0cs00737d.

[7] C. Kranz and M. Wachtler, "Characterizing photocatalysts for water splitting: From atoms to bulk and from slow to ultrafast processes," *Chemical Society Reviews*. 2021. doi: 10.1039/d0cs00526f.

[8] M. A. Kozuleva, B. N. Ivanov, D. V. Vetoshkina, and M. M. Borisova-Mubarakshina, "Minimizing an Electron Flow to Molecular Oxygen in Photosynthetic Electron Transfer Chain: An Evolutionary View," *Frontiers in Plant Science*. 2020. doi: 10.3389/fpls.2020.00211.

[9] T. Mirkovic, E. E. Ostroumov, J. M. Anna, R. Van Grondelle, Govindjee, and G. D. Scholes, "Light absorption and energy transfer in the antenna complexes of photosynthetic organisms," *Chemical Reviews*. 2017. doi: 10.1021/acs.chemrev.6b00002.

[10] F. Müh and A. Zouni, "Structural basis of light-harvesting in the photosystem II core complex," *Protein Science*. 2020. doi: 10.1002/pro.3841

CHAPTER 24

AN ALTERNATIVE PATHWAY OF THE CITRIC ACID CYCLE; GLYOXYLATE PATHWAY

Dr.Dhyuti Gupta, Demonstrator
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- dhyuti92@rediffmail.com

ABSTRACT:

In the glyoxysomes of plant cells, the glyoxylate process takes place. The glyoxylate pathway is also referred to as the alternate citric acid cycle route. Isocitrate lyase and malate synthase are two extra enzymes found in this route. This cycle is primarily triggered in plant seeds that are germination and in a small number of microbes that use acetate as their only carbon source. The stages of the glyoxylate pathway were discussed in this paper.

KEYWORDS:

Acetyl CoA, Acetate Assimilation, Gene Expression, Glyoxylate Cycle, Isocitrate Lyase.

INTRODUCTION

A variant of the tricarboxylic acid cycle called the glyoxylate cycle is a metabolic mechanism that can be found in fungus, microbes, protists, and plants. In order to synthesize carbs, acetyl-CoA is converted to succinate in the glyoxylate cycle. When basic carbohydrates like glucose or fructose are not accessible, the glyoxylate cycle in microbes enables cells to use two carbons (C2 molecules) such as acetate to fulfill cellular carbon needs. Animals are typically thought to lack the cycle, with the exception of worms during the early phases of development. However, in recent years, the discovery of malate synthase (MS) and isocitrate lyase (ICL), two essential enzymes in the glyoxylate cycle, in some animal tissue has called into question the evolutionary relationship between enzymes in bacteria and animals. This finding also raises the possibility that animals may encode alternative enzymes of the cycle that are distinct from the MS and ICL found in non-metazoan species. Acetate can be used as a source of carbon for the synthesis of carbon molecules by plants, some cyanobacteria, and microbes.

The glyoxylate cycle, a variant of the TCA cycle used by both plants and microbes, converts two carbon acetate molecules into four-carbon dicarboxylic acids. The glyoxylate cycle directly transforms isocitrate through isocitrate lyase and malate synthase into malate and succinate, skipping the two oxidative decarboxylation processes of the TCA cycle in the process. Five of the eight enzymes involved in the tricarboxylic acid cycle citrate synthase, aconitase, succinate dehydrogenase, fumarase, and malate dehydrogenase are involved in the glyoxylate cycle. Isocitrate is transformed by the enzyme isocitrate lyase (ICL) into glyoxylate and succinate in the glyoxylate cycle as opposed to -ketoglutarate in the other cycle. By passing the citric acid cycle's (TCA cycle) decarboxylation stages, basic carbon compounds can now be used in the subsequent production of polymers like glucose. Malate synthase then uses glyoxylate to join with acetyl-CoA to create malate (Figure. 1). Succinate dehydrogenase and fumarase work together to simultaneously make malate from succinate [1].

Alternative carbon usage, such as the production of fatty acids, ethanol, and acetate via acetyl-CoA, also produces malate, which refills the TCA cycle, which produces energy. In summary, the glyoxylate cycle intermediary allows for the absorption of substitute carbon

sources while acting as a connection between anabolic and catabolic fungus metabolism during glucose shortage. Isocitrate lyase and malate synthase are frequently regarded as the distinguishing features of this anaplerotic route because they are the only enzymes necessary for a working glyoxylate cycle. These crucial glyoxylate cycle enzymes are also very conserved in fungus, microbes, and plants. Both important enzymes are typically peroxisomal in fungus, with the notable exception of S. cerevisiae cytoplasmic isocitrate lyase. In phagocytosed *C. albicans* exposed to human blood, both important glyoxylate cycle enzyme genes, ICL1 (isocitrate lyase) and MLS1 (malate synthase), are greatly up-regulated. ICL1 has been shown to be necessary for the complete pathogenicity of this internal disease. Similar observations have also been made in Candida (NCAC) taxa other than *C. albicans*, such as *C. glabrata*. In a mouse model of invasive candidiasis, it has been found that the glyoxylate cycle is crucial for the metabolic adaptability and pathogenicity of C. glabrata. Loss of ICL1 caused much more abnormalities in *C. albicans* than in *C. glabrata* because it interferes with the development of glycerol as well as other carbon sources like ethanol, acetate, and fatty acids.

The glyoxylate cycle's use of different carbon sources in both species of Candida also caused numerous metabolic alterations linked to the fungi's virulence. Alternative carbon sources, for instance, had a significant impact on C. albicans' cell wall characteristics, resilience to external stressors and antimycotic drugs, immunological detection, fitness, and pathogenicity in vivo. The growth of *C. glabrata* on different carbon sources, such as glucose, acetate, lactate, ethanol, glycerol, and oleic acids, changed the fungus' cell wall (-glucan and chitin), promoted the development of biofilms, and made it more resistant to antifungal and oxidizing agents. Additionally, *C. glabrata's* contact with macrophages is greatly impacted by the absorption of acetic acid as an additional carbon source. The ICL1 and MLS1 homologs in Aspergillus species go by the names acuD and acuE, respectively.

It has been hypothesized that *A. fumigatus* does not rely on fatty acids and acetate as one of the main carbon sources for survival and invasive growth, as the acuD mutant is still capable of strong hyphal formation, in contrast to Candida species like *C. albicans* and *C. glabrata*, where the glyoxylate shunt is essential. After phagocytosis, A. fumigatus may briefly depend on other carbs and proteins while prioritizing forceful hyphal growth to aid in the destruction of macrophages and escape from the immune cells. Because of this, it is conceivable that different carbon usage routes (such as the methyl citrate cycle) will cooperate and make up for the absence of a functioning glyoxylate cycle. The deletion of the critical genes for the glyoxylate cycle also prevents *C. neoformans* from utilizing some carbon sources, including ethanol and acetate. Like *A. fumigatus*, loss of ICL1 and MLS1 does not seem to have an impact on *C. neoformans'* pathogenicity. Because of the lengthy history of connection and development between this fungus disease and the host, the glyoxylate cycle is only essential for the pathogenicity of Candida species.

While Candida species can only be found in mammalian hosts and lacks any obvious environmental reservoir, Aspergillus and Cryptococcus species can both be found in the environment and within a host, which may help to explain the different strategies used by these fungal pathogens in alternative carbon utilization [2]. The possibility of introducing different biochemical pathways into animals that do not naturally have them is a subject that interests bioengineers greatly today. One of the processes that technologists have tried to modify in human cells is the glyoxylate cycle. Engineers are mainly interested in this in order to boost sheep output of fleece, which is constrained by access to glycogen reserves. The huge stockpiles of acetate in cells could be used to make glucose through the cycle by bringing the route into sheep, enabling greater output of fleece. Isocitrate lyase and malate

synthase, two enzymes required for the cycle to occur, are absent in mammals, which prevents them from carrying out the process. However, some people think that the genes that make these enzymes are pseudogenic in animals, which means that the gene is not actually missing but is simply "turned off."

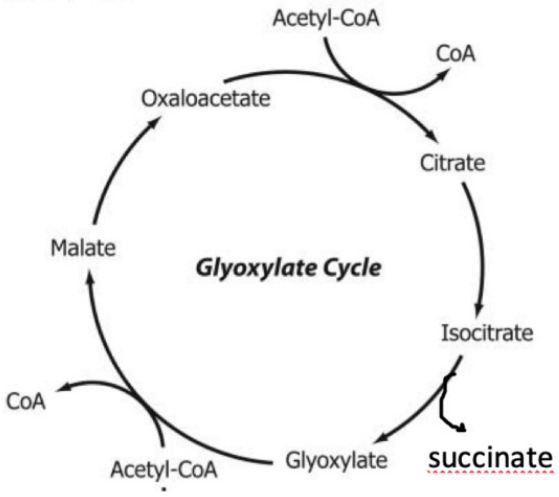

Figure 1: Glyoxylate cycle: Diagram showing the steps involved in the Glyoxylate cycle (Chegg).

The genes encoding the enzymes had to be isolated and sequenced to engineer the pathway into cells. This was accomplished using the bacteria E. coli, from which the AceA gene, which codes for isocitrate lyase, and the AceB gene, which codes for malate synthase, were sequenced. The AceA and AceB genes have been successfully inserted into mammalian cells in culture by engineers. The cells were able to translate and transcribe the genes into the appropriate enzymes, demonstrating that the genes could be successfully incorporated into the cell's DNA without endangering the cell's functionality or health. However, it has been challenging for researchers to design the route into mutant rodents. Although the DNA has been detected in some test animal organs, such as the liver and small intestine, the amount of translation is not statistically relevant. Engineers would need to combine the gene with regulators that could be controlled to raise the amount of expression and have the translation in the appropriate cells, such as epithelium cells, to effectively design the process.

Engineering the way into more complicated creatures, like sheep, has not been successful. This shows the need for much more study on the subject and raises the possibility that the cell's metabolism might not be able to support a high manifestation of the cycle in mammals. Scientists will be able to study and access the route for functional integration within the genome before the cycle is transferred to animals thanks to advancements in nuclear transfer technology. However, the lack of the cycle in rodent cells may have some advantages. The cycle is found in pathogenic microbes but missing in animals, such as humans. The possibility of creating antibiotics that target the glyoxylate cycle is very likely. These drugs would kill disease-causing microorganisms that depend on the cycle for survival while being safe for use in humans in the absence of the cycle and the enzymes the antibiotic would target.

DISCUSSION

The main reproductive resource in many higher plants is oil. This resource is activated following sprouting to promote growth during the first stages of embryo development. This biochemical mechanism depends on the glyoxylate cycle. It permits the production of

carbohydrates using acetyl-CoA produced by the decomposition of stored lipids. Recently, essential glyoxylate cycle enzyme-deficient Arabidopsis variants have been discovered. The first chance to examine the metabolic and physiological properties of the glyoxylate cycle in vivo in a soybean species came from an isocitrate lyase mutant [3].

A variant of the tricarboxylic acid cycle called the glyoxylate cycle is a metabolic mechanism that can be found in fungi, microbes, protists, and plants. To synthesize carbs, acetyl-CoA is converted to succinate in the glyoxylate cycle. The glyoxylate cycle in microbes enables cells to use basic carbon molecules as a carbon source in the absence of complicated sources like glucose. Animals are typically thought to lack a pattern. However, the discovery of malate synthase (MS) and isocitrate lyase (ICL), two essential enzymes in the glyoxylate cycle, in some animal tissue in recent years has called into question the evolutionary relationship between enzymes in bacteria and animals. It also suggests that animals encode alternative enzymes of the cycle that are functionally distinct from MS and ICL, which are known as MS and ICL in nonmetazoan species. The tricarboxylic acid cycle combines many of its intermediary stages with the glyoxylate cycle, which employs three of the five enzymes connected to it[4].

The majority of fungus infections in immunosuppressed individuals are caused by Candida albicans, a typical member of the gut microbiota of mammals. Neutrophils and macrophages typically phagocytose Candida, which causes it to release hormones and start growing hyphae. Patients with neutropenia, who lack these immune cells, are especially vulnerable to systemic candidiasis. Here, we use the genome-wide transcript patterns of the closely related yeast Saccharomyces cerevisiae to derive a hallmark of the fungi' internal processes in response to a human macrophage's consumption. Genes for the glyoxylate cycle, a metabolic process that allows the use of two-carbon molecules as carbon sources, are activated in live S. cerevisiae cells that have been separated from the phagolysosome. Isocitrate lyase (ICL1) and malate synthase, the two key enzymes of the glyoxylate cycle, are also increased by phagocytosis in *C. albicans*. ICL1-deficient variants of Candida albicans are significantly less pathogenic in rodents than in the normal type. The wide-ranging importance of the glyoxylate cycle in microbial disease is shown by these results in fungus and reports that isocitrate lyase is both elevated and necessary for the pathogenicity of Mycobacterium TB [5].

In soybean plants, the glyoxylate cycle is thought to be crucial for post germinative development and embryo establishment. Two allelic Arabidopsis mutants, designated icl-1 and icl-2, are deficient in the glyoxylate cycle due to a loss of the essential enzyme isocitrate lyase. These mutations show that germination can occur without the glyoxylate cycle. Additionally, during post germinative development, photosynthesis can make up for the lack of the glyoxylate cycle; plant settlement is only jeopardized when light levels or day duration are reduced. Exogenous carbohydrates can help to make up for this development deficit. The glyoxylate cycle is critical for seedling life and recuperation after extended dark circumstances that simulate development in nature, as shown by the icl mutants. Surprisingly, mutated plants can degrade stored lipids despite not being able to initiate the net conversion of acetate to glucose. According to the findings, lipids can be used as a source of carbon for respiration in oilseeds that are in the process of germination, and products of fatty acid degradation can move from the peroxisome to the mitochondrion without the assistance of the glyoxylate cycle. However, lipid decomposition and plant development call for an extra anaplerotic supply of carbon. In the lack of the glyoxylate cycle, foreign sugar or photosynthesis can serve as this supply [6].

By converting C2-units to C4-precursors for biosynthesis, the glyoxylate cycle enables the development on fatty acids and C2-compounds. It is no longer true, as was once believed, that plants and fungi's peroxisomes house the glyoxylate cycle. Enzymes involved in the glyoxylate cycle can be found both inside and outside the peroxisome. Therefore, several compounds must be transported across the peroxisomal membrane for the glyoxylate cycle to function. The development of the glyoxylate cycle also depends on mitochondrial respiration. Further research into the involved metabolite transporters in the peroxisomal membrane will be necessary to comprehend the operation, control, and interaction of the glyoxylate cycle with cellular metabolism [7].

We have earlier suggested that the control of cucumber malate synthase (MS) and isocitrate lyase (ICL) gene expression during plant growth depends on the metabolic state. In this study, we used a cell culture method to show that both of these genes' transcript levels are influenced by intracellular metabolic state. When sugar was added back to the culture media after starving cucumber cell cultures, the coordinated increase of the expression of the MS and ICL genes was undone. Although there was no correlation between the increase of gene expression and a reduction in respiratory rate, it was tightly associated with intracellular amounts of sugar, glucose, and fructose falling below cutoff levels. The suppression of MS and ICL also occurred in the presence of glucose, fructose, or raffinose in the culture media. In contrast to a third glyoxylate cycle gene, malate dehydrogenase, 2-deoxyglucose, and mannose, which hexokinase phosphorylates but does not further process, specifically suppressed the expression of MS and ICL. However, neither MS nor ICL was suppressed by the presence of 3-methyl glucose, a glucose derivative that is not phosphorylated. It is suggested that the content of hexose sugars inside cells or the flow of hexose sugars into glycolysis is the source of the signal that causes a shift in gene expression [8].

Acetyl-CoA can be transformed into anapleurotic and gluconeogenic substances using the glyoxylate cycle, which was first discovered by Kornberg et al. in 1957. Studies of several bacteria that can develop using C2 molecules as their only carbon source has shown that they lack the essential glyoxylate cycle enzyme isocitrate lyase, indicating that these bacteria have an alternative route or pathways for the absorption of acetate. Intriguing and intricate novel routes for acetate assimilation without isocitrate lyase have been discovered in recent investigations of acetate assimilation in methylotrophs and purple phototrophs. This MicroCommentary discusses the specifics of these novel paths[9].

It can be difficult for organisms to make all of the components of their cells from this C2-unit when they are growing on organic materials that are processed by acetyl-CoA. The solution, known as the glyoxylate cycle, was found in Kornberg's key study. The essential enzyme for this process, isocitrate lyase, is known not to be present in many microbes. Rhodobactersphaeroides was cultivated in acetate to solve this issue. The locus for -ketothiolase was impacted by an acetate-deficient mutation, which produced acetoacetyl-CoA from two acetyl-CoA molecules. This mutation, which grew on acetoacetate and acetate + glyoxylate, lacked this enzyme function. The gene for a suspected mesaconyl-CoA hydratase, an enzyme that catalyzes the hydration of mesaconyl-CoA to -methylmalyl-CoA, was impacted by a second acetate/acetoacetate-minus mutation. Glyoxylate and propionyl-CoA are produced by further cleavage of -methylmalyl-CoA. These findings, along with the discovery of proteins that are increased in response to acetate using two-dimensional gel electrophoresis, led to the suggestion of a novel route for acetate absorption. In the first section, which is mutated, two molecules of acetyl-CoA and one molecule of CO2 are changed to glyoxylate and propionyl-CoA via acetoacetyl-CoA and mesaconyl-CoA. In a

subsequent step, glyoxylate and propionyl-CoA are transformed into l-malyl-CoA and succinyl-CoA along with a further molecule of acetyl-CoA and CO2[10].

We discuss the extraction and identification of ICL1, a gene that produces isocitrate lyase, one of the key enzymes of the glyoxylate cycle, from the rice blast fungus *Magnaporthe grisea*.ICL1 exhibits increased expression during the formation of infectious structures and epidermis penetration, and a targeted gene substitution revealed that the gene is necessary for *M. grisea* to function at its highest level of pathogenicity. We discovered that loss of the glyoxylate cycle is particularly detrimental to the prepenetration stage of growth, which occurs before entrance into plant tissue. In icl1 mutations, epidermis invasion, infection-related growth, and germination are delayed. Recent studies have demonstrated the significance of the glyoxylate cycle in the pathogenicity of the bacterial disease *Mycobacterium TB* and the human infectious fungus *Candida albicans*. Our findings show that the glyoxylate cycle is significant in this plant's pathogenic fungus, highlighting the pathway's broad applicability in microbial disease[11].

The two glyoxylate cycle enzymes, isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2), were seen in promastigotes of five different types of *Leishmania.* (*L. brasiliensis, L. donovani, L. mexicana, L. tarentolae, and L. tropica*). Cells developed in a solution containing 10 mM glucose had both enzymes. The enzyme levels were not increased when 20 mM acetate was substituted for glucose. The cells quickly absorbed and processed acetate. A functioning glyoxylate cycle and its activity in gluconeogenesis/glyconeogenesis are indicated by the spread of labels from acetate into different intermediate molecules. The production of glyoxylate, the starting material for glycine biosynthesis, may also depend on the glyoxylate cycle, alanine-glyoxylate aminotransferase, and glyoxylate-aspartate aminotransferase [12].

CONCLUSION

Mammals do not have the glyoxylate cycle, also known as the glyoxylate shunt, but fungi, plants, and microbes do. The cycle serves an anaplerotic function in the supply of substrates for biosynthesis and is crucial for the development of two-carbon molecules like ethanol and acetate. The acetyl-CoA generated by the ß-oxidation of fatty acids is converted into succinate by the glyoxylate cycle, which takes place in the peroxisomes. The TCA cycle is then used to transform succinate into malate. The glyoxylate cycle is crucial for species that produce sugars from two-carbon sources like ethanol or acetate as well as for plant embryos that must produce sugars from triacylglycerols that have been conserved before they can germinate. Isocitrate lyase (ICL) and malate synthase (MS), two essential glyoxylate cycle enzymes, have been found in the livers of rodents given alloxan treatment.

REFERENCES:

[1] “Glyoxylate cycle,” *Wikipedia*. Feb. 28, 2020. Accessed: Mar. 25, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Glyoxylate_cycle&oldid=1142152591

[2] S. Y. Chew and L. T. L. Than, “Glucose Metabolism and Use of Alternative Carbon Sources in Medically-Important Fungi,” in *Encyclopedia of Mycology*, Ó. Zaragoza and A. Casadevall, Eds. Oxford: Elsevier, 2021, pp. 220–229. doi: 10.1016/B978-0-12-819990-9.00068-8.

[3] P. J. Eastmond and I. A. Graham, "Re-examining the role of the glyoxylate cycle in oilseeds," *Trends Plant Sci.*, vol. 6, no. 2, pp. 72–78, Feb. 2001, doi: 10.1016/S1360-1385(00)01835-5.

[4] H. Beevers, "4 - The Role of the Glyoxylate Cycle," in *Lipids: Structure and Function*, P. K. Stumpf, Ed. Academic Press, 1980, pp. 117–130. doi: 10.1016/B978-0-12-675404-9.50010-2.

[5] M. C. Lorenz and G. R. Fink, "The glyoxylate cycle is required for fungal virulence," *Nature*, vol. 412, no. 6842, Art. no. 6842, Jul. 2001, doi: 10.1038/35083594.

[6] P. J. Eastmond, V. Germain, P. R. Lange, J. H. Bryce, S. M. Smith, and I. A. Graham, "Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle," *Proc. Natl. Acad. Sci.*, vol. 97, no. 10, pp. 5669–5674, May 2000, doi: 10.1073/pnas.97.10.5669.

[7] M. Kunze, I. Pracharoenwattana, S. M. Smith, and A. Hartig, "A central role for the peroxisomal membrane in glyoxylate cycle function," *Biochim. Biophys. Acta BBA - Mol. Cell Res.*, vol. 1763, no. 12, pp. 1441–1452, Dec. 2006, doi: 10.1016/j.bbamcr.2006.09.009.

[8] I. A. Graham, K. J. Denby, and C. J. Leaver, "Carbon Catabolite Repression Regulates Glyoxylate Cycle Gene Expression in Cucumber.," *Plant Cell*, vol. 6, no. 5, pp. 761–772, May 1994, doi: 10.1105/tpc.6.5.761.

[9] S. A. Ensign, "Revisiting the glyoxylate cycle: alternate pathways for microbial acetate assimilation," *Mol. Microbiol.*, vol. 61, no. 2, pp. 274–276, 2006, doi: 10.1111/j.1365-2958.2006.05247.x.

[10] B. E. Alber, R. Spanheimer, C. Ebenau-Jehle, and G. Fuchs, "Study of an alternate glyoxylate cycle for acetate assimilation by Rhodobactersphaeroides," *Mol. Microbiol.*, vol. 61, no. 2, pp. 297–309, 2006, doi: 10.1111/j.1365-2958.2006.05238.x.

[11] Z.-Y. Wang, C. R. Thornton, M. J. Kershaw, L. Debao, and N. J. Talbot, "The glyoxylate cycle is required for temporal regulation of virulence by the plant pathogenic fungus Magnaporthe grisea," *Mol. Microbiol.*, vol. 47, no. 6, pp. 1601–1612, 2003, doi: 10.1046/j.1365-2958.2003.03412.x.

[12] M. W. Simon, E. Martin, and A. J. Mukkada, "Evidence for a functional glyoxylate cycle in the leishmaniae," *J. Bacteriol.*, vol. 135, no. 3, pp. 895–899, Sep. 1978, doi: 10.1128/jb.135.3.895-899.1978.

CHAPTER 25

THE HATCH-SLACK PATHWAY, AND THE CAM PATHWAY, ARE TWO DIFFERENT PATHWAYS FOR ASSIMILATING CARBON

Dr Pooja Agarwal, Assistant Professor
Department Of Pharmacology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email id- poojaagr.sgrrr@gmail.com

ABSTRACT:

Plants used a distinct biochemical pathway the C_4 pathway under various conditions to effectively repair CO_2 at low concentrations. When a plant was thriving in an arid environment, it used a variety of mechanisms to close its stomata during the day and sequester carbon at night. These plants can take more CO_2 from a given quantity of air thanks to various kinds of carbon fixation pathways that were assessed during evolution for the ability to do so, which helps them avoid water loss in arid regions. We outlined the various carbon absorption pathways in this paper, along with their importance.

KEYWORDS:

Bundle Sheath, C4 Photosynthesis, Carbon Fixation, Organic Acids, PEP Carboxylase.

INTRODUCTION

The Hatch-Slack pathway, also known as C4 carbon fixation, is one of three recognized photosynthesis carbon fixation mechanisms in plants. The labels are a result of Marshall Davidson Hatch and Charles Roger Slack's 1960 finding that some plants first attach the 14C marker to four-carbon compounds when given $^{14}CO_2$. In addition to the more typical and ancestor C_3 carbon fixation, C4 fixation also occurs. RuBisCO, the primary carboxylation enzyme in C_3 photosynthesis, catalyzes two separate processes using either CO2 (carboxylation) or oxygen (oxygenation) as a substrate. The useless process of photorespiration is created by the latter process, oxygenation. By clustering CO_2 around RuBisCO, C4 photosynthesis lowers photorespiration. C4 leaves typically distinguish two partly segregated sections known as mesophyll cells and bundle-sheath cells to guarantee that RuBisCO functions in an atmosphere with a lot of carbon dioxide and very little oxygen.

The enzyme PEP carboxylase fixes CO_2 at first in the mesophyll cells by reacting the three-carbon phosphoenolpyruvate (PEP) with the gas to create the four-carbon oxaloacetic acid (OAA). Chemically, OAA can be transmuted into aspartate or decreased to malate. As a result of these intermediates diffusing to the bundle sheath cells and being decarboxylated there, RuBisCO is surrounded by a CO_2-rich atmosphere, which inhibits photorespiration. The resulting pyruvate (PYR) diffuses back to the mesophyll along with about half of the phosphoglycerate (PGA) created by RuBisCO. To finish the reductive pentose phosphate cycle, PGA is chemically reduced and diffuses back to the bundle membrane (RPP). The functioning of C4 photosynthesis depends on this interchange of molecules. On the one hand, these extra processes demand more ATP energy to replenish PEP.

However, focusing CO_2 makes it possible for photosynthesis to occur at rapid rates even at high temps. Henry's rule states that a higher quantity surpasses a decreasing gas solubility with temperature. High variations of CO_2 content across the stomatal openings are also maintained by the CO_2 focusing process. As a result, C_4 plants typically have decreased stomatal permeability, less water loss, and better overall water use efficiency. Since RuBisCO is much more expensive to produce than PEP carboxylase, C_4 plants are also more

effective at using nitrogen. However, the C_3 route is more effective in situations where photorespiration is restricted, usually at low temps and in the shadow, as it does not require additional energy for the replenishment of PEP [1].

Kranz anatomy, named after the German term for circle, is a distinctive leaf architecture that is frequently found in C_4 plants. Two bands of cells encircle their arterial bundles; the inner ring, known as the bundle sheath cells, has chloroplasts abundant in glucose but missing grana, which is different from the chloroplasts found in the mesophyll cells present in the exterior ring. The chloroplasts are therefore referred to as dimorphic. Kranz anatomy's main purpose is to offer a location where CO_2 can be collected around RuBis CO, preventing photorespiration. Numerous intracellular bands are known as plasmodesmata, whose transparency at the leaf level is known as bundle sheath conductance, link mesophyll, and bundle sheath cells. To lessen the apoplastic passage of CO_2, suberin is frequently deposited at the level of the middle lamella (tangential contact between mesophyll and bundle sheath) (called leakage).

The C_4 plants' carbon concentration process sets them apart from other photosynthesis species in terms of their isotopic profile. Even though the majority of C_4 plants have a kranz structure, some species only have a partial C_4 cycle and lack any recognizable bundle sheath tissue. Terrestrial plants called chenopods, such as *Suaedaaralocaspica*, *Bienertia cyclopean*, *Bienertiasinuspersici*, and *Bienertiakavirense*, live in arid, saline depressions in Middle Eastern wastes. It has been demonstrated that these plants use single-cell C_4 CO_2-concentrating mechanisms, which are distinct from other C_4 mechanisms. Although the morphology of the two species is slightly different, the fundamental idea is the same: the cell is split into two distinct regions using fluid-filled vacuoles. Decarboxylase enzymes and RuBisCO in the chloroplasts are isolated from carboxylation enzymes in the cytoplasm. Between the cytoplasm and the chloroplasts, which house RuBisCO, is a diffusive barrier. As a result, a mesophyll-type region and a bundle-sheath-type area can develop within a single cell. Although a small C_4 cycle can run as a result, it is not very effective. It is common for CO_2 to escape from the area around RuBisCO. Hydrilla verticillata, a non-kranz marine macrophyte, has also shown signs of induced C_4 photosynthesis in temperate environments, though it is presently unknown how CO_2 leaking from the area around RuBisCO is minimized.

In C_3 plants, the enzyme RuBisCO fixes CO_2 to create 3-phosphoglycerate as the first stage in the light-independent processes of photosynthesis. RuBisCo, on the other hand, has both carboxylase and oxygenase action. The process of photorespiration occurs when oxygenation causes a portion of the substrate to be oxidized rather than carboxylated, resulting in substrate loss and energy consumption. The respective concentrations of oxygen and CO_2 affect how quickly the reactions proceed because oxygenation and carboxylation are competing processes. C_4 plants raise the CO_2 content around RuBisCO to slow the rate of photorespiration. Mesophyll and the bundle sheath, two partly separated regions within leaves, distinguish in this manner. Instead of being directly fixed by RuBisCO, CO_2 is first converted into a four-carbon organic acid in the mesophyll, either malate or aspartate. The organic acids then enter the bundle sheath cells via plasmodesmata. They are decarboxylated there, producing a CO_2-rich atmosphere. The bundle sheath cells' chloroplasts use the traditional C_3 route to turn this CO_2 into carbs. The molecular characteristics of C_4 absorption are highly variable, and they are typically divided into three subgroups (NADP-malic enzyme, NADP-ME; NAD-malic enzyme, NAD-ME; and PEP carboxykinase, PEPCK) based on the primary enzyme used for decarboxylation. It was suggested to divide the metabolic diversity into two categories because PEPCK is frequently recruited on top of

NADP-ME or NAD-ME. For instance, millet primarily employs NAD-ME and *Megathyrsus maximus*, while maize and sugarcane use a mixture of NADP-ME and PEPCK[2].

NADP-ME:The enzyme pyruvate phosphate dikinase converts pyruvate (Pyr) to phosphoenolpyruvate (PEP), which is the first stage in the NADP-ME type C4 pathway.(PPDK). PEP, AMP, and inorganic pyrophosphate are produced by this process, which calls for inorganic phosphate, ATP + pyruvate (PPi) (Figure. 1). The PEP carboxylase enzyme (PEPC) carboxylates PEP in the following process, resulting in the production of oxaloacetate. The mesophyll cells carry out both of these actions:

PEP + AMP + PPi = pyruvate + Pi + ATP

CO2 + PEP = oxaloacetate

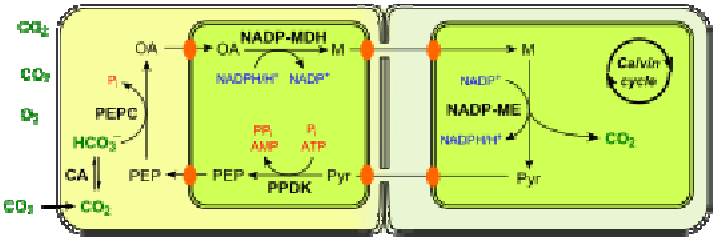

Figure 1: NADP-ME: Diagramed showing the NADP-ME pathway (Wikipedia)

PEPC has a low KM for HCO_3, which results in a strong affinity. It is also unaffected by O_2, allowing it to function even at low CO2 amounts. Malate (M), which diffuses to the bundle-sheath cells encircling a neighboring capillary, is the typical form in which the product is transformed. The NADP-malic enzyme (NADP-ME) decarboxylates it here, resulting in the production of CO_2 and pyruvate. While the pyruvate is carried back to the mesophyll cell along with about half of the phosphoglycerate, the CO_2 is fixed by RuBisCo to create phosphoglycerate (PGA). After being chemically reduced in the mesophyll, this PGA diffuses back to the bundle sheath and starts the Calvin cycle's conversion phase. The malate shuttle transmits two electrons for every CO_2 molecule transferred to the bundle sheath, decreasing the need for reducing power in the bundle sheath.

NAD-ME: Aspartate aminotransferase transforms the OAA generated by PEPC into aspartate (ASP), which diffuses to the bundle sheath. ASP is transaminated once more to OAA in the bundle sheath, where it then proceeds through fruitless reduction and oxidative decarboxylation to produce CO_2. Pyruvate that results from this process is transaminated to alanine and diffuses to the mesophyll (Figure. 2).

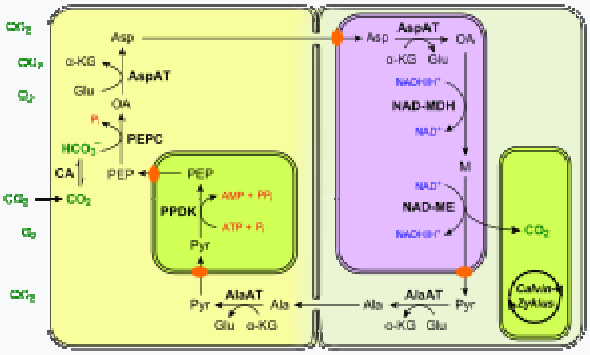

Figure 2: NAD-ME: Diagramed showing the NAD -ME pathway (Wikipedia)

Finally, pyruvate (PYR), which can be converted back into PEP by PPDK in the mesophyll chloroplasts, is formed from alanine through transamination. This cycle does not transmit reducing equivalents to the bundle sheath because it avoids the mesophyll's malate dehydrogenase process.

PEPCK: In this variation, PEPCK decarboxylases the OAA that aspartate aminotransferase produces in the bundle sheath into PEP. PEP's future is still up for discussion. The most straightforward answer is that PEPC would migrate back to the mesophyll to act as a medium (Figure. 3). The recycling of PEP through PEPCK would potentially improve this subtype's photosynthesis efficacy because PEPCK consumes just one ATP molecule, but this has never been observed.

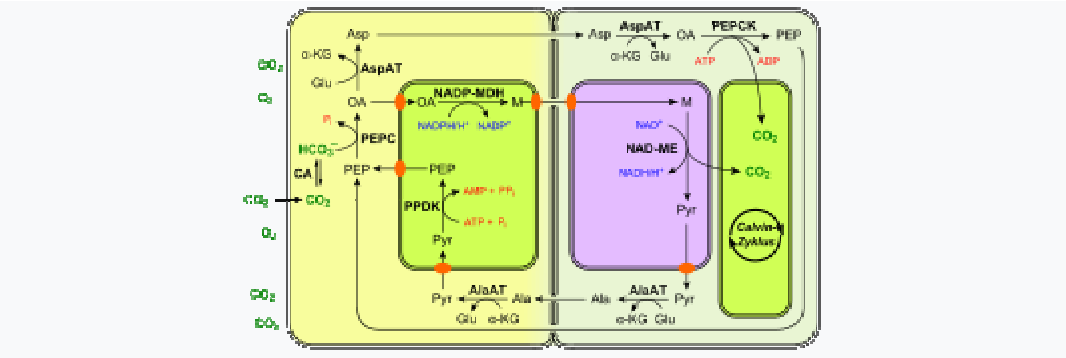

Figure 3: PEPCK: Diagramed showing the PEPCK pathway (Wikipedia)

Low light has been associated with a rise in PEPCK relative expression, which has been suggested to aid in regulating the energy needs of the bundle sheath and mesophyll. As a response to dry circumstances, some plants developed a carbon fixation process known as crassulacean acid metabolism, or CAM photosynthesis, which enables a plant to photosynthesize during the day but only trade gases at night. The stomata in the leaves of a plant that uses complete CAM close during the day to lessen evapotranspiration, but open at night to capture carbon dioxide (CO_2) and enable it to percolate into the mesophyll cells. During the day, the malate is carried to chloroplasts where it is transformed back to CO_2, which is then utilized during photosynthesis. At night, the CO_2 is kept as four-carbon malic acid in vacuoles. The enzyme RuBisCO is surrounded by a concentration of pre-collected CO_2, which improves photosynthesis efficacy. Plants in the Crassulaceae family were the ones that first revealed this process of acid digestion. Since CAM is a modification for more effective water use, it is frequently found in plants that thrive in dry environments. CAM is present in nearly all of the cacti that produce palatable flowers and in over 99% of the 1700 varieties of Cactaceae that are currently recognized.

In the evening: A CAM-using plant leaves its stomata open at night, enabling CO_2 to enter and be fixed as organic acids through a PEP reaction resembling the C_4 pathway. As the Calvin cycle requires ATP and NADPH, byproducts of light-dependent processes that do not occur at night, to function, the resulting organic acids are kept in vacuoles for later use (Figure. 4).

In the daytime: The mesophyll cells' vacuoles discharge the organic acids that store CO_2 during the day as the stomata shut to preserve water. The CO_2 is released by an enzyme in the membrane of chloroplasts, where it joins the Calvin cycle to enable photosynthesis. The C4 route and CAM are comparable in that they both work to consolidate CO_2 around RuBisCO, improving RuBisCO's effectiveness. By supplying CO_2 during the day and not at night, when breathing is the main response, CAM focuses it temporally. Contrarily, C_4 plants

geographically concentrate CO_2, flooding a RuBisCO reaction center in a "bundle sheath cell" with CO_2. In terms of PGA synthesis, C_4 carbon fixation is more effective due to the dormancy demanded by the CAM mechanism. Some species, like *Peperomia camptotricha*, *Portulaca oleracea*, and *Portulaca grandiflora*, are C_4/CAM intermediates. The two photosynthetic routes cannot pair and can only occur side by side in the same leaves, but they cannot occur in the same cells. Plants with CAM must manage the spatial and temporal distribution of CO_2 accumulation and its conversion to branched carbs.

When CAM-using plants open their stomata at low temps (often at night), CO_2 molecules migrate into the internal regions of the porous mesophyll before entering the cytosol. Here, they can come into contact with the modified triose phosphoenolpyruvate (PEP). PEP carboxylase kinase (PEP-C kinase), a protein that the plants are making at this time, can have its production suppressed by elevated temps (often during the day) and the presence of malate. The target enzyme PEP carboxylase is phosphorylated by PEP-C kinase. (PEP-C). The capacity of the enzyme to cause the production of oxaloacetate, which can then be converted into malate by NAD+ malate dehydrogenase, is markedly improved by phosphorylation. The vacuole is where malate is transformed into the storing form of malic acid after being carried there by malate shuttles. In contrast to PEP-C kinase, PEP-C is constantly produced but is almost completely blocked during the daytime, either by malate binding or dephosphorylation via PEP-C phosphatase. Since malate is effectively carried into the vesicle and PEP-C kinase easily inverts dephosphorylation, the latter is not possible at low temps.

Plants that use CAM in the daytime shut their guard cells and release malate, which is then carried into chloroplasts. Depending on the type of plant, the malic enzyme or PEP carboxykinase cleaves it there into pyruvate and CO_2. The Calvin cycle, a linked and self-recovering enzyme mechanism used to create branched carbs, is then activated with CO_2. The mitochondrial citric acid cycle can further decompose the byproduct pyruvate, producing more CO_2 molecules for the Calvin Cycle. Pyruvate phosphate dikinase is a high-energy process that uses pyruvate and another phosphate to restore PEP from pyruvate. PEP is ultimately transported into the cytosol the following cold night, where it participates in carbon dioxide fixation via malate [3].

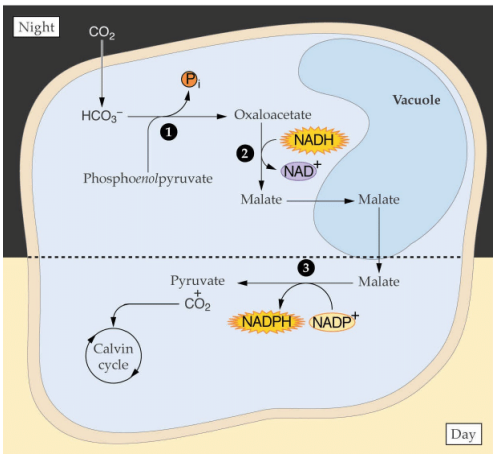

Figure 4: CAM Pathway:Diagramed showing the day and night cycle of the CAM pathway (Wikipedia).

The majority of CAM-producing plants are either epiphytes (e.g., orchids, bromeliads) or succulent xerophytes (e.g., cacti, *cactoid Euphorbias*), but it can also be found in hemiepiphytes (e.g., Clusia), lithophytes (e.g., *Sedum, Sempervivum*), terrestrial bromeliads,

wetland plants (*Sesuviumportulacastrum*). Only plants in the family Clusia, whose varieties are distributed throughout Central America, South America, and the Caribbean, are capable of CAM. In Clusia, species that live in harsher, dryer biological regions exhibit CAM, whereas those that reside in colder upland woodlands typically exhibit C_3. Additionally, facultative CAM refers to the ability of some Clusia species to briefly transition from C_3 to CAM photosynthesis metabolism. This enables these plants to take advantage of the higher development rates of C_3 photosynthesis during the wet season and the arid tolerance of CAM during the dry season. *Portulacariaafra*, also known as the dwarf jade plant, typically uses C_3 fixation but can switch to CAM if it is under drought stress, and Portulaca oleracea, also known as purslane, which typically uses C_4 fixation but has the ability to switch to CAM when under drought stress. Many times, CAM has developed convergently. Although this is believed to be a significant underestimation, it is found in 16,000 species or about 7% of plants, which are divided into more than 300 groups and roughly 40 families. Angiosperms (flowering plants) make up the vast bulk of CAM-using plants, but it can also be found in ferns, Gnetopsida, and quillworts (club moss cousins). The use of CAM by the first quillwort genome discovered in 2021 (*I. taiwanensis*) was interpreted as another instance of convergent evolution.

DISCUSSION

When circumstances favor high rates of photorespiration, C4 photosynthesis is a sequence of morphological and metabolic changes that consolidate CO2 around the carboxylation enzyme Rubisco and boost photosynthetic effectiveness. One of the most converging evolutionary events is the C4 pathway, which separately developed over 45 times in 19 families of angiosperms. With at least 30 families, the dicots are where C4 photosynthesis originated the majority of the time. In grasses, C4 photosynthesis likely first developed during the Oligocene period (24–35 million years ago). However, the majority of C4 dicot groups are thought to have emerged comparatively lately, possibly less than 5 million years ago. The oldest C4 dicots are most likely Chenopodiaceae individuals going back 15–21 million years. The beginning of C4 photosynthesis in dicots is associated with low-latitude dry areas, suggesting that salt, heat, and/or dehydration were significant factors in C4 development. Because it is necessary for high rates of photorespiration, low ambient CO2 is a major contributory element. C4 plants have consistently appeared in the genetic record during times of rising worldwide aridification and decreasing atmospheric CO2. The primary processes for producing C4 chromosomes are gene amplification, neo- and neofunctionalization, and selection for carbon conservation characteristics in environments with high photorespiration. This is what ultimately led to the genesis of C4 photosynthesis [4].

The development of meadows in temperate climes during the Late Miocene was facilitated by the emergence of the C4 photosynthesis pathway from the primordial C3 pathway in grasses. (8 to 3 million years ago). This was a significant turning point in the evolution of plants, and the high rates of leaf production they exhibited allowed for high animal consumption rates. Significant progress has been made in our knowledge of the ecology of C4 grasslands over the past ten years, and as a result, we now have a plethora of information on the ancient past of these habitats and a much greater grasp of the evolution of grasses. Examine this interdisciplinary field of study and make an effort to integrate new findings regarding the development of grass species in the framework of plant and habitat ecology [5].

In this study, we link C_4 photosynthesis' metabolic importance to natural plant growth. The physiological effects of the C_4 pathway on photosynthesis are first discussed, after which we move on to the ecophysiological performance of C_4 plants in various settings. The efficacy of

C_3 and C_4 plants in coexisting C_3 and C_4 environments is then compared. Finally, the spread of C_4 photosynthesis concerning the physical world, lineage, and life form is discussed[6].

The fundamental processes of this reaction are still mainly unknown, despite growing proof that C4 plants can accrue more biomass at higher CO_2 partial pressures ($p(CO_2)$). The present status of information regarding the rection of C4 plants to increased p(CO2) is reviewed in this paper, along with the most probable processes. We pinpoint two key mechanisms by which increased $p(CO_2)$ can promote the development of both well-watered and water-stressed C_4 plants. First, through accelerated intercellular p-mediated leaf CO_2 absorption rates (CO_2). Second, through decreased leaf evaporation rates as a result of decreased stomatal permeability. By preserving soil water, enhancing the relationship between the stalk and water, and raising leaf warmth, reduced evaporation rates can promote leaf CO_2 absorption and development rates. We contend that the high CO_2 responsiveness of C_4 photosynthesis cannot be explained by bundle sheath leakiness, direct CO_2 absorption in the bundle sheath, or the existence of C3-like photosynthesis in juvenile C_4 leaves. Important topics for pressing study include the effects of increased $p(CO_2)$, leaf temperature, and stem water relations on the development and photosynthesis of C_4 plants [7].

A notable example of adaptation in blooming plants is the development of crassulacean acid metabolism (CAM) photosynthesis in dry and/or low CO2 environments. The genetic and chromosomal resources of pineapple have been created over many years as it is the most significant product that uses CAM photosynthesis. The two taxa Ananas and Pseudananas and nine species have been reclassified into one genus Ananas and two species, *A. comosus,* and *A. macrodontes*, with five plant variants in *A. comosus* as a result of genetic diversification research using different kinds of DNA markers. Using F1 or F2 populations, five genetic maps have been created, and high-density genetic maps produced by genotype sequencing are crucial tools for decoding and compiling the pineapple genome as well as marker-assisted selection. While pineapple has few genetic sequences, there are many sites for transcript sequence tags. Only a small number of CAM plants from which the genes implicated in the CAM pathway have been studied are pineapple plants. The generation of a pineapple reference genome will hasten genetic and genomic study on this important CAM product.

This pineapple reference genome offers the basis for research into the development and regulation mechanisms of CAM photosynthesis, as well as the chance to assess the categorization of Ananas species and horticultural varieties [8].Plants have been found to possess THREE distinct types of photosynthesis pathways1,2: Three different photosynthesis pathways are present in most plants: (1) the reductive pentose phosphate or C3 pathway, where CO2 is incorporated into ribulose-1,5-diphosphate (RuDP) to produce two molecules of 3-phosphoglyceric acid, a three-carbon compound; (2) the C4 mode, where the first photosynthetic products are four-carbon dicarboxylic acids like oxaloacetate and malate formed following CO2 incorporation into In the final, stomatal opening and net CO2 absorption happen at night, with CO2 being converted into organic acids by PEP carboxylase. When the inwardly produced CO2 is stopped from exiting by closed stomata during the day, the organic acids are decarboxylated, causing the tissue acidity to drop.

Since the nighttime water vapor concentration differential between the tissue and surrounding air is smaller, CAM plants' nighttime stomatal opening results in total water saving. For instance, in natural circumstances, the average water loss per CO2 fixed is approximately six times higher for C4 plants and ten times higher for C3 ones than for CAM plants2. CAM plants tend to develop more slowly than C3 or C4 plants because their total daily CO2 absorption is lower[9].In contrast to C3 and C4 photosynthesis, crassulacean acid metabolism

(CAM) is a specialized type of photosynthesis that maximizes water use efficiency by shifting CO2 uptake to the night, when evapotranspiration rates are low. This is already a proven mechanism for drought resilience. To characterize the cellular behavior underlying CAM, a systems-level knowledge of timing molecular and biochemical regulation is required. Here, we describe high-resolution time patterns of RNA, protein, and chemical abundances throughout a CAM diel cycle and, where appropriate, relate the findings to those of the well-known C3 model plant Arabidopsis. A molecular discovery was that CAM works with an altered diel redox equilibrium in comparison to Arabidopsis. Additionally, we discover the extensive rescheduled expression of genes linked to signal transmission systems that control stomatal opening/closing. A timing system to control cellular activity is represented by the controlled synthesis and decay of transcripts and proteins. However, it is unclear how this molecular timekeeping controls CAM. In this paper, we offer fresh perspectives on the intricate post-transcriptional and translational networks that control CAM in Agave. These data sets serve as a resource for attempts to design commercially beneficial C3 cultivars with more effective CAM characteristics [10].

CONCLUSION

Plants known as C_4 and CAM use specific unique chemicals to capture carbon dioxide during photosynthesis. These substances enable these plants to increase the amount of CO_2 they can take from a particular volume of air, which helps them avoid water loss in arid regions. This pathway's function is to deliver carbon dioxide to the RPP pathway and for any carbon dioxide produced as a result of photorespiration. As a consequence, it reduces the energy loss brought on by the oxygenase activity of the RuBisCO enzyme in C_3 plants. Plants have modified their CAM system to conduct photosynthesis in stressful situations. Photorespiration is decreased by the CAM system. To minimize water loss during the day, CAM plants have open stomata at night, where they collect carbon dioxide. C_4 plants are more productive than C_3 plants because of their higher rate of photosynthesis and a lower rate of photorespiration. RuBisCO is the primary carbon-fixing enzyme in the Calvin cycle. It exhibits a preference for both CO_2 and O_2.

REFERENCES:

[1] "C_4 carbon fixation," *Wikipedia*. Mar. 18, 2021. Accessed: Mar. 25, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=C4_carbon_fixation&oldid=1145266044

[2] C. Bellasio and H. Griffiths, "Acclimation of C4 metabolism to low light in mature maize leaves could limit energetic losses during progressive shading in a crop canopy," *J. Exp. Bot.*, vol. 65, no. 13, pp. 3725–3736, Jul. 2014, doi: 10.1093/jxb/eru052.

[3] "Crassulacean acid metabolism," *Wikipedia*. Feb. 10, 2020. Accessed: Mar. 25, 2020. [Online]. Available: https://en.wikipedia.org/w/index.php?title=Crassulacean_acid_metabolism&oldid=1138574476

[4] "The evolution of C4 photosynthesis - Sage - 2004 - New Phytologist - Wiley Online Library." https://nph.onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8137.2004.00974.x (accessed Mar. 25, 2021).

[5] E. J. Edwards *et al.*, "The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science," *Science*, vol. 328, no. 5978, pp. 587–591, Apr. 2010, doi: 10.1126/science.1177216.

[6] R. W. Pearcy and J. Ehleringer, "Comparative ecophysiology of C3 and C4 plants," *Plant Cell Environ.*, vol. 7, no. 1, pp. 1–13, 1984, doi: 10.1111/j.1365-3040.1984.tb01194.x.

[7] "The growth response of C4 plants to rising atmospheric CO2 partial pressure: a reassessment - Ghannoum - 2000 - Plant, Cell & Environment - Wiley Online Library." https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-3040.2000.00609.x (accessed Mar. 25, 2021).

[8] J. Zhang, J. Liu, and R. Ming, "Genomic analyses of the CAM plant pineapple," *J. Exp. Bot.*, vol. 65, no. 13, pp. 3395–3404, Jul. 2014, doi: 10.1093/jxb/eru101.

[9] T. L. Hartsock and P. S. Nobel, "Watering converts a CAM plant to daytime CO2 uptake," *Nature*, vol. 262, no. 5569, Art. no. 5569, Aug. 1976, doi: 10.1038/262574b0.

[10] P. E. Abraham *et al.*, "Transcript, protein and metabolite temporal dynamics in the CAM plant Agave," *Nat. Plants*, vol. 2, no. 12, Art. no. 12, Nov. 2016, doi: 10.1038/nplants.2016.178.