

## Role of Long-Chain Hydroxy Fatty Acids in Modulating Membrane Fluidity And Cellular Signalling

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### Abstract:

*Triacyl glycerides and phospholipids are examples of more complex lipids that include natural fatty acids; these lipids provide structural and energy storage functions in cells. So, fatty acids, both synthetic and natural, have the potential to influence cell signaling via influencing lipid membrane structure, microdomain organization, and other physical features. Membrane lipid therapy is an approach that aims to regulate the of cell membranes by means of medication delivery, which may reverse pathogenic processes by means of structural re-adaptation of cell membranes. Membrane phospholipids' biophysical characteristics and activities are dictated by the three kinds of fatty acids that are incorporated into them. Through the integration of Through the use of functional two-photon microscopy (fTPM) of cellular membranes and lipidomic characterization of membrane phospholipids, this study aimed to examine how fatty acids affect the biophysical properties of membranes under different dietary and clinical situations.*

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### Introduction:

The structurally most basic kind of lipids are fatty acids (FAs), which consist of a hydrocarbon chain with anywhere from four to thirty-six carbon atoms with a carboxylic group (COOH) attached to one end. The majority of FAs contain a carbon chain with an even number of carbon atoms. Most biological lipids have this basic structure, which may be either a single chain or a network of branches that allow for the synthesis of more complex lipids. Some hydrocarbon chains have no hydrogen or carbon atoms in any of the available bonds; these chains Saturated fatty acids are what they're termed. In contrast, mono-unsaturated fatty acids (MUFAs) are hydrocarbon chains that include more than one double bond.

Infertility, dermatitis, and stunted development may result from a lack of fatty acids (FAs), which are essential for proper physiology and can be categorized according to the length of their carbon chains. It has been shown that shorter fatty acids (C4-10) stimulate leukocytes via signaling pathways and are rapidly absorbed for energy usage. The majority of fatty acid species found in mammalian cells are long chained fatty acids (LCFAs), which have a carbon chain length ranging from ten to twenty carbon atoms. A lot of the time, LCFAs serve as building blocks for more useful, more complicated structural molecules that cells may use. If they are not generated further, LCFAs may be used for energy during exercise, leading to a significant increase in LCFA metabolism in muscle.

In contrast to FAs with shorter carbon chains, which are more prevalent in the skin, retina, testes, and brain, fatty acids with very long carbon chains, or FAs with a chain length more than 20—are far rarer. A number of vital inflammatory processes, including sphingolipid biosynthesis, vesicular transport, and inflammation resolution, rely on VLCFAs, which LCFAs are unable to replicate. “Indeed, dietary ALA is a crucial dietary source of fatty acids and its dietary inclusion is critical for maintaining tissue long chain levels.” This is supported by growing evidence in humans, even though there is still debate about how much ALA guarantees the preservation of DHA and other long-chain polyunsaturated fatty acids in human newborns.

One way to get DHA is from dietary sources; another is the liver’s conversion of ALA to DHA, which is then transported to the bloodstream attached add an unsaturated fatty acid (FA) to serum albumin or esterify it as DHA-Lys phosphatidylcholine. It is crucial to maintain health by consuming a balanced amount of  $\omega 6$  and  $\omega 3$  polyunsaturated fatty acids (PUFA), which are both required for human nutrition. Atherosclerosis, obesity, and diabetes are more common in humans because of the prothrombotic and proinflammatory implications of an imbalanced diet that favors PUFA. Dietary reference values for lipids have been established in response to the importance of FA intake in humans. This includes recommending as little saturated and trans fats as possible, setting an adequate intake (AI) of 0.4 percent for ALA and 4% for LA as a proportion of total caloric intake, with EPA and DHA dosages ranging from 100 to 250 mg for children, adults, and women of childbearing age, respectively.

### Literature Review:

**de Carvalho, Carla et.al. (2018).** There is a wide range of chemical diversity among the molecules that make up lipids. The presence of fatty acids (FA) in the bulk of these substances makes them useful probes for investigations ranging from the microscopic to the macroscopic. Among FA’s many activities are structural support as phospholipids, lipids, the “building blocks” of cellular membranes; neutral lipid storage inside cells; and cell signaling via FA derivatives. Many scientific disciplines place a premium on studying FA and their metabolism. These include ecology, human nutrition, health, bacteriology, and biology. Biomarkers based on specific FA and their ratios in cell membranes might reveal relationships in food webs, research bacterial cell adaptability to harmful chemicals and environmental factors, and allow for the identification of species. This study focuses on the use of FA analysis to understand ecological processes and covers the different functions of FA in prokaryotes and eukaryotic organisms.

**Maulucci, Giuseppe et.al. (2016).** All living things have intricate and tightly controlled systems for maintaining metabolic homeostasis of fatty acids. Saturated fatty acid (SFA) biosynthesis in mammals supplies building blocks for  $\beta$ -oxidation and the creation of ATP. Desaturases add a methylene group to saturated fatty acids (SFA) in a cis-geometry, resulting in MUFA, or monounsaturated fatty acids for short. Both n-6 and n-3 polyunsaturated fatty acids (PUFA) are produced when the necessary Stretched out is linoleic acid and desaturated is  $\alpha$ -linolenic acid. As a result of liver processing, ingested fatty acids may be distributed and stored in peripheral tissues. and exports them in lipoproteins. Membrane phospholipids’ biophysical characteristics and activities are dictated by the three kinds of fatty acids that are incorporated

into them. Through the integration of Through the use of functional two-photon microscopy (fTPM) of cellular membranes and lipidomic characterization of membrane phospholipids, this study aimed to examine how fatty acids affect the biophysical properties of membranes under different dietary and clinical situations.

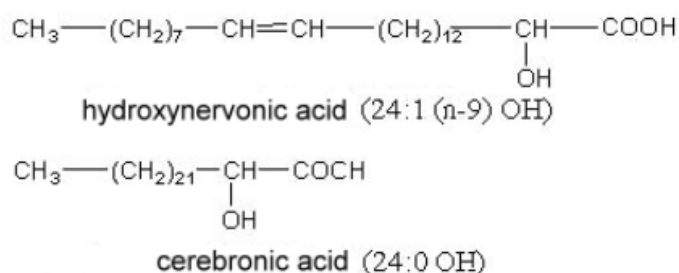
**Erdbrügger, Pia et.al. (2020).** The molecular properties of fatty acids (FAs) may vary greatly, including chain length, the amount of double bonds, and the locations of hydroxylation. VLCFAs are fatty acids having 22 carbon atoms or more in their chain. At the endoplasmic reticulum stands the of VLCFA synthesis, where enzymes embedded in membranes carry out a four-step elongation cycle. The production of sphingolipids (SLs) and glycerophospholipids begins with acids with a very long carbon chain (VLCFAs). The role of VLCFAs extends beyond that of lipid components; they are also present as lipid mediator precursors. Several hereditary disorders, including ichthyosis, myopathies, and demyelination, may be caused by an imbalance in VLCFA metabolism. Enzymes that have a role in very long chain fatty acid production have remained mostly unchanged throughout evolution, and *Saccharomyces cerevisiae* was the subject of several ground-breaking investigations. More and more evidence point to complex regulation of VLCFA metabolism as a means of lipid balance maintenance. With an emphasis on budding yeast, this review will outline the regulation of VLCFA metabolism, including its synthesis, transport, and degradation. We will go over the effects of VLCFAs on lipid metabolism and membrane characteristics, as well as the physiological consequences of biosynthetic gene alterations. Furthermore, we will provide a concise overview of illnesses that arise from the dysregulation of VLCFAs in human cells.

**He, Qiburi et.al. (2023).** The importance of essential fatty acids (FAs) in sustaining and enhancing development and health in mammals. Saturated LCFAs have at least one double bond in their carbon chain, whereas unsaturated LCFAs do not. This allows for easy categorization of LCFAs. Diet is the main source of essential fatty acids for humans and other animals, and mammals absorb long chain fatty acids (LCFAs) from extracellular sources. Passive diffusion and protein-mediated translocation across the plasma membrane are two pathways by which LCFAs enter cells. In the latter process, proteins such as FA translocase (FAT/CD36), FABPpm, FATP, and caveolin-1 are thought to play important roles. In order to convert them into acyl-CoA, the LCFAs that cells take up attach to FA-binding proteins known as FABPs). The correct organelles get them thereafter to target certain metabolic processes. An assortment of specialized lipids, including cholesteryl ester, triacylglycerol, and phospholipids, may be formed by esterifying LCFA-CoAs. It is preferred to store molecules of triacylglycerol containing free fatty acids that have not been esterified. Fatty acid catabolism mostly happens in mitochondria and peroxisomes via  $\beta$ -oxidation.

**Batsale, Marguerite et.al. (2021).** Very long-chain fatty acids (VLCFAs) are crucial chemicals that play an important role in plant structure and physiological processes. VLCFAs are fatty acids that include more than 18 carbon atoms. Essential for maintaining membrane homeostasis, VLCFA are found in particular in a number of membrane lipids. The proper functioning of the plasma membrane in intercellular communication is critically dependent on their unique accumulation in the sphingolipids of its outer leaflet. Phosphatidylserine and phosphatidylethanolamine are two examples of phospholipids that include VLCFA; these molecules may be involved in the coupling of interleaflet and the organization of membrane domains. Precursors of the plant cuticle's cuticular waxes, which play a pivotal role in many Vegetatively linked long-chain fatty acids (VLCFAs) are found in epidermal cells. Root suberin barrier, of which VLCFA are important components, is essential for maintaining nutritional homeostasis and allowing plants to adapt to harsh environments. Last but not least, VLCFA are stored by certain plants in the triacylglycerols of their seeds, where they function as an essential component in the germination process.

## Monohydroxy Fatty Acids:

Various plant and animal sources include  $\alpha$ -hydroxy acids, such as specialized tissues, the brain, skin lipids, wool waxes being the most abundant. The Labiateae *Thymus vulgaris* seed oil contained 13% 2-hydroxylinolenic acid. *Salvia nilotica*, another member of the Labiateae family, also contained 2-hydroxylinoleic and 2-hydroxyoleic. The polar lipids of the French alga *Grateloupia turuturu* contain several 2-hydroxy fatty acids. Cerebrosides are glycosphingolipides mostly present in nerve tissue and in very small amounts in plants. Cerebronic acid and the ceramide part of cerebrosides consists of two components: hydroxy nervonic acid.

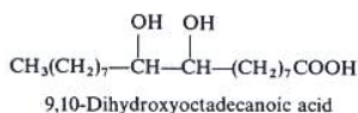


Sphingomyelin, bound to acid n-6 tetra- and pentaenoic 2-hydroxylation, is present in swine and rat testis and spermatozoa. These acids have very lengthy carbon chains, sometimes reaching 34 carbon atoms. These lipids may have a function in fertilization, according to certain theories.

## Polyhydroxy Fatty Acids

The seed oil of the Euphorbiaceae plant *Baliospermum axillare* contains an ester of 11,13-dihydroxy-tetracos-9t-enoic acid, a long-chain dihydroxy fatty acid. Axillarenic acid was its official name. Oils extracted from flowers include two types of dihydroxy fatty acids. A class of fatty acids known as tetrapedic acids was formed from 3Docosanoic and eicosanoic acids that are 7,2-dihydroxyphenolic. One of these compounds that might be di-acetylated is byrsonic acid (3,7-dihydroxy-docosanoic acid). Another finding was that a molecule of mono-acylated glycerol was being acylated by these fatty acids. In order to entice pollination insects, some floral structures called elaiophores create these oily substances. From the acid hydrolysis of the *Nannochloropsis* cell remains, two dihydroxy fatty acids, 15,16-dihydroxy-30:0 and 16,17-dihydroxy-33:0, have also been obtained.

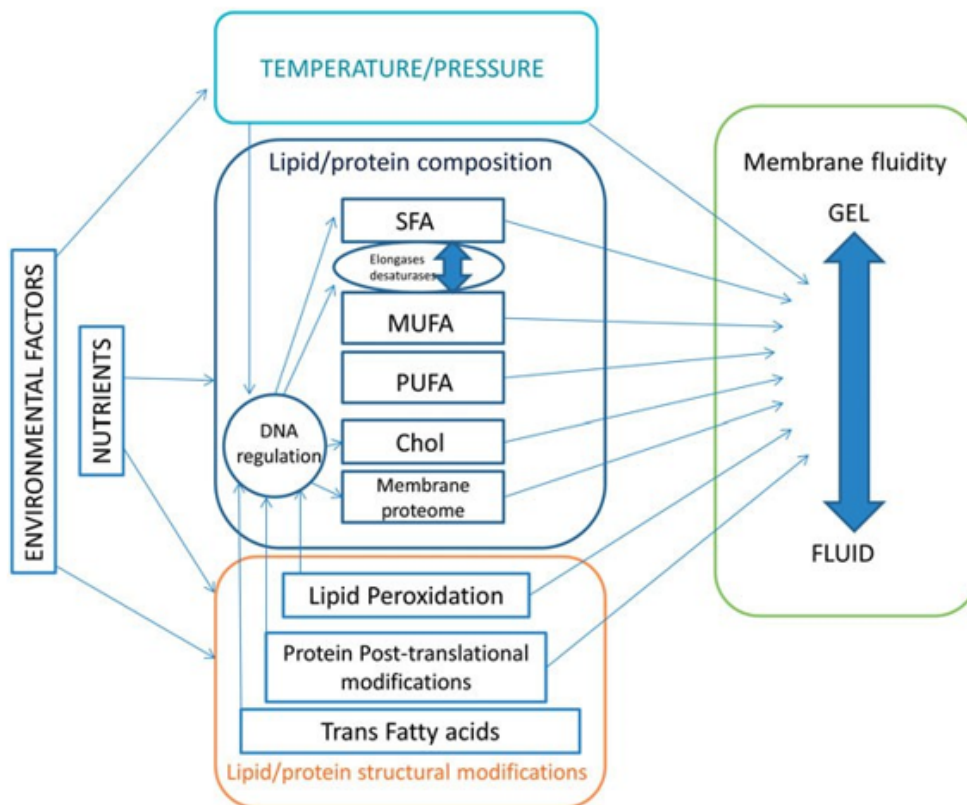
Approximately 25% of the oil in *Cardamine impatiens* seed oil is a combination of long-chain vicinal dihydroxy fatty acids; this plant is in the Cruciferae family of the Brassicaceae. In phytochemistry, this unusual structure is still unmatched. The following is an illustration using a C18 chain. Some compounds in the series include C20, C22, or C24 carbon atoms, but their hydroxyl groups stay in the same position relative to the methyl group at the end. Additionally, the seed oil of the Rutaceae plant *Feronia elephantum* contained 11% 9,10-dihydroxyoctadecanoic acid.



Rutaceae *peganium harmala* seed oil contains an isomeric form of 9,14-dihydroxyoctadecanoic acid, which is a combination comprising  $\omega$ -hydroxy acids with lengthy C16 and C18 chains with secondary sites for hydroxyl or epoxy groups. This form does not include vicinal hydroxy groups.

## Changes in Cellular Membrane Fluidity Induced By Fatty Acids:

The following items were procured from Biological Industries: L-glutamine, fetal calf serum (FCS), standard RPMI 1640 medium without glucose. Part V of bovine serum albumin and DMSO were supplied by Sigma-Aldrich (St. Louis, MO and Rehovot, Israel, respectively) From Eugene, OR's Molecular Probes, Inc., Laurdan was bought.



**Figure 1. Variables that cause variations in membrane fluidity. Nutrients and environmental variables change the fluidity of cell membranes via modifying:**

(1) environmental factors including pressure and temperature, (2) the makeup of lipids and proteins, and (3) the induction of changes to these components. Enzymatic modification of lipid content is the primary mechanism for maintaining the balance of fluidity across cell membranes.

## Effects of Natural And Synthetic Fatty Acids on Membrane Structure

Both free fatty acids and their phospholipid and triacyl glyceride partners may influence membrane structure. It just takes a few minutes for them to interact with membranes and be incorporated into more complicated molecules. It follows that the vesicle enlargement caused by externally injected OA in solutions of large unilamellar vesicles containing phosphatidylcholine (PC) occurs within three minutes, suggesting that the insertion of the FFA into the membrane structure occurs during that time. In a separate investigation, researchers found that enormous unilamellar vesicles made of palmitoyl-oleoyl-three minutes of incubation with OA, ARA, and DHA were unstable. It is worth noting that the same outcome was seen when their synthetic analogues 2-hydroxyoleic (2OHOA), 2-hydroxyarachidonic (2OHARA), and 2-hydroxydocosahexaenoic (2OHDHA) acid were used. Within 5 minutes of intravenous administration, the omega-3 eicosatetraenoic acid may be found in phospholipids and triacylglycerols in the myocardium, brain, and liver of rats, which is relevant information about Fatty acid esterification in nature.



It has been shown that U118 human glioma cells can detect the synthetic lipid 2OHOA in phosphatidylinositol, phosphatidylserine, PC, and PE 2–24 hours after incubation with this lipid. Ultimately, these acyl chains are able to alter the rapid intracellular movement of FFA and their incorporation into bigger molecules affect the structure of lipid bilayers. Atomic force microscopy, light scattering, electrophoresis, and differential scanning calorimetry (DSC), fluorescence spectroscopy, electron spin resonance, and differential thermal analysis are some of the methods that have been used to study how fatty acids influence the shape of model membranes since the seventies. The combined results of these investigations demonstrate that the transition temperature ( $T_m$ ) from Short-chain or cis-unsaturated fatty acids reduce the  $T_m$ , whereas long-chain saturated fatty acids boost the gel-to-fluid phase ( $L\beta$ -to- $L\alpha$ ) of phospholipid bilayers. Thus, the effect of naturally occurring FFAs on membrane lipid structure is dependent on their length and unsaturation degree.

Phase behavior, permeability, fusion, lateral pressure, and flip-flop dynamics are all aspects of fluidity. are all altered as a result of the lipid structural perturbations caused by these fatty acids in the membrane. An example of this would be the hypothesis that FFAs disrupt the interactions between proteins and lipids on human red blood cell membranes and the lipid bilayer. Since the lipid environment regulates the action of membrane proteins, membrane fluidity is crucial for cellular processes. Here, lipids may alter the biophysical characteristics of the membrane, which in turn affects the ideal shape for proteins to carry out their catalytic activities. An example of this would be the idea that FFAs influence the lipid bilayer via non-specific interactions.

**Incubation of phospholipids with various hydroxylated and non-hydroxylated fatty acids changes their phase transition temperature (Table 1).**

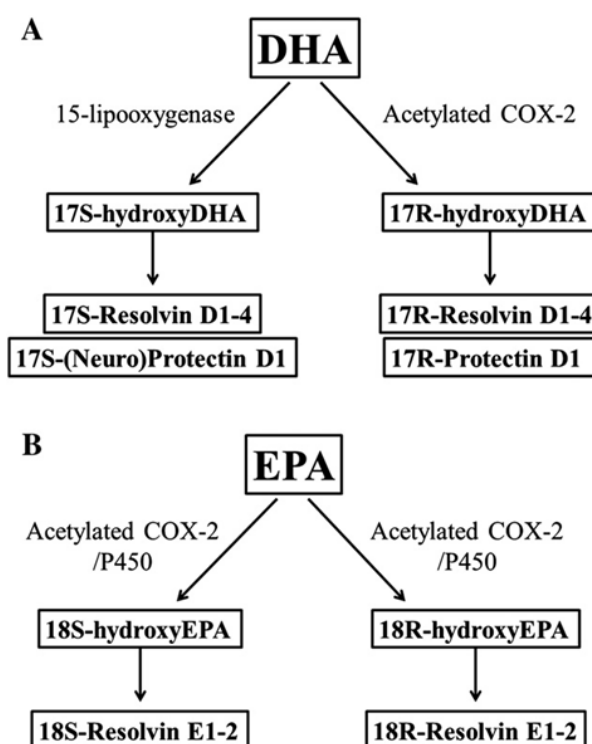
Lipid under study	Fatty acid	Effect	Technique
DPPC $L\beta$ -to- $L\alpha$ 40.8 °C	30 mol% SA	41.3 °C	<sup>a</sup> DSC, FL
	30 mol% OA	37.5 °C	
	30 mol% EPA	36.3 °C	
	30 mol% DHA	36.2 °C	
DEPE $L\alpha$ -to- $H_{II}$ 65.5 °C	5 mol% SA	66 °C	XRD
	5 mol% OA	53 °C	XRD, <sup>31</sup> P NMR
	5 mol% EA	59 °C	XRD
	5 mol% 2OHOA	55 °C	DSC, XRD
POPE $L\alpha$ -to- $H_{II}$ 70 °C	2.5 mol% OA	51 °C	XRD
DMPC $L\beta$ -to- $L\alpha$ 23.4 °C	5 mol% SA	24.3 °C	DSC
	5 mol% OA	22.6 °C	
	5 mol% (+)- Ricinoleic acid	22.0 °C	
	5 mol% R/S-2-OH octadecanoic acid	24.3 °C	
	5 mol% R/S-2-OH hexadecanoic acid	24.5 °C	
	5 mol% R/S-3-OH hexadecanoic acid	24.3 °C	

causing alterations in the shape and permeability of the membrane, which ultimately results in modifications to the sodium pump function inside human erythrocytes. Surface tension, which is influenced changes the

activity of human neutral sphingomyelinase in red blood cells due to changes in the bilayer's lipid composition, as has previously been shown.

### Natural Fatty Acids in Human Health

The fat in food items includes fatty acids, which give them taste, make them thicker, and make you feel full after eating. Fatty acids and other forms of fat not only aid in the absorption of vitamins A, D, E, and K, and they also provide a substantial quantity of energy. People cannot convert omega-6 fatty acids into omega-3 fatty acids or vice versa, and they also lack the enzymes  $\Delta 12$ - and  $\Delta 15$ -desaturase, which are in charge of creating a double bond at the carbon 12 and carbon 15 positions of an acyl chain, respectively. Thus, the omega-3 fatty acid  $\alpha$ -LNA and the omega-6 fatty acid are the two fatty acids that are essential for human good functioning and are consumed by diet. Additionally, LA. Human cells may use several metabolic processes to create additional fatty acids from these (Fig.2). Such processes include chain elongation reactions and desaturation,



**Fig. 2. Omega-3 and omega-6 fatty acid metabolism. Alternative omega-3**

The production of elongation of the essential  $\alpha$ -LNA fatty acid is involved in omega-6 fatty acid the desaturation of the LA fatty acid. In the endoplasmic reticulum, double bonds are added and acyl chains are elongated. In the peroxisome, the final step in making A single process involving  $\beta$ -oxidation is involved in the production of omega-3 DHA and omega-6 docosopentaenoic acid. Problems such as diabetes, cancer, eye problems, heart disease, mental illness, inflammatory diseases, renal disease, liver disease, aging, and neuromuscular issues are some of these conditions.

In addition to their many other vital roles, Essential fatty acids in the diet are necessary for healthy maturation, expansion, and equilibrium. There is mounting evidence that compounds produced from fatty acids may mediate important physiological processes, such as the control of cell communication and the activation and expression of genes. The pathophysiology of these diseases and the development of effective preventative interventions may be better understood if we know how fatty acids work biologically. Diets high in the omega-3 fatty acids EPA and DHA have been associated with a decreased risk of coronary heart

disease, for example. It was noted that the death rate of Greenland Eskimos due to coronary heart disease was 10% lower than that of Caucasians, which sparked interest in these lipids. The omega-3 polyunsaturated fatty acids were plentiful in the diet of aboriginal Eskimos, even though the body fat percentage in both groups was comparable. Asthma, psoriasis, and rheumatoid arthritis were less common among Eskimo people, together with the prevalence of CHD was also lower in this ethnic group.

Omega-3 fatty acid dietary supplements, known as nutraceuticals, have gained popularity due to the positive effects of these lipids. In contrast, saturated and unsaturated fatty acid consumption raises the danger of coronary heart disease, which promotes the growth of low-density lipoprotein (LDL) cholesterol. In addition, a number of nations' nutritional labeling regulations now require businesses to list the quantity of trans fat in each serving. Despite the fact that obesity has been a growing problem in recent decades, especially in industrialized nations, and is thought in order to significantly increase the likelihood of getting diabetes, heart disease, and cancer, among other diseases and conditions, very few studies have shown a direct correlation to obesity and the use of trans fats in the diet.

### **Therapeutic Approaches Using Synthetic Fatty Acids and Related Lipid Derivatives**

Glycogen metabolic syndrome has been successfully treated with sepiotanic acid and the triacyl glyceride triheptanoin, which are linked. The acyl chain moiety with seven carbon atoms goes through  $\beta$ -oxidation metabolism to produce propionyl-CoA, which is then carboxylated to succinyl-CoA. Anaplerosis, or the tricarboxylic acid cycle refilling, occurs in the metabolic route) when the latter, a metabolite of the Krebs cycle, is incorporated. It is believed that  $\beta$ -ketopentanoate and/or  $\beta$ -hydroxypentanoate, which are C5 ketones, may reach the brain after being converted to heptanoate in the liver.

For example, this chemical has shown promise in the treatment of Adult Polyglucan Body Disease (APBD), a subtype of glycogen metabolic syndrome IV. Adults of Ashkenazi Jewish descent are more likely to be affected with APBD, a rare neurodegenerative condition that normally manifests in the fourth or fifth decade of life and is marked by neurogenic bladder, gradual difficulties mobility, and issues with sensation in the lower extremities. As part of the pathophysiology of the illness, intracellular polyglucosan bodies (amylopectin-like polysaccharides) accumulate in cells of the central nervous system and peripheral nervous system. In many cases, this is accompanied by a partial deficiency of glycogen branching enzyme, a protein that helps with the  $\alpha$  (1  $\rightarrow$  6) glycosidic bond creation in glycogen. Theoretically, nerve cells experience an energy shortage as a result of reduced glycogen breakdown.

So, to fix the energy deficit, anaplerotic treatment could provide the citric acid cycle substrates. Patients with APBD who took the synthetic triglyceride triheptanoin as a dietary supplement showed a halt in disease development and, in the majority of instances, only partial functional improvement, according to recent research. Another possible therapy for epilepsy is being shown to be an effective anticonvulsant in models of both acute and chronic epilepsy, oral triheptanoin seizures in mice. The medicinal potential of bacterial lipids has also been investigated.

Antitumoral and somewhat endotoxic, Lipid A has a synthetic derivative called ONO-4007. Composed of two glucosamine units connected by acyl chains and often one phosphate group on each carbohydrate, lipid A is an endotoxin in Gram-negative bacteria. Oddly enough, it failed to inhibit the growth of any TNF-resistant cell lines tested, while its synthetic analogue, ONO-4007, showed promise against KDH-8, a rat hepatoma cell line that is susceptible to tumor necrosis factor (TNF). It seems that the mechanism of action of ONO-4007 is connected to increased production of TNF $\alpha$  in tumor tissues, and these findings imply that it might be therapeutically beneficial for treating malignancies that are susceptible to TNF $\alpha$ .

### **Conclusion:**



It is now known that fatty acids may do more than only provide energy or make up complex lipids; they may also influence cellular processes via influencing membrane modifications. There is evidence that both synthetic and naturally occurring fatty acids may alter biophysical properties of membranes, including their permeability, fluidity, and domain formation. Additionally, these acids can influence cellular processes like antiproliferative control, membrane protein activities, and cell fusion transmission. The seed oil of the Euphorbiaceae plant *Baliospermum axillare* contains a long-chain dihydroxy fatty acid known as 11,13-dihydroxy-tetracos-9t-enoic acid was found. Axillarenic acid was its official name.

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