Lipids: An insight into the neurodegenerative disorders

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S U M M A R Y
Brain development is a sequential anatomical process characterised by specific well-defined stages of growth and maturation. One of the fundamental and necessary events in the normal development of the central nervous system in vertebrates is the formation of a myelin sheath. This process is influenced by dietary lipids. A number of researches have indicated that the administration of a diet, deficient in essential fatty acids during development causes hypomyelination in the brain. Brain lipids determine the localization and function of proteins in the cell membrane and in doing so regulate synaptic signalling in neurons. Lipids may also function as transmitters and relay signals from the membrane to intracellular compartments or to other cells. Several experimental studies have suggested a crucial role of n-3 polyunsaturated fatty acids in membrane formation, as well as clinical role of glycerolipids, glycerophospholipids, and sphingolipids in the attenuation of depression- and anxiety-related behaviours. Hence it can be assumed that polyunsaturated fatty acids may also offer new treatment options (for example, targeted dietary supplementation or pharmacological interference with lipid-regulating enzymes). These lipids could be exploited for improved prevention and treatment. A very interesting and emerging approach in this direction is through 'Lipidomics' which is a relatively recent research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational...
Lipids play a pivotal role in normal physiological function of the neurons and structural development of the brain. The lipid composition of the brain highly governs mood, perception and emotional behaviour of the subject. There are eight different classes of lipids that form the central nervous system [1]: Fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides.

The lipids have variety of functions like formation of lipid bilayers that form the structure and provide necessary channel for protein function, function as an energy reservoir (for example triglycerides) and serve as precursors for various secondary messengers such as arachidonic acid (ArAc), docosahexaenoic acid (DHA), ceramide, 1,2-diacylglycerol (DAG), phosphatidic acid and lysophosphatidic acid. The normal functions of these lipids govern the overall normal physiology of the brain. Any abnormal deviation from the normal function of brain, either due to any mechanical injury or due to pathological changes in neurons, leads to different types of neurodegenerative diseases, mental disorders, stroke and CNS traumas. Currently there exists no cure for these CNS injuries and disorders, resulting in a huge impact on quality of life. The crucial role of lipids in tissue physiology and cell signalling is demonstrated by the many neurological disorders. Both, neurological disorders and neurodegenerative diseases involve unregulated lipid metabolism. Altered lipid metabolism is also believed to be a key event which contributes to CNS injury [2].
1.1. Lipids and neurodegenerative disorders

In order to understand the link between lipids and neurodegenerative disorders we need to first focus and understand role of different lipids in the development of these diseases, the following section of the paper highlights the most common neurodegenerative disorders with role of lipids involved at different stages of each disease.

1.2. Alzheimer’s disease (AD)

It is the most common form of dementia, a progressive brain disorder affecting regions of the brain that control memory and cognitive functions, gradually destroying a person’s memory and ability to learn, reason, communicate and carry out daily activities. AD is broadly divided into sporadic or late onset AD (90–95% of AD) and early onset (occurring in persons under age 65, 5–10% of AD). Biochemically, cholesterol plays a very important role in the aetiology of this disease. Apolipoprotein E, the most abundantly found apolipoprotein in plasma is the principal carrier protein of cholesterol in brain. Identification of the gene encoding the variant ApoE4 (APOE e4 allele) as a significant risk factor for AD provided evidence for a role of cholesterol in the pathogenesis of AD [1–5].

It has been reported that AD is characterised by overproduction of a protein named Amyloid-β protein which has 2 subunits; Aβ42 and Aβ40. This protein leads to the formation of plaques in the brain causing neuritic atrophy. Aβ40 inhibits HMG-CoA reductase, an enzyme important for cholesterol and lipid synthesis while Aβ42 activates neutral sphingomyelinase (N-SMase) and increases ceramide production, which can accelerate the neurodegenerative process. On these grounds, it may be presumed that if lipid rich dietary supplement along with Aβ40 & Aβ42 antagonist based approach is designed for palliative treatment of Alzheimer’s it may prove efficacious [6–14].

2. Role of oxidative stress and lipid peroxidation in Alzheimer’s disease

A number of studies illustrate increased lipid peroxidation in AD. Lipid peroxidation results in formation of acrolein, the strongest electrophile among all α, β-unsaturated aldehydes [15–19].

It reacts with DNA bases including guanine, adenine, cytosine and thymidine to form cyclic adducts, the major exocyclic adduct being acrolein-deoxyguanosine. Increased levels of acrolein-deoxyguanosine adducts were recently demonstrated in brain tissue from AD patients. Reactive oxygen species (ROS) may also play a role in amyloid deposition in AD as oxidizing conditions cause protein cross-linking and aggregation of Aβ peptides. Aβ aggregation has been shown to induce accumulation of ROS, which may lead to cyclic or self-propagating oxidative damage. AD and Mild Cognitive Disorder subjects also showed lower levels of antioxidant defence systems. Thus, this signifies that oxidative stress plays a pathologically important role in disease progression [20–23].

2.1. Parkinson’s disease (PD)

This disease is characterized by selective degeneration of dopaminergic neurons present in the substantia nigra, a basal ganglia structure located in the midbrain that plays an important role in movement, leading to symptoms like bradykinesia, tremor and rigidity. The level of dopamine in brain falls due to hyperactivity of an enzyme Monoamine oxidase-B. This results in build up of oxidative stress caused by free radical generation and lipid peroxidation. One of the factors responsible for this stress is believed to be phospholipases activation in substantia nigra. Some recent research suggests that PD is also associated with formation of Lewy bodies in brain, which are small agglomerates of protein named α-synuclein. These lewy bodies remain soluble in brain, but few aggregates that were associated with polyunsaturated fatty acids (PUFA) remained insoluble, making them asymptomatic [24–26].

Although, there is a line of treatment for PD which includes use of drugs like Levodopa, Carbidopa and MAO inhibitors but their extra pyramidal side effects (like, bradykinesia) remains a loophole of these drug therapy. A fatty acid named docosahexaenoic acid has been reported to stimulate oligomerization of α-synuclein. Recent studies have shown increased levels of docosahexaenoic acid (DHA)
in PD brains compared to controls, suggesting that DHA could have a role in formation of insoluble α-synuclein aggregates which do not show PD symptoms. Interestingly, DHA reduced levodopa-induced dyskinesia indicating that DHA can reduce the severity or delay the development of levodopa-induced dyskinesia. Hence, it could be concluded that DHA may represent a new approach to improve the quality of life of Parkinson’s disease patients [27–29].

3. Role of oxidative stress and lipid peroxidation in Parkinson’s disease

In PD, the accelerated metabolism of dopamine by monoamine-oxidase-B may result in excessive reactive oxygen species formation. The oxidative stress in PD is marked by increase in 8-hydroxy-2’-deoxyguanosine, a hydroxyl radical-damaged guanine nucleotide commonly used to evaluate oxidative damage to DNA. Furthermore, several markers of lipid peroxidation were also found to be significantly increased in PD brain regions such as the concentration of poly unsaturated fatty acids in the substantia nigra is decreased, while that of malondialdehyde, a marker of lipid oxidation, is increased. Additional evidence of lipid oxidation in PD is provided by the demonstration of an increase in 4-hydroxy-2-nonenal, a lipophilic product of the peroxidation of membrane bound arachidonic acid. Thereby, this indicates that pathogenesis of Parkinson’s involves peroxidation of lipids which accelerated the metabolism of dopamine [30,31].

3.1. Multiple sclerosis (MS)

It is an inflammatory demyelinating autoimmune disease affecting the CNS. Symptoms range from relatively benign to severely disabling, in which communication between the brain and other parts of the body is disrupted, rendering a person unable to write, speak, or walk. In MS, the immune system attacks the myelin sheath of nerve cell fibres in the brain and spinal cord. MS is predominantly a T lymphocyte mediated disorder, and cytokines may therefore have a key role in the pathogenesis of the disease. MS is marked by increased levels on pentane and ethane in urine; both are the degradation products of unsaturated fatty acids. Thiobarbituric acid reactive substances and F-isoprostane levels were shown to be elevated in CSF of MS patients. Thus, both these evidences and pathological findings in urine as well as CSF shows that peroxidation of lipids is an important biomarker in pathogenesis of MS [32–36].

3.2. Huntington’s disease (HD)

Huntington disease is an autosomal dominant neurological disorder characterized by behavioural abnormalities, cognitive decline, and involuntary movements that lead to a progressive decline in functional capacity, independence, and ultimately death. The pathophysiology of Huntington disease is linked to an expanded trinucleotide repetition of cytosine-adenine-guanine (CAG) amino acids in the IT-15 gene on chromosome 4. There is no disease-modifying treatment for Huntington disease, and novel pathophysiological insights and therapeutic strategies are needed. Lipids are vital to the health of the central nervous system, and research in animals and humans has revealed that cholesterol metabolism is disrupted in Huntington disease. This lipid dysregulation has been linked to specific actions of the mutant huntingtin gene on sterol regulatory element binding proteins. This results in lower cholesterol levels in affected areas of the brain with evidence that this depletion is pathologic. Huntington disease is also associated with a pattern of insulin resistance characterized by a catabolic state resulting in weight loss and a lower body mass index than individuals without Huntington disease. Insulin resistance appears to act as a metabolic stressor attending disease progression. The fish-derived omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, have been examined in clinical trials of Huntington disease patients. Drugs that combat the dysregulated lipid milieu in Huntington disease may help treat this perplexing and catastrophic genetic disease [44–47].

A group of British researchers conducted a 6-month randomized, placebo-controlled pilot study of the ethyl-ester of eicosapentaenoic acid (ethyl-EPA) carried out in seven in-patients with advanced (stage III) Huntington’s disease. Out of seven patients four were on placebo and three on ethyl-EPA and there was no significant difference between age and sex between the groups. After 6 months study
patients treated with ethyl-EPA improved on the orofacial component of the United Huntington’s Disease Rating Scale while all the patients on placebo deteriorated on this scale \( (p < 0.03) \). In the follow-up 3D MRI brain scans placebo patients showed progressive cerebral atrophy. Researchers inferred that the patients treated with ethyl-EPA showed beneficial motor and MRI changes \[48\]. As an extension of the above work Puri and colleagues completed a multicenter, double-blind, randomized, placebo-controlled trial on 135 patients received either 2 g/day ethyl-EPA or placebo. The primary end point was outcome at 12 months on the Total Motor Score 4 subscale (TMS-4). Out of 135 patients 121 patients conducted the study and 83 completed the study without protocol violations (PP cohort). Intent-to-treat (ITT) analysis revealed no significant difference between ethyl-EPA and placebo for TMS-4. In the PP cohort, ethyl-EPA proved better than placebo on the \( \chi^2 \) test on TMS-4 \( (p < 0.05) \), but missed significance on ANCOVA \( (p = 0.06) \). Secondary end points (ITT cohort) showed no benefit of ethyl-EPA but a significantly worse outcome in the behavioural severity and frequency compared with placebo. Hence further investigations are required to assess the efficacy of EPA \[45\]. However significant TMS-4 improvement is observed in patients with fewer CAG \[49\].

In a recent study conducted on apolipoprotein E deficiency \( (\text{ApoE}^{-/-}) \) mice for investigating lipid composition in atherosclerosis-induced dyslipidemia inferred disturbance on sphingolipid and glycerophospholipid metabolism particularly involving 6 phosphatidylcholines (PCs) 1 sphianganine (SP) and 3 sphingomyelins (SMs) The lipid species were profiled utilizing chromatography coupled with time of flight mass spectrometry \( (\text{UPLC-Q/TOF-MS}) \). However lipidomics should be further applied to low-density lipoprotein \( (\text{LDL}) \) receptor knockout mice to extensively investigate the atherosclerotic dyslipidemia in plasma \[50\].

Lipidomics provide a deep insight in the cellular mechanisms involving dysregulation of lipid metabolism disorders such as obesity and type 2 diabetes \( (\text{T2D}) \). The main transgressor in lipid induced insulin resistance is DAG. In muscle accumulated DAG species were delineated to recruit novel PKC isoform nPKC\( \beta \) to plasma membrane resulting to its activation, sequent inhibitory phosphorylation of IRS1, resulting in inhibiting insulin signaling and subsequently glucose uptake \[51–53\]. Similarly insulin signalling is affected in liver by inhibiting the activation of IRS proteins via recruitment of nPKCe in a DAG dependent manner \[54\]. Moreover accumulated ceramide species reduce insulin sensitivity through inhibition of AKT activity by activation of protein phosphatase 2A \( (\text{PP2A}) \) resulting in its inhibitory dephosphorylation \[55\]. Further investigations inferred reduction in insulin sensitivity due to activation of inflammatory pathways resulting due to binding of saturated fatty acids to toll-like receptor 4 \( (\text{TLR4}) \) \[56\]. Analogously accumulation of acylcarnitines resulting due to decrement or impairment of \( \beta \)-oxidation of fatty acids in mitochondria results in decreased insulin sensitivity \[57\].

### 3.3. Schizophrenia and bipolar disorders

Schizophrenia is marked by disturbances in thinking, emotional reactions, social behaviour, with delusions and hallucinations. Drugs that block dopamine receptors alleviate symptoms of schizophrenia, indicative of excess dopaminergic function, while agents that block glutamate receptors induce some of the symptoms of schizophrenia in otherwise normal persons. Recent theories on the neurological deficits of schizophrenia have focused on abnormalities in phospholipid metabolism, particularly increased activity of PLA enzymes and reduced activity of the system which incorporates PUFAs into phospholipids (a simultaneous increase in phospholipid hydrolysis and decrease in synthesis). Docosahexaenoic acid \( (\text{DHA}) \) and eicosapentaenoic acid \( (\text{EPA}) \) are two important fatty acids for monoamine neurotransmission, brain development, and synaptic functioning. It is well known that monoamine oxidation is responsible for development of neurological disorders, BHA \& EPA help in enhancing monoamine mediated neurotransmission and prevent disease progression. Hence, supplementation with essential fatty acids may alleviate symptoms of schizophrenia \[57–60\].

All the neurodegenerative disorders discussed in this review have one thing in common and that is the role of lipids in their pathogenesis. The oxidative stress generated by the peroxidation of lipids is the key pathological event of most of the disorders; hence the next part of this review focuses on the descriptive classification of lipid family and discussion of those lipids in detail that are specifically associated with the neurodegenerative diseases along with the importance of ‘Lipidomics’ in identification of these lipids as potent biomarkers.
3.4. The biomarker lipids for neurodegenerative disorders

Fahy and co-workers (2005) proposed a classification system for lipids with a universal platform compatible with bioinformatics requirements. Recently, the classification system has been revised to include lipid structures from bacteria, fungi and plants [1]. As a result, lipids are regrouped under the following eight categories that cover eukaryotic and prokaryotic sources:

1. **Fatty acyls** (fatty acids, hydroxyl-fatty acids, and so on),
2. **Glycerophospholipids** (phosphatidylcholines [PC], phosphatidylethanolamines [PE], phosphatidylglycerols, phosphatidylserines [PS], phosphatidylinositols, phosphatidic acids [PA], cardiolipins, and so on),
3. **Glycerolipids** (monogalactosyldiacylglycerol, diacylglycerides, digalactosyldiacylglycerol, triacylglycerides, and so on), sphingolipids (sphingosines, sphingosine-1-phosphates, ceramides [Cer], sphingomyelins [SM], ganglioside mannoside 3 [GM3], and so on),
4. **Sphingolipids** (Phosphosphingolipids and ceramides)
5. **Prenol lipids** (quinines, polypreols, isoprenoids, and so on),
6. **Sterol lipids** (cholesteryl esters [CE], cholesterols [Chol], cholesteryl sulfates [CS], and so on),
7. **Saccharolipids**
8. **Polyketides**

3.5. Fatty acyls; Omega-3 and Omega-6 poly unsaturated fatty acids

Essential polyunsaturated fatty acids (PUFAs) are critical nutritional lipids that must be obtained from the diet to sustain homeostasis. Omega-3 and Omega-6 PUFAs are key components of biomembranes and play important roles in cell integrity, development, maintenance, and function. The essential omega-3 fatty acid family member docosahexaenoic acid (DHA) is avidly retained and uniquely concentrated in the nervous system, particularly in photoreceptors and synaptic membranes. DHA plays a key role in vision, neuroprotection, successful ageing, memory, and other functions. In addition, DHA displays anti-inflammatory and inflammatory resolving properties in contrast to the proinflammatory actions of several members of the omega-6 PUFAs family. This review discusses DHA signalolipidomics, comprising the cellular/tissue organization of DHA uptake, its distribution among cellular compartments, the organization and function of membrane domains rich in DHA-containing phospholipids, and the cellular and molecular events revealed by the uncovering of signalling pathways regulated by DHA and docosanoids, the DHA-derived bioactive lipids, which include neuroprotectin D1 (NPD1), a novel DHA-derived stereoselective mediator. NPD1 synthesis agonists include neurotrophins and oxidative stress; NPD1 elicits potent anti-inflammatory actions and prohemostatic bioactivity, is anti-angiogenic, promotes corneal nerve regeneration, and induces cell survival. In the context of DHA signalolipidomics, this review highlights ageing and the evolving studies on the significance of DHA in Alzheimer's disease, macular degeneration, Parkinson's disease, and other brain disorders. DHA signalolipidomics in the nervous system offers emerging targets for pharmaceutical intervention and clinical translation [61–65].

**Docosahexaenoic acid** (DHA) is the most abundant polyunsaturated fatty acid in the brain, and is present in the form of aminophospholipids in cell membranes. Although the underlying mechanisms of its essential function are not clear, emerging evidence suggests that unique metabolism of DHA plays an important role [88]. The studies on the role of Phospholipase A2 (PLA2) and Phospholipase A (PLA) hydrolysis products, accidentally identified the DHA metabolites, resolvins and protectins including 10,17-docosatriene (Neuroprotectin D1) following cerebral ischaemia/reperfusion in mouse. Neuroprotectin D1 was found to serve an endogenous neuroprotective role by inhibiting apoptotic DNA damage, upregulating anti-apoptotic proteins Bcl-2 and BclxL 2, and down-regulating expression of pro-apoptotic genes: *Bax* and *Bad*. Neuroprotectin D1 also inhibited oxidative stress-induced caspase-3 activation and IL-1β-stimulated COX-2 expression. Administration of albumin causes systemic mobilization of n-3 PUFAs including DHA, and provides substantial neuroprotection in models of brain ischaemia and trauma. DHA-albumin administration increased Neuroprotectin D1 in the ipsilateral brain after transient cerebral ischemia in rat. Some studies also revealed that Neuroprotectin D1 promoted neuronal survival by the
induction of neuroprotective gene expression and anti-apoptotic pathways that attenuated Aβ42-induced neurotoxicity [66–74].

3.6. Glycerophospholipids

Neural membranes contain several classes of glycerophospholipids which turnover at different rates with respect to their structure and localization in different cells and membranes. The glycerophospholipid composition of neural membranes greatly alters their functional efficacy. The length of glycerophospholipid acyl chain and the degree of saturation are important determinants of many membrane characteristics including the formation of lateral domains that are rich in polyunsaturated fatty acids. Receptor-mediated degradation of glycerophospholipids by phospholipases A (1), A (2), C, and D results in generation of second messengers such as arachidonic acid, eicosanoids, platelet activating factor and diacylglycerol. Thus, neural membrane phospholipids are a reservoir for second messengers. They are also involved in apoptosis, modulation of activities of transporters, and membrane-bound enzymes. Marked alterations in neural membrane glycerophospholipid composition have been reported to occur in neurological disorders. These alterations result in changes in membrane fluidity and permeability. These processes along with the accumulation of lipid peroxides and compromised energy metabolism may be responsible for the neurodegeneration observed in neurological disorders [75].

3.7. Glycerolipids (DAG)

Diacyl glycerolipids (DAG) is an important membrane signalling lipid in the brain. DAG is primarily generated through the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C (PLC). Once generated, DAG can activate numerous intracellular proteins such as protein kinase C (PKC), Ras guanyl nucleotide-releasing protein and the transient receptor potential cation channel. DAG signalling is terminated by diacylglycerol kinases (DGKs), which convert DAG to the lipid second messenger, phosphatidic acid (PA). DGKs modulate intracellular lipid signalling by terminating DAG’s effects and by producing PA.

DGKβ is a member of the DGK family and is widely distributed in the brain. A high density of DGKβ was found in the neurons of brain areas associated with emotion such as the olfactory bulb, Nac, amygdale and hippocampus. In the hippocampus, DGKβ was found in the postsynaptic regions of projection neurons as well as in GABAergic interneurons. Membrane-bound DGKβ controls the lipid activity that regulates long-term potentiation, dendrite outgrowth and spine maturation in hippocampal CA1 neurons. The elimination of DGKβ activity in a KO mouse model resulted in attention deficit and memory impairment. However, these mice also showed psychomotor deficits. During the active period of the day, DGKβ KO mice showed significantly increased locomotor activity in their home cages.

When tested in the open field, general activity was increased; however, the time spent in the centre also increased, suggesting reduced levels of anxiety. These effects were confirmed in the EPM test in which DGKβ KO mice also showed reduced levels of anxiety and increased locomotor activity. Interestingly, sensorimotor gating (as measured via prepulse inhibition) and social interaction were not disturbed in DGKβ KO mice. The reduced anxiety and hyperactivity observed could be reduced using the mood stabilizer lithium ion DGKβ KO mice. These findings suggest a role for the intracellular lipids DAG and PA in the control of anxiety related behaviours.

Chronic unpredictable stress, which induces depression-like behaviour in the FST and oxidative stress in the brains of mice, was shown to alter the brain’s phospholipid content. Stress significantly reduced the levels of PI but increased the levels of PC. The increase in PE was not significant. These effects were observed throughout the entire brain. A more recent study suggests a brain area-specific regulation of phospholipid levels. Chronic unpredictable stress for 4 weeks led to a significant increase in hippocampal PI levels and a decrease in PE levels in the PFC of rats. No effects on phospholipid levels were observed in the amygdala or cerebellum. These findings suggest that brain areas need to be considered separately in functional analyses. Antidepressant drug treatment does not only affect monoaminergic systems but may also have profound effects on the phospholipid levels in the brain. PIPs, also called phosphoinositides, are involved in various downstream signalling pathways including
DAG-regulated PKC activation, nPI-3-kinase (PI3K)/Akt signalling, and inositol triphosphate mediated calcium signalling. Cytidine diphosphate diacylglycerol (CDP-DAG) is an intermediate in the synthesis of PI. Imipramine, paroxetine, and maprotiline increased the levels of CDP-DAG and PI in neuron-like PC12 cells. Several classes of antidepressant drugs dose-dependently increased CDP-DAG levels in the hippocampus, PFC, and striatum of rat brain slices; these classes include classical antidepressants (imipramine and desipramine), selective 5-HT reuptake inhibitors (SSRIs) (fluoxetine and paroxetine), and atypical agents (maprotiline and nomifensine). Other psychotropic compounds, such as the antipsychotic agents sulpiride, chlorpromazine, and haloperidol, or the anxiolytic agents diazepam and Phenobarbital had no effect on CDP-DAG levels. The increased CDP-DAG levels were found to translate to an increased synthesis of PI and an accumulation of inositol phosphates in these brain regions.

To test whether this effect has any relevance in the antidepressant action of the investigated compounds, animals were pre-treated with neomycin (a PIP-inactivating polyhydroxylated amino-glycoside) and tested in the modified FST. Neomycin reduced the accumulation of newly synthesized PIP as well as the formation of inositol phosphates and blocked the antidepressant effects of imipramine, fluoxetine, and maprotiline. Fluoxetine, imipramine and maprotiline also increased CDP-DAG levels in the FC and hippocampus of mice. This effect was at least partially 5-HT-dependent for fluoxetine but not for imipramine. Overall, these studies suggest an important role for PIP in the action of antidepressant drugs. PC are the most abundant phospholipids in membrane bilayers.

Lysophosphatidylcholines (LPC) also have important roles in membrane permeability and fluidity. A lipidomic study in mice suggested that a daily intraperitoneal treatment with the antidepressant drugs maprotiline and paroxetine decreased PC species and increased LPC species in the PFC, which suggests an increase in PLA activity.

Interestingly, these effects were region-specific and were not observed in the hippocampus, striatum or cerebellum. These effects were suggested to lead to the release of DHA which has independently demonstrated antidepressant effects [76–81].

3.8. Sphingolipids

Sphingolipids are minor biomolecules present in mammalian cell membranes. Owing to their polar nature, they play a crucial role in transmembrane exchange of small molecules. Sphingolipids are highly enriched in nervous cells, where they exert important biological functions. They deeply affect the structural and geometrical properties and the lateral order of cellular membranes, modulate the function of several membrane-associated proteins, and give rise to important intra- and extracellular lipid mediators. The differentiation and development of nervous system regulates the metabolism of sphingolipids and the expression of a peculiar spatially and temporarily regulated sphingolipid pattern is essential for the maintenance of the functional integrity of the nervous system: sphingolipids in the nervous system participate to several signalling pathways controlling neuronal survival, migration, and differentiation, responsiveness to trophic factors, synaptic stability and synaptic transmission, and neuron–glia interactions, including the formation and stability of central and peripheral myelin. In several neurodegenerative diseases, sphingolipid metabolism is deeply deregulated, leading to the expression of abnormal sphingolipid patterns and altered membrane organization that participate to several events related to the pathogenesis of these diseases. The most inquisitive result of this deregulation is symbolised by anomalous sphingolipid–protein interactions that are at least, in part, responsible for the misfolding events that cause the fibrillogenic and amyloidogenic processing of disease-specific protein isoforms, such as amyloid β peptide in Alzheimer’s disease, huntingtin in Huntington’s disease, α-synuclein in Parkinson’s disease, and prions in transmissible encephalopathies. Targeting sphingolipid metabolism presents an underexploited field but practically feasible opportunity to design novel therapeutic strategies for the intervention in these diseases [82–106] Table 1.

4. Role of lipidomics: the advanced diagnostic techniques for identification of disease biomarkers

Lipids exhibit immense combinatorial and structural diversity. There is a diverse range of lipids in the biological system which encode for distinct cellular and membrane functions but studying their
Table 1
List of reported clinical trial on lipids having protective effect of neurodegenerative disorder.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Status</th>
<th>Study title</th>
<th>Conditions</th>
<th>Interventions</th>
<th>Study type</th>
<th>Sponsor/ Collaborators</th>
<th>Outcome measures</th>
<th>Study completion</th>
<th>Locations</th>
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<td>High Dose Omega-3 Fatty Acids in the Treatment of Sport Related Concussions</td>
<td>Mild Concussion Brain Concussion Cerebral Concussion</td>
<td>Dietary Supplement: Docosahexaenoic acid Drug: Placebo</td>
<td>Interventional</td>
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<td>Number of days to return to full unrestricted athletic participation Number of days for balance and cognition to return to baseline</td>
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<td>DHA (Docosahexaenoic Acid), an Omega 3 Fatty Acid, in Slowing the Progression of Alzheimer’s Disease</td>
<td>Alzheimer’s Disease Drug: DHA (Docosahexaenoic Acid) Drug: Placebo</td>
<td>Intervventional Alzheimer’s Disease Cooperative Study (ADCS) National Institute on Aging (NIA) DSM Nutritional Products, Inc.</td>
<td>Rate of Change on the ADAS-Cog 11. Rate of Change on CDR-SOB ADCS-ADL Neuropsychiatric Inventory (NPI)</td>
<td>May-09</td>
<td>University of Alabama, Birmingham, Alabama, United States Banner Alzheimer’s Institute Phoenix, Arizona, United States</td>
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<td>4</td>
<td>Active, not recruiting</td>
<td>Docosahexaenoic Acid (DHA) To Prevent Development of Cognitive Dysfunction Due to Chemotherapy</td>
<td>Cognitive Dysfunction</td>
<td>Drug: DHA (Docosahexaenoic Acid) Drug: Placebo</td>
<td>Interventional</td>
<td>Carol Fabian, MD DSM Nutritional Products, Inc. University of Kansas Medical Center</td>
<td>Number of potentially eligible subjects who consent to participate in the study. Number of enrolled subjects who complete all cognitive assessments at all three defined timepoints. Number of enrolled subjects who take at least 70% of prescribed study agent.</td>
<td>Dec-18</td>
<td>Decatur Memorial Hospital Decatur, Illinois, United States University of Kansas Medical Center Kansas City, Kansas, United States</td>
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<td>Subarachnoid Haemorrhage. Cerebral Vasospasm</td>
<td>Drug: Eicosapentaenoic acid ethyl ester</td>
<td>Interventional</td>
<td>Yamaguchi University Hospital, Nakamura Memorial Hospital, Iwate Medical University</td>
<td>Cerebral vasospasms: Symptomatic vasospasm defined as documented arterial vasospasm consistent with new neurological deterioration. New low-density areas on CT scans associated with vasospasm Patient's Glasgow Outcome Scale (GOS). Change in Extrapyramidal Symptom Rating Scale (ESRS) dyskinesia score from baseline to week 12. Change in ESRS for parkinsonism, dystonia, akathisia, and total scores from baseline to week 12. The proportion of subjects in each group who achieve a 30% reduction in ESRS total scores at week 12</td>
<td>Dec-08</td>
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<td>Completed</td>
<td>Ethyl-Eicosapentaenoic Acid and Tardive Dyskinesia</td>
<td>Dyskinesia Schizophrenia</td>
<td>Drug: eicosapentaenoic acid</td>
<td>Interventional</td>
<td>University of Stellenbosch Stanley Medical Research Institute</td>
<td>Change in Extrapyramidal Symptom Rating Scale (ESRS) dyskinesia score from baseline to week 12. Change in ESRS for parkinsonism, dystonia, akathisia, and total scores from baseline to week 12. The proportion of subjects in each group who achieve a 30% reduction in ESRS total scores at week 12</td>
<td>Mar-05</td>
<td>Department of Psychiatry, Department of Health sciences, University of Stellenbosch Cape Town, Western Cape, South Africa</td>
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<td>9</td>
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<td>Ethyl-EPA Treatment of Prodromal Patients</td>
<td>Drug: ethyl-eicosapentaenoic acid</td>
<td>Interventional Yale University</td>
<td>Conversion to psychosis Aug-05 Yale University</td>
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<td>Alpha-linolenic acid</td>
<td>Effect of Fatty Acids on Memory Performance of Toddlers</td>
<td>Dietary Supplement: Flaxseed oil, Dietary Supplement: corn oil</td>
<td>Interventional University of North Carolina, Chapel Hill</td>
<td>Change in declarative memory performance Jan-11 UNC at Chapel Hill Nutrition Research Institute Kannapolis, North Carolina, United States</td>
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<td>11</td>
<td>Recruiting</td>
<td>Omega-3 fatty acid</td>
<td>Effect of Fatty Acids</td>
<td>Dietary Supplement: Flaxseed oil, Dietary Supplement: corn oil</td>
<td>Interventional Gregor Berger, Psychiatric University Hospital, Zurich, Swiss National Science Foundation</td>
<td>Symptomatic improvement in declarative memory performance Dec-20 Psychiatric University Clinics, Department of Child and Adolescent Psychiatry Zurich, ZH, Switzerland</td>
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<td>12</td>
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<td>Omega-3 fatty acid</td>
<td>Depression</td>
<td>Drug: Omega 3 fatty acid, Drug: Placebo oil</td>
<td>Interventional Region Skane University of California, San Francisco</td>
<td>Reduction in depressive symptom responses May-19 Lund University, Dept of Psychiatry Lund, Sweden</td>
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<td>13</td>
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<td>Irish Omega-3 Study</td>
<td>Psychotic Disorders</td>
<td>Dietary Supplement: 1000 mg of eicosapentaenoic acid and 1000 mg docosahexaenoic acid</td>
<td>Interventional University College Cork</td>
<td>To ascertain the effectiveness of Omega-3 fatty acid supplements in reducing transition to psychosis in individuals who are at ultra high risk of developing psychosis. Feb-18 Clinical Research Facility, Cork, Ireland</td>
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<td>Omega-3 Fatty Acids for Autism Treatment</td>
<td>Autism</td>
<td>Dietary Supplement: Omega-3 Fatty Acids</td>
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<td>University of California, San Francisco</td>
<td>Group-specific and Comparison of Change in Aberrant Behaviour Checklist-Hyperactivity Subscale Score (Active Omega-3 Group Only, Placebo Group Only and Comparison Between Groups) Change in Percentage of Serum Omega-3 Fatty Acids Change in Serum TNFα (Cytokine) Level</td>
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<td>UC San Francisco</td>
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<td>The Influence of Omega-3 Fatty Acid on the Violence of Schizophrenia Patient and the Possible Mechanism</td>
<td>Schizophrenia</td>
<td>Biological: omega-3 fatty acid Biological: placebo Device: positron emission tomography (PET)</td>
<td>Interventional</td>
<td>Shanghai Mental Health Center</td>
<td>Violent behaviour of Participants as Assessed by Modified Overt Aggression Scale (MOAS) Psychiatry symptoms of participants as assessed by Positive and Negative Syndrome Scale (PANSS) Blood level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), noradrenalin (NE), dopamine (DA) and serotonin (5-HT) of participants The density of 5-HT1A receptor in brain tested by positron emission tomography (PET)</td>
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<td>16</td>
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<td>The Role Of Omega-3 Fatty Acids In Adolescent Depression</td>
<td>Depressive Disorder, Major</td>
<td>Drug: Omega 3 Fatty Acids Dietary Supplement: Corn oil</td>
<td>Interventional</td>
<td>Icahn School of Medicine at Mount Sinai</td>
<td>Children’s Depressive Rating Scale - Revised (CDRS-R) Clinician’s Global Improvement Scale (CGI)</td>
<td>Jun-13</td>
<td>Icahn School of Medicine at Mount Sinai New York City, New York, United States</td>
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<td>Omega-6/Omega-3 Ratio and Neural Impaired Psychomotor Development</td>
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<td>Dietary Supplement:</td>
<td>Interventional</td>
<td>Ayham alshweki Health Research</td>
<td>Levels of fatty acids in blood Psychomotor development</td>
<td>Sep-14</td>
<td>Health Research Institute of Santiago</td>
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**Omega 6 Fatty Acid**
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<td>Development in Preterm Infants. Omega-3/Omega-6 Fatty Acids for Attention-Deficit/Hyperactivity Disorder (ADHD): A Trial in Children and Adolescents</td>
<td>ADHD Reading/Writing Disorder</td>
<td>Institute of Santiago Göteborg University Vifor Pharma</td>
<td>ADHD-Rating Scale, Investigator-rated Clinical Global Impression-Severity Scale</td>
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<td>Polyunsaturated Fatty Acids (PUFA) Supplementation of Polyunsaturated Fatty Acids in Children With Attention Deficit/Hyperactivity Disorder (ADHD)</td>
<td>ADHD</td>
<td>Medical University of Warsaw</td>
<td>Assessment of intensity of ADHD symptoms using neuropsychological tests and parents and teacher questionnaires in treated group according to placebo intervention. Assessment of adverse events. Psychiatric interview Continuous Performance Test (CPT). Wechsler Intelligence Scale for Children-3rd edition (WISC-III) Cambridge Neuropsychological Test Automated Batteries (CANTAB) the Clinician’s Interview-Based Impression of Change Scale (CIBIC-plus) the cognitive portion of the Alzheimer’s Disease Assessment Scale (ADAS-cog) Mini Mental Status Examination (MMSE) scores</td>
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<td>Effects of Polyunsaturated Fatty Acids on the Visual Memory of Children With Attention Deficit Hyperactivity Disorder</td>
<td>Attention-deficit/Hyperactivity Disorder</td>
<td>National Taiwan University Hospital</td>
<td>Assessment of intensity of ADHD symptoms using neuropsychological tests and parents and teacher questionnaires in treated group according to placebo intervention. Assessment of adverse events. Psychiatric interview Continuous Performance Test (CPT). Wechsler Intelligence Scale for Children-3rd edition (WISC-III) Cambridge Neuropsychological Test Automated Batteries (CANTAB) the Clinician’s Interview-Based Impression of Change Scale (CIBIC-plus) the cognitive portion of the Alzheimer’s Disease Assessment Scale (ADAS-cog) Mini Mental Status Examination (MMSE) scores</td>
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<td>21</td>
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<td>Preliminary Study of Fish Oil and Dementia</td>
<td>Alzheimer's Disease Mild Cognitive Impairment</td>
<td>Taipei City Psychiatric Center, Taiwan Department of Health, Executive Yuan, R.O.C., (Taiwan)</td>
<td>the Clinician’s Interview-Based Impression of Change Scale (CIBIC-plus) the cognitive portion of the Alzheimer’s Disease Assessment Scale (ADAS-cog) Mini Mental Status Examination (MMSE) scores</td>
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<td>22</td>
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<td>Arachidonic acid Dietary Fatty Acids Improve Social Impairment in Autism Spectrum Disorders</td>
<td>Other: Aravita including arachidonic acid and docosahexaenoic acid</td>
<td>Ashiya University</td>
<td>A aberrant Behaviour Checklist-community (ABC) Social Responsiveness Scale (SRS)</td>
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role is a bit complicated given to their structural diversity and considerable technical challenges associated with distinguishing between pathogenic and non-pathogenic lipid species within samples which contain numerous lipid isoforms. Therefore, the large-scale analysis of lipid profiling is the first requirement to illustrate the complex lipid—lipid and lipid—protein interactions and dynamics of lipids during cellular disturbances, and this needs analytical techniques to detect and monitor delicate changes in the diverse array of lipid species in cells and tissues. The term Lipidomics was coined by Han and Gross (2003), it is the large-scale study and complete characterisation of network of cellular lipids including their interaction with proteins and gene regulation in biological systems. The word “lipidome” is used to describe the complete lipid profile within a cell, tissue, organism, or ecosystem and is a subset of the “metabolome” which also includes the three other major classes of biological molecules: proteins/amino-acids, sugars and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly expanding field complements the huge progress made in genomics and proteomics, all of which constitute the family of systems biology. Lipidomics research involves the identification and quantification of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Investigators in lipidomics examine the structures, functions, interactions, and dynamics of cellular lipids and the changes that occur during perturbation of the system [107].

The emerging field of lipidomics tries to define the crucial role of lipids in the cell; the research is aimed at mapping the entire lipid population in a biological system, describing the composition and biological function. The main progress that has spurred recent advances in lipid analysis was the development of new mass spectroscopic techniques, particularly the “soft ionization” techniques electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). Lipidomics seeks to provide a molecular signature to a certain pathway or a disease condition. The role of lipids in formation of cell membranes makes them both ligand and substrate for proteins, suggesting that advances in lipidomics could have far reaching implications in genomic, proteomic and metabolomic fields. Impressive achievements and advancements have surfaced during the last few years. Lipidomics holds the promise of characterizing complex mixtures of lipids, identifying previously unknown changes in lipid metabolism [108].

4.1. Lipid extraction

A well defined lipid profile is the first and foremost requirement of lipidomics study, in this view, the extraction of lipids becomes a crucial step. Owing to high solubility of hydrocarbon chains in organic solvents the lipids are extracted with immiscible solvents leading to partitioning of lipids in the hydrophobic phase which is the fundamental of lipid extraction method. The type of analytical matrix forms the basis of different extraction methods employed in lipidomics. Typically, liquid—liquid extraction and solid phase extraction (SPE) are the two mostly preferred extraction methods for lipids extraction in lipidomics. SPE is a rapid extraction method and can attenuate degradation with setting up automatic pre-analytical facilities for a simultaneous preparation of numerous samples. The method utilizes solute affinity for stationary phase which not only minimizes solvent consumption, analysis time but also provides better extraction efficiency and specificity in targeted lipidomics.

5. Analytical techniques utilized in lipidomics

The diversity of lipid structures presents important challenges to full lipidome analysis. It is almost impossible to collect data for all classes of lipids in many biological samples by a single extraction, chromatography and detection method. Complete lipidome analysis suffers a major drawback due to versatility in lipid structures. The analysis for all classes of lipids having varying chemical structures is infeasible utilizing a single extraction procedure or chromatographic technique. However mass spectrometry plays a key role in lipid analysis since the development of soft ionisation techniques such as ESI and MALDI complements lipid analysis by overcoming volatility of lipids along with excellent
detection limits. Moreover compatibility with MS methods such as direct infusion ESI-MS makes it less time consuming and is widely used in lipidomics.

RNA interference (RNAi) takes advantage of a naturally occurring process to degrade RNA. RNAi selectively and temporarily switches off specific genes, providing a powerful tool to analyse protein function and biochemical pathways. Lipidomic analyses together with RNAi may provide a powerful tool to elucidate the specific roles of lipid intermediates in cell signalling. These approaches have provided crucial information that was previously unachievable. A deeper knowledge of the complexity of lipid signalling will elevate our understanding of the role of lipid metabolism in various CNS disorders, opening new opportunities for drug development and therapies for neurological diseases [109–115].

Lipid homoeostasis is fundamental to health maintenance. It is becoming increasingly clear that lipid metabolic disorders or abnormalities play a critical role in many human diseases such as metabolic syndromes, neurodegenerative diseases, cancer and infectious diseases. The advancement in above-mentioned MS or LC-MS technologies allows sensitive and highly selective analysis of lipids with diverse chemical compositions in complex biological samples, which opens a new door for lipids and their interacting partners in various diseases. Both targeted and nontargeted lipidomics from different physiological conditions are especially powerful in illuminating lipid cell biology and discovering novel biomarkers and drug for various diseases. Comparative lipidomics can identify lipid-related metabolic pathways that are specifically altered in different pathological states and from which key lipid metabolites operating important biological function in different diseases can be concluded. These lipid biomarkers provide a new perspective for acquiring better diagnosis in preclinical experiments by mammal models and in human clinical presentations [116–135] Table 2.

**Conflict of interest**

None.

**References**


