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Role of Lipids in Brain Injury and Diseases

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Summary

Lipid metabolism is of particular interest due to its high concentration in CNS. The importance of lipids in cell signaling and tissue physiology is demonstrated by many CNS disorders and injuries that involve deregulated metabolism. The long suffering lipid field is gaining reputation and respect as evidenced through the Center of Biomedical Research Excellence in Lipidomics and Pathobiology (COBRE), Lipid MAPS (Metabolites And Pathways Strategy) Consortium sponsored by NIH, European initiatives for decoding the lipids through genomic approaches, and Genomics of Lipid-associated Disorder (GOLD) project initiated by Austrian government. This review attempts to provide an overview of the lipid imbalances associated with neurological disorders (Alzheimer's, Parkinson's; Niemann-Pick; Multiple sclerosis, Huntington, amyotrophic lateral sclerosis, schizophrenia, bipolar disorders and epilepsy) and CNS injury (Stroke, traumatic brain injury; and spinal cord injury) and a few provocative thoughts. Lipidomic analyses along with RNA silencing will provide new insights into the role of lipid intermediates in cell signaling and hopefully open new avenues for prevention or treatment options.

Keywords

Alzheimer's disease; Arachidonic acid; CDP-choline; CNS injury; Docosahexaenoic acid; Huntington disease; Lipid peroxidation; Neurodegenerative diseases; Phospholipases; Phospholipids; Stroke

Lipids and the Central Nervous System (CNS)

Phospholipids are important components of all mammalian cells and have a variety of biological functions: 1) formation of lipid bilayers that provide structural integrity necessary for protein function 2) function as an energy reservoir (for example triglycerides) and 3) serve as precursors for various second messengers such as arachidonic acid (ArAc), docosahexaenoic acid (DHA), ceramide, 1,2-diacylglycerol (DAG), phosphatidic acid, and lyso-phosphatidic acid. Lipids comprise a large number of chemically distinct molecules arising from combinations of fatty acids with various backbone structures. Overall, mammalian cells may contain ~1000-2000 lipid species [1]. Lipids are classified into eight categories (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides) [2]. Subtypes of these eight lipid classes are given in Table 1. Lipid

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metabolism may be of particular importance for the CNS, as this organ has a high concentration of lipids, second only to adipose tissue.

Neurodegenerative diseases, mental disorders, stroke and CNS traumas are problems of vast clinical importance. Currently there exists no cure for these CNS injuries and disorders, resulting in a huge impact on quality of life and an economic burden on society (Table 2, based on the US population statistics). The crucial role of lipids in tissue physiology and cell signaling is demonstrated by the many neurological disorders, including bipolar disorders and schizophrenia, and neurodegenerative diseases such as Alzheimer's, Parkinson's, Niemann-Pick and Huntington diseases, that involve deregulated lipid metabolism [1]. Altered lipid metabolism is also believed to be a key event which contributes to CNS injury [3].

Reactive Oxygen Species (ROS) and Lipid Peroxidation

ROS including superoxide anion radical and hydrogen peroxide are produced by a number of cellular oxidative metabolic processes including oxidative phosphorylation by the mitochondrial respiratory chain, xanthine oxidase, NAD(P)H oxidases, monoamine oxidases and metabolism of ArAc by cyclooxygenases/lipoxygenases (COX/LOX) [4]. However, recent literature suggests that COX does not directly produce ROS during ArAc oxidative metabolism, but does form free radicals (i.e., carbon-centered radicals on ArAc). Although there are many reports in the literature on ROS production by COX, this is probably due to secondary ROS generation induced by various eicosanoids. Disruption of mitochondria during COX-2-associated apoptosis is a likely source of ROS production as has been established for a number of different cells [5-7]. ROS then cause oxidative damage to nucleic acids, proteins, carbohydrates, and lipids. Beyond damage to membranes, lipid peroxides give rise to reactive α,β -unsaturated aldehydes including malondialdehyde, 4-hydroxynonenal (HNE) and acrolein. These aldehydes covalently bind to proteins through reaction with thiol groups and alter their function. Although there are intracellular defenses against ROS, increased production of ROS or loss of antioxidant defenses leads to progressive cell damage and decline in physiological function. "Oxidative stress" results when generation of ROS exceeds the cell's capacity to detoxify them. The brain is believed to be particularly vulnerable to oxidative stress as it contains high concentrations of PUFAs that are susceptible to lipid peroxidation, consumes relatively large amounts of oxygen for energy production, and has lower antioxidant defenses compared to other organs.

Lipids in CNS Disorders

A summary of CNS disorders, their symptoms and lipid systems involved is presented in Table 3 as a quick reference guide.

Alzheimer's Disease (AD), the most common form of dementia, is a progressive brain disorder affecting regions 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). One of the hallmarks of AD is overproduction of a 4-kDa peptide, amyloid β -peptide or A β , resulting in the formation of plaques. AD encompasses a range of phenotypes, extending from cases with exclusively aggregated A β 42 to cases with, in addition, large quantities of insoluble A β 40 species [8]. A two-step cleavage of the neuronal membrane protein amyloid precursor protein (APP) [9] results in two products, A β 40 and A β 42. Strong evidence for the role of A β in the pathogenesis of AD was provided by the observation that, in familial or early onset AD, mutations in APP or the enzymes that cleave it lead to overproduction of A β 42 and rapid progression of the disease [10]. Late onset AD is also characterized by accumulation of A β and formation of plaques but is not inherited in any simple

pattern. The second hallmark of AD is formation of neurofibrillary tangles due to hyperphosphorylation of tau protein.

There has been debate concerning the roles of A β vs tau protein in AD. Transgenic mouse models of AD require two or more mutations to reproduce all the physical features of AD (A β plaques and tau tangles), however these models have not provided any leads to the relationship between A β plaques and tau tangles. Transgenic mice that over-express APP do not form neurofibrillary tangles and more closely resemble age-related memory impairment than AD. Some tau models express little amyloid but develop severe memory problems associated with AD [10]. So far most of the efforts in developing treatments for AD are directed to attenuating A β plaques. Mouse models based on over-expression of A β may be useful in developing strategies to limit formation of A β plaques, but these models are considered limited/incomplete models for AD. APP and APP/PS1 mutant mouse models develop amyloid deposition at a young age; but fail to develop neurofibrillary tangles that are essential hall mark of AD. Neuritic atrophy is found in some transgenics, but of nearly one dozen mouse models, only one has reported loss of neurons that are characteristic of AD [11], which may limit the usefulness of these models for developing therapeutic strategies for AD [12]. Development of therapeutic strategies directed to tau pathology will require identifying which phosphorylation sites are linked to tau aggregation and filament formation, and the specific kinases and phosphatases involved [13,14].

While the etiology of late-onset AD is not clearly understood, there is growing evidence that cholesterol is of particular importance in development and progression of the disease. Apolipoprotein E (ApoE) is one of the major apolipoproteins in plasma and the principal cholesterol carrier protein in the brain. Identification of the gene encoding the variant ApoE4 (APOE ϵ 4 allele) as a significant risk factor for late-onset AD provided evidence for a role of cholesterol in the pathogenesis of AD [15]. Elevated cholesterol levels increase A β in cellular and animal models, and drugs such as statins that inhibit cholesterol synthesis lower A β levels [14,16]. Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that initiates cholesterol and isoprenoid lipid synthesis. Cholesterol is needed to make the cellular membrane micro-domains referred to as lipid rafts. APP, β -secretase, γ -secretase complex, and neutral sphingomyelinase (N-SMase) are present in the lipid rafts that are rich in cholesterol and sphingomyelin (SM). Genetic mutations in APP or presenilins (part of the γ -secretase complex) increase production of A β 42. Recent studies suggest that A β 40 inhibits HMG-CoA reductase while A β 42 activates N-SMase and increases ceramide production, which can accelerate the neurodegenerative process [17,18]. However it is unclear what regulates the cleavage to A β 42 vs A β 40 and whether the ratio of A β 42 to A β 40 can be lowered such that attenuation of HMG-CoA reductase and N-SMase will be simultaneously achieved. Prospective trials evaluating statin therapy did not demonstrate improvement in cognitive function in AD patients [19]. It is also unknown whether genetic differences such as presence of the APOE ϵ 4 allele affects clinical outcome.

Recent studies have shown that mRNA expression of pro-inflammatory secretory phospholipase A₂ IIA (sPLA₂ IIA) was up-regulated in AD brains compared to non-dementia elderly brains and also found sPLA₂ IIA immunoreactive astrocytes in AD hippocampus that were associated with A β plaques [20].

AD, oxidative stress and lipid peroxidation: A number of studies demonstrating increased lipid peroxidation in AD provide increasing evidence supporting a role for oxidative damage in this disorder [21]. Recent studies demonstrated increased levels of HNE and acrolein [21] in the brain tissue from patients affected by mild cognitive disorder and early AD, indicating that lipid peroxidation occurs early in the pathogenesis of AD [21]. Acrolein, by far the strongest electrophile among all α,β -unsaturated aldehydes, 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 [22]. ROS may also play a role in amyloid deposition in AD as oxidizing conditions cause protein cross-linking and aggregation of A β peptides, and also contribute to tau protein aggregation [23]. A β 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 defense systems [23].

There may not be any simple answer for the complexity of AD. Are the familial and sporadic forms of AD distinct? Is the research field on a wrong track studying the familial form and not paying more attention to sporadic form? Are the animal models good enough to provide concrete answers? Mouse models are based on mutations in familial AD (the rare form of the disease) and may not model the more common sporadic form. Furthermore, most models do not exhibit the extent of neurodegeneration seen in AD patients [10]. The amyloid vaccine AN-1792 effectively cleared plaques and improved memory in animal model, while phase 2 trials were abandoned after some participants developed meningoencephalitis, a potentially fatal inflammation of the brain. Are these treatments (for eg; AN-1792) more compatible with the familial form compared to sporadic? The triple transgenic mouse (APP/PS1/tau) studies raise the possibility that a multi-antibody-based approach (i.e. one targeted against A β and one against tau) may provide the most significant clinical benefit for the treatment of AD [24]. The real issue will be how the patients respond to the combination treatment without developing clinical complications.

Parkinson's Disease (PD) is characterized by selective degeneration of dopaminergic neurons of the substantia nigra, resulting in bradykinesia, tremor and rigidity. The etiology of PD is unknown. Oxidative stress caused by free radical generation and lipid peroxidation plays a significant role in pathogenesis of PD. One of the factors responsible for this stress is believed to be phospholipases activation in substantia nigra, supported by the fact that cPLA₂ null mice are resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurotoxicity [25]. The MPTP metabolite MPP⁺ is taken up by nigrostriatal neurons where it inhibits mitochondrial oxidative phosphorylation and causes neuronal death. MPTP neurotoxicity has been used as an animal model for PD. *It should be noted that, while MPTP is capable of producing virtually all the signs and symptoms of PD, strictly speaking, it is not PD and is a separate disease.*

Treatment of PD: No therapy has been demonstrated to slow the loss of dopamine neurons in PD. The dopamine prodrug levodopa remains the gold standard for the treatment of PD. However, long-term levodopa therapy leads to development of dyskinesia. Alternatives to the use of levodopa in early PD include monoamine oxidase B inhibitors, dopamine agonists, catechol-*O*-methyltransferase inhibitors, and amantadine [26]. The mechanism of action of amantadine remains unknown; however, it has been suggested to have anticholinergic properties in addition to acting as a NMDA receptor antagonist to increase dopamine release and inhibit its reuptake. These treatments, however, remain focused on achieving symptomatic relief while minimizing adverse effects.

PD, oxidative stress and lipid peroxidation: In PD, the accelerated metabolism of dopamine by monoamine-oxidase-B may result in excessive ROS formation. A role for oxidative stress in PD was demonstrated by marked increases 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 found to be significantly increased in PD brain regions [23].

PD and α -synuclein: PD is associated with the presence of Lewy bodies containing insoluble aggregates of α -synuclein in association with other proteins. Recently the presence of polyunsaturated fatty acids (PUFA) was linked to the appearance of soluble oligomers of α -synuclein that ultimately promote the formation of insoluble α -synuclein aggregates [27]. Docosahexaenoic acid (DHA) was shown to stimulate oligomerization of α -synuclein. Recent studies have shown increased levels of docosahexaenoic acid (DHA) in PD brains compared to controls [28], suggesting that DHA could have a role in formation of α -synuclein aggregates. Interestingly, DHA reduced levodopa-induced dyskinesias in MPTP-treated monkeys, indicating that DHA can reduce the severity or delay the development of levodopa-induced dyskinesias [29]. It was suggested that DHA may represent a new approach to improve the quality of life of Parkinson's disease patients. This apparent discrepancy for the role of DHA in PD (beneficial or harmful?) could be due to the use of MPTP animal models and warrants further investigation.

Niemann-Pick (NP) Diseases are genetic pediatric neurodegenerative conditions characterized by specific disorders in lipid metabolism and are categorized as types A, B and C. NP Types A and B (NPA and NPB) are caused by deficiencies in acidic sphingomyelinase (A-SMase) [30]. NPA, the most common type, is caused by a nearly complete lack of A-SMase (less than 1% of normal activity) and is characterized by jaundice, an enlarged liver, and profound brain damage. Infants with NPA rarely live beyond 18 months. Since A-SMase is localized to the lysosomes, NPA results in accumulation of sphingomyelin (SM) and is classified as a lysosomal storage disorder [31]. People with NPB have approximately 10% of the normal level of A-SMase, generally have little or no neurologic involvement and may survive into late childhood or adulthood. Tricyclodecan-9-yl potassium xanthate (D609), a widely known phosphatidylcholine-phospholipase C (PC-PLC) inhibitor, also inhibits SM synthase [32] and may help prevent accumulation of SM in NPB, a therapeutic possibility not yet explored.

NP Type C (NPC) also is always fatal but it differs from NPA and NPB at the biochemical and genetic level. NPC is caused by mutations in either the *NPC1* gene (95% of cases) or *NPC2* gene (5% of cases) [33]. While the precise functions of the NPC1 and NPC2 proteins are not clear, these are involved in transport of lipids, particularly cholesterol, from the late endosomes/lysosomes [34]. Deficiencies in these proteins result in lysosomal accumulation of cholesterol and other lipids. In NPC, cholesterol accumulates in all tissues except the brain, where cholesterol levels decrease with age [33]. Since 70-80% of cholesterol in the brain is contained in myelin, the extensive demyelination that occurs in NPC probably accounts for the loss of cholesterol in the brain, which would likely mask accumulation of cholesterol in neurons or astrocytes. Currently there are no treatments for NPC. Despite the accumulation of cholesterol in late endosomes/lysosomes, neither cholesterol lowering agents nor dietary measures slowed the progression of the disease. Neurosteroids and enzymes involved in steroid synthesis were significantly reduced in NPC1-deficient mice. Administration of the neurosteroid allopregnanolone alleviated progression of the disease [33], suggesting that neurosteroid therapy might be a treatment option for NPC. Since NP diseases have a known genetic origin, it is possible that gene therapy to replace the defective genes may eventually provide a cure since that would be directed to the root causes of the diseases.

Multiple Sclerosis (MS) is an inflammatory demyelinating autoimmune disease affecting the CNS, but its underlying cause remains elusive. 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 fibers 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 the only neurological disorder where therapeutic manipulation of the

cytokine system influences development of the disease [35]. Thiobarbituric acid reactive substances and F₂-isoprostane levels were shown to be elevated in CSF of MS patients, and HNE was associated with MS lesions, indicative that lipid peroxidation also occurs in MS [36].

Experimental autoimmune encephalomyelitis (EAE) can be induced in animals by immunizing against myelin antigens [37] and is an animal model for MS due to similarities in its histopathology and clinical course. Recent studies demonstrated a key role for cPLA₂ in EAE [37,38]. cPLA₂, which can be induced by the cytokine tumor necrosis factor- α (TNF- α) [39,40], was highly expressed in EAE lesions. Blocking cPLA₂ showed a remarkable decrease in both the onset and progression of the disease [37], indicating that cPLA₂ has a significant role in both the induction and effector phases of EAE. A second study showed that cPLA₂ null mice were resistant to EAE [38]. It should be noted that these studies were conducted using C57BL/6 or SV127 mouse strains which are naturally deficient in inflammatory PLA₂ or sPLA₂ IIA [4]. Treatment of EAE rats with the nonapeptide sPLA₂ inhibitor CHEC-9 (CHEASAAQC) significantly attenuated sPLA₂ activity, EAE symptoms, and ED-1 positive microglia/macrophages [41]. Recently, MS patients were shown to have elevated sPLA₂ activity [41]. These studies suggest that both cPLA₂ as well as sPLA₂ inhibition may be treatment options for MS.

Previous studies have focused on the immunological aspects of MS and the immune system's involvement in the disease has been elucidated. Future studies seeking deeper insights into the molecular mechanisms of T cell activation (what causes the immune system to attack the myelin?) and the signaling cascades responsible for neurodegeneration will help to further identify molecular targets for efficient treatment strategies [42].

Huntington's Disease (HD) is a relatively rare (8 per 100,000) inherited neurological disorder characterized by abnormal body movements (chorea, or jerky, random, uncontrollable movements) and a lack of coordination; cognition and personality may also be affected. HD is caused by a trinucleotide repeat expansion in the Huntingtin (*Htt*) gene. The normal gene has fewer than 36 repeats, whereas the mutated *Htt* gene has 40 or more CAG repeats. Since CAG is the codon for glutamine, HD is one of the polyglutamine diseases.

Endocannabinoids are endogenous agonists of cannabinoid receptors comprised of amides, esters and ethers of long chain PUFAs. *N*-Arachidonylethanolamine (AEA, anandamide) and 2-arachidonylglycerol (2-AG) are well characterized lipid mediators of the endocannabinoid system [43]. The endocannabinoid system has been found to have an important neuroprotective role in CNS injury and neurodegenerative diseases and an extensive review of these studies was recently published [44].

Endocannabinoids act as retrograde messengers and, upon release from postsynaptic neurons, regulate further neurotransmitter release by activating presynaptic cannabinoid receptors [45]. Cannabinoid receptor 1 (CB₁) expression in the brain is highest in the basal ganglia, globus pallidus, and substantia nigra; moderate in the cerebellum, hippocampus, caudate nucleus, putamen, hypothalamus, and amygdala. The endocannabinoid system is hypoactive in HD [43], which may underlie the neurotransmission abnormalities of HD and may be the cause of the clinical manifestations of the disease. Inhibition of fatty acid amide hydrolase, monoacylglycerol lipase or the endocannabinoid membrane transporter can enhance endocannabinoid levels and counteract neurochemical deficits and the hyperkinetic effects of HD [46]. Other studies have shown that dietary supplementation with essential fatty acids protected against motor deficits in a transgenic mouse model of HD [47]. Recent studies using ethyl-eicosapentaenoate (Ethyl-EPA, LAX-101 or Miraxion) showed promise in clinical trials and its action is presumed to be through c-Jun amino-terminal kinase (JNK) pathway [48]. HD

is associated with up-regulated transglutaminase activity in selectively vulnerable brain regions and transglutaminase-catalyzed cross-links co-localize with huntingtin (htt) protein aggregates [49]. Combination therapy using minocycline and coenzyme Q₁₀ (CoQ₁₀) in R6/2 transgenic HD mouse model also provided benefit by acting synergistically (minocycline attenuated microglia proliferation and CoQ₁₀ reduced htt protein aggregation)[50].

HD and lipid peroxidation [23]: In HD, one study reported no increase in 8-hydroxy-2'-deoxyguanosine or other markers of DNA oxidation, and no change in lipid peroxidation (as measured by thiobarbituric acid reactive substances, a relatively non-specific marker for lipid peroxidation). In contrast, other studies have shown increases in the lipid peroxidation markers F₂-isoprostane and malondialdehyde in HD.

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive loss of spinal cord and cortical motorneurons and is usually fatal within 2-5 years of diagnosis. Approximately 10% of ALS cases are familial (inherited), with the remaining 90% of cases being sporadic in origin [51]. The underlying cause of sporadic ALS still remains elusive. Of the familial cases, approximately 20% (i.e., 2% of all ALS) are due to mutations in the gene for the cytosolic copper-zinc superoxide dismutase (SOD1). SOD is responsible for detoxifying superoxide radicals to hydrogen peroxide. Expression of a mutant SOD1 protein, with or without residual SOD activity, is necessary to cause ALS phenotype, suggesting a dominant negative mechanism [51]. There is strong evidence that the toxicity of mutant SOD1 in ALS is not due to loss of activity, but to the gain of one or more toxic functions that are independent of SOD1 activity [52]. It is hypothesized that mutant SOD1 stimulates oxidative stress and induces mitochondrial dysfunction, excitotoxicity, inflammation, and protein aggregation [51].

The role of glutamate-mediated excitotoxicity in ALS was supported by the efficacy of riluzole, (a benzothiazole derivative that acts by reducing glutamate excitotoxicity) in slowing the progression of ALS [51]. Riluzole is the only available treatment for ALS, but is now known to have limited therapeutic benefits with minimal effects on survival [53]. Several inflammatory markers such as caspase 1, cyclooxygenase 2 (COX-2) and TNF- α are increased in spinal cord tissue in transgenic mouse models of ALS. Inhibition of COX-2 reduced spinal neurodegeneration and prolonged the survival of ALS transgenic mice [54]. The role of COX-2 in ALS as well as the presence of TNF- α suggests that cPLA₂ and/or sPLA₂ may also be up-regulated in ALS to supply the ArAc substrate to the COX pathway. TNF- α induces cPLA₂, sPLA₂ as well as COX-2. This suggests that anti-TNF- α therapy could attenuate the progression of ALS, an option that does not appear to have been tested.

ALS and lipid peroxidation: Evidence of increased oxidative DNA damage in ALS was indicated by elevated levels of 8-hydroxy-2'-deoxyguanosine in plasma, urine and CSF [23]. Several studies have shown increased lipid peroxidation and DNA damage in transgenic mice expressing mutant SOD1 (an animal model for ALS) and in neural tissue or sera from ALS patients [55,56].

Although studies demonstrate that oxidative damage occurs in a number of neurodegenerative diseases, in chronic conditions such as these it is difficult to determine if this damage relates directly to the underlying cause of the disease or represents secondary damage in cells with otherwise compromised function [55]. Interpretation of data from patients is complicated by the fact that observations are usually obtained at the end stage of neurological disease and thus the time course of oxidative damage relative to the progression of the disease is not obtained. Also, these oxidative modifications are not disease specific but occur in a number of neurodegenerative diseases.

Schizophrenia, Epilepsy, and Bipolar disorders. Schizophrenia is marked by disturbances in thinking, emotional reactions, social behavior, 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 [57].

Recent theories on the neurological deficits of schizophrenia have focused on abnormalities in phospholipid metabolism, particularly increased activity of PLA₂ enzymes and reduced activity of the system which incorporates PUFAs into phospholipids (a simultaneous increase in phospholipid hydrolysis and decrease in synthesis) [57,58]. Neither abnormality alone produces schizophrenia but the presence of both does [57]. These abnormalities lead to changes in membrane structure and thus the function of membrane-bound proteins, availability of cell signaling molecules, and the behavior of neurotransmitter systems. This hypothesis is supported by animal studies demonstrating that application of PLA₂ into the brain produces alterations in the dopamine system [57]. Also, since phospholipid metabolism has a crucial role in neuronal and synaptic growth and remodeling, it is plausible that defects in this system result in failure of normal neurodevelopment in schizophrenia. There is also evidence that schizophrenia is associated with alterations in lipid transport proteins and membrane phospholipid composition (increase in phosphatidylserine and decrease in phosphatidylcholine and phosphatidylethanolamine) [58]. Genome studies have found that several genes involved in myelination have decreased expression levels in schizophrenia [59].

From a therapeutic perspective, a number of reports indicate that at least a portion of schizophrenic patients have reduced levels of PUFAs, particularly ArAc and DHA, in red cell phospholipids, with low levels particularly associated with negative symptoms [57]. ArAc, DHA and eicosapentaenoic acid (EPA) are important for monoaminergic neurotransmission, brain development, and synaptic functioning [58]. This suggests that supplementation with essential fatty acids could alleviate symptoms of schizophrenia. In preliminary studies, however, DHA essentially had no effect and ArAc appeared to worsen symptoms in some schizophrenia patients. Unexpectedly, EPA provided significant improvement, comparable in magnitude to that produced by new atypical antipsychotic drugs, without any of the side effects characteristic of drug treatment. The combination of EPA and DHA was also beneficial in **bipolar disorder** (manic-depressive illness) [57].

Epilepsy is a neurological disorder characterized by recurrent spontaneous seizures due to an imbalance between cerebral excitability and inhibition, with a tendency towards uncontrolled excitability [60]. Recurrent severe seizures can lead to death of brain cells. The usual treatment for epilepsy is administration of anti-epileptic drugs. Phenytoin (Dilantin, Phenytek, US brand names) is the generic name of a widely used anti-seizure medicine [61]. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited possibly by promoting sodium efflux from neurons. Phenytoin tends to stabilize the threshold against hyper-excitability caused by excessive stimulation. The current status of new (second generation) antiepileptic drugs has been recently reviewed [61-63].

The ketogenic diet is an established and effective non-pharmacological symptomatic treatment for epilepsy that has been in clinical use for more than 80 years [60,64,65]. The ketogenic diet is a high fat, low protein and low carbohydrate diet in which >90% of calories are derived from fat and dietary availability of glucose is minimal [60,64,65]. The hallmark feature of the ketogenic diet is production of ketone bodies (β -hydroxybutyrate, acetoacetate, and acetone) in the liver with a concomitant rise in plasma levels. Since glucose (the preferred source of energy, particularly in the brain) is severely restricted, the ketone bodies are used as the energy source in extrahepatic tissues [64]. Despite its many years of use, there is still considerable

debate over how the ketogenic diet works; several major hypotheses have been advanced, but none are widely accepted.

Lipids in CNS Injury

Focal Cerebral Ischemia (Stroke), caused by obstruction of blood flow (ischemia) to the brain, is the first leading cause of long-lasting disability and third leading cause of death. Presently, tissue plasminogen activator (tPA) is the only FDA approved drug for the treatment of ischemic stroke. tPA administration beyond 3 hr presents hemorrhagic risk. The recent failure of NXY-059 (a nitron spin trap agent developed by AstraZeneca) in phase III stroke clinical trials emphasizes the need for careful pre-clinical scrutiny of test agents before embarking into mega clinical trials [66].

The initial event in cerebral ischemia is energy failure, resulting in excessive release of the neurotransmitters such as dopamine and glutamate [67]. Over-stimulation of glutamate receptors results in elevated intracellular Ca^{++} and activation of phospholipases (A_2 , C, and D) (Fig. 1A) and sphingomyelinases (Fig. 1B). Activation of these phospholipases causes hydrolysis of membrane phospholipids and release of second messengers [4,68,69]. TNF-alpha and IL-1, major pro-inflammatory cytokines, are rapidly up-regulated in brain after cerebral ischemia and activate phospholipases [70-73]. The nature of the inflammatory response after stroke suggests that cytokines, particularly TNF-alpha and IL-1alpha/beta, affect the phospholipid metabolism and subsequent production of eicosanoids, ceramide and free radicals (ROS, RNS and other radicals) that may potentiate brain injury [74].

CDP-choline, the rate-limiting intermediate in phosphatidylcholine (PC) biosynthesis, has shown beneficial effects in cerebral ischemia, TBI, hypoxia, AD, PD, learning and memory disorders, alcoholism, drug addiction, amblyopia and glaucoma, and has undergone several clinical trials for stroke treatment worldwide [67] (see the boxed text “**CDP-choline and stroke: An issue to be revisited**”). An extensive review of CDP-choline studies was recently published [75]. Due to the complexity of stroke biochemistry, targeting a single pathway may never prove efficacious. Drug cocktails or combinational therapies have been suggested but have not yet been initiated in clinical trials. In experimental paradigms, CDP-choline in combination with urokinase, basic fibroblast growth factor, or nimodipine provided synergistic benefits [67]. The failure of NXY-059 SAINT II trials [76] reiterates need for combinational treatment. CDP-choline is a safe drug (virtually no side effects even when given at 2 grams/day in clinical trials) and should be considered as one component in combinational therapy.

Stroke, ROS and lipid peroxidation: Until now, it has been generally accepted that the oxidative metabolism of ArAc through COX generates prostaglandins and ROS. Recent studies have clarified that COX-2 produces tyrosyl radicals on the protein and carbon centered radicals on the substrate ArAc, but does not generate ROS [5,6]. It has been shown recently that ROS production was elevated in a stroke model but that COX-2 inhibition did not attenuate ROS production. Also, ROS generation was not reduced in COX-2 deficient mice [7]. These studies indicated that NADPH oxidase was a significant source of ROS in the stroke model [7]. The role of ROS in activation of various signaling pathways such as p38, JNK, p53, ERK1/2, Akt, NF- κ B [77], matrix metalloproteinases [78] and stroke injury [4,79] have been recently reviewed.

Generation of HNE has been demonstrated by us and others in brain tissue after stroke [4]. Recently, elevated levels of an acrolein-protein conjugate (N^{ϵ} -(3-formyl-3,4-dehydropiperidino)-lysine, FDP-lysine) were demonstrated in plasma of stroke patients [80]. The time-course of alterations in lipid metabolism and formation of lipid metabolites and lipid peroxidation products after transient cerebral ischemia is presented in Fig. 2.

Traumatic Brain Injury (TBI) is usually associated with significant neuropsychological deficits, primarily in the domains of attention, executive functioning and memory. In TBI, the initial traumatic event is shearing, laceration, and/or contusion of brain tissue resulting from a physical impact. Secondary injury after the initial trauma results from ischemia, alterations in ion and neuromodular levels, oxidative stress caused by ROS, edema and axonal swelling [81]. Similar to stroke, pathways of secondary injury that occur subsequent to the initial trauma present targets for therapeutic intervention. Corticosteroids have been proposed as therapies to reduce the secondary injuries following TBI. Corticosteroids inhibit the PLA₂/COX/LOX pathways, thus limiting ArAc release and metabolism, down-regulating pro-inflammatory cytokines and attenuating the inflammatory response. However, large scale clinical trials of corticosteroids and lizaroids (21-aminosteroids) for treatment of TBI have either failed to demonstrate efficacy or found increased risk of mortality [81].

TBI and ApoE. While the APOE ε4 allele was first implicated as a significant risk factor for AD, a number of clinical studies have indicated that performance on neuropsychological tasks is worse in TBI patients with the APOE ε4 allele than those without it [82]. While other clinical studies did not find an association of APOE ε4 allele with poorer outcome after TBI, these differences could be due to the severity of the TBI (no association in some studies with predominantly mild TBI), the neurological evaluation methods that assess the involvement of different brain regions, and the evaluation time post injury (up to 25 years after TBI). Fluid percussion brain injury up-regulated both ApoE mRNA and protein expression in rats [83]. Both human postmortem and experimental studies have shown Aβ deposition and tau pathology after TBI [84]. The development of AD-like neuropathological and biochemical changes after severe TBI suggested that TBI may be a risk factor for subsequent development of dementia [85]. Epidemiological studies have provided discrepant findings and the relationship between TBI and dementia remains a topic for further investigation. It has been suggested that TBI may lower the threshold for dementia among predisposed individuals. In other studies, the presence of the APOE ε4 allele has been associated with poorer outcome after cardiopulmonary resuscitation and intracerebral hemorrhage, but not after ischemic stroke [86]. Statins have shown benefit in experimental TBI [87], but it is unknown if statin treatment affected Aβ levels.

ApoE is an important mediator of cholesterol and lipid transport in the brain and is encoded by the polymorphic gene APOE. ApoE has been shown to reduce glial activation and CNS inflammatory response. While it may be reasonable to relate effects of ApoE to cholesterol transport, the mechanism whereby ApoE elicits these effects has not been elucidated. This action is isoform-specific with the ApoE4 isoform being less effective at down-regulating inflammatory cytokines [88]. These studies suggest that ApoE could be a therapeutic target for treatment of TBI, although administration of the full protein would be impractical due to its large size and inability to cross the blood-brain barrier. A small peptide apoE(133-149) was created from the receptor binding region that retains the ability of the native protein in down-regulating inflammatory responses. Administration of apoE(133-149) was shown to significantly improve histological and functional outcome after experimental TBI [88].

Spinal Cord Injury (SCI). Similar to TBI, SCI is the result of an initial physical trauma followed by a secondary degenerative process. The majority of SCI do not involve physical transaction of the spinal cord; instead, the cord is injured as a result of contusive, compressive, or stretch injury. Residual white matter containing portions of sensory and motor tracts remain intact, suggestive that pharmacological interventions aimed at the secondary injury cascade could promote neurological recovery and preserve function [89]. The initial event after SCI is depolarization and opening of voltage-dependent ion channels, and consequent massive release of neurotransmitters including glutamate. This leads to accumulation of intracellular calcium, initiating a number of damaging events: mitochondrial dysfunction, activation of nitric oxide

synthase (NOS) and PLA₂. PLA₂ releases ArAc into the COX/LOX pathways to generate eicosanoids. One consequence of mitochondrial dysfunction, activation of NOS, and COX/LOX activity is generation of free radicals (comprised of different species including reactive nitrogen species (RNS), ROS and other radicals) and subsequent lipid peroxidation, believed to be a major pathway of secondary injury in SCI [89].

The glucocorticoid steroids dexamethasone and methylprednisolone have been extensively used in clinical treatment of SCI. The initial rationale was that, since these compounds reduced brain edema in brain tumor patients, they would also reduce edema in SCI. With the knowledge of the lipid peroxidation mechanism, later attention focused on the potential of glucocorticoid steroids to inhibit post-SCI lipid peroxidation, based on their ability to intercalate into artificial membranes and limit propagation of lipid peroxidation chain reactions [89]. In animal studies, it was demonstrated that high dose of methylprednisolone did inhibit post-traumatic lipid peroxidation in spinal cord tissue. Beneficial effects secondary to inhibition of lipid peroxidation included preservation of ion homeostasis, mitochondrial energy metabolism, and attenuation of delayed glutamate release [89]. It is believed that inhibition of lipid peroxidation is the principle neuroprotective mechanism of high-dose methylprednisolone and that glucocorticoid receptor-mediated anti-inflammatory effects have only a minor role [89]. In clinical trials, high-dose methylprednisolone was shown to improve outcome in SCI if treatment was initiated within 8 hr of the initial trauma.

Another agent that has undergone Phase III clinical trials for SCI is GM1 ganglioside. Since high-dose methylprednisolone had become widely accepted for treatment of SCI, GM1 was administered after the completion of the 24 hr methylprednisolone dosing protocol. The results indicated that GM1 did not provide greater functional improvement compared to methylprednisolone alone [89].

DHA generates anti-inflammatory resolvins and protectins including Neuroprotectin D1

Release of lipid second messengers after neuronal injury can either promote further neuronal injury or neuroprotection. Cerebral ischemia results in rapid accumulation of free fatty acids, including ArAc (20:4) and DHA (22:6). Through the COX/LOX pathways, ArAc is metabolized to pro-inflammatory and vasoactive eicosanoids (prostaglandins, leukotriens, thromboxanes). ArAc is also metabolized to anti-inflammatory lipoxins through the LOX pathway (Fig. 1A).

DHA is the most abundant polyunsaturated fatty acid in the brain, and is concentrated in aminophospholipids of 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 [90]. Studies on the role of PLA₂ and PLA₂ hydrolysis products have identified the DHA metabolites resolvins and protectins including 10,17*S*-docosatriene (Neuroprotectin D1) following cerebral ischemia/reperfusion in mouse [91] (Fig. 1A). Neuroprotectin D1 was found to serve an endogenous neuroprotective role by inhibiting apoptotic DNA damage, up-regulating anti-apoptotic proteins Bcl-2 and Bcl_{xL}, and down-regulating pro-apoptotic Bax and Bad expression [92]. Neuroprotectin D1 also inhibited oxidative stress-induced caspase-3 activation and IL-1 β -stimulated COX-2 expression [93 and references cited therein]. Administration of albumin causes systemic mobilization of n-3 PUFAs including DHA, and provides substantial neuroprotection in models of brain ischemia and trauma. DHA-albumin administration increased Neuroprotectin D1 in the ipsilateral brain after transient cerebral ischemia in rat [94]. Recent studies also showed that Neuroprotectin D1 promoted neuronal survival by the induction of neuroprotective gene expression and anti-apoptotic pathways that attenuated A β 42-induced neurotoxicity [95].

Future Trends: Lipidomics and RNA Silencing

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 [1,96,97]. 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 [91]. Lipidomics holds the promise of characterizing complex mixtures of lipids, identifying previously unknown changes in lipid metabolism [67].

RNA interference (RNAi) [98-100] takes advantage of a naturally occurring process to degrade RNA. RNAi selectively and temporarily switches off specific genes, providing a powerful tool to analyze 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 signaling. These approaches have provided crucial information that was previously unachievable [95]. A deeper knowledge of the complexity of lipid signaling 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.

CDP-choline and Stroke: An Issue to be Revisited

The therapeutic action of CDP-choline is *thought* to be due to stimulation of phosphatidylcholine (PC) synthesis in the brain after stroke. However to the best of our knowledge there were no reports in stroke models demonstrating that loss of PC occurs, and CDP-choline treatment restores PC levels. CDP-choline has also been termed a ‘*membrane stabilizer*’, ‘*free radical scavenger*’ and ‘*antioxidant*’ without any experimental evidence. Recent research on this drug revealed some interesting findings [68,101].

PC is a major membrane phospholipid and its loss threatens cell viability. PC homeostasis is regulated by the balance between hydrolysis and synthesis [67]. PC is hydrolyzed by phospholipase A₂ (PLA₂) [4], PC-phospholipase C (PC-PLC) and phospholipase D (PLD). PC synthesis is rate-limited by CTP:phosphocholine cytidyltransferase (CCT) that makes CDP-choline. Pro-inflammatory cytokines TNF- α and IL-1 β are up-regulated after stroke and activate phospholipases and sphingomyelinases (SMases), generating arachidonic acid (ArAc) and pro-apoptotic ceramide, and also down-regulate CCT and sphingomyelin (SM) synthase (Fig. 3).

In rat stroke model, the up-regulation of phospholipases and down-regulation of CCT collectively resulted in the loss of PC [68]. CDP-choline treatment significantly attenuated phospholipase induction, loss of CCT and PC, and infarction volume. CDP-choline also attenuated cytokine expression (TNF- α and IL-1 β) after stroke [102], suggesting that CDP-choline may affect PC synthesis and hydrolysis through proinflammatory pathways.

CDP-choline in stroke clinical trials. Clinical trials in Japan and Europe used intravenous administration in contrast to oral route in US trials, which gave ambiguous results [67, 101]. It was unclear why the oral route was chosen in USA trials (to the best of our knowledge, this is the only drug given orally to stroke patients; if the patient was incapable of swallowing the drug was administered nasally). Animal studies have shown brain uptake

of CDP-choline increased to 2% when administered intravenous compared to 0.5% by oral route. The following points are worth considering in future CDP-choline clinical trials:

CDP-choline efficacy may depend on the formulation and route of delivery [103]. Liposome encapsulation of CDP-choline provided more efficient delivery of the drug to the brain and significantly decreased infarction (by 62%) compared to the equivalent dose of free CDP-choline (26% reduction) after stroke [101]. Blood-brain barrier permeability after stroke allows passage of liposomes <100 nm directly through the compromised vasculature [101]. Advantages of CDP-choline liposomes in stroke include: 1) effective at low doses (18 mg/kg in CDP-choline liposomes vs 500 mg/kg free CDP-choline), 2) the presence of GM1 ganglioside confers long-circulating properties by suppressing phagocytosis, 3) brain uptake of the drug is increased to ~23% of the injected dose, and 4) exogenous CDP-choline is rapidly hydrolyzed and absorbed as cytidine and choline and needs to be re-synthesized by CCT [101]. However it should be noted that CCT expression is significantly reduced after stroke [68]. CDP-choline liposomes deliver the agent intact to the brain, circumventing the rate-limiting CCT step [101] (Fig. 4).

Conclusions: CDP-choline beneficial effects could be attributed to interactions at TNF- α /IL-1 mediated events, differentially affecting phospholipases and CCT [102]. This limits phospholipid hydrolysis and increases synthesis, thereby restoring PC and SM levels (Fig. 3). Our recent studies also showed that PC loss is a *cause* rather than a *result* of cell death [32]. Since all cells (neurons, astrocytes, and endothelial cells etc., called the 'neurovascular unit') contain PC as a membrane building block, CDP-choline protection may arise from stabilizing the 'neurovascular unit'. These studies provide an integration of CDP-choline effects with cytokine biology and lipid metabolism in stroke [67,74,102].

IVAX Corporation, a Florida based company has entered into a licensing agreement with Grupo Ferrer, Barcelona, Spain, for the final development of CDP-choline in oral neuroprotective therapy for the treatment of stroke.

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List of abbreviations

Aβ	Amyloid beta
AD	Alzheimer's disease
ALS	Amyotrophic Lateral Sclerosis
APP	Amyloid precursor protein
ArAc	Arachidonic acid
CDP-choline	Cytidine-5'-diphosphocholine
CNS	Central Nervous System
COX/LOX	Cyclooxygenase/lipoxygenase
DAG	

	1,2-diacylglycerol
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acid
HD	Huntington disease
HNE	4-Hydroxynonenal
MS-EAE	Multiple sclerosis-Experimental Autoimmune Encephalomyelitis
NP	Niemann-Pick disease
PC	Phosphatidylcholine
PD	Parkinson's disease
PLA₂	Phospholipase A ₂
PLC	Phospholipase C
PUFA	Polyunsaturated fatty acid
ROS	Reactive Oxygen Species
SCI	Spinal Cord Injury
SM	Sphingomyelin
SMase	Sphingomyelinase
TBI	Traumatic Brain Injury

Figure 1. A) Lipid metabolism in ischemic neuronal death

Activation of phospholipases (PLA₂, PC-PLC, PI-PLC and PLD) following cerebral ischemia results in release of lipid second messengers 1,2-diacylglycerol (DAG), phosphatidic acid (PA), lyso-phosphatidic acid (lyso-PA), docosahexaenoic acid (DHA), and arachidonic acid (ArAc). PA and DAG can be readily inter-converted by phosphohydrolases and DAG-kinases. ArAc undergoes further metabolism by cyclooxygenases/lipoxygenases (COX/LOX) to generate important signaling and vasoactive eicosanoids. Free radicals are formed during ArAc metabolism by COX/LOX and free radical generation can be induced by eicosanoids. ArAc generates pro-inflammatory prostaglandins, leukotrienes, and thromboxanes as well as LOX-generated anti-inflammatory lipoxins. Through the LOX pathway, DHA is metabolized to anti-inflammatory resolvins and protectins, including 10,17S-docosatriene (Neuroprotectin D1), an endogenous neuroprotectant. **B).** Glycerophospholipid and sphingolipid relationship. Tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) activate neutral sphingomyelinase (N-SMase) and acidic sphingomyelinase (A-SMase) through stimulation of PLA₂ and PC-PLC and release of ArAc and DAG, respectively. N-SMase and A-SMase hydrolyze sphingomyelin (SM) to liberate ceramide. SM synthase transfers the phosphocholine head-group of PC to ceramide to form SM and DAG.

Figure 2. Time course of changes related to cytokines, lipid metabolism and oxidative stress after transient brain ischemia

↑=increase, ↓=decrease, compared to control. ArAc: arachidonic acid; CCT: cytidine triphosphate:phosphocholine cytidyltransferase; HNE: 4-hydroxynonenal; IL-1β: interleukin-1β; MDA: malondialdehyde; •OH: hydroxyl radical; PLA₂: phospholipase A₂; PC-PLC: PC-phospholipase C; PLD2: phospholipase D2; PLA₂ enzyme activity, sPLA₂ mRNA and protein expression, PC-PLC activity and PLD2 protein expression were increased after stroke. CCT catalyzes the rate-limiting step in the biosynthesis of PC. CCT activity and protein expression decreased following stroke. Activation of phospholipases and loss of CCT collectively resulted in loss of PC.

Figure 3. CDP-choline: Integration of cytokine response and lipid metabolism after stroke
 [74] Upregulation of TNF- α and IL-1 differentially affects phospholipases, SMases; CCT and SM synthase in collectively causing loss of PC and SM and release of ArAc and ceramide. CDP-choline partially prevents loss of these phospholipids by attenuating the inflammatory response.

Figure 4. CDP-choline and PC synthesis

CCT is down-regulated after stroke, impeding PC synthesis. CDP-choline liposomes deliver the drug intact to the brain, bypassing the CCT step. **CCT**: cytidine triphosphate:phosphocholine cytidyltransferase. **CPT**: CDP-choline:1,2-diacylglycerol cholinephosphotransferase. The Western blot shows loss of CCT protein after stroke.

Table 1

The major lipid classes and representative subtypes. A comprehensive listing of lipids is provided in Fahy, *et al.* [2].

Lipid class	Subtypes
Fatty acyls	Free fatty acids and conjugates; eicosanoids; docosanoids; fatty alcohols, aldehydes and esters
Glycerolipids	Mono-, di-, and triacylglycerols
Glycerophospholipids	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol
Sphingolipids	Ceramide, sphingobases, sphingomyelin, glycosphingolipids (gangliosides)
Sterol lipids	Sterols including cholesterol, steroids, bile acids
Prenol lipids	Isoprenoids, polyprenols, quinones, hopanoids
Saccharolipids	acylamminosugars, acylaminosugar glycans
Polyketides	Macrolide and Aromatic polyketides

Table 2
Annual cost* of selected CNS disorders** and injuries in USA

CNS disorders or injury	Cases (in millions)	Costs/year (in \$ billions)
Neurological illnesses	50	\$ 400
Mental disorders, (in addition to neurological illnesses above but excluding alcohol and drug problems)	44	\$ 148
Alzheimer's Disease (AD)	4.5	\$ 100
Stroke	4.7	\$ 57
Traumatic Brain Injury (TBI)	5	\$ 56.3
All Depressive Disorders	20.5	\$ 44
Schizophrenia	2	\$ 32.5
Spinal Cord Injury (SCI)	0.25	\$ 10
Multiple Sclerosis (MS)	2.5	\$ 9.5
Parkinson's Disease (PD)	1	\$ 5.6
Huntington's Disease (HD)	0.3	\$ 2
Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease)	0.03	-

Table 3

Lipid systems affected by the CNS disorders and injuries

Disorder/injury	Symptoms/pathologic features	CNS region(s) affected	Mechanism of damage	Possible Treatments
Alzheimer Disease [10]	Memory loss/dementia Difficulties in communicating Problems learning, thinking, reasoning Difficulty with familiar tasks Disorientation	Amygdala Hippocampus Cerebral cortical areas controlling reasoning, learning, and language	Altered cholesterol [104] and lipid [105] homeostasis APP cleavage in lipid rafts [9] DHA levels ↓; upregulation of PLA ₂ , increased lipid peroxidation [21,25,106] sPLA ₂ -IIA expression increased [20]	Ganglioside treatments prevent neuronal death [25] ApoE protects against oxidative injury by mediating Aβ [105] Statins [16]
Parkinson's Diseases [27]	Amyloid plaques and tau protein aggregation [13,14] Movement disorder Resting tremors Muscle rigidity Bradykinesia (slow movements) Impaired posture, balance, coordination Lewy bodies/ α -synuclein aggregates	Substantia nigra	Although a direct role of PLA ₂ in PD is not yet clearly demonstrated, cPLA ₂ knock out mice showed protection against MPTP toxicity [25] PUFAs promote α -synuclein aggregation [27]	PLA ₂ inhibition [25] Ganglioside treatments [25] Quinacrine [25]
Niemann-Pick Disease [30,31,33]	Type A: progressive loss of early motor skills, early fatality typically within the first few Years Type B: enlarged liver, spleen; and respiratory problems Type C: Inability to move eyes up and down. Learning and cognitive impairment	Lysosomal storage disorder that leads to SM and cholesterol accumulation.	Type A: <1% of normal A-SMase activity leads to lysosomal sphingomyelin accumulation Type B: <10% of normal A-SMase Type C: defect in cholesterol transport with secondary defect in A-SMase activity Excessive accumulation of cholesterol and sphingolipids [107] Lipid peroxidation products from ROS cPLA ₂ is highly expressed in EAE [25] sPLA ₂ levels increased prior to onset of symptoms T cells and auto-antibodies to Lipids [109]	Enzyme replacement was shown to be effective in a mouse model for Type B [108]. De09, a SM synthase inhibitor may be an option not yet explored [32] Inhibition of glycosphingolipid synthesis prolonged lifespan in mouse model of type C [33]
Multiple Sclerosis-Experimental Autoimmune Encephalomyelitis (MS-EAE) [38,109]	Unpredictable and varies from person to person Loss of balance Loss of muscle coordination, resulting in tremors, bladder problems and slurred speech Problems with memory, attention, cognitive functions	Demyelination of axons		Antioxidants [36] sPLA ₂ inhibition by CHEC-9 blocks inflammation [41] Ganglioside treatments prevent neuronal death
Huntington Disease [43,110]	Movement disorder	Striatal (caudate and putamen) neurons	DHA levels ↓ [106] Trimucleotide CAG repeat expansion in the Huntingtin (Htt) gene is	Inhibiting 1) fatty acid amide hydrolase (FAAH), 2) monoacylglycerol lipase

Disorder/injury	Symptoms/pathologic features	CNS region(s) affected	Mechanism of damage	Possible Treatments
Amyotrophic Lateral Sclerosis	Uncontrollable muscle movements	Degeneration of medium spiny striato-efferent GABAergic neurons, atrophy of caudate nucleus.	responsible for This polyglutamine disease.	(MAGL or 3) endocannabinoid membrane transporter (EMT) provided relief to HD patients [43,46]
	Lack of concentration			Ethyl- eicosapentaenoic acid (EPA) [48]
	Memory loss			Combination therapy (minocycline + Coenzyme Q ₁₀) [50]
Schizophrenia	Nuclear and cytoplasmic inclusion/Huntingtin	Progressive loss of cortical and spinal cord motoneurons	Lipid peroxidation [56]	Riluzole is the only available clinical agent [51]
	Limb and muscle weakness, twitching and cramping of muscles			COX-2 inhibition was beneficial in mouse model [54]
	Lewy body like hyaline inclusion/SOD1 mutation			Antipsychotic drugs
Bipolar disorder (manic depression illness)	Disturbances in thinking, emotional reactions, and social behavior	Dorsolateral prefrontal cortex	Altered lipid metabolism may be responsible for defects in neurological development [57,58]	Eicosapentaenoic acid supplementation [57]
	Mood disorders characterized by episodes of mania and major depression			Combination of EPA and DHA [57]
	Wide range of severity			
Epilepsy	Violent convulsions	Focal cortical area, later transferred to the Thalamus	DHA levels ↓ [106]	Ketogenic diet [64]
	Loss of consciousness			Phenytoin [61]
	Minimal or no movements			Second generation antiepileptic drugs [62]
Stroke [69]	Sudden weakness on one side of the body, loss of balance and coordination, trouble with cognition	Cerebral cortical Areas Striatum	Activation of Phospholipases (A ₂ , C, D) [4,68], increased sPLA ₂ [111] cPLA ₂ knock out mice showed protection [4 and references Cited therein] DHA levels ↓ [106]	CDP-choline attenuated sPLA ₂ [68]
				Neuroprotectin D1 reduces infarct in MCAO model [91]
				sPLA ₂ inhibitors [112]
Traumatic Brain Injury [113]		Loss of CA ₃ hippocampal neurons	Aβ deposition, tau pathology [84]	Corticosteroids [81]
				apoE mimetic peptide showed benefit in experimental TBI [88]
				High-dose methylprednisolone in clinical use [89]; DHA treatment is beneficial [106]
Spinal Cord Injury [89]	Weakness and sensory loss; paralysis		Activation of PLA ₂ , COX/LOX pathways. Corticosteroids inhibit These activations [81]	