Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Lysophosphatidylcholine-Omega3

Evidence Summary

LPC-bound forms of DHA/EPA may allow for enhanced brain uptake and utilization. Clinical trials are needed to confirm neuroprotection and the safety profile.

Neuroprotective Benefit: LPC-DHA/EPA forms are expected to have greater brain uptake, and offer preferential benefits for ApoE4 carriers, relative to traditional supplements, but clinical studies are needed to validate these preclinical findings.

Aging and related health concerns: LPC-DHA/EPA forms may have preferential access to certain tissues, such as the liver, whereas other forms may offer greater heart uptake, suggesting that a mixture may be ideal to maximally benefit all organ systems.

Safety: LPC-DHA/EPA supplements are expected to have a safety profile similar to currently available phospholipid, triglyceride, and ethyl ester-bound DHA/EPA supplements, including an increased risk for bleeding, but confirmatory clinical trials are needed.

Availability: Contained in fish and fish roe. LPC-DHA/EPA supplements are not currently commercially available. Lipase-treated krill oil has been used to generate LPC-DHA/EPA enriched preparations in preclinical studies.	**Dose**: Not established
Half-life: Plasma half-life of free DHA ~30 s Plasma half-life of LPC-DHA ~5-10 min Tissue DHA half-life is substantially longer	**BBB**: Penetrant via Mfsd2a transporter
Clinical trials: LPC-DHA formulations have not yet been clinically tested.	**Observational studies**: Higher levels of phospholipid-DHA have been associated with higher fish intake and lower risk for dementia.

What is it?

Lysophosphatidylcholine-bound omega-3 polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are forms of PUFAs that are thought to have preferential access to the brain [1]. Omega-3 PUFAs are essential for brain structure and function, especially DHA, which comprises >30% of the lipids in the brain. Omega-3 PUFAs are bound to phospholipids in tissues, whereas they are esterified to phospholipids, triglycerides or cholesterol esters in lipoprotein carriers such as chylomicrons or high-density lipoprotein (HDL) particles, or as free albumin-bound PUFA in circulation [2]. The PUFA is typically esterified at the sn-2 position. As a result, following ingestion, esterified PUFAs are broken down by pancreatic phospholipase 2 (PLA_2) to generate free fatty acid, which can then undergo further metabolism to generate the various circulating forms. Lipases on the lipoproteins, in the liver, and on endothelial cells can also generate different forms of PUFAs [3]. PUFAs that are bound to phospholipids can be converted into lysophosphatidylcholine (LPC)-bound forms, which can be taken up into the brain and retina via the major facilitator superfamily domain-containing protein 2a (Mfsd2a) transporter expressed on barrier endothelial cells [1]. Preclinical studies suggest that supplementation directly with LPC-PUFAs (DHA/EPA) allows for superior brain uptake relative to traditional omega-3 supplements which only generate a small fraction of LPC-bound

forms after metabolic processing *in vivo*. Some clinical studies have been conducted using phospholipid-bound PUFAs, but not with preparations specifically enriched for LPC-bound PUFAs. Head-to-head clinical trials will be needed to determine whether LPC-bound omega-3 PUFA supplements will offer superior impacts to cognition relative to currently available omega-3 supplements.
A few formulations of LPC-DHA/EPA have been tested in preclinical studies, but are not yet commercially available.

AceDoPC® is a stabilized form of LPC-DHA developed at the Institut National des Sciences Appliquees de Lyon [4].

LYSOVETA™ is an LPC-DHA/EPA enriched form of krill oil developed by Aker Biomarine.

Neuroprotective Benefit: LPC-DHA/EPA forms are expected to have greater brain uptake, and offer preferential benefits for ApoE4 carriers, relative to traditional supplements, but clinical studies are needed to validate these preclinical findings.

Types of evidence:

- 2 biomarker analyses from clinical trials assessing phospholipid-DHA levels and brain measures
- 2 observational studies for phospholipid-DHA levels and dementia risk
- 1 clinical study assessing red blood cell levels of AceDoPC®
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Omega-3 PUFA are essential for brain structure and function, with DHA comprising approximately 30% of the lipids in the brain. Diets rich in omega-3 PUFA, especially fish-rich diets, have been associated with reduced risk for dementia, however, to-date, fish oil/omega-3 supplements have not been associated with the same degree of neuroprotection [5]. This suggests that there may be different brain bioavailability of the omega-3 PUFAs in foods, particularly fish, relative to the most common types of fish oil/omega-3 supplements.
PUFA can exist in different forms, and recent evidence from animal models suggests that there are different brain uptake mechanisms for the different forms, and that one particular form, which is

esterified to lysophosphatidylcholine (LPC), may have preferential access to the brain. A clinical study in premenopausal women (n=11) found that the alterations to the plasma lipidome differed following supplementation with krill oil and fish oil, which differ in the molecular form composition of omega-3 PUFAs [6]. Krill oil led to greater changes in levels of phosphatidylcholine-bound omega-3 PUFAs, which is consistent with krill oil containing a high percentage of phosphatidylcholine-bound DHA/EPA compared with fish oil, which is primarily comprised of triglyceride-bound DHA/EPA.

Only albumin-bound forms of DHA are transported into the brain. Albumin-bound DHA can exist in a non-esterified form as free fatty acid or in an esterified form to lysophosphatidylcholine (LPC-DHA). The free DHA is thought to enter the brain primarily via passive diffusion, though some transporters have also been implicated [2]. The efficiency of this mechanism is dependent on the integrity of the BBB. In contrast, there is a specific transporter on BBB endothelial cells, Mfsd2a, which facilitates the selective transport of LPC-PUFA, such as LPC-DHA. This suggests that formulations of PUFA that are enriched in LPC forms, or forms which readily convert to LPC-containing forms may be most effective in reaching the brain. While these studies comparing the brain incorporation of different forms of PUFA have been conducted in rodent models, these head-to-head comparisons have not yet been done in humans. However, there is some evidence to suggest that this uptake pathway is also relevant in humans.

One study comparing the bioavailability of 13-C labeled versions of a stabilized form of LPC-DHA called AceDoPC® with traditional triglyceride-DHA (TAG-DHA) in healthy older men (n=3) (NCT02168738) found that there was a greater enrichment of DHA-containing phospholipids with AceDoPC® (5,386 pmol/mL) compared with TAG-DHA (3,247 pmol/mL) in erythrocyte membranes, which is suggestive of higher potential brain bioavailability, but is not definitive [7].

The loss of Mfsd2a activity during neurodevelopment is associated with microcephaly. Children with homozygous missense mutations in Msfd2a show evidence of microcephaly, dystonia, spasticity, hypotonia, global neurodevelopmental delay, enlarged lateral ventricles, and reduced white matter [8]. Levels of plasma LPCs are also elevated due to the defect in CNS transport. Additionally, prenatal Zika virus infection is associated with the loss of Msfd2a on brain vascular endothelial cells, impaired brain lipid homeostasis, and microcephaly [9; 10].

Although the associations of circulating LPCs with disease states are complex because some LPC species are associated with pro-inflammatory effects, while others have been associated with anti-inflammatory effects, reductions in levels of circulating LPCs have generally been associated with chronic disease states characterized by systemic low-level inflammation, which are considered risk factors for dementia [11]. Reduced levels of LPCs in blood, CSF, and postmortem brain tissue have also been reported in dementia patients, relative to controls [11]. Notably, loss of function of the cholesterol transporter

ABCA7 is associated with increased risk for Alzheimer's disease (AD), and ABCA7 has also been shown to act as a transporter for LPCs [11].

In a cross-sectional observational study including 53 cognitively normal older adults (mean age 64.7 years), serum free DHA levels were positively associated with entorhinal cortex volume in non-ApoE4 carriers, while LPC-DHA levels were positively associated with hippocampal volume in non-ApoE4 carriers [12]. In ApoE4 carriers, there was an inverse association between circulating DHA levels and spatial cognition, suggesting that, in this population, elevated serum DHA levels may be an indication of impaired brain uptake of DHA, possibly due to BBB dysfunction. Similarly, a pilot RCT testing the impact of 2,152 mg per day of oral algal DHA for six months on lipid species in the entorhinal cortex and CSF in cognitively normal older adults (n=22) (NCT02541929) found that changes in phosphatidylcholine-DHA (PC-DHA) showed the strongest association with changes in entorhinal cortex thickness [13]. Although the association for PC-DHA was stronger than for LPC-DHA, this could be a reflection of the relative levels, since PC-DHA (PC 38:6) showed the largest increase in the CSF following supplementation. Additionally, the levels of LPC-DHA were highly correlated with the levels of PC-DHA in plasma and CSF suggesting that there may be a rapid interconversion between these different phospholipid-bound forms, in which case the PC-DHA within the CNS compartment could be a reflection of DHA that entered the CNS as LPC-DHA. This study also found an elevation of lipids in the density of HDL in the CSF following supplementation, suggesting that particles with HDL density could be carriers of DHA into the brain. Additionally, or alternatively, the HDL particles may be a relevant source of LPC-DHA. The predominant form of DHA in fish and algal oils is triglyceride-bound DHA (TAG-DHA), which then gets released as free DHA in the digestive tract, and solubilized into chylomicrons. DHA can also be taken up by lipoproteins, including HDL. Phospholipid-bound DHA on HDL can be converted to LPC-DHA due to the activity of the lipases LCAT and hepatic lipase. As a result, HDL-bound DHA in the blood could represent a population of DHA with the capacity to enter the brain, and serve as a biomarker of DHA brain levels following supplementation.

As a precursor of LPC-DHA, PC-DHA levels may also be indicative of DHA brain levels. A prospective observational study including 899 participants from the Framingham Heart Study who were cognitively normal at baseline, found that individuals in the highest quartile of plasma PC-DHA levels had a reduced risk for all-cause dementia (Relative Risk [RR] 0.53, 95% Confidence Interval [CI] 0.29 to 0.97) [14]. These individuals in the upper quartile had a mean DHA intake of 18 g per day and a mean fish intake of three servings per week. Similarly, an observational study including 135 participants found that dietary omega-3 intake was a positive predictor of PC-PUFA levels, and that plasma PC-DHA and PC-EPA levels were positive predictors of memory function in this cohort [15].

Human research to suggest benefits to patients with dementia:

Supplements specifically enriched in LPC-PUFAs have not yet been tested in dementia patients.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

DHA metabolism: Different forms of DHA (PUFA) are preferentially incorporated into different tissue types because they are broken down via different pathways following ingestion. DHA supplements typically include a form of DHA that is esterified to triglycerides (TAG-DHA) or to phospholipids, such as phosphatidylcholine (PC-DHA), at the sn-2 position. Fish oil and algal oil supplements contain PUFA in a TAG form, while krill oil supplements contain a mix of TAG and PC forms. In the digestive tract, the DHA will be cleaved at the sn-2 position by phospholipase A2 (PLA_2), and released as free fatty acid [2]. The DHA is then re-esterified to TAG, and incorporated into chylomicrons, which transport the lipids to peripheral tissues, including adipose tissue, skeletal muscle, and the heart, and then the remnants go to the liver. As a result, TAG-DHA preferentially gets incorporated into adipose, muscle, and heart tissue. A small fraction of the free DHA can also be taken up into HDL lipoprotein particles. PC-DHA in which the DHA is esterified at the sn-1 position is resistant to pancreatic PLA_2 cleavage, gets absorbed as LPC-DHA and is more readily (~two-fold) taken up into HDL particles and into circulation [16]. Within the HDL particles the PC-DHA can be converted to LPC-DHA via lipases, including LCAT and hepatic lipase. Hepatic lipase also appears to play a role in the synthesis of LPC-DHA from the liver [3]. PC-DHA in chylomicron remnants goes to the liver where it can be converted to LPC-DHA by hepatic lipase and then secreted into circulation. TAG-DHA can be coupled to albumin or lipoprotein, and the free DHA can then be transported into the brain via passive diffusion through the outer membrane leaflet of the BBB [17]. In contrast, the albumin-bound LPC-DHA can be actively transported into the inner membrane leaflet of the BBB by the transporter Mfsd2a. CNS entry via active transport is more efficient than passive diffusion, thus LPC-DHA forms appear to have preferential access. This suggests that an enrichment of phospholipid-DHA forms fosters increased brain access and incorporation [16]. Thus, higher levels of circulating PC-DHA, especially HDL-bound DHA, may be an indication of the proportion of brain penetrant DHA, and a biomarker of DHA brain uptake. This would be consistent with observational studies in humans showing associations with higher plasma PC-DHA levels with higher fish intake and lower rates of dementia [14].

Enhanced brain uptake: Msfd2a was identified as a transporter located on endothelial cells of the BBB and blood-retinal-barrier (BRB), to facilitate uptake of LPC-PUFA into the brain and retina, respectively [18]. Based on this finding, it has been hypothesized that PUFAs bound to LPCs may have preferential access to the CNS. A variety of studies conducted in rodents suggests that, at least in the species tested, LPC-PUFAs show superior incorporation into brain tissues relative to other forms of PUFAs. In male rats, TAG-DHA was released as free DHA or absorbed as TAG in chylomicrons, from which the DHA preferentially went to adipose and heart tissue [19]. LPC-DHA was preferentially incorporated into brain tissue. PC-DHA that is esterified at the sn-1 position can be readily transformed into the LPC-DHA form. Di-PC-DHA, in which there was an equal mix of species where phosphatidylcholine was esterified to the sn-1 and sn-2 positions of DHA, was half as efficient as LPC-DHA at incorporating into brain tissue. The incorporation of DHA into brain tissue was associated with an increase in BDNF levels and an enhancement of performance on the Morris water maze. Although not yet commercially available, there have been efforts from research groups to develop formulations of LPC-DHA, or related analogs, that enhance DHA uptake into the brain.

Lipase-treated krill oil: Fish oil contains DHA in the form of TAG-DHA, whereas krill oil contains a proportion of phospholipid bound DHA [20]. These largely consist of DHA esterified at the sn-2 position. *In vivo*, PC-DHA that is esterified at the sn-2 position will be cleaved by the pancreatic lipase PLA_2, similar to TAG-DHA, to generate free DHA which can bind to albumin or lipoproteins. PC-DHA esterified at the sn-1 position is resistant to this cleavage by PLA_2, and thus can be readily converted into the LPC-DHA form. Treating PC-PUFA (PC-DHA + PC-EPA) with a lipase that cleaves at the sn-1 position can generate LPC-DHA and LPC-EPA. Pre-treatment of krill oil with lipase (Mucor meihei lipase) was shown to increase the brain levels of DHA and EPA by 5-fold and 70-fold, respectively in male mice, relative to untreated krill oil [20]. Lipase treatment of fish oil did not impact its brain exposure. DHA and EPA levels in the prefrontal cortex and hippocampus were significantly increased with lipase treated krill oil, but not by fish oil. BDNF levels were greatly increased with the lipase treated krill oil, and slightly increased with the fish oil. A separate study confirmed that a similar effect was seen with lipase-treated krill oil in female mice [21].

LPC-EPA: Although the contribution to total brain lipid of EPA (<1%) is far lower than DHA (>30%), the maintenance of CNS EPA levels is important for brain structure and function, but may play a more selective role. Dietary EPA has been found to be more effective for depression than DHA, which may be related to differences in the mitigation of inflammatory signaling [22]. However, currently available EPA supplements lead to minimal brain enrichment. Preclinical studies suggest that LPC-EPA enhances levels

of both EPA and DHA in the brain, since a large percentage of LPC-EPA gets converted into DHA after entering the CNS. Levels of LPC-EPA in the plasma are much lower than those of LPC-DHA, and may be a limiting factor for EPA brain entry. Mice treated with 3.3 µmol/day of LPC-EPA or free EPA showed a 100-fold increase in brain EPA levels with LPC-EPA as well as a two-fold increase in brain DHA levels [22]. The change in brain EPA levels was accompanied by an increase in brain BDNF, CREB, and 5-HT1A, along with a decrease in TNFα. LPC-EPA, but not free EPA, also led to an increase in EPA and DHA in the retina. The preference for LPC-EPA was selective for the brain/retina and was not seen in other tissues. Enrichment of EPA in adipose tissue occurred only with free EPA, while the LPC-EPA and free EPA forms led to roughly equal EPA enrichment in the heart and liver.
PC-EPA has been shown to be neuroprotective in rodent models of AD. In a rat model of Aβ-induced neurotoxicity, PC-EPA reduced NLRP3 inflammasome activation and protected against spatial memory loss, whereas ethyl ester-EPA supplementation did not protect cognition [23]. PC-EPA also protected against cognitive decline, enhanced antioxidant activity, and reduced pro-inflammatory mediators in the SAMP8 mouse model [24].

AceDoPC®: Endogenous LPC-DHA, primarily produced in the liver, typically has LPC esterified to DHA at the sn-2 position, however, at physiological pH, there is migration of the unsaturated acyl group from the sn-2 to the sn-1 position. AceDoPC® is a synthetic analog of LPC-DHA in which there is an acetyl group at the sn-1 position, which prevents the migration of DHA from the sn-2 position [4]. LPC-DHA esterified at the sn-2 position is purported to be the more physiologically relevant isomer for tissue uptake and use. Since supplementation with both sn-1 and sn-2 forms of LPC-DHA has been shown to enhance brain DHA levels, it is unclear whether preventing this isomerization confers an advantage over administration of non-stabilized LPC-DHA. Preclinical studies indicate that supplementation with AceDoPC® led to increases in brain DHA levels that were 2- to 2.5-fold higher than with free non-esterified DHA, though AceDoPC® led to less incorporation of DHA into the heart [25]. AceDoPC® promoted neurogenesis in cell culture in physiological and hypoxic conditions and induced an antioxidant response, whereas non-esterified DHA only promoted neurogenesis under pathological/hypoxic conditions [26]. AceDoPC® and PC-DHA both attenuated LPS-induced pro-inflammatory IL-6 production in the hippocampus of mice [27]. Both AceDoPC® and DHA reduced lesion size, lipid peroxidation, and neurological deficits in a cerebral ischemic injury model (MCAO) in rats, though AceDoPC® showed greater neuroprotection in this study [28]. It has not yet been clinically tested whether AceDoPC® offers superior enhancement of human brain DHA levels relative to other commercially available DHA supplements.

APOE4 interactions: POTENTIAL BENEFIT

Clinical studies have indicated that ApoE4 carriers show less neuroprotective benefit from currently available omega-3 PUFA supplements, relative to non-ApoE4 carriers [5]. Observational studies have found that while a fish-rich diet can impact cognition in ApoE4 carriers, clinically tested DHA/EPA supplements have failed to show a similar benefit. Additionally, circulating DHA levels positively corelate with measures of brain volume and cognition in non-ApoE4 carriers, but not in ApoE4 carriers, and in some cases, there is an inverse association, suggesting that there is an impairment of DHA brain entry in ApoE4 carriers [12; 13]. To overcome this deficit in brain entry, clinical trials are testing whether supplementation with higher levels of DHA (~2 g/day) can allow for sufficient brain uptake [13]. It is hypothesized that due to the preferential brain access of the LPC-bound form, supplementation with LPC-DHA could allow for more reliable brain uptake at a lower concentration [5].

The reduction in DHA brain uptake in ApoE4 carriers is thought to be related to a reduction in BBB integrity, as it compromises the passive diffusion of free DHA [5]. As a result, elevated serum levels can be indicative of lower levels of brain uptake in this population [12]. Mice engineered to express the human form of ApoE4 have lower levels of hippocampal DHA [21]. Similar to what has been observed in some human studies, AD model mice expressing ApoE4 had elevated plasma levels of LPC-DHA, but lower brain levels, relative to those expressing ApoE3 [29]. In mice containing the human form of ApoE3 or Apoe4, supplementation with lipase-treated krill oil, which is enriched in LPC-bound forms (2.6 µmol LPC-EPA + 1.0 µmol LPC-DHA per 3 g of diet) starting at four months of age increased total plasma levels of LPC-DHA in both ApoE3 (7.1-fold) and ApoE4 (6.6-fold) mice, though the increase was slightly greater in ApoE3 mice. Similarly, hippocampal DHA levels were enriched following supplementation in both genotypes, but the effect was larger in ApoE3 mice (1.8-fold vs. 1.5-fold). Supplementation with non-lipase treated krill oil, which is not enriched for LPC-bound forms, led to hippocampal enrichment of DHA (+12%) only in ApoE3 mice. The lipase-treated krill oil also significantly increased plasma EPA levels (15–34-fold) and hippocampal EPA levels, with a stronger effect in ApoE3 mice and in females. This study suggests that supplementation with LPC-DHA/EPA may allow for greater brain uptake in the context of ApoE4.

It is unclear whether there is also a deficit in the production of LPC-PUFA or its uptake via the Mfsd2a transporter in ApoE4 carriers. Levels of LPC-DHA/EPA levels available for brain uptake could be impacted by changes in PUFA metabolism, including differences in the activity of lipases that act on PUFAs. An increase in PLA_2 activity in the presence of primarily sn-2 esterified DHA/EPA, which appears to be the dominant physiological form and what is found in supplements, could lead to an increased proportion of free DHA [12]. The activity of hepatic lipase, an enzyme involved in the generation of LPC-DHA from PC-

DHA on lipoproteins (i.e. HDL) and in the liver appears to be impacted by the ApoE isoform [3]. Hepatic lipase is activated by ApoE, but ApoE4 is less effective at activating this lipase, which could lead to a reduction in the generation of LPC-DHA. The expression of the Mfsd2a transporter appears to be impacted by levels of LPC-DHA, at least in preclinical models [30]. Thus, lower levels of LPC-DHA production could potentially lead to lower levels of Mfsd2a expression. In mice, Mfsd2a was shown to be involved in promoting the integrity of the BBB [30], thus a reduction in Mfsd2a could exacerbate BBB integrity, and further reduce free DHA transport. Supplementation with LPC-DHA directly would be expected to circumvent these issues [5]. Studies in humanized ApoE mice suggest that in the context of ApoE4, direct supplementation with LPC-DHA/EPA offers preferential benefit relative to triglyceride-bound forms, but that individuals with other isoforms of ApoE benefit also as much, or more, than ApoE4 carriers, suggesting it offers brain benefits irrespective of ApoE status [21]. Observational studies suggest that LPC-DHA is preferentially associated with brain volume measures in certain regions, whereas other regions, such as the entorhinal cortex are more associated with free DHA levels [12; 13]. These differences may be related to differences in transporter expression in different regions of the brain, such that certain brain regions may be preferentially impacted by changes in LPC-DHA levels. Ultimately, head-to-head studies are needed comparing different forms of DHA in ApoE4 carriers to determine whether LPC-DHA enriched supplements offer better brain uptake and cognitive outcomes.

Aging and related health concerns: LPC-DHA/EPA forms may have preferential access to certain tissues, such as the liver, whereas other forms may offer greater heart uptake, suggesting that a mixture may be ideal to maximally benefit all organ systems.

Types of evidence:

- 1 clinical trial testing krill oil in exercise
- 1 biomarker analysis from a clinical trial testing fish oil and phytosterols in NAFLD
- Numerous laboratory studies

Non-alcoholic fatty liver disease: POTENTIAL BENEFIT (Preclinical)
Preclinical studies suggest that phospholipid forms of PUFAs, such as DHA, may offer better access to the liver, potentially because phospholipid-PUFA contained on chylomicron remnants preferentially traffics to the liver [2; 20; 31]. A placebo-controlled RCT testing a combination of fish oil capsules (PronovaPure 150:500TG, 450 mg EPA + 1500 mg DHA) in combination with phytosterol-enriched soymilk powder (3.3 g of phytosterol Vegapure 67 WDP) for 12 weeks in 96 patients with non-alcoholic

fatty liver disease (NAFLD) found that, while not significant, the combination group showed the greatest reduction in measures of hepatic steatosis relative to those in single agent or placebo groups [32]. Metabolomic analysis indicated that levels of phosphatidylcholine (PC) and lysophosphosphatidylcholine (LPC) were inversely associated with the degree of hepatic steatosis [33]. Following supplementation, there was a shift from more pro-inflammatory n-6 LPCs (C18:2, C20:2, C22:4, C22:5) toward more anti-inflammatory n-3 LPCs (C20:5 and C22:6).

The supplementation of PC-DHA derived from *Clupea harengus* roe (100 mg/kg bw) for ten weeks significantly reduced levels of serum aminotransaminases (ALT, AST), serum lipid levels, hepatic pro-inflammatory markers (IL-6, IL-1β, and TNF-α), and hepatic oxidative stress markers (MDA, SOD, GSH-Px, and CAT) in male mice with high-fat diet-induced NAFLD [34]. Additionally, PC-DHA supplementation mitigated intestinal dysbiosis stemming from a high-fat diet, which was accompanied by changes in the species profile of the gut microbiome.

PC-DHA supplementation (300 mg/kg bw) also protected against obesity-induced osteoporosis in male mice [35]. While both PC-DHA and TAG-DHA promoted the osteogenic differentiation and inhibited the adipogenic differentiation of bone-marrow mesenchymal stem cells *in vitro*, PC-DHA was more effective in improving bone formation rates and inhibiting bone marrow fat accumulation *in vivo*, which is expected to be due to the higher bioavailability of PC-DHA.

Exercise: POTENTIAL BENEFIT DURING RECOVERY PHASE

Plasma choline levels have been shown to be reduced in response to strenuous physical exercise. Krill oil contains phosphatidylcholine-bound PUFAs. Supplementation with 2.5 g/day of Neptune krill oilTM (550 mg EPA/DHA and 150 mg choline) for 12 weeks in power training athletes led to increases in n-3 PUFAs DHA (from 4.41 to 5.31%) and EPA (from 0.41 to 1.34%) levels in red blood cell membranes, coupled with a decrease in n-6 PUFA arachidonic acid levels [36]. A study in healthy volunteers previously found that plasma choline levels were increased following a single 8 g dose of krill oil [37]. While krill oil supplementation did not impact the degree of choline depletion following one-hour of high-intensity exercise in this study, it did shorten the recovery time for choline repletion [36]. Krill oil treatment also mitigated the post-exercise decline in total antioxidant capacity (-8% vs -21%). It has not been established whether lipase pre-treated krill oil, which would be enriched for LPC-DHA and LPC-EPA would offer further benefit, relative to untreated krill oil.

Cerebrovascular injury: POTENTIAL BENEFIT (Preclinical)

Preclinical studies have found that free DHA/EPA generating forms, such as triglyceride-bound forms lead to preferential accumulation of these omega-3 PUFAs in heart tissue, relative to LPC-bound forms

[19], suggesting that currently available supplement formulations may be well-suited for cardiovascular benefit. However, LPC-bound forms may be best suited toward minimizing neurological injury in the context of a heart attack or stroke. In patients with cardiac arrest, plasma levels of LPC-DHA were positively associated with neurological outcomes, such that those with higher levels (cutoff point >0.43 µmol/L) had better outcomes [38]. Similarly, plasma LPC-DHA levels were significantly higher for patients with a higher gray to white matter radiological signal intensity, indicative of reduced brain injury. In rats, LPC-DHA levels were found to be reduced following cardiac arrest. Supplementation with LPC-DHA (i.v.) reduced levels of neuronal death, inflammation, and neurological deficits following the ischemic event. Consumption of an LPC-DHA rich diet also reduced neuronal loss and neurological impairment in a mouse model of hypoxic-ischemic brain injury [30].

Cancer: POTENTIAL BENEFIT (Preclinical)
The intake of omega-3 PUFAs has been associated with reduced risk for some forms of cancer [39]. Cell culture studies suggest that different forms of DHA have differential cytotoxic potency toward cancer cells, and that phospholipid bound forms may be the most potent. In 95D non-small-cell lung cancer (NSCLC) cells, phosphatidylcholine-DHA (PC-DHA) inhibited cancer cell growth by 53.7%, while triglyceride-DHA (TAG-DHA) inhibited growth by 33.8%, and ethyl ester-DHA did not significantly impact cancer cell proliferation [40]. The PC-DHA and TAG-DHA forms promoted cancer cell death via the upregulation of PPARγ, RXRα, Bax, and caspase-3, as well as the inhibition of NF-κB and bcl-2. In triple negative MDA-MB-231 breast cancer cells, LPC-DHA had the strongest effect at reducing cell viability (IC_{50} = 23.7 µM), followed by PC-DHA (IC_{50} = 67 µM), whereas TAG-DHA did not inhibit breast cancer cell viability in this assay [41]. The preferential cytotoxicity of LPC-DHA was related to better absorption and incorporation of DHA into cancer cell membranes from the LPC-DHA form. While *in vivo* studies are needed, these *in vitro* studies suggest that preparations of omega-3 PUFAs enriched in LPC-bound forms may offer preferential anti-cancer benefit.

Retinal degeneration: POTENTIAL BENEFIT (Preclinical)
The omega-3 PUFA transporter, Msfd2a, which is selective for LPC-bound forms of PUFAs, has been identified on endothelial cells in the blood-retinal-barrier [18]. Mice lacking Msfd2a undergo a slow progressive form of retinal degeneration, as the uptake of LPC-DHA is essential for the long-term survival of photoreceptors [42]. LPC-DHA, but not TAG-DHA, was shown to increase retinal DHA levels (+96%), which was associated with a preservation of retinal structure and function in the 5X FAD mouse model [43].

Safety: LPC-DHA/EPA supplements are expected to have a safety profile similar to currently available phospholipid, triglyceride, and ethyl ester-bound DHA/EPA supplements, including an increased risk for bleeding, but confirmatory clinical trials are needed.

Types of evidence:

- Reviews of omega-3 supplementation safety
- Numerous laboratory studies

Omega-3 PUFA preparations that are enriched for the LPC-bound forms have been tested in preclinical models, but they have not yet been clinically tested in humans. The safety profile for supplements containing this form of omega-3 PUFA is expected to be similar to that of currently available supplements, which are generally in the form of triglyceride-bound omega-3 PUFAs (see Omega-3 Fatty Acids report) [44]. Phospholipid-bound forms of omega-3 PUFAs are currently ingested during the consumption of fish, fish roe, and krill oil. Common side effects with omega-3 PUFA supplementation include gastrointestinal effects and an increased bleeding risk (WebMD).

Drug interactions: The interactions for LPC-PUFAs are expected to be similar to those for currently available supplements derived from fish oil or algal oil, in the form of triglyceride-PUFAs, or krill oil, which is in the form of phospholipid-bound PUFAs (Drugs.com). Omega-3 PUFA supplements can increase the risk for bleeding when combined with blood thinners. Omega-3 PUFAs derived from fish/shellfish should be avoided by those with fish/shellfish allergies.

Sources and dosing:

Omega-3 PUFA supplements specifically enriched for the LPC-PUFA form have not yet been clinically tested and are not currently commercially available. It is hypothesized that the dosing of LPC-DHA/EPA supplements for the purpose of brain and eye health would be lower than what is typically used with the current TAG-DHA supplements, due to enhanced access to the CNS [5].

Aker Biomarine, a Norwegian company that manufactures Superba krill oil for use in supplements has developed an LPC-DHA/EPA formulation derived from krill oil) that is not yet commercially available. called LYSOVETA™.

AceDoPC®, a stabilized synthetic form of LPC-DHA, was shown to promote the enrichment of phospholipids bound to DHA in plasma and red blood cells in a pilot clinical study (n=3) (NCT02168738). The patent for AceDoPC® is held by the Institut National des Sciences Appliquees de Lyon. All of the studies conducted thus far have been academic in nature, and it is unclear whether it will be developed as a commercial product.
Currently, the best source of phospholipid-bound DHA/EPA, potentially including LPC-bound forms, is through the consumption of fresh raw fish, with the highest concentration in fish roe [5]. An analysis of natural aquatic products found that the highest levels of phospholipid-bound DHA/EPA were found in Antarctic krill (2574.69 µg/g), mackerel (2330.11 µg/g), salmon (2109.91 µg/g), and Farrer's scallop (1883.59 µg/g) [45]. Processing, such as cooking, freezing, and fermenting, reduces the concentration of phospholipid-bound PUFAs in the fish [46].

Krill oil contains a mixture of DHA/EPA that is bound to phospholipids (~35%) and to triglycerides [5]. However, both forms are bound to DHA/EPA at the sn-2 position, which means that they will be cleaved by PLA_2 in the digestive tract and form free DHA/EPA. There have been some claims that a small proportion of the phospholipid-bound DHA/EPA in krill oil may be in the sn-1 position, and thus generate LPC-DHA/EPA [11].
Lipase treatment of krill oil has been used to generate LPC-DHA/EPA for preclinical research studies [20]. It has been reported that the lipases Lipozyme RM-IM®, Lipozyme TL-IM®, and Novozym 435® generate the highest yields of LPC-bound forms [46].

There are also some supplements containing phospholipid-bound DHA/EPA derived from fish roe.

Additionally, there have been clinical studies testing whether combining DHA with choline supplementation can increase levels of LPC-DHA. A study testing choline chloride (480 or 930 mg/day) plus DHA (200 mg) in women (n=71) (NCT01127022) found that this combination was associated with an increase in levels of d3-LPC-DHA, indicating an increase in LPC-DHA production via the phosphatidylethanolamine N-methyltransferase (PEMT) pathway of phosphatidylcholine biosynthesis [47]. The effect was modified by reproductive status, such that the increase was not seen in pregnant and lactating women, which could be due to altered metabolism and/or enhanced placental/mammary uptake of LPC-DHA. A related study found that the combined supplementation of DHA with 550 mg/day choline during the third trimester of pregnancy did boost levels of d3-PC and improved hepatic DHA export by boosting PEMT activity [48].

Research underway:

Preclinical studies testing formulations of LPC-DHA/EPA in animal models have been published with increasing frequency, however, it is not clear when clinical studies testing these formulations will be conducted.

Search terms:

Pubmed, Google: Lysophosphatidylcholine-DHA/EPA

- Alzheimer's disease, neurodegeneration, aging, cardiovascular, cancer, clinical trial, safety

Websites visited for LPC-DHA/EPA:

- Clinicaltrials.gov (AceDoPC®)
- Examine.com (Fish oil)
- Drugs.com (Fish oil)
- WebMD.com (DHA)

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