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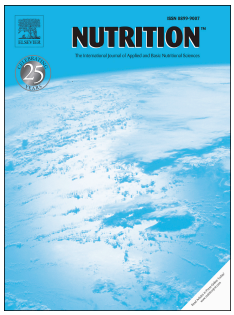

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Phosphatidylserine and the Human Brain

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Abstract

Objective: The roles and importance of phosphatidylserine, an endogenous phospholipid and dietary nutrient, in human brain biochemistry, physiology, and function were assessed.

Methods: A scientific literature search was conducted on MEDLINE (National Library of Medicine, Bethesda, MD, USA) for relevant articles regarding phosphatidylserine and the human brain published prior to June 2014. Additional publications were identified from references provided in original papers. 127 articles were selected for inclusion in this review.

Results: A large body of scientific evidence describes the interactions among phosphatidylserine, cognitive activity, cognitive aging, and retention of cognitive functioning ability.

Conclusion: Phosphatidylserine is required for healthy nerve cell membranes and myelin. Aging of the human brain is associated with biochemical alterations and structural deterioration that impair neurotransmission. Exogenous phosphatidylserine (300 mg to 800 mg daily) is absorbed efficiently in humans, crosses the blood-brain barrier, and safely slows, halts or reverses biochemical alterations and structural deterioration in nerve cells and supports human cognitive functions, including the

formation of short-term memory, the consolidation of long-term memory, the ability to create new memories, the ability to retrieve memories, the ability to learn and recall information, the ability to focus attention and concentrate, the ability to reason and solve problems, language skills and the ability to communicate, and locomotor functions, especially rapid reactions and reflexes.

Keywords: phosphatidylserine; neurotransmission; cognitive function; cognitive decline; cognitive aging

Phosphatidylserine is the major acidic phospholipid in human membranes and constitutes 2% to 20% of the total phospholipid mass of adult human plasma and intracellular membranes [1-3]. Within the healthy human brain, myelin is enriched in phosphatidylserine [4,5] and the phosphatidylserine content of grey matter doubles from birth to age 80 years [4]. Throughout the human body, phosphatidylserine is a structural component of endoplasmic reticulum, nuclear envelopes, Golgi apparati, inner (cytosolic) leaflets of plasma membranes, outer mitochondrial membranes, and myelin [1-9].

About 20% to about 30% of the phosphatidylserine in human grey matter is in the form of 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoserine [4,10-13]. The docosahexaenoic acid (DHA) content of neuronal phosphatidylserine is of functional importance [12]; in the cortex of the brain, a reduction in the DHA content of phosphatidylserine is associated with the progression of mild cognitive impairment to Alzheimer's disease [14]. Consequently, the incorporation of phosphatidylserine into human membranes is sensitive to the availability of both phosphatidylserine and DHA [4,10,11]. In addition, fatty acid recycling at the *sn*-1 and *sn*-2 positions of phosphatidylserine is frequent, rapid and energy-consuming, allowing co-accumulation of DHA and phosphatidylserine [10,11,15] and facilitating DHA enrichment of phosphatidylserine molecules within membranes [11].

Phosphatidylserine Synthesis and Incorporation into Membranes

Most phosphatidylserine that is synthesized *de novo*, including that synthesized within the central nervous system, results from the phosphatidylserine synthase 1- (PSS1-)

83 catalyzed substitution of serine for choline on phosphatidylcholine within mitochondria-
84 associated membrane (MAM) domains of the endoplasmic reticulum [13,16-25]. Some
85 newly synthesized phosphatidylserine is transported from the endoplasmic reticulum to
86 the inner (cytosolic) leaflet of the plasma membrane [1], where thermodynamic barriers
87 minimize its movement to the outer (extracellular) leaflet of the plasma membrane; all
88 healthy human cells exhibit phosphatidylserine-rich cytosolic plasma membrane leaflets
89 and phosphatidylserine-poor extracellular leaflets [1,26-31]. Maintenance of
90 transmembrane phosphatidylserine asymmetry is critical to cell survival; active
91 translocation of phosphatidylserine to the extracellular leaflet is a required and
92 irreversible signal for the initiation of phagocytic engulfment of apoptotic cells [16,32-
93 40]. In order to avoid inappropriate engulfment, healthy cells devote up to 4% of all ATP
94 consumption to maintaining transmembrane phosphatidylserine asymmetry [15,41].
95
96 Most newly synthesized phosphatidylserine is actively transported from MAM domains
97 of the endoplasmic reticulum to the outer leaflet of the mitochondrial inner membrane [6-
98 8,19-23,42-48]. Phosphatidylserine synthesizing MAM domains of the endoplasmic
99 reticulum tether transiently to the cytosolic leaflet of the mitochondrial outer membrane
100 via interactions involving MAM domains, the mitochondrial outer membrane and the
101 endoplasmic reticulum-mitochondria encounter structure (ERMES), a complex of 5
102 proteins (Mmm1, Mdm10, Mdm12, Mdm34 and mitofusin2) that forms a molecular
103 bridge between the endoplasmic reticulum and mitochondrion [49-52]. Movement from
104 the cytosolic leaflet of the outer mitochondrial membrane to the outer leaflet of the inner
105 mitochondrial membrane requires metabolic energy, is very rapid and typically depletes

the outer mitochondrial membrane of phosphatidylserine [6-8,48], generating a requirement for nearly continuous replenishment from the endoplasmic reticulum [42,43,52,53]. Once within the inner mitochondrial membrane, phosphatidylserine is converted rapidly to another major membrane phospholipid, phosphatidylethanolamine, by phosphatidylserine decarboxylase-1 in a reaction that produces most of a cell's phosphatidylethanolamine [1,20,42,47,53-56]. As intracellular phosphatidylethanolamine content reaches a steady-state, a small amount is transported across ERMES into MAM domains of the endoplasmic reticulum for reconversion into phosphatidylserine by phosphatidylserine synthase 2 (PSS2) [10,18-21]. The expression of PSS2 is greatest in the phosphatidylserine-enriched brain and testes [24,25].

Oral phosphatidylserine is highly bioavailable in humans [57] and readily crosses the blood-brain barrier [57,58]. The amount of exogenous phosphatidylserine that is incorporated into human cell membranes and is transported from the plasma membrane's outer leaflet to its inner leaflet by a phosphatidylserine-specific ATP-dependent aminophospholipid translocase ("flippase") [19,21,27-30,41,59,61] increases as phosphatidylserine intake increases [30,41,57-60]. As intracellular phosphatidylserine content increases, the activities of PSS1 and PSS2 decrease, conserving phosphatidylcholine and phosphatidylethanolamine [17,19,40,56,62].

Phosphatidylserine and Neurotransmission

The incorporation of phosphatidylserine into neuronal cell membranes influences the metabolism of the neurotransmitters acetylcholine, norepinephrine, serotonin and

dopamine [63-65]. Adequate amounts of DHA-enriched phosphatidylserine are required for the fusion of intraneuronal secretory granules with the presynaptic membrane, the subsequent release of neurotransmitter molecules into the synaptic cleft during the intracellular transmission of action potentials and proper postsynaptic neurotransmitter-receptor interactions [12,66]. In addition, exogenous phosphatidylserine stimulates electroencephalographic evidence of increased cholinergic neurotransmission in healthy men and women [57].

The neurotransmitter-driven postsynaptic activation of the signal transducer, calcium/calmodulin-dependent protein kinase C, requires an interaction between postsynaptic membrane-associated *sn*-1,2-diacylglycerol/protein kinase C complexes and postsynaptic membrane-bound phosphatidylserine [67-69]. The binding of *sn*-1,2-diacylglycerol (originating from the acetylcholine-triggered catabolism of either phosphatidylinositol or phosphatidylcholine [70,71]) to the membrane targeting domain of protein kinase C increases the affinity of protein kinase C for the negatively-charged serine-rich head groups of postsynaptic membrane-bound phosphatidylserine (but not for other phospholipids). The ionic attraction of these phosphatidylserine-specific clusters of negative charge is required for the attraction of cytosolic calmodulin-associated Ca^{2+} ions to protein kinase C [18,27-29,72,73]. The formation of a *sn*-1,2-diacylglycerol/Ca^{2+} ion/phosphatidylserine/protein kinase C complex induces a de-inhibiting conformational change in the catalytic site of protein kinase C that activates the enzyme; subsequent downstream phosphorylations of intracellular proteins by activated protein kinase C and

the biochemical consequences of those phosphorylations “translates” the presynaptic message into specific responses within the postsynaptic cell [74].

Aging and Deterioration of the Human Brain

Aging of the human brain is associated with loss of neurons, dendritic atrophy, loss of synaptic connections, decreased synaptic density, decreased synthesis of acetylcholine and other neurotransmitters, abnormal neuronal membrane lipid composition (especially decreased membrane phosphatidylserine content and increased membrane cholesterol content), and reduced sensitivity of postsynaptic membranes to acetylcholine [63,64,75-81]. A decrease in the ratio of phosphatidylserine to cholesterol within neuronal membranes causes neurochemical changes which can contribute to an increase in the viscosity of cellular membranes, thus reducing enzymatic activities that require optimum fluidity. These cell membrane changes can be indirectly responsible for alterations in enzymatic activities, receptor functions, membrane carriers and neuronal electrical characteristics, and can result in functional impairments [63,75,80].

Phosphatidylserine in the Deteriorating Brain

In intact aged rats, ingested phosphatidylserine increases interneuronal communication by increasing the fluidity of cell membranes [59,63,64], eliminates the typical age-dependent decreases in stimulus-evoked acetylcholine release, cholinergic functioning and cognitive problem-solving [82-84], and stimulates enhanced performance on tasks that test learning ability and short-term memory [82,85-87]. These beneficial outcomes have been associated with rapid incorporation of supplemental phosphatidylserine into neuronal cell

membranes [75], increases in cell membrane-associated ATPase activity and in the synthesis of acetylcholine and dopamine in the cerebral cortex [75,83,84,87-89], increased cholinergic neurotransmission and signal transduction [83,84,89,90], deceleration of the rate of loss of dendritic connections (prolonging the maintenance of pyramidal dendritic spine density) in the hippocampus [91], attenuation of the rate of loss of receptors for nerve growth factor in the hippocampus [91] (which might facilitate the ability of nerve growth factor to stimulate effective remodeling of interneuronal connections, possibly restoring dendritic spine density [91]), arrest of atrophy of cholinergic cells in the basal forebrain [92], increased resistance to pro-apoptotic stimuli [66], and reduced frequency of the normal rodent age-associated episodes of erratic electroencephalographic patterns [85].

In humans, the incorporation of exogenous phosphatidylserine into brain structures is functionally relevant; for example, human studies using positron emission tomography (PET) to investigate brain glucose utilization in patients with Alzheimer's disease have noted evidence of significantly increased glucose utilization in response to supplementation with phosphatidylserine, especially in the temporo-parietal areas which are specifically affected by this disease [93-96]. Such biochemical responses to phosphatidylserine supplementation elicit physiological processes that produce functional manifestations reflecting the impact of exogenous phosphatidylserine on neuronal membranes in the central nervous system.

In open-label trials, elderly subjects with mild degrees of decline in cognitive function have responded to 60 days of dietary supplementation with 300 mg of oral phosphatidylserine (100 mg, t.i.d.) with significantly improved performance on tests of verbal learning, verbal recall, verbal fluency, visual learning, attention, communication skills, initiative, socialization and self-sufficiency [97,98]. Similar results were obtained in similar subjects following 90 days of the same level of daily supplementation; in addition, the abilities to recall names and recognize faces also were improved [99]. Other groups of elderly men and women with subjective memory complaints have experienced significantly improved abilities to sustain attention and to recall words after 6 weeks [100], 12 weeks [101], or 15 weeks [102] of supplemental phosphatidylserine (100 mg t.i.d. [100,101] or 100 mg daily [103]). Significant improvements in verbal learning, verbal recall, attention span and ability to concentrate, vigilance, initiation, socialization and self-sufficiency also were observed in elderly adults with more severe cognitive impairment, following 2 months of oral supplementation with phosphatidylserine (100 mg, t.i.d.) [104,105]. The improvements observed after 15 weeks of daily supplementation with 300 mg of phosphatidylserine were sustained for another 15 weeks by continued dietary supplementation with 100 mg of phosphatidylserine daily [102].

The effectiveness of oral phosphatidylserine supplementation also has been studied in double-blind placebo-controlled randomized clinical trials. Elderly men and women over 60 years of age exhibiting mild memory loss have been given placebo or oral phosphatidylserine (100 mg, t.i.d.) for 90 days [106]. Compared to the effects of placebo, which was ineffective, phosphatidylserine supplementation produced significant

219 improvements in short-term recall, immediate memory, vocabulary skills and ability to
220 recall words, attention and vigilance. More severe deterioration of cognitive functions
221 (such as attention, concentration, learning ability, and ability to perform daily activities),
222 but without dementia or pseudodementia, also has responded to supplementation with
223 oral phosphatidylserine (100 mg, t.i.d., for 2 months), with significantly greater
224 improvements in verbal recall, initiation, withdrawal, apathy and overall cognitive
225 functioning than those produced by placebo [107]. Similar results were obtained when
226 elderly adults with moderately severe cognitive impairment were supplemented with oral
227 phosphatidylserine (100 mg, t.i.d.) for 6 months [63]. In addition, long-term memory and
228 ability to perform the activities of daily living were improved significantly.
229
230 In one study of elderly subjects with memory impairments, there were no responses to 12
231 weeks of daily dietary supplementation with phosphatidylserine (200 mg, t.i.d.) [108].
232 However, this study used a preparation of mixed phospholipids that had been produced
233 by enzymatic transesterification of soybean-derived phosphatidylcholine. The crude
234 nature of this formulation may have affected the outcome of the trial; the investigators
235 speculated that the absorption of phosphatidylserine from this preparation may have been
236 minimal.
237
238 Patients exhibiting symptoms of chronic depression also have responded to
239 phosphatidylserine supplementation (100 mg, t.i.d., for 1 to 6 months) with decreased
240 apathy, withdrawal and sleep disturbances and increases in motivation and interest in
241 others [63,107,109]. These beneficial effects have been accompanied by improved

memory performance [109], increases in electroencephalographic alpha rhythm that are indicative of increased acetylcholinergic activity [98], and positron emission tomography (PET) evidence of increased brain glucose utilization [94,95].

In addition to enhancing cognition in healthy humans, the daily consumption of 300 mg of phosphatidylserine (100 mg, t.i.d.) has been effective in retarding, arresting or reversing cognitive deterioration by interrupting cognitive decline and, therefore, in reducing the risk of later development of dementia [65,93,96,110,111]. Most studies have employed phosphatidylserine that was extracted from bovine or porcine sources; however, in one study, phosphatidylserine of plant origin was equally effective [99].

In one placebo-controlled randomized double-blind trial of nondemented elderly patients with mild degrees of accelerated cognitive deterioration, 8 weeks of supplemental phosphatidylserine (100 mg t.i.d.) was accompanied by improved ability to perform executive functions and electroencephalographic evidence of normalization of some brain functions; these improvements persisted for at least 16 weeks (the extent of follow-up) after discontinuation of supplementation [103]. However, in a placebo-controlled randomized double-blind trial of elderly patients with more severe memory loss and cognitive decline, although 6 weeks of daily supplemental phosphatidylserine (100 mg t.i.d.) stabilized cognitive function, with improvements in recall, long-term memory, pattern recognition and ability to perform the activities of daily living that were significantly greater than those produced by placebo, discontinuation of

phosphatidylserine supplementation was followed by resumption of pre-supplementation rates of cognitive deterioration [111].

Elderly patients diagnosed with Alzheimer's disease also have benefitted from supplemental phosphatidylserine. For example, in one placebo-controlled randomized double-blind trial of elderly patients with severe cognitive impairments secondary to Alzheimer's disease who were given supplemental phosphatidylserine (200 mg daily for 3 months), the investigators reported significantly greater improvements in memory, information processing and the ability to perform activities of daily living than those produced by placebo [110]. In another trial in which oral phosphatidylserine (400 mg daily) was administered to patients with Alzheimer's disease, the addition of phosphatidylserine supplementation to a cognitive training program for 16 weeks resulted in significantly greater improvements in performance on neuropsychological tests than did cognitive training alone [94]. However, the progression of disease was not halted by phosphatidylserine, with deterioration of performance noted in most patients four months later despite continued phosphatidylserine supplementation. It is not known whether larger phosphatidylserine intakes may have attenuated disease progression in these patients. In other trials that have studied patients with confirmed Alzheimer's disease, improvements in cognitive function associated with phosphatidylserine supplementation (300 mg to 400 mg daily) generally have been greatest in the least severely impaired patients [65,93,95].

The ability of dietary supplementation with phosphatidylserine to support cognition and interrupt cognitive deterioration was recognized by the U.S. Food and Drug Administration in its approval of the qualified health claims, “Consumption of phosphatidylserine may reduce the risk of dementia in the elderly” and “Consumption of phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly” [112].

Phosphatidylserine also may protect cell membranes from oxidative damage. In cell culture studies, human neurons cultured in the presence of phosphatidylserine (25 μM) exhibited significant reductions in electric shock-induced ROS production [113] and phosphatidylserine supplementation has been reported to inhibit the oxidation of cell membrane phospholipids by ROS generated by xanthine oxidase [114,115]. Concurrent with inhibition of oxidation of cell membrane phospholipids was reduction in the rate of free radical-induced cell death. Anti-oxidant defenses are bolstered by phosphatidylserine; rats fed phosphatidylserine upregulated antioxidant enzyme activities in the brain (SOD and catalase) and liver (SOD and glutathione peroxidase) [113] and the capacity of human HDL particles to prevent the oxidation of circulating LDL particles is proportional to the phosphatidylserine content of the HDL particles [116,117].

Increased circulating concentrations of phosphatidylserine also attenuate the endocrine responses to exercise-induced acute stress. When healthy men received single intravenous infusions of either placebo or phosphatidylserine just prior to the initiation of a strenuous workout on a stationary cycle, the typical exercise-induced stress response (increases in plasma adrenocorticotropin (ACTH) and cortisol concentrations) [118]

occurred only following infusions of placebo and not after acute administration of phosphatidylserine [119]. Oral phosphatidylserine also attenuates the "stress response;" daily supplementation with 300 mg of phosphatidylserine for 1 month [120], 400 mg for 21 days [121], 600 mg for 21 days [121], 600 mg for 10 days [122], 800 mg for 10 days [123], 800 mg for 21 days [121], or 800 mg for 14 days [124] suppressed the typical exercise-induced spikes in the serum concentrations of ACTH and cortisol that accompanied the initiation of cycling exercise in healthy young physically-conditioned men [123,124] or exposure to acute psychological stress in healthy young men and women [120,121]. In one study, supplementation with phosphatidylserine increased subjects' exercise capacity [125]. Together these findings indicate that supplemental phosphatidylserine interacts with neuronal cell membranes within the human brain to blunt the typical pituitary ACTH secretory response to hypothalamic stimuli, reduce resting serum cortisol concentrations, and attenuate the expected hypersecretion of cortisol during and after exercise [118-125].

The Safety of Dietary Supplementation with Phosphatidylserine

In addition to the absence of reports in the published scientific literature of adverse reactions concerning oral supplementation with phosphatidylserine, the safety of dietary supplementation with phosphatidylserine has been demonstrated in many human clinical trials[57,63,65,93-112,119-127] and has been documented in detail by several investigators [63,102,105,126,127]. The U.S. Food and Drug administration also endorsed the safety of daily dietary supplementation with up to 300 mg of phosphatidylserine [112].

Conclusions

Phosphatidylserine is required for healthy nerve cell membranes and myelin. Oral phosphatidylserine is absorbed efficiently in humans and crosses the blood-brain barrier following its absorption into the bloodstream, increasing the supply of phosphatidylserine to the brain. Increasing the supply of phosphatidylserine increases the incorporation of phosphatidylserine into neuronal cell membranes. The incorporation of adequate amounts of phosphatidylserine within nerve cell membranes is required for efficient neurotransmission throughout the human nervous system.

Aging of the human brain during adulthood is associated with biochemical alterations and structural deterioration that impair neurotransmission. Exogenous phosphatidylserine slows, halts or reverses biochemical alterations and structural deterioration in nerve cells and supports human cognitive functions, including the formation of short-term memory, the consolidation of long-term memory, the ability to create new memories, the ability to retrieve memories, the ability to learn and recall information, the ability to focus attention and concentrate, the ability to reason and solve problems, language skills and the ability to communicate, and locomotor functions, especially rapid reactions and reflexes. Increasing the supply of phosphatidylserine to the human central nervous system through dietary supplementation with 300 mg to 800 mg of phosphatidylserine daily safely attenuates the increase in cortisol secretion that is induced by acute stressors, including moderate- to high-intensity exercise.

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