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Review

Phosphatidylserine and the human brain

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ABSTRACT

Objective: The aim of this study was to assess the roles and importance of phosphatidylserine (PS), an endogenous phospholipid and dietary nutrient, in human brain biochemistry, physiology, and function.
Methods: A scientific literature search was conducted on MEDLINE for relevant articles regarding PS and the human brain published before 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 PS, 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 PS (300–800 mg/d) is absorbed efficiently in humans, crosses the blood–brain barrier, and safely slows, halts, or reverses biochemical alterations and structural deterioration in nerve cells. It 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. It also supports locomotor functions, especially rapid reactions and reflexes.

Introduction

Phosphatidylserine (PS) 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 PS [4,5] and the PS content of gray matter doubles from birth to age 80 y [4]. Throughout the human body, PS 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 30% of the PS in human gray matter is in the form of 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoserine [4,10–13]. The docosahexaenoic acid (DHA) content of neuronal PS is of functional importance [12]; in the cortex of the brain, a reduction in the DHA content of PS is associated with the progression of mild cognitive impairment to Alzheimer's disease [14]. Consequently, the incorporation of PS into human membranes is sensitive to the availability of both PS and DHA [4,10,11]. Additionally, fatty-acid recycling at the *sn*-1 and *sn*-2 positions of PS is frequent, rapid and energy-consuming, allowing co-accumulation of DHA and PS [10,11,15] and facilitating DHA enrichment of PS molecules within membranes [11].

Phosphatidylserine synthesis and incorporation into membranes

Most PS that is synthesized de novo, including that synthesized within the central nervous system, results from the PS synthase 1- (PSS1-) catalyzed substitution of serine for choline on PS within mitochondria-associated membrane (MAM) domains of the endoplasmic reticulum (ER) [13,16–25]. Some newly synthesized PS is transported from the ER to the inner (cytosolic) leaflet of the plasma membrane [1], where thermodynamic barriers minimize its movement to the outer (extracellular) leaflet of the plasma membrane; all healthy human cells exhibit

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PS-rich cytosolic plasma membrane leaflets and PS-poor extracellular leaflets [1,26–31]. Maintenance of transmembrane PS asymmetry is critical to cell survival; active translocation of PS to the extracellular leaflet is a required and irreversible signal for the initiation of phagocytic engulfment of apoptotic cells [16,32–40]. To avoid inappropriate engulfment, healthy cells devote up to 4% of all adenosine triphosphate (ATP) consumption to maintaining transmembrane PS asymmetry [15,41].

Most newly synthesized PS is actively transported from MAM domains of the ER to the outer leaflet of the mitochondrial inner membrane [6–8,19–23,42–48]. Phosphatidylserine-synthesizing MAM domains of the ER tether transiently to the cytosolic leaflet of the mitochondrial outer membrane via interactions involving MAM domains, the mitochondrial outer membrane, and the ER-mitochondria encounter structure (ERMES), a complex of 5 proteins (Mmm1, Mdm10, Mdm12, Mdm34, and mitofusin2) that forms a molecular bridge between the ER and mitochondrion [49–52]. Movement from the cytosolic leaflet of the outer mitochondrial membrane to the outer leaflet of the inner mitochondrial membrane requires metabolic energy, is very rapid, and typically depletes the outer mitochondrial membrane of PS [6–8,48], generating a requirement for nearly continuous replenishment from the ER [42,43,52,53]. Once within the inner mitochondrial membrane, PS is converted rapidly to another major membrane phospholipid, phosphatidylethanolamine (PE), by PS decarboxylase-1 in a reaction that produces most of a cell's PE [1,20,42,47,53–56]. As intracellular PE content reaches a steady-state, a small amount is transported across ERMES into MAM domains of the ER for reconversion into PS by PS synthase 2 (PSS2) [10,18–21]. The expression of PSS2 is greatest in the PS-enriched brain and testes [24,25].

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

Phosphatidylserine and neurotransmission

The incorporation of PS into neuronal cell membranes influences the metabolism of the neurotransmitters acetylcholine (ACh), norepinephrine, serotonin, and dopamine [63–65]. Adequate amounts of DHA-enriched PS 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]. Additionally, exogenous PS stimulates electroencephalographic (EEG) 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 (PKC), requires an interaction between postsynaptic membrane-associated *sn*-1,2-diacylglycerol (DAG)/PKC complexes and postsynaptic membrane-bound PS [67–69]. The binding of DAG (originating from the ACh-triggered catabolism of either phosphatidylinositol or phosphatidylcholine [70,71]) to the membrane targeting domain of PKC increases the affinity of PKC for the negatively charged serine-rich head groups of postsynaptic membrane-bound PS (but not for other phospholipids). The ionic attraction of these PS-specific clusters of negative charge is required for the attraction of cytosolic calmodulin-associated Ca^{2+} ions to PKC [18,27–29,72,73]. The formation of a DAG/Ca^{2+} ion/PS/PKC complex induces a de-inhibiting conformational change in the catalytic site of PKC that activates the enzyme; subsequent downstream phosphorylations of intracellular proteins by activated PKC 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 ACh and other neurotransmitters, abnormal neuronal membrane lipid composition (especially decreased membrane PS content and increased membrane cholesterol content), and reduced sensitivity of postsynaptic membranes to ACh [63,64,75–81]. A decrease in the ratio of PS to cholesterol within neuronal membranes causes neurochemical changes that 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 PS increases interneuronal communication by increasing the fluidity of cell membranes [59,63,64], eliminates the typical age-dependent decreases in stimulus-evoked ACh 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 PS into neuronal cell membranes [75], increases in cell membrane-associated ATPase activity and in the synthesis of ACh 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 proapoptotic stimuli [66], and reduced frequency of the normal rodent age-associated episodes of erratic EEG patterns [85].

In humans, the incorporation of exogenous PS 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 PS, especially in the temporoparietal areas that are specifically affected by this disease [93–96]. Such biochemical responses to PS supplementation elicit physiological processes that produce functional manifestations reflecting the impact of exogenous PS on neuronal membranes in the central nervous system.

In open-label trials, older individuals with mild degrees of decline in cognitive function have responded to 60 d of dietary supplementation with 300 mg of oral PS (100 mg three times daily) 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 individuals following 90 d of the same level of daily supplementation; additionally, the abilities to recall names and recognize faces also were improved [99]. Other groups of older men and women with subjective memory complaints have experienced significantly improved abilities to sustain attention and to recall words after 6 wk [100], 12 wk [101], or 15 wk [102] of supplemental PS (100 mg three times daily [100,101] or 100 mg/d [103]). Significant improvements in verbal learning, verbal recall, attention span and ability to concentrate, vigilance, initiation, socialization, and self-sufficiency also were observed in older adults with more severe cognitive impairment, following 2 mo of oral supplementation with PS (100 mg three times daily) [104,105]. The improvements observed after 15 wk of daily supplementation with 300 mg of PS were sustained for another 15 wk by continued dietary supplementation with 100 mg/d of PS [102].

The effectiveness of oral PS supplementation also has been studied in double-blind placebo-controlled randomized clinical trials. Men and women age >60 y exhibiting mild memory loss were given placebo or oral PS (100 mg three times daily) for 90 d [106]. Compared with the effects of placebo, which was ineffective, PS supplementation produced significant improvements in short-term recall, immediate memory, vocabulary skills and ability to recall words, attention, and vigilance. More severe deterioration of cognitive functions (such as attention, concentration, learning ability, and ability to perform daily activities), but without dementia or pseudo-dementia, also has responded to supplementation with oral PS (100 mg three times daily for 2 mo), with significantly greater improvements in verbal recall, initiation, withdrawal, apathy, and overall cognitive functioning than those produced by placebo [107]. Similar results were obtained when older adults with moderately severe cognitive impairment were supplemented with oral PS (100 mg three times daily) for 6 mo [63]. Additionally, long-term memory and ability to perform activities of daily living (ADLs) improved significantly.

In one study of elderly individuals with memory impairments, there were no responses to 12 wk of daily dietary supplementation with PS (200 mg three times daily) [108]. However, this study used a preparation of mixed phospholipids that had been produced by enzymatic transesterification of soybean-derived phosphatidylcholine. The crude nature of this formulation may have affected the outcome of the trial; the investigators speculated that the absorption of PS from this preparation may have been minimal.

Patients exhibiting symptoms of chronic depression also have responded to PS supplementation (100 mg three times daily, for 1–6 mo) with decreased apathy, withdrawal, and sleep disturbances and increased motivation and interest in others [63,107,109]. These beneficial effects have been accompanied by improved memory performance [109], increases in EEG alpha rhythm that are indicative of increased acetylcholinergic activity [98], and PET evidence of increased brain glucose utilization [94,95].

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

In one placebo-controlled randomized double-blind trial of nondemented older patients with mild degrees of accelerated cognitive deterioration, 8 wk of supplemental PS (100 mg three times daily) was accompanied by improved ability to perform executive functions and EEG evidence of normalization of some brain functions; these improvements persisted for at least 16 wk (the extent of follow-up) after discontinuation of supplementation [103]. However, in a placebo-controlled randomized double-blind trial of older patients with more severe memory loss and cognitive decline, although 6 wk of daily supplemental PS (100 mg three times daily) stabilized cognitive function, with improvements in recall, long-term memory, pattern recognition, and ability to perform ADLs that were significantly greater than those produced by placebo, discontinuation of PS supplementation was followed by resumption of presupplementation rates of cognitive deterioration [111].

Older patients diagnosed with Alzheimer's disease also have benefited from supplemental PS. For example, in one placebo-controlled randomized double-blind trial of older patients with severe cognitive impairments secondary to Alzheimer's disease who were given supplemental PS (200 mg/d for 3 mo), the investigators reported significantly greater improvements in memory, information processing, and the ability to perform ADLs than those produced by placebo [110]. In another trial in which oral PS (400 mg/d) was administered to patients with Alzheimer's disease, the addition of PS supplementation to a cognitive training program for 16 wk resulted in significantly greater improvements in performance on neuropsychological tests than did cognitive training alone [94]. However, PS did not halt progression of the disease and deterioration of performance was noted in most patients 4 mo later despite continued PS supplementation. It is not known whether larger PS 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 PS supplementation (300–400 mg/d) generally have been greatest in the least severely impaired patients [65,93,95].

The ability of dietary supplementation with PS to support cognition and interrupt cognitive deterioration was recognized by the FDA 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 PS (25 μM) exhibited significant reductions in electric shock-induced reactive oxygen species (ROS) production [113], and PS 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, there was reduction in the rate of free radical-induced cell death. Antioxidant defenses are bolstered by PS; rats fed PS up-regulated antioxidant enzyme activities in the brain (superoxide dismutase and catalase) and liver (superoxide dismutase and glutathione peroxidase) [113] and the capacity of human HDL particles to prevent the oxidation of circulating LDL particles is proportional to the PS content of the HDL particles [116,117].

Increased circulating concentrations of PS also attenuate the endocrine responses to exercise-induced acute stress. When healthy men received single intravenous infusions of either placebo or PS just before the initiation of a strenuous workout on a stationary bike, 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 PS [119]. Oral PS also attenuates the "stress response"; supplementation with PS 300 mg/d for 1 mo [120], 400 mg for 21 d [121], 600 mg for 21 d [121], 600 mg for 10 d [122], 800 mg for 10 d [123], 800 mg for 21 d [121], or 800 mg for 14 d [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 PS increased subjects' exercise capacity [125]. Together these findings indicate that supplemental PS 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].

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 PS, the safety of dietary supplementation with PS 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 FDA also endorsed the safety of daily dietary supplementation with up to 300 mg of PS [112].

Conclusions

Phosphatidylserine is required for healthy nerve cell membranes and myelin. Oral PS is absorbed efficiently in humans and crosses the blood–brain barrier following its absorption into the bloodstream, increasing the supply of PS to the brain. Increasing the supply of PS increases the incorporation of it into neuronal cell membranes. The incorporation of adequate amounts of PS 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 PS 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 PS to the human central nervous system through dietary supplementation with 300 to 800 mg of PS daily safely attenuates the increase in cortisol secretion that is induced by acute stressors, including moderate- to high-intensity exercise.

References

[1] van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 2008;9:112–24.
[2] Kobayashi T, Beuchat MH, Chevallier J, Makino A, Mayran N, Escola JM, et al. Separation and characterization of late endosomal membrane domains. J Biol Chem 2002;277:32157–64.
[3] Coniglio JG, Grogan WM Jr, Rhamy RK. Lipids of human testes removed at orchidectomy. J Reprod Fertil 1974;41:67–73.
[4] Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 1968;9:570–9.
[5] Hayes LW, Jungalwala FB. Synthesis and turnover of cerebrosides and phosphatidylserine of myelin and microsomal fractions of adult and developing rat brain. Biochem J 1976;160:195–204.
[6] Voelker DR. Disruption of phosphatidylserine translocation to the mitochondria in baby hamster kidney cells. J Biol Chem 1985;260:14671–6.
[7] Voelker DR. Phosphatidylserine translocation to the mitochondrion is an ATP-dependent process in permeabilized animal cells. Proc Natl Acad Sci U S A 1989;86:9921–5.
[8] Voelker DR. Characterization of phosphatidylserine synthesis and translocation in permeabilized animal cells. J Biol Chem 1990;265:14340–6.
[9] Omori T, Mihara H, Kurihara T, Esaki N. The distribution of phosphatidyl-D-serine in the rat. Biosci Biotechnol Biochem 2010;74:1953–5.
[10] Kimura AK, Kim HY. Phosphatidylserine synthase 2: high efficiency for synthesizing phosphatidylserine containing docosahexaenoic acid. J Lipid Res 2013;54:214–22.
[11] Retterstol K, Haugen TB, Tran TN, Christophersen BO. Studies on the metabolism of essential fatty acids in isolated human testicular cells. Reproduction 2001;121:881–7.
[12] Tanaka K, Farooqui AA, Siddiqi NJ, Alhomida AS, Ong WY. Effects of docosahexaenoic acid on neurotransmission. Biomol Ther 2012;20:152–7.
[13] Kim HY, Bigelow J, Kevala JH. Substrate preference in phosphatidylserine biosynthesis for docosahexaenoic acid containing species. Biochemistry 2004;43:1030–6.
[14] Cunnane SC, Schneider JA, Tangney C, Tremblay-Mercier J, Fortier M, Bennett DA, et al. Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2012;29:691–7.
[15] Purdon AD, Rapoport SI. Energy requirements for two aspects of phospholipid metabolism in mammalian brain. Biochem J 1998;335:313–8.
[16] Kay JG, Grinstein S. Phosphatidylserine-mediated cellular signaling. Adv Exp Med Biol 2013;991:177–93.
[17] Kuge O, Saito K, Nishijima M. Control of phosphatidylserine synthase II activity in Chinese hamster ovary cells. J Biol Chem 1999;274:23844–9.
[18] Stone SJ, Vance JE. Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated membranes. J Biol Chem 2000;275:34534–40.
[19] Vance JE, Steenbergen R. Metabolism and functions of phosphatidylserine. Prog Lipid Res 2005;44:207–34.
[20] Vance JE, Tasseva G. Formation and function of phosphatidylserine and phosphatidylethanolamine in mammalian cells. Biochim Biophys Acta 2013;1831:543–54.
[21] Schenkel LC, Bakovic M. Formation and regulation of mitochondrial membranes. Int J Cell Biol 2014;2014:709828.
[22] Osman C, Voelker DR, Langer T. Making heads or tails of phospholipids in mitochondria. J Cell Biol 2011;192:7–16.
[23] Stone SJ, Vance JE. Cloning and expression of murine liver phosphatidylserine synthase (PSS)-2: differential regulation of phospholipid metabolism by PSS1 and PSS2. Biochem J 1999;342:57–64.
[24] Garcia MC, Ward G, Ma YC, Salem N Jr, Kim HY. Effect of docosahexaenoic acid on the synthesis of phosphatidylserine in rat brain in microsomes and C6 glioma cells. J Neurochem 1998;70:24–30.
[25] Sturbois-Balcerzak B, Stone SJ, Sreenivas A, Vance JE. Structure and expression of the murine phosphatidylserine synthase-1 gene. J Biol Chem 2001;276:8205–12.
[26] Voelker DR. Organelle biogenesis and intracellular lipid transport in eukaryotes. Microbiol Rev 1991;55:543–60.
[27] Daleke DL. Regulation of transbilayer plasma membrane phospholipid asymmetry. J Lipid Res 2003;44:233–42.
[28] Daleke DL. Phospholipid flippases. J Biol Chem 2007;282:821–5.
[29] Yamaji-Hasegawa A, Tsujimoto M. Asymmetrical distribution of phospholipids in biomembranes. Biol Pharm Bull 2006;29:1547–53.
[30] Martin OC, Pagano RE. Transbilayer movement of fluorescent analogs of phosphatidylserine and phosphatidylethanolamine at the plasma membrane of cultured cells. Evidence for a protein-mediated and ATP-dependent process(es). J Biol Chem 1987;262:5890–8.
[31] Connor J, Pak CC, Schroit AJ. Exposure of phosphatidylserine in the outer leaflet of human red blood cells. Relationship to cell density, cell age, and clearance by mononuclear cells. J Biol Chem 1994;269:2399–404.
[32] Shiratsuchi A, Umeda M, Ohba Y, Nakanishi Y. Recognition of phosphatidylserine on the surface of apoptotic spermatogenic cells and subsequent phagocytosis by Sertoli cells of the rat. J Biol Chem 1997;272:2354–8.
[33] Nakanishi Y, Shiratsuchi A. Phagocytic removal of apoptotic spermatogenic cells by Sertoli cells: mechanisms and consequences. Biol Pharm Bull 2004;27:13–6.

[34] Condorelli R, Calogero AE, La Vignera S. Relationship between testicular volume and conventional or nonconventional sperm parameters. Int J Endocrinol 2013;2013:145792.
[35] Kawasaki Y, Nakagawa A, Nagaosa K, Shiratsuchi A, Nakanishi Y. Phosphatidylserine binding of class B scavenger receptor type I, a phagocytosis receptor of testicular sertoli cells. J Biol Chem 2002;277:27559–66.
[36] Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: Inhibition by overexpression of Bcl-2 and Abl. J Exp Med 1995;182:1545–56.
[37] Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA. Phosphatidylserine receptor is required for clearance of apoptotic cells. Science 2003;302:1560–3.
[38] Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL. Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. J Biol Chem 2001;276:1071–7.
[39] Hoffmann PR, deCathelineau AM, Ogden CA, Leverrier Y, Bratton DL, Daleke DL, et al. Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. J Cell Biol 2001;155:649–59.
[40] Sambrano GR, Steinberg D. Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. Proc Natl Acad Sci U S A 1995;92:1396–400.
[41] Seigneuret M, Devaux PF. ATP-dependent asymmetrical distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes. Proc Natl Acad Sci U S A 1984;81:3751–5.
[42] Voelker DR. Phosphatidylserine functions as the major precursor of phosphatidylethanolamine in cultured BHK-21 cells. Proc Natl Acad Sci U S A 1984;81:2669–73.
[43] Voelker DR. Reconstitution of phosphatidylserine import into rat liver mitochondria. J Biol Chem 1989;264:8019–25.
[44] Voelker DR, Frazier JL. Isolation and characterization of a Chinese hamster ovary cell line requiring ethanolamine or phosphatidylserine for growth and exhibiting defective phosphatidylserine synthase activity. J Biol Chem 1986;261:1002–8.
[45] Kuge O, Nishijima M, Akamatsu Y. Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. II. Isolation and characterization of phosphatidylserine auxotrophs. J Biol Chem 1986;261:5790–4.
[46] Schumacher MM, Choi JY, Voelker DR. Phosphatidylserine transport to the mitochondria is regulated by ubiquitination. J Biol Chem 2002;277:51033–42.
[47] Shiao YJ, Lupo G, Vance JE. Evidence that phosphatidylserine is imported into mitochondria via a mitochondria-associated membrane and that the majority of mitochondrial phosphatidylethanolamine is derived from decarboxylation of phosphatidylserine. J Biol Chem 1995;270:11190–8.
[48] Zborowski J, Dygas A, Wojtczak L. Phosphatidylserine decarboxylase is located on the external side of the inner mitochondrial membrane. FEBS Lett 1983;157:179–82.
[49] Annunziata I, d'Azzo A. Interorganellar membrane microdomains: dynamic platforms in the control of calcium signaling and apoptosis. Cells 2013;2:574–90.
[50] Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, et al. An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 2009;325:477–81.
[51] Kornmann B, Walter P. ERMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology. J Cell Sci 2010;123:1389–93.
[52] Shiao YJ, Balcerzak B, Vance JE. A mitochondrial membrane protein is required for translocation of phosphatidylserine from mitochondria-associated membranes to mitochondria. Biochem J 1998;331:217–23.
[53] Vance JE. Newly made phosphatidylserine and phosphatidylethanolamine are preferentially translocated between rat liver mitochondria and endoplasmic reticulum. J Biol Chem 1991;266:89–97.
[54] Shiao YJ, Vance JE. Evidence for an ethanolamine cycle: differential recycling of the ethanolamine moiety of phosphatidylethanolamine derived from phosphatidylserine and ethanolamine. Biochem J 1995;310:673–9.
[55] Wilson JD, Gibson KD, Udenfriend S. Studies on the conversion in vitro of serine to ethanolamine by rat liver and brain. J Biol Chem 1960;235:3539–43.
[56] McCaman RE, Cook K. Intermediary metabolism of phospholipids in brain tissue. 3. Phosphocholine-glyceride transferase. J Biol Chem 1966;241:3390–4.
[57] Rosadini G, Sannita WG, Nobili F, Cenacchi T. Phosphatidylserine: quantitative EEG effects in healthy volunteers. Neuropsychobiology 1990–1991;24:42–8.
[58] Aporti F, Borsato R, Calderini G, Rubini R, Toffano G, Zanotti A, et al. Age-dependent spontaneous EEG bursts in rats: effects of brain phosphatidylserine. Neurobiol Aging 1986;7:115–20.
[59] Tsakiris S, Deliconstantinos G. Influence of phosphatidylserine on ($Na^+ + K^+$)-stimulated ATPase and acetylcholinesterase activities of dog brain synaptosomal plasma membranes. Biochem J 1984;220:301–7.
[60] Sebastian TT, Baldridge RD, Xu P, Graham TR. Phospholipid flippases: Building asymmetrical membranes and transport vesicles. Biochim Biophys Acta 2012;1821:1068–77.
[61] Nishijima M, Kuge O, Akamatsu Y. Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. I. Inhibition of de novo phosphatidylserine biosynthesis by exogenous phosphatidylserine and its efficient incorporation. J Biol Chem 1986;261:5784–9.
[62] Kuge O, Hasegawa K, Saito K, Nishijima M. Control of phosphatidylserine biosynthesis through phosphatidylserine-mediated inhibition of phosphatidylserine synthase I in Chinese hamster ovary cells. Proc Natl Acad Sci U S A 1998;95:4199–203.
[63] Cenacchi T, Bertoldin T, Farina C, Fiori MG, Crepaldi G. Cognitive decline in the elderly: a double blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. Aging Clin Exp Res 1993;5:123–33.
[64] Crook TH, Tinklenberg J, Yesavage J, Petrie W, Nunzi MG, Massari DC. Effects of phosphatidylserine in age-associated memory impairment. Neurology 1991;41:644–9.
[65] Crook T, Petrie W, Wells C, Massari DC. Effects of phosphatidylserine in Alzheimer's disease. Psychopharmacol Bull 1992;28:61–6.
[66] Kim HY, Akbar M, Kim YS. Phosphatidylserine-dependent neuroprotective signaling promoted by docosahexaenoic acid. Prostaglandins Leukot Essent Fatty Acids 2010;82:165–72.
[67] Zeisel S. Choline phospholipids: signal transduction and carcinogenesis. FASEB J 1993;7:551–7.
[68] Giorgione JR, Lin JH, McCammon JA, Newton AC. Increased membrane affinity of the C1 domain of protein kinase Cδ compensates for the lack of involvement of its C2 domain in membrane recruitment. J Biol Chem 2006;281:1660–9.
[69] Wrenn RW, Katoh N, Wise BC, Kuo JF. Stimulation by phosphatidylserine and calmodulin of calcium-dependent phosphorylation of endogenous proteins from cerebral cortex. J Biol Chem 1980;255:12042–6.
[70] Parekh DB, Ziegler W, Parker PJ. Multiple pathways control protein kinase C phosphorylation. EMBO J 2000;19:496–503.
[71] Nishizuka Y. Turnover of inositol phospholipids and signal transduction. Science 1984;225:1365–70.
[72] Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J Biol Chem 1979;254:3692–5.
[73] Nash HA, Tobias JM. Phospholipids membrane model: importance of phosphatidylserine and its cation exchanger nature. Proc Natl Acad Sci U S A 1964;51:476–80.
[74] Toffano G, Battistella A, Orlando P. Pharmacokinetics of radiolabeled brain phosphatidylserine. Clin Trials J 1987;24:18–24.
[75] Cohen SA, Muller WE. Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. Brain Res 1992;584:174–80.
[76] Ball MJ. Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. A quantitative study. Acta Neuropath 1977;37:111–8.
[77] Scheibel ME, Lindsay RD, Tomiyasu U, Scheibel AB. Progressive dendritic changes in the aging human limbic system. Exp Neurol 1976;53:420–30.
[78] Lippa AS, Critchett DJ, Ehlert F, Yamamura HI, Enna SJ, Bartus RT. Age-related alterations in neurotransmitter receptors: an electrophysiological and biochemical analysis. Neurobiol Aging 1981;2:3–8.
[79] Fox NC, Warrington EK, Agnew SK, Rossor MN. Presymptomatic cognitive deficits in individuals at risk of familial Alzheimer's disease. A longitudinal prospective study. Brain 1998;121:1631–9.
[80] Marra C, Silveri MC, Gainotti G. Predictors of cognitive decline in the early stage of probable Alzheimer's disease. Dement Geriatr Cogn Disord 2000;11:212–8.
[81] Petersen RC, Jack CR Jr, Xu YC, Waring SC, O'Brien PC, Smith GE, et al. Memory and MRI-based hippocampal volumes in aging and AD. Neurology 2000;54:581–7.
[82] Corwin J, Dean RL, Bartus RT, Rotrosen J, Watkins DL. Behavioral effects of phosphatidylserine in the aged Fischer 344 rat: amelioration of passive avoidance deficits without changes in psychomotor task performance. Neurobiol Aging 1985;6:11–5.
[83] Casamenti F, Scali C, Pepeu G. Phosphatidylserine reverses the age-dependent decrease in cortical acetylcholine release: a microdialysis study. Eur J Pharmacol 1991;194:11–6.
[84] Toffano G, Leon A, Mazzari S, Savoini G, Teolato S, Orlando P. Modification of noradrenergic hypothalamic system in rat injected with phosphatidylserine liposomes. Life Sci 1978;23:1093–102.
[85] Zanotti A, Aporti F, Toffano G, Valzelli L. Effects of phosphatidylserine on avoidance relearning in rats. Pharmacol Res Commun 1984;16:485–93.
[86] Drago F, Canonico PL, Scapagnini U. Behavioral effects of phosphatidylserine in aged rats. Neurobiol Aging 1981;2:209–13.

[87] Suzuki S, Yamatoya H, Sakai M, Kataoka A, Furushiro M, Kudo S. Oral administration of soybean lecithin transphosphatidylated phosphatidylserine improves memory impairment in aged rats. J Nutr 2001;131:2951–6.
[88] Casamenti F, Mantovani P, Amaducci L, Pepeu G. Effect of phosphatidylserine on acetylcholine output from the cerebral cortex of the rat. J Neurochem 1979;32:529–33.
[89] Vannucchi MG, Pepeu G. Effect of phosphatidylserine on acetylcholine release and content in cortical slices from aging rats. Neurobiol Aging 1987;8:403–7.
[90] Filburn CR. Dietary supplementation with phospholipids and docosahexaenoic acid for age-related cognitive impairment. J Am Nutraceutical Assoc 2000;3:45–55.
[91] Nunzi MG, Milan F, Guidolin D, Toffano G. Dendritic spine loss on hippocampus of aged rats. Effect of brain phosphatidylserine administration. Neurobiol Aging 1987;8:501–10.
[92] Nunzi MG, Milan F, Guidolin D, Polato P, Toffano G. Effects of phosphatidylserine administration on age-related structural changes in the rat hippocampus and septal complex. Pharmacopsychiat 1989;22:125–8.
[93] Heiss WD, Szelies B, Kessler J, Herholz K. Abnormalities of energy metabolism in Alzheimer's disease studied with PET. Ann NY Acad Sci 1991;640:65–71.
[94] Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K. Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer's disease. Dementia 1994;5:88–98.
[95] Heiss WD, Kessler J, Slansky I, Mielke R, Szelies B, Herholz K. Activation PET as an instrument to determine therapeutic efficacy in Alzheimer's disease. Ann NY Acad Sci 1993;695:327–31.
[96] Klinkhammer P, Szelies B, Heiss WD. Effect of phosphatidylserine on cerebral glucose metabolism in Alzheimer's disease. Dementia 1990;1:197–201.
[97] Sinforiani E, Agostinis C, Merlo P, Gualtieri S, Mauri M, Mancuso A. Cognitive decline in ageing brain. Therapeutic approach with phosphatidylserine. Clin Trials J 1987;24:115–25.
[98] Caffara P, Santamaria V. The effects of phosphatidylserine in patients with mild cognitive decline. An open trial. Clin Trials J 1987;24:109–14.
[99] Schreiber S, Kampf-Sherf O, Gorfine M, Kelly D, Oppenheim Y, Lerer B. An open trial of plant-source derived phosphatidylserine for treatment of age-related cognitive decline. Isr J Psychiatry Relat Sci 2000;37:302–7.
[100] Richter Y, Herzog Y, Cohen T, Steinhart Y. The effect of phosphatidylserine-containing omega-3 fatty acids on memory abilities in subjects with subjective memory complaints: a pilot study. Clin Interv Aging 2010;5:313–6.
[101] Richter Y, Herzog Y, Lifshitz Y, Hayun R, Zchut S. The effect of soybean-derived phosphatidylserine on cognitive performance in elderly with subjective memory complaints: a pilot study. Clin Interv Aging 2013;8:557–63.
[102] Vakhapova V, Cohen T, Richter Y, Herzog Y, Kam Y, Korczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in nondemented elderly individuals with memory complaints: results from an open-label extension study. Dement Geriatr Cogn Disord 2014;38:39–45.
[103] Engel RR. Double-blind crossover study of phosphatidylserine versus placebo in subjects with early cognitive deterioration of the Alzheimer type. Eur Neuropsychopharmacol 1992;2:149–55.
[104] Granata Q, DiMichele J. Phosphatidylserine in elderly patients. An open trial. Clin Trials J 1987;24:99–103.
[105] Allegro L, Favaretto V, Ziliotto G. Oral phosphatidylserine in elderly patients with cognitive deterioration. An open study. Clin Trials J 1987;24:104–8.
[106] Villardita C, Grioli S, Salmeri G, Nicoletti F, Pennisi G. Multicentre clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. Clin Trials J 1987;24:84–93.
[107] Palmieri G, Palmieri R, Inzoli MR, Lombardi G, Sottini C, Tavolato B, et al. Double-blind controlled trial of phosphatidylserine in patients with senile mental deterioration. Clin Trials J 1987;24:73–83.
[108] Jorissen BL, Brouns F, Van Boxtel MP, Ponds RW, Verhey FR, Jolles J, et al. The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. Nutr Neurosci 2001;4:121–34.
[109] Maggioni M, Picotti GB, Bondiolotti GP, Panerai A, Cenacchi T, Nobile P, et al. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. Acta Psychiatr Scand 1990;81:265–70.
[110] Amaducci L. Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. Psychopharmacol Bull 1988;24:130–4.
[111] Delwaide PJ, Gyselynck-Mambourg AM, Hurlet A, Ylieff M. Double-blind randomized controlled study of phosphatidylserine in senile demented patients. Acta Neurol Scand 1986;73:136–40.
[112] Taylor CL. Letter regarding phosphatidylserine and cognitive dysfunction and dementia. Bethesda, MD: US Food and Drug Administration; 2003.
[113] Chaung HC, Chang CD, Chen PH, Chang CJ, Liu SH, Chen CC. Docosahexaenoic acid and phosphatidylserine improves the antioxidant activities in vitro and in vivo and cognitive functions of the developing brain. Food Chem 2013;138:342–7.
[114] Latorraca S, Piersanti P, Resco G, Piacentini S, Amaducci L, Sorbi S. Effect of phosphatidylserine on free radical susceptibility in human diploid fibroblasts. J Neural Transm 1993;6:73–7.
[115] Amaducci L, Crook TH, Lippi A, Bracco L, Baldereschi M, Latorraca S, et al. Use of phosphatidylserine in Alzheimer's disease. Ann NY Acad Sci 1991;640:245–9.
[116] Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. Arterioscler Thromb Vasc Biol 2003;23:1881–8.
[117] Camont L, Lhomme M, Rached F, Le Goff W, Nègre-Salvayre A, Salvayre R, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, antiinflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol 2013;33:2715–23.
[118] Singh A, Petrides JS, Gold PW, Chrousos GP, Deuster PA. Differential hypothalamic-pituitary-adrenal axis reactivity to psychological and physical stress. J Clin Endocrinol Metab 1999;84:1944–8.
[119] Monteleone P, Beinat L, Tanzillo C, Maj M, Kemali D. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. Neuroendocrinology 1990;52:243–8.
[120] Benton D, Donohoe RT, Sillance B, Nabb S. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. Nutr Neurosci 2001;4:169–78.
[121] Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, et al. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress 2004;7:119–26.
[122] Starks MA, Starks SL, Kingsley M, Purpura M, Jäger R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Int Soc Sports Nutr 2008;5:11.
[123] Monteleone P, Maj M, Beinat L, Natale M, Kemali D. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. Eur J Clin Pharmacol 1992;41:385–8.
[124] Fahey TD, Pearl MS. The hormonal and perceptive effects of phosphatidylserine administration during 2 wk of resistive exercise-induced overtraining. Biol Sport 1998;15:135–44.
[125] Kingsley MI, Miller M, Kilduff LP, McEneny J, Benton D. Effects of phosphatidylserine on exercise capacity during cycling in active males. Med Sci Sports Exerc 2006;38:64–71.
[126] Cenacchi T, Baggio C, Palin E. Human tolerability of oral phosphatidylserine assessed through laboratory examinations. Clin Trials J 1987;24:125–31.
[127] Vakhapova V, Richter Y, Cohen T, Herzog Y, Korczyn AD. Safety of phosphatidylserine containing omega-3 fatty acids in non-demented elderly: a double-blind placebo-controlled trial followed by an open-label extension. BMC Neurol 2011;11:79.