Neuroscience and Biobehavioral Reviews 111 (2020) 183–193

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review article

Dietary phospholipids: Role in cognitive processes across the lifespan

Marina Schverer[a,b], Siobhain M. O'Mahony[a,b], Kenneth J. O'Riordan[a], Francisco Donoso[a,c], Bernard L. Roy[d], Catherine Stanton[a,e], Timothy G. Dinan[a,c], Harriët Schellekens[a,b,*], John F. Cryan[a,b,**]

[a] APC Microbiome Ireland, University College Cork, Cork, Ireland
[b] Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland
[c] Department of Psychiatry and Neurobehavioural Sciences, University College Cork, Cork, Ireland
[d] Cremo SA, Fribourg, Switzerland
[e] Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland

ARTICLE INFO

Keywords:
Dietary phospholipids
Cognition
Brain health
Stress
Ageing
Gut microbiota

ABSTRACT

Chronic stress and ageing are two of the most important factors that negatively affect cognitive processes such as learning and memory across the lifespan. To date, pharmacological agents have been insufficient in reducing the impact of both on brain health, and thus, novel therapeutic strategies are required. Recent research has focused on nutritional interventions to modify behaviour and reduce the deleterious consequences of both stress and ageing. In this context, emerging evidence indicate that phospholipids, a specific type of fat, are capable of improving a variety of cognitive processes in both animals and humans. The mechanisms underlying these positive effects are actively being investigated but as of yet are not fully elucidated. In this review, we summarise the preclinical and clinical studies available on phospholipid-based strategies for improved brain health across the lifespan. Moreover, we summarize the hypothesized direct and indirect mechanisms of action of these lipid-based interventions which may be used to promote resilience to stress and improve age-related cognitive decline in vulnerable populations.

1. Introduction

Lipids are crucial for proper nervous system function and health, and make up 60% of the dry brain weight (Lauritzen et al., 2001). Among these, phospholipids are a class of fat composed of two different parts linked by a phosphate group. They are one of three major classes of membrane lipids, the others being glycolipids and cholesterol (McCabe and Green, 1977). Phospholipids have a hydrophobic tail containing two fatty acids, and a hydrophilic head containing a polar group (Fig. 1A). This composition confers amphiphilic properties to the phospholipids, enabling the spontaneous formation of a bilayer in an aqueous solution (Li et al., 2015). The polar head can be choline, ethanolamine, serine, or inositol, forming respectively phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, or phosphatidylinositol (Fig. 1A). Sphingomyelin is a phospholipid belonging to the sphingolipid family and is composed of a sphingosine, a fatty acid and a phosphorylcholine group (Fig. 1A) (Fredman, 1998). Phospholipids are omnipresent as they represent the major components of all biological membranes, and as such, are crucial for membrane function and integrity (Fig. 2A) (Watson, 2015). Interestingly, phospholipids are abundant in the nervous system (Bozek et al., 2015; Sastry, 1985) and have long been suspected of being involved in brain maturation and function (Gozzo et al., 1982). This partly explains the rationale for the approach to use phospholipids to enhance or preserve brain health and brain processes.

There is an increasing interest in dietary means to support brain health and cognition across the lifespan (Davis et al., 2017; Lassale et al., 2019; Sandhu et al., 2017; Scott et al., 2017). Phospholipids are available from a variety of food sources. In addition, some foods are particularly enriched with phospholipids, such as dairy products, soybean and egg yolk (Fig. 1C) (Küllenberg et al., 2012). An imbalance in phospholipid metabolism, signalling and transport has been reported in some neurological conditions, such as stress-related disorders, dementia, schizophrenia and Parkinson's disease (Farooqui et al., 2000; Müller et al., 2015; Zhao et al., 2018). In this context, a growing number of studies are focusing on diets enriched in phospholipids as

* Corresponding author at: Dept. of Anatomy and Neuroscience, University College Cork, Rm. 3.85, Western Gateway Bldg., Cork, Ireland.
** Corresponding author at: Dept. of Anatomy and Neuroscience, University College Cork, Rm. 3.86, Western Gateway Bldg., Cork, Ireland.
E-mail addresses: h.schellekens@ucc.ie (H. Schellekens), j.cryan@ucc.ie (J.F. Cryan).

https://doi.org/10.1016/j.neubiorev.2020.01.012
Received 7 November 2019; Received in revised form 16 December 2019; Accepted 10 January 2020
Available online 13 January 2020
0149-7634/

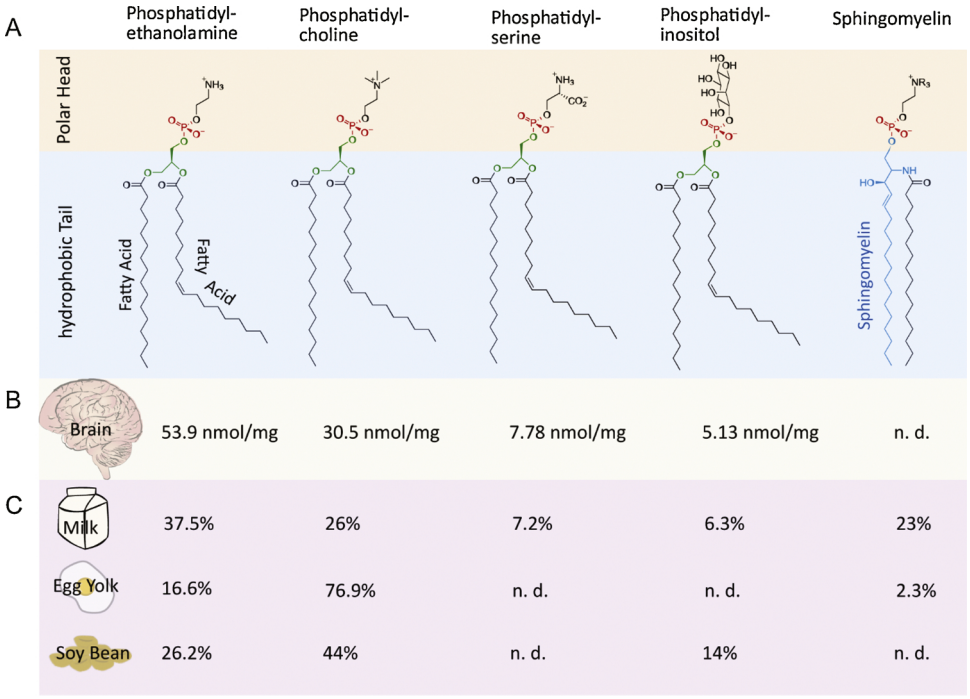

Fig. 1. Different classes of phospholipids. A: structure of the different phospholipids. B: Brain distribution of phospholipids per mg of tissue. C: Distribution of phospholipids in the main dietary sources, expressed in % of total phospholipids (Choi et al., 2018).

means to impact behaviour in general, and more precisely cognitive processes, particularly during times of stress and in ageing (see Küllenberg et al., 2012; Wehrmüller, 2008 for reviews).

Here we review both preclinical and human studies that have investigated phospholipid-mediated effects on brain health from infancy to old age. Furthermore, we highlight the potential direct and indirect mechanisms of action of these lipid-based interventions to promote cognitive resilience from factors such as stress and ageing in vulnerable populations. Together, accumulating evidence suggests that phospholipid-enriched diets may be developed as novel nutritional strategies to treat impaired cognition at different stages of life and under stress.

2. Distribution and source of phospholipids

2.1. Phospholipid content within the brain

The brain and the rest of the nervous system are particularly enriched in phospholipids and show a more diverse lipid composition compared to other bodily tissues (Bozek et al., 2015; Sastry, 1985). The varying concentrations of phospholipids in the entire rat brain include: approximately 54 nmol/mg of tissue of phosphatidylethanolamine, 31 nmol/mg of phosphatidylcholine, 8 nmol/mg of phosphatidylserine and 5 nmol/mg of phosphatidylinositol (Fig. 1B) (Choi et al., 2018). The levels of sphingomyelin are still not well-established but are thought to be similar to those of phosphatidylethanolamine, at least in the hippocampus and the prefrontal cortex of adult male rats (Oliveira et al., 2016). To date, the levels of phospholipids are unknown in the human brain, and further studies are needed to know if they are comparable to those of rodents. At the cellular level, there are differences in the amount of phospholipids in the plasma membranes, given that the inner leaflet of the membrane, facing the cytoplasm, is rich in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, whereas the outer part of the bilayer contains mostly phosphatidylcholine and sphingomyelin. These levels can vary due to different conditions and across different brain regions. For example, chronic stress has been shown to increase phosphatidylcholine and phosphatidylethanolamine levels in the whole brain of a mouse model of depression (Faria et al., 2014), while no change was reported due to ageing in both rats and humans (Fillerup and Mead, 1967; Zhang et al., 1996). Interestingly, when the content of individual phospholipids was measured in specific brain areas of rats, stress was found to decrease the amounts of phosphatidylethanolamine and sphingomyelin in the prefrontal cortex, and of sphingomyelin in the hippocampus (Oliveira et al., 2016). Similarly, old age seems to be accompanied with reduced levels of phosphatidylcholine and phosphatidylethanolamine in the hippocampus and the frontal cortex in humans, as measured by high performance liquid chromatography in individuals aged from 89 to 92 years old (Söderberg et al., 1991, 1990).

Aligned with this evidence, it has been noted that diets enriched in phospholipids can modulate behaviour including cognitive processes (See Section 3). However, whether these modified diets lead to changes in phospholipid concentration levels in the brain *per se* has only partly been investigated. Indeed, phospholipid supplementation restored the deficits in phospholipid composition in multiple brain regions in a mouse model of n–3 polyunsaturated fatty acid deficiency, and this was associated with improvement in learning (Carrié et al., 2000). Another study has shown that rat maternal supplementation with a milk enriched in phospholipids during pregnancy and lactation led to changes in neonatal brain lipid composition in rats (Gustavsson et al., 2010). Specifically, at postnatal day 2, offspring from mothers receiving the supplemented diet had a higher brain weight, but with unexpectedly lower phospholipid levels compared to control offspring rats, showing that the amount of phospholipids was not correlated with brain weight in this study. However, the altered phospholipid levels were no longer observed after weaning (at postnatal day 21), and the behaviour tested in adulthood was similar between groups (Gustavsson et al., 2010). Furthermore, when provided to mice from birth to adulthood, a diet supplemented with phospholipids from bovine milk had no impact on brain phospholipid composition, or the behaviour parameters assessed in adulthood, indicating that the phospholipid-mediated effects may not be long-lasting (Schipper et al., 2016). Although those studies suggest an ability to recalibrate phospholipid levels in the brain following maturation, it is currently unknown why such beneficial effects from phospholipids are not persistent. In contrast, piglets receiving

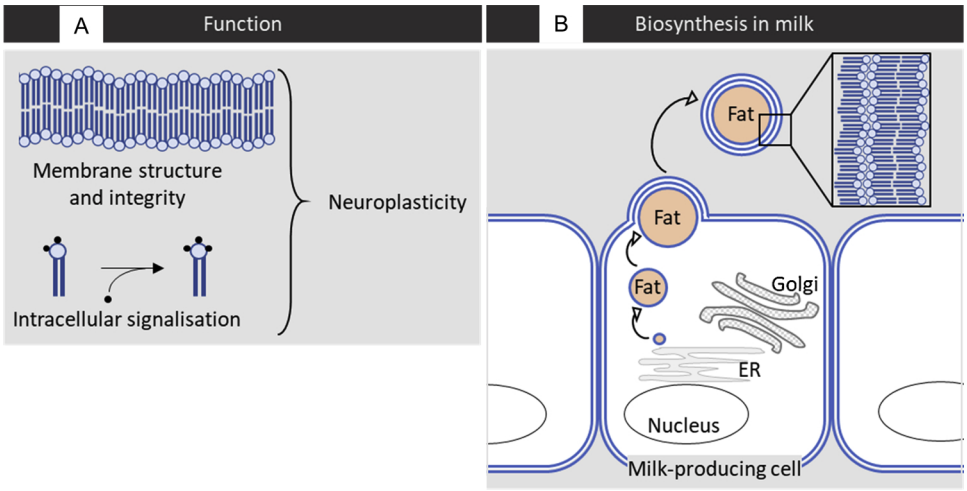

Fig. 2. A: Main functions of phospholipids. Phospholipids modulate neuroplasticity through the plasma membrane function or intracellular signalisation. The dot stands for a phosphate ion. B: Biosynthesis of phospholipids in milk producing cells. The synthesis starts in the endoplasmic reticulum (ER) and after exocytosis, the fat is surrounded by a trilayer membrane called milk fat globule membrane.

phospholipid supplementation throughout life had better memory performance associated to higher brain weight and increased amounts of both grey and white matter (Liu et al., 2014). It is unclear if this last finding could be due to an increase in central phospholipid content in those animals, nevertheless this study demonstrated a positive effect of a phospholipid-enriched diet on cognitive processes.

Thus, while evidence suggests that the effects of dietary phospholipid intake on cognitive behaviour may be associated with brain phospholipid concentrations, it is likely that the supplementation needs to be maintained for the effects to be observed. Another hypothesis could be that the experimental design itself was not ideal to see changes with these specific diets. For example, it is possible that the timing, the dose of phospholipids, or perhaps the measured outcomes could yet be optimized. Further investigations into the required duration of dietary phospholipid supplementation for optimum brain health are now warranted in preclinical and human studies.

2.2. Metabolism of phospholipids

Phospholipids can be synthesized *de novo* in almost all tissues, including the brain. The reaction mostly takes place in the endoplasmic reticulum, with phosphatidic acid and diacylglycerol as precursors (see (Tracey et al., 2018) for review). As phospholipids are major constituents of biological membranes, they are also present in almost all types of food, being particularly abundant in egg yolk or soybean, in addition to dairy products (Fig. 1C) (Küllenberg et al., 2012). Phospholipids are broken down by enzymatic processes in the small intestine, with the pancreatic phospholipase A2 metabolising phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and the alkaline sphingomyelinase acting on sphingomyelin (Sun et al., 2009). This activity leads to the hydrolysis of one acyl derivative, conducting to lysophospholipids, which are absorbed by enterocytes in the gastrointestinal tract. Then, a re-esterification into phospholipids occurs within chylomicrons, themselves released by exocytosis into blood circulation via lymphatic vessels (Sun et al., 2009). However, the mechanism by which phospholipids can reach the brain to exert their actions is still unknown and needs further investigation.

2.3. Dietary sources of phospholipids

In both bovine and human mammary glands, milk is released by the milk-producing cells as a globule surrounded by a lipid trilayer (Fig. 2B), called milk fat globule membrane (MFGM) (Mather, 2011). MFGM, has been associated with beneficial effects, particularly with respect to brain development and function, which may be due to its high phospholipid content (Mudd et al., 2016). This unique and complex structure of MFGM, is described as a source of several bioactive compounds, including phospholipids, that represent 60%–70% of the different components of MFGM (Huang et al., 2019; Verardo et al., 2017). The concentration of phospholipids in milk varies as function of the stage of lactation, season, breed of animal, and may also be influenced by maternal diet (Verardo et al., 2013; Wehrmüller, 2008). Nevertheless, it is well established that the major phospholipids present in milk are phosphatidylethanolamine (35.7%), phosphatidylcholine (26%) and sphingomyelin (23%), whereas phosphatidylserine (7.2%) and phosphatidylinositol (6.3%) are in smaller amounts, as measured using high-performance liquid chromatography with an evaporative light-scattering detector (Fig. 1C) (Wehrmüller, 2008). Egg yolk contains high amounts of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylinositol, that represent respectively 76.9%, 16.6%, 2.3% of the total phospholipid content (Fig. 1C) (Wehrmüller, 2008). In addition, both lysophosphatidylcholine (3.3%) and lysophosphatidylethanolamine (1.1%) are also present in egg yolk (Blesso, 2015). The phospholipids present in soybean are phosphatidylcholine (44%), phosphatidylethanolamine (26.2%), and phosphatidylinositol (14%) (Fig. 1C) (Küllenberg et al., 2012; Wehrmüller, 2008).

It is noteworthy that some bacteria of the human gut microbiota also produce phospholipids as metabolites. This is the case for the commensal bacteria from the Bacteroidetes phylum that synthesize sphingolipids (Brown et al., 2019). These particular bacteria-derived phospholipids, have recently been characterized as essential for gut homeostasis, since a lack in their expression resulted in increased intestinal inflammation, illustrating their critical role in intestinal health (Brown et al., 2019). Interestingly, the brain and the gut are able to influence each other through a system called the microbiota-gut-brain axis (see Section 4.**3**) (Cryan et al., 2019). Accumulating evidence is highlighting the plethora of functional metabolites derived from the human gut bacteria (Chen et al., 2019; Cryan et al., 2018) and further studies are now warranted on bacteria-derived phospholipids and their potential impact on brain health.

3. Phospholipids as cognition enhancers across the lifespan

Dietary phospholipids have shown several benefits in health, including improvements in cognition across the lifespan (Huang et al., 2019; Küllenberg et al., 2012). It is noteworthy that in humans, cognition encompasses all biological processes that include attention, learning, memory, reasoning, judgment, decision making, problem solving, understanding, eventually leading to knowledge formation and use. In rodents, cognition is assessed mainly by measuring learning, memory and attention (Wallace et al., 2015).

Table 1
Early life supplementation with dairy phospholipids enhances cognitive processes across the lifespan.

Compound	Phospholipid intake: dose and duration	Species	Model and cognitive function tested	Limitations	Reference
Phosphatidylcholine derivate	~8 g/kg per day for 120 days	Mouse	Adult; ↑ maze learning abilities	- Only one behavioural test used - Brain phospholipid composition not measured	Lim and Suzuki (2008)
Large lipid droplets coated with milk phospholipids	~33 mg/kg per day for 28 days	Mouse	Adolescent and adult; ↑ spatial memory, short term recognition memory	- Supplementation given from postnatal day 16 and not from birth - Control mice had poor recognition memory performances - Locomotor activity of animals is lacking	Schipper et al. (2016)
Milk lipids derived from MFGM	~1 g/kg per day for 70 days	Rat	Adults; ↑ recognition memory, spatial memory, operant conditioning	- Control mice had poor recognition memory performances - Locomotor activity of animals is lacking	Vickers et al. (2009)
Krill phosphatidylserine	50 – 100 mg/kg	Rat	Young adults; ↑ spatial memory	- Immunohistochemistry method used to measure growth factor expression not very quantitative	Park et al. (2013)
Complex milk lipids	~683.5 mg/kg per day for 60 days	Rat	Young adults; ↑ spatial memory	- Measure of the level of proteins involved in neuroplasticity in the hippocampus is lacking	Guan et al. (2015a)
Lacprodan PL-20 (Arla Food Ingredients)	235 mg/kg per day for 26 days	Pig	Infants; ↑ spatial memory	- Brain phospholipid composition not measured although changes in structure volumes are reported	Liu et al. (2014)
Complex milk lipids	Formula enriched in phospholipids (235% versus 220%) for 4 months	Human	Infants (24 weeks old); ↑ performances in the Griffiths Mental Development Scale	- Serum ganglioside levels do not reflect the behavioural improvements - Long-term effects are not measured	Gurnida et al. (2012)
Sphingomyelin	Formula enriched in sphingomyelin (20 % of all phospholipids, versus 13 %) for 18 months	Human	Infants (until 18 months old); ↑ performances in the Bayley Scales of Infant and Toddler Development, Second Edition	- Long-term effects are not measured	Tanaka et al. (2013)
Lacprodan MFGM-10 (Arla Food Ingredients)	Formula enriched in phospholipids (70% versus 30%) for 3–4 months	Human	Infants (12 months old); ↑ performances in the Bayley Scales of Infant and Toddler Development, Third Edition	- Long-term effects are not measured	Timby et al. (2014)

3.1. Early life

The impact of dietary phospholipids on cognitive processes in early life has been investigated in only a few studies conducted in both animals and humans (Table 1). Postnatal diets enriched with phospholipids were shown to impact cognition through enhancement of spatial memory in piglets (Liu et al., 2014) and young rats (Guan et al., 2015a; Park et al., 2013; Vickers et al., 2009), and recognition memory in adult rats (Vickers et al., 2009). Mice fed with a phospholipid-enriched diet from early life through adolescence exhibited improved working memory in adolescence, whereas recognition memory was unchanged (Schipper et al., 2016). However, when the same animals were tested again in adulthood, no change was observed in working memory, while recognition memory was enhanced in mice receiving the diet enriched in phospholipids (Schipper et al., 2016). This last finding is questionable because the animals receiving the control diet had poor memory performances as shown by their inability to discriminate the novel object (Schipper et al., 2016). Nevertheless, the discrepancies between the observed effects in adolescence versus adulthood may be explained by the fact that the diet was stopped before adulthood. As mentioned in Section 2.1, the offspring of mothers undergoing phospholipid dietary supplementation exhibited changes in brain phospholipid composition, compared to pups from control mothers, which were no longer seen in adulthood (Gustavsson et al., 2010). This suggests that phospholipid supplementation may not have a long-term effect on behaviour *per se*. Indeed, it is likely that those effects are only observed when animals are receiving the phospholipid-enriched diets, which ceases when supplementation stops. It is possible that phospholipids are not stored in a long-term manner in cells and the excess is eliminated from the organism, which is not unusual as phospholipids in bilayer membranes are rapidly turned over (Suetsugu et al., 2014). This needs to be studied further, as to date, the long-term fate of phospholipids remains unknown.

Early phospholipid supplementation has also been investigated in humans. In two randomised double-blind controlled trials, infants were given a formula, either supplemented with gangliosides from complex milk lipid (Gurnida et al., 2012), or with MFGM (Timby et al., 2014). The infants receiving the phospholipid-enriched diets displayed higher cognitive performances than infants fed with standard formula, as measured with IQ tests (Gurnida et al., 2012), or using Bayley Scales of Infant and Toddler Development, Third Edition (Timby et al., 2014). Similarly, MFGM supplemented formula was associated with lower behavioural problems in school going children as reported by parents (Farooqui et al., 2000; Veereman-Wauters et al., 2012; Wang et al., 2018; Zhao et al., 2018). Interestingly, there was no difference in cognitive performances between breast-fed infants and those fed with phospholipid-enriched milk (Gurnida et al., 2012; Timby et al., 2014). This further suggests that phospholipids present in human breast milk may contribute to cognitive health. Moreover, infants provided with a diet enriched only with sphingomyelin had higher score in tests assessing the rate of infant intelligence compared to controls (Tanaka et al., 2013), suggesting a particular high potency for this type of phospholipid. The long-term effects of dietary phospholipids have not been well studied in humans yet. It would be of great interest to evaluate cognition in adults that received an early life supplementation of phospholipids. Nevertheless, infant studies have highlighted the positive impact of dietary phospholipids on behaviour and cognition in early life.

It is noteworthy that the positive effects on cognitive processes of phospholipids taken in early life may depend on intake postnatally, since maternal supplementation with phospholipids during pregnancy and lactation did not alter cognitive performances of mouse pups (Gustavsson et al., 2010). This has been confirmed in humans, as supplementing pregnant women with phosphatidylcholine did not result in an enhanced cognitive function in the offspring (Cheatham et al., 2012).

3.2. Adulthood

Few studies evaluated the impact of phospholipid enriched diets on cognitive processes in adulthood, hence their potential therapeutic benefits remain to be fully explored. Nevertheless, phosphatidylcholine was demonstrated to improve maze-learning abilities in adult mice (Lim and Suzuki, 2008). This may be partly due to the conversion of phosphatidylcholine into acetylcholine, because phosphatidylcholine intake can restore memory deficits induced by low brain levels of acetylcholine in mice (Moriyama et al., 1996).

In adult humans, healthy subjects receiving a supplementation with a milk phospholipid concentrate, had a tendency toward lower reaction time when tested in a working memory task, highlighting better cognitive performances associated with the intake of phospholipids (Hellhammer et al., 2010).

3.3. Stress

Learning and memory can be altered in some pathological conditions, such as in stress-related disorders (McEwen and Sapolsky, 1995; Roca et al., 2015). In humans, the cognitive deficits related to chronic stress exposure include disruption of attention, memory and executive functions (Fjell et al., 2014; Iosifescu, 2012), which can be assessed in animal models (Hölter et al., 2015; Tanila, 2018). In addition to the effect of phospholipids on cognition in healthy individuals (Table 1), the impact of phospholipid supplementation in a context of stress has

Table 2
Supplementation with dairy phospholipids improves disrupted cognition in stress and ageing.

Compound	Dose and duration	Species	Model and cognitive function tested	Limitations	Reference
1,2-dilinoleoyl-sn-glycero-3-phosphocholine	1 mg/kg per day for 7 days	Mouse	Chronic restraint stress in adults; ↑ spatial memory	- The depressive-like behaviour could be measured by other means than just forced swim test	Kanno et al. (2014)
Complex milk lipids	~850 mg/kg per day for 60 days	Rat	Old rats; ↑ spatial memory	- Locomotor activity of animals is lacking	Guan et al. (2015b)
MFGM	~715 mg/kg per day for 10–11 weeks	Rat	Maternal separation; ↑ spatial memory	- Effects of treatments on specific neuroplasticity measures not assessed	O'Mahony et al. (2019)
Bovine milk-derived phospholipid drink	2.7 g per day for 6 weeks	Human	Adults with high stress-load; ↑ attention-switching	- No effect on cortisol levels	Boyle et al. (2019)
Phosphatidylserine	300 mg per day for 6 months	Human	Geriatric subjects with cognitive impairments; ↑ learning and verbal memory	- Levels of cortisol are not consistent throughout the study - The generalizability of "high-perfectionist men" to other chronic stress states unclear	Cenacchi et al. (1993)
Phosphatidylserine	300 mg per day for 16 weeks	Human	Old subjects with mild primary degenerative dementia according to DSM-III; ↑ immediate nonverbal memory	- Dementia and mood were not changed - The positive benefits of phospholipids only rely on the clinical global impression scale.	Engel et al. (1992)

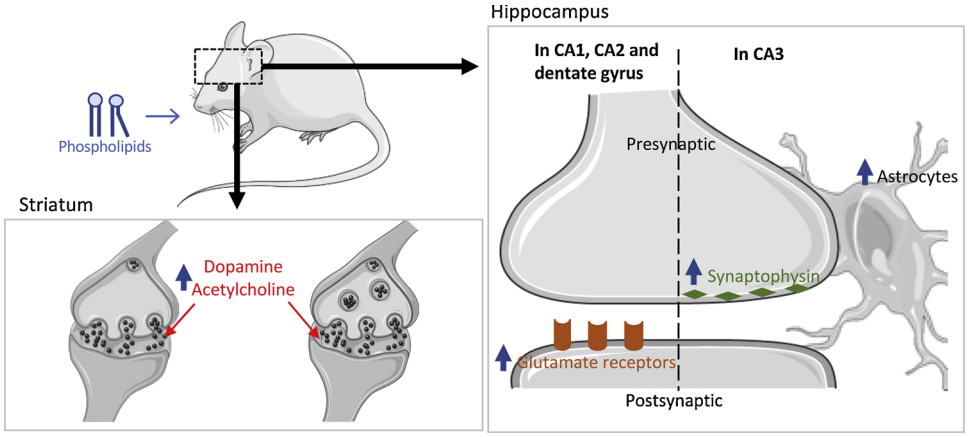

Fig. 3. Effects of phospholipid-enriched diets on neuroplasticity in rodents. A phospholipid dietary supplementation was demonstrated to increase the levels of dopamine and acetylcholine in the striatum (left panel), glutamate receptors in CA1, CA2 and the dentate gyrus, the expression of synaptophysin and the number of astrocytes in the hippocampus (right panel).

also been investigated (Table 2). A diet enriched in phospholipids was shown to reduce stress-induced cognitive deficits.

Rats submitted to maternal separation and receiving an MFGM-enriched diet after weaning had greater spatial memory performance in the Morris water maze test compared to rats receiving the control diet (O'Mahony et al., 2019). The benefits of MFGM may at least in part be due to phosphatidylcholine, one of the major phospholipids present in milk and brain. Indeed, a diet supplemented with phosphatidylcholine given to mice subjected to restraint stress, reduced the depressive-like behaviour as assessed in the forced swim test (Kanno et al., 2014). This finding was replicated in another mouse model of depression, one generated by the inoculation of the human papillomavirus vaccine, or its adjuvant aluminium hydroxide. In female mice, this resulted in a depressive-like behaviour and cognitive impairments being attenuated by a phospholipid intake (Inbar et al., 2017; Kivity et al., 2017).

These results have been replicated in humans, where subjects with a high stress-load, and receiving a diet supplemented with milk phospholipids, exhibited better memory performance in an attention-switching task (**Table 2**) (Boyle et al., 2019). In addition, supplementing MFGM in the diet is related to changes in gut bacteria and further lessened the impact of stress on sleep in children (Thompson et al., 2017). Another study conducted in men with high self reported stress levels also suggested that a diet supplemented with phospholipids may attenuate stress-induced memory impairments (Schubert et al., 2011). The specific mechanisms of action of phospholipid mediated amelioration of stress-induced cognitive deficits remains to be elucidated. Taken together, these data suggest that the effects of phospholipids may be related to activity in the stress pathways, hence leading to potential therapeutic effects on stress-related conditions.

3.4. Elderly

The effects of a diet rich in phospholipids on cognitive deficits related to ageing were explored in preclinical studies. One study used a diet supplemented with complex milk lipid concentrate rich in phospholipids (Guan et al., 2015b), and another used an oral administration of phosphatidylserine isolated from krill or soy (Lee et al., 2010). In both studies, phospholipids improved the spatial memory of aged rats tested in the Morris water maze (Guan et al., 2015b; Lee et al., 2010), which could be due to modulation of cholinergic neurotransmission, as suggested by the authors, although direct evidence remains elusive.

Early studies showed that phosphatidylserine intake could have a compensatory effect on cognitive deficits related to the elderly and Alzheimer's Disease in humans (Table 2) (Cenacchi et al., 1993; Engel et al., 1992). More precisely, phosphatidylserine was able to improve behaviour and cognition in patients with cognitive impairments (Cenacchi et al., 1993), and normalized electroencephalogram measures in patients with mild primary degenerative dementia (Engel et al., 1992). This was the first evidence showing that phospholipids could be serious candidates for the improvement of cognition in pathological and neurodegenerative conditions. In terms of age-induced neurochemical changes, the phospholipid content was shown to be reduced in several parts of the brain of aged subjects, including the white and grey matters and the hippocampus (Söderberg et al., 1990), whereas no change was reported in the frontal and the temporal cortices (Svennerholm et al., 1994). Despite these studies, it is not yet established whether a decrease in phospholipid levels and metabolism could play a critical role in the onset of cognitive decline associated with ageing.

4. Mechanism of action of phospholipids

Neuroplasticity is one of the key biological substrates for cognition and can be defined as the adaptive response of brain cells to internal or external stimuli, occurring throughout life in key brain regions such as the hippocampus and the prefrontal cortex. These changes involve membrane-related processes, leading to growth or regression of cellular processes (Lefebvre et al., 2015), remodelling of dendritic spines (Hering and Sheng, 2001), and activation of intracellular signalling pathways (Kumar, 2005). Moreover, both neuroplasticity and cognition are under the influence of several systems, including the hypothalamic-pituitary-adrenal (HPA) and the microbiota-gut-brain axes, and little evidence has shown these systems can be influenced by phospholipids.

4.1. Impact of phospholipids on neuronal transmission

Early studies have shown that neurotransmitter receptor activity could be modulated by phosphoinositides, which are phospholipid derivatives from phosphatidylinositol (Downes, 1983). Moreover, neurotransmitter receptors can recruit phosphoinositides as a signal-transduction system (Nicoletti et al., 1986). These data were the first to directly link phospholipid metabolism to neuronal transmission.

Interestingly, the intake of phospholipid-enriched diets during postnatal brain development was found to increase the number of striatal terminals (Fig. 3) (Guan et al., 2015a). More precisely, elevated nigral dopamine expression was measured following phospholipid intake, along with a rise in dopamine and acetylcholine outputs in the striatum (Fig. 3) (Guillermo et al., 2015). Acetylcholine can be synthesized from phosphatidylcholine, phosphatidylethanolamine, or sphingomyelin (Jope and Jenden, 1979; Zeisel et al., 1991). Given the role of acetylcholine and cholinergic neurotransmission in both brain development and cognition (Picciotto et al., 2012; Wilson and Fadel, 2017), it is possible that phospholipid-supplemented diets provide more substrate for acetylcholine production. In addition, phosphoinositides are essential not only for synaptic vesicle trafficking at the presynaptic compartment, but also for receptor endocytosis and exocytosis at the postsynaptic side, together with regulation of the spine morphology

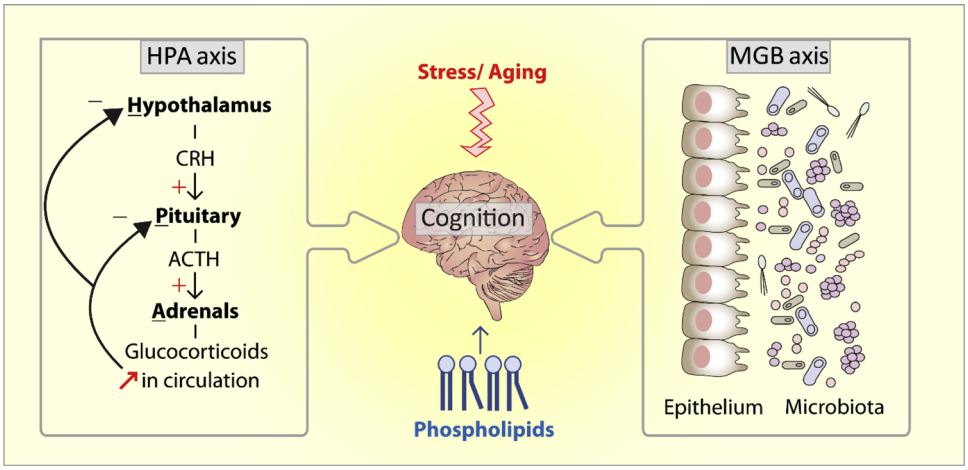

Fig. 4. The HPA axis and the gut microbiota modulate cognition. Stress and ageing can disrupt cognition and alter the HPA axis and the microbiota-gut-brain axis, while phospholipids can positively modulate them. HPA: hypothalamic-pituitary-adrenal. MGB: microbiota gut brain.

(Frere et al., 2012; Lauwers et al., 2016; Ueda, 2014). Phospholipid-enriched diet induced an increase in glutamate receptor density in CA1-2 and the dentate gyrus (Fig. 3) (Guan et al., 2015a), but not in CA3 (Guillermo et al., 2015). Therefore, phospholipid supplementation during early life could act on glutamate neurotransmission, but only in specific hippocampal subregions. In contrast, the number of astrocytes and synaptophysin expression in CA3 were increased following dietary phospholipid intake (Fig. 3) (Guillermo et al., 2015). Synaptophysin is a presynaptic protein involved in the release of vesicles containing neurotransmitters. Therefore, these data indicate that phospholipid supplementation during early life could regulate glutamate neurotransmission and synaptophysin expression in specific hippocampal subregions, suggesting that they are able to activate different neuroplasticity processes in a context-dependent manner.

4.2. Impact of phospholipids on intracellular signalling

Membrane-bound phospholipids are important for intracellular signalling pathways (Berridge and Irvine, 1984), given that they can be converted into second messengers. Indeed, phosphatidylinositol can be reversibly phosphorylated at the positions 3, 4, or 5 of the inositol hydroxyl group, by diverse kinases, resulting in seven different species located heterogeneously in the cells (Dickson and Hille, 2019). One of these enzymes is the phosphoinositide 3-kinase (PI3-kinase), activated by phosphorylation at the plasma membrane either by an active growth factor receptor, with a tyrosine kinase activity, or following G protein-coupled receptor activation (Cantley, 2002). The PI3-kinase converts phosphatidylinositol-4,5-bisphosphate (PIP2), which is located at the plasma membrane, into phosphatidylinositol-3,4,5-trisphosphate, which eventually stimulates the phosphorylation of Akt by phosphoinositide-dependent kinase 1 (Lawlor and Alessi, 2001). Once Akt is activated, it triggers the phosphorylation of a range of proteins affecting numerous cell processes, including cycle entry, growth and survival (Brunet et al., 2001; Cantley, 2002). A large body of evidence has demonstrated the involvement of nerve growth factor and cholinergic neurotransmission in memory processes. Indeed, nerve growth factor is critical for the maintenance of cholinergic neurotransmission in ageing (Claudio Cuello et al., 2019), and can improve memory in aged rats via increasing acetylcholine levels in the parietal cortex and the hippocampus (Scali et al., 1994). As mentioned in the Section 4.1., acetylcholine can be synthetized from phospholipids. There is therefore a plausible direct connection between the phospholipid metabolism and growth factor signalling. Further studies are required to disentangle the relative contribution of such interactions to cognitive enhancing effects of phospholipids.

The PI3-kinase signalling pathway can be activated by the tyrosine kinase activity of growth factor receptors, so in addition to cholinergic pathways, phospholipid activity on cognitive processes could be due to the recruitment of the PI3-kinase (Kitagishi et al., 2012; O'Neill, 2013). In the context of stress, it was reported that the activation of this kinase was reduced in the prefrontal cortex of depressed patients that committed suicide (Karege et al., 2011), and that antidepressants could increase the activation of the PI3-kinase/Akt pathway by potentiating the synthesis of phosphatidylinositol (Müller et al., 2015). In addition, when mice are submitted to restraint stress, hypothalamic activation of Akt is reduced, which was reversed by a phosphatidylcholine-enriched diet (Kanno et al., 2014), suggesting that phosphatidylcholine could also modulate the PI3-kinase pathway. Given these data, diet supplementation with phospholipids could provide more substrate for this pathway, which may contribute to improved cognition in stressed individuals.

4.3. The hypothalamic-pituitary-adrenal axis

Humans and rodents subjected to stress exhibit disrupted cognition depending on the duration, chronicity and temporality of the stressor. When a stressor is perceived by the organism, the hypothalamic-pituitary-adrenal (HPA) axis is activated, leading to the production of glucocorticoids (cortisol in humans and corticosterone in rodents), the levels of which are eventually reduced at the end of the stress (Fig. 4). The activity of the HPA axis can be assessed by measuring the level of plasma glucocorticoids after an acute stress exposure. In addition, high levels of glucocorticoids are reported in both stress-related disorders and ageing (Lupien et al., 1998; Zunszain et al., 2011), and it is well accepted that they could be responsible for the reported altered cognition (Buchanan and Tranel, 2008; Lupien et al., 2007). Lysophosphatidylcholine, a metabolite of phosphatidylcholine, has been shown to be positively correlated to corticosterone concentration in blood in rats (Oliveira et al., 2016), giving evidence of a potential link between the activity of the HPA axis and phospholipid metabolism. In the rat maternal separation model, a diet supplemented with MFGM normalised the HPA axis response to restraint stress (O'Mahony et al., 2019). Moreover, early work suggests that phospholipid intake could reduce stress-induced elevation of plasma cortisol in healthy men (Monteleone et al., 1992), which was replicated in a more recent study (Starks et al., 2008). This blunted response to stress was also seen in subjects with high stress load as a result of exposure to a psychosocial stress, leading to reduced serum and salivary cortisol levels after milk phospholipid consumption (Hellhammer et al., 2010). Interestingly, some studies also investigated cognition in this context, and revealed that phospholipids

increased working memory in humans tested in the Trier Social Stress Test (Hellhammer et al., 2010; Schubert et al., 2011). On the other hand, a study in men considered as perfectionist (as assessed with questionnaires) have a higher cortisol responsivity when exposed to an acute stressor than control subjects (Wirtz et al., 2007), and this was unchanged after a chronic phospholipid intake, although enhanced memory was reported (Boyle et al., 2019).

Taken together, human studies strongly suggest that a diet supplemented with phospholipids could alleviate some cognitive deficits related to a stress state. However, the chronic intake of phospholipids does not always reduce the activity of the HPA axis *per se*, showing that other systems may be involved in phospholipid-mediated improvements on cognition.

4.4. The microbiota-gut-brain axis

The brain and the gut are able to communicate in a bidirectional way, through a system called the microbiota-gut-brain axis (Cryan et al., 2019; Sherwin et al., 2016). In addition, the gut microbiota is known to contribute to the normal development of the brain (Foster et al., 2016; Heijtz et al., 2011). It has long been postulated that the major establishment of gut microbiota starts at birth and continues to vary throughout life, being under the influence of both internal and environmental factors (Cryan et al., 2019; Dinan and Cryan, 2017). The first bacteria that thrive are aerobic or facultative anaerobic bacteria that will consume the oxygen present, to allow the growth of anaerobic bacteria that become predominant in the mature gut (Le Hurou-Luron et al., 2010). A large body of evidence highlights that the gut microbiota could be involved in cognition in both humans and rodents (Bravo et al., 2011; Cryan and Dinan, 2012; Desbonnet et al., 2015; Gareau, 2014; Messaoudi et al., 2011; Savignac et al., 2015). Studies conducted in germ-free animals or normal mice receiving either an antibiotic treatment, or colonized with *Citrobacter rodentium,* found that these animals exhibited impaired memory performances compared to controls (Fig. 4) (Fröhlich et al., 2016; Gareau et al., 2011). Interestingly, these deficits were reversed by the administration of specific probiotic strains, which are live beneficial bacteria that have a positive impact on health (Gareau et al., 2011), further showing that modulation of the gut microbiota can impact cognitive functions. In addition, the combination of dairy lipids and probiotics in formula given to piglets was shown to contribute to the maturation of the gut microbiota (Lemaire et al., 2018). Given these data, and since phospholipids have a positive impact on cognition, it could be hypothesized that phospholipids act through gut microbiota to exert their positive activity on cognition. The gut microbiota composition was also reported to be altered by a phospholipid-enriched diet. Indeed, piglets fed with a mixture of bioactive components from cow milk, including phospholipids, had higher relative abundance of Bacteroidetes and lower proportion of Proteobacteria in the ascending colon compared to controls (Berding et al., 2016). Another study showed that a diet enriched in milk-derived sphingomyelin could alter distal gut microbiota phylogenetic abundance as well as Gram-negative bacteria (Norris et al., 2016). This suggests a potential involvement of gut microbiota in the mechanism of action of phospholipids in the context of cognition enhancement, and further investigation is warranted to shed light on this exciting area of diet-microbiota-host interaction for cognitive function and brain health.

5. Future directions

5.1. Mechanism of action

Although the body of evidence regarding the positive impact of phospholipids on cognitive processes is growing, further questions still need to be addressed. First, it is unclear if a phospholipid supplementation in diet leads to an increase in their brain levels, and if dietary phospholipids actually cross the blood-brain barrier, reach the brain to exert their action on cognition. It would also be interesting to measure the actual levels of phospholipids in both plasma and brain after a supplementation in diet, and perhaps tag those phospholipids to study their fate.

On the other hand, the activity of phospholipids on the microbiota-gut-brain axis is still poorly characterised. Few published experiments have begun to elucidate their action on the brain, and in particular neuroplasticity. However, these studies need to be broadened to better understand the consequences of an intake of dietary phospholipids on brain and intermediate systems such as the gut microbiota, neurotransmission, and the HPA axis. In addition to the mechanism of action of phospholipids, other systems that might be influenced by phospholipids have to be examined.

5.2. Phospholipids as nutritional strategy

Reports showed that phospholipids were able to promote cognition in some situations. However, it is probable that the protocol of phospholipid supplementation still needs to be optimized. First, future studies should also consider adding females on their protocols, since almost all the work conducted to date has been done in males only. In addition, as discussed above, the behavioural benefits of a diet enriched in phospholipids could not be permanent, since once the supplementation stops, the abilities of subjects are no longer different than those from controls (see Section 3.1). So, it is possible that this strategy should be maintained in order to have positive effects on cognition. Intervention in adulthood need also to be studied more in depth. Indeed, only few works were conducted, suggesting a potential therapeutic benefit that still needs to be demonstrated.

Regarding human studies, further work needs to be done including the effect of a diet enriched in phospholipids on different clinical populations in which cognitive deficits are present, including patients with stress-related disorders, mild cognitive impairments, or even schizophrenia. Brain imaging could also help to see the functional consequences of a chronic intake in phospholipids through the diet.

6. Concluding remarks

Fat is often primarily seen as a negative thing. However, there is a growing understanding of the effects of dietary phospholipids and other fats on brain health. This is most notable in studies investigating the ability of phospholipids to improve cognition across the lifespan in both physiological and pathological conditions. This supports the idea that dietary interventions are useful and easy tools to manipulate brain functions.

Declaration of Competing Interest

APC Microbiome Ireland has conducted studies in collaboration with several companies, including GSK, Pfizer, Cremo, Suntory, Wyeth, Mead Johnson, Nutricia, 4D Pharma, and DuPont. B. L. Roy is an employee of Cremo SA. T. G. Dinan has been an invited speaker at meetings organized by Servier, Lundbeck, Janssen, and AstraZeneca and has received research funding from Mead Johnson, Cremo, Nutricia, and 4D Pharma. J. F. Cryan has been an invited speaker at meetings organized by Mead Johnson, Yakult, Alkermes, and Janssen and has received research funding from Mead Johnson Nutrition, Cremo, Nutricia, DuPont, and 4D Pharma.

Acknowledgements

This work was supported, in part, by research grants from Science Foundation Ireland (SFI) to APC Microbiome Ireland through the Irish Government's National Development Plan (Grants SFI/12/RC/2273 and SFI/12/RC/ 2273_P2).

References

Berding, K., Wang, M., Monaco, M., Alexander, L., Mudd, A., Chichlowski, M., et al., 2016. Prebiotics and bioactive milk fractions affect gut development, microbiota, and neurotransmitter expression in piglets. J. Pediatr. Gastroenterol. Nutr. 63, 688–697. https://doi.org/10.1097/MPG.0b013e318228574e.

Berridge, M.J., Irvine, R.F., 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312, 315–321. https://doi.org/10.1038/312315a0.

Blesso, C.N., 2015. Egg phospholipids and cardiovascular health. Nutrients 7, 2731–2747. https://doi.org/10.3390/nu7042731.

Boyle, N.B., Dye, L., Arkbåge, K., Thorell, L., Frederiksen, P., Croden, F., et al., 2019. Effects of milk-based phospholipids on cognitive performance and subjective responses to psychosocial stress: a randomized, double-blind, placebo-controlled trial in high-perfectionist men. Nutrition 57, 183–193. https://doi.org/10.1016/j.nut.2018.05.002.

Bozek, K., Wei, Y., Yan, Z., Liu, X., Xiong, J., Sugimoto, M., et al., 2015. Organization and evolution of brain lipidome revealed by large-scale analysis of human, chimpanzee, macaque, and mouse tissues. Neuron 85, 695–702. https://doi.org/10.1016/j.neuron.2015.01.003.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., et al., 2011. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Nat. Acad. Sci. U.S.A 108, 16050–16055. https://doi.org/10.1073/pnas.1102999108.

Brown, E.M., Ke, X., Hitchcock, D., Jeanfavre, S., Avila-Pacheco, J., Nakata, T., et al., 2019. Bacteroides-derived sphingolipids are critical for maintaining intestinal homeostasis and symbiosis. Cell Host Microbe 25, 668–687. https://doi.org/10.1016/j.chom.2019.04.002.

Brunet, A., Datta, S.R., Greenberg, M.E., 2001. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr. Opin. Neurobiol. 11, 297–305. https://doi.org/10.1016/S0959-4388(00)00211-7.

Buchanan, T.W., Tranel, D., 2008. Stress and emotional memory retrieval: effects of sex and cortisol response. Neurobiol. Learn. Mem. 89, 134–141. https://doi.org/10.1016/j.nlm.2007.07.003.

Cantley, L.C., 2002. The phosphoinositide 3-kinase pathway. Science 296, 1655–1657. https://doi.org/10.1126/science.296.5573.1655.

Carrié, I., Clément, M., De Javel, D., Francès, H., Bourre, J.M., 2000. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. J. Lipid Res. 41, 473–480.

Cenacchi, T., Bertoldin, T., Farina, C., Fiori, M.G., Crepaldi, G., Azzini, C.F., et al., 1993. Cognitive decline in the elderly: a double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. Aging Clin. Exp. Res. 5, 123–133. https://doi.org/10.1007/BF03324139.

Cheatham, C.L., Goldman, B.D., Fischer, L.M., Da Costa, K.A., Reznick, J.S., Zeisel, S.H., 2012. Phosphatidylcholine supplementation in pregnant women consuming moderate-choline diets does not enhance infant cognitive function: a randomized, double-blind, placebo-controlled trial. Am. J. Clin. Nutr. 96, 1465–1472. https://doi.org/10.3945/ajcn.112.037184.

Chen, H., Nwe, P.K., Yang, Y., Rosen, C.E., Bielecka, A.A., Kuchroo, M., et al., 2019. A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology. Cell 177, 1217–1231. https://doi.org/10.1016/j.cell.2019.03.036.e18.

Choi, J., Yin, T., Shinozaki, K., Lampe, J.W., Stevens, J.F., Becker, L.B., et al., 2018. Comprehensive analysis of phospholipids in the brain, heart, kidney, and liver: brain phospholipids are least enriched with polyunsaturated fatty acids. Mol. Cell. Biochem. 442, 187–201. https://doi.org/10.1007/s11010-017-3203-x.

Claudio Cuello, A., Pentz, R., Hall, H., 2019. The brain NGF metabolic pathway in health and in Alzheimer's pathology. Front. Neurosci. 13, 1–8. https://doi.org/10.3389/fnins.2019.00062.

Cryan, J.F., Clarke, G., Dinan, T.G., Schellekens, H., 2018. A microbial drugstore for motility. Cell Host Microbe 23, 691–692. https://doi.org/10.1016/j.chom.2018.05.020.

Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712. https://doi.org/10.1038/nrn3346.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., et al., 2019. The microbiota-gut-Brain Axis. Physiol. Rev. 99, 1877–2013. https://doi.org/10.1152/physrev.00018.2018.

Davis, E.C., Wang, M., Donovan, S.M., 2017. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. Gut Microbes 8, 143–171. https://doi.org/10.1080/19490976.2016.1278104.

Desbonnet, L., Clarke, G., Traplin, A., O'Sullivan, O., Crispie, F., Moloney, R.D., et al., 2015. Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. Brain Behav. Immun. 48, 165–173. https://doi.org/10.1016/j.bbi.2015.04.004.

Dickson, E.J., Hille, B., 2019. Understanding phosphoinositides: rare, dynamic, and essential membrane phospholipids. Biochem. J. 476, 1–23. https://doi.org/10.1042/bcj20180022.

Dinan, T.G., Cryan, J.F., 2017. The microbiome-gut-Brain Axis in health and disease. Gastroenterol. Clin. North Am. 46, 77–89. https://doi.org/10.1016/j.gtc.2016.09.007.

Downes, C.P., 1983. Inositol phospholipids and neurotransmitter-receptor signalling mechanisms. Trends Neurosci. 6, 313–316. https://doi.org/10.1016/0166-2236(83)90146-7.

Engel, R.R., Satzger, W., Günther, W., Kathmann, N., Bove, D., Gerke, S., et al., 1992. Double-blind cross-over study of phosphatidylserine vs. Placebo in patients with early dementia of the Alzheimer type. Eur. Neuropsychopharmacol. 2, 149–155. https://doi.org/10.1016/0924-977X(92)90025-4.

Faria, R., Santana, M.M., Aveleira, C.A., Simões, C., Maciel, E., Melo, T., et al., 2014. Alterations in phospholipidomic profile in the brain of mouse model of depression induced by chronic unpredictable stress. Neuroscience 273, 1–11. https://doi.org/10.1016/j.neuroscience.2014.04.042.

Farooqui, A.A., Horrocks, L.A., Farooqui, T., 2000. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem. Phys. Lipids 106, 1–29.

Fillerup, D.L., Mead, J.F., 1967. The lipids of the aging human brain. Lipids 2, 295–298. https://doi.org/10.1007/BF02532114.

Fjell, A.M., McEvoy, L., Holland, D., Dale, A.M., Walhovd, K.B., 2014. What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. Prog. Neurobiol. 117, 20–40. https://doi.org/10.1016/j.pneurobio.2014.02.004.

Foster, J.A., Lyte, M., Meyer, E., Cryan, J.F., 2016. Gut microbiota and brain function: an evolving field in neuroscience. Int. J. Neuropsychopharmacol. 19, 1–7. https://doi.org/10.1093/ijnp/pyv114.

Fredman, P., 1998. Sphingolipids and cell signalling. J. Inherit. Metab. Dis. 21, 472–480. https://doi.org/10.1023/A:1005454602454.

Frere, S.G., Chang-Ileto, B., Di Paolo, G., 2012. Role of phosphoinositides at the neuronal synapse. Subcell. Biochem. 59, 131–175. https://doi.org/10.1007/978-94-007-3015-1.

Fröhlich, E.E., Farzi, A., Mayerhofer, R., Reichmann, F., Jačan, A., Wagner, B., et al., 2016. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav. Immun. 56, 140–155. https://doi.org/10.1016/j.bbi.2016.02.020.

Gareau, G., 2014. Microbiota-gut-brain axis and cognitive function. In: In: Lyte, M., Cryan, J.F. (Eds.), Advances in Experimental Medicine and Biology 817. pp. 357–371. https://doi.org/10.1007/978-1-4939-0897-4.

Gareau, M.G., Wine, E., Rodrigues, D.M., Cho, J.H., Whary, M.T., Philpott, D.J., et al., 2011. Bacterial infection causes stress-induced memory dysfunction in mice. Gut 60, 307–317. https://doi.org/10.1136/gut.2009.202515.

Gozzo, S., Oliverio, A., Salvati, S., Serlupi-Crescenzi, G., Tagliamonte, B., Tomassi, G., 1982. Effects of dietary phospholipids and odd-chain fatty acids on the behavioural maturation of mice. Food Chem. Toxicol. 20, 153–157.

Guan, J., Macgibbon, A., Fong, B., Zhang, R., Liu, K., Rowan, A., et al., 2015a. Long-term supplementation with beta serum concentrate (BSC), a complex of milk lipids, during post-natal brain development improves memory in rats. Nutrients 7, 4526–4541. https://doi.org/10.3390/nu7064526.

Guan, J., MacGibbon, A., Zhang, R., Elliffe, D.M., Moon, S., Liu, D.-X., 2015b. Supplementation of complex milk lipid concentrate (CMLc) improved the memory of aged rats. Nutr. Neurosci. 18, 22–29. https://doi.org/10.1179/1476830513Y.0000000096.

Guillermo, R.B., Yang, P., Vickers, M.H., McJarrow, P., Guan, J., 2015. Supplementation with complex milk lipids during brain development promotes neuroplasticity without altering myelination or vascular density. Food Nutr. Res. 59. https://doi.org/10.3402/fnr.v59.25765.

Gurnida, D.A., Rowan, A.M., Idjradinata, P., Muchtadi, D., Sekarwana, N., 2012. Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants. Early Hum. Dev. 88, 595–601. https://doi.org/10.1016/j.earlhumdev.2012.01.003.

Gustavsson, M., Hodgkinson, S.C., Fong, B., Norris, C., Guan, J., Krageloh, C.U., et al., 2010. Maternal supplementation with a complex milk lipid mixture during pregnancy and lactation alters neonatal brain lipid composition but lacks effect on cognitive function in rats. Nutr. Res. 30, 279–289. https://doi.org/10.1016/j.nutres.2010.04.005.

Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., et al., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Nat. Acad. Sci. U.S.A 108, 3047–3052. https://doi.org/10.1073/pnas.1010529108.

Hellhammer, J., Waladkhani, A.R., Hero, T., Buss, C., 2010. Effects of milk phospholipid on memory and psychological stress response. Br. Food J. 112, 1124–1137. https://doi.org/10.1108/00070701011080258.

Hering, H., Sheng, M., 2001. Dendritic spines: structure, dynamics and regulation. Nat. Rev. Neurosci. 2, 880–888.

Hölter, S.M., Garrett, L., Einicke, J., Sperling, B., Dirscherl, P., Zimprich, A., et al., 2015. Assessing cognition in mice. Curr. Protoc. Mouse Biol. 5, 331–358. https://doi.org/10.1002/9780470942390.mo150068.

Huang, Z., Zhao, H., Guan, W., Liu, J., Brennan, C., Kulasiri, D., et al., 2019. Vesicle properties and health benefits of milk phospholipids: a review. J. Food Bioactives 5, 31–42. https://doi.org/10.31665/JFB.2019.5176.

Le Hurou-Luron, I., Blat, S., Boudry, G., 2010. Breast- v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects. Nutr. Res. Rev. 23, 23–36. https://doi.org/10.1017/S0954422410000065.

Inbar, R., Weiss, R., Tomljenovic, L., Arango, M.T., Deri, Y., Shaw, C.A., et al., 2017. Behavioral abnormalities in female mice following administration of aluminum adjuvants and the human papillomavirus (HPV) vaccine Gardasil. Immunol. Res. 65, 136–149. https://doi.org/10.1007/s12026-016-8826-6.

Iosifescu, D.V., 2012. The relation between mood, cognition and psychosocial functioning in psychiatric disorders. Eur. Neuropsychopharmacol. 22, S499–S504. https://doi.org/10.1016/j.euroneuro.2012.08.002.

Jope, R.S., Jenden, D.J., 1979. Choline and phospholipid metabolism and the synthesis of acetylcholine in rat brain. J. Neurosci. Res. 4, 69–82. https://doi.org/10.1002/jnr.490040110.

Kanno, T., Jin, Y., Nishizaki, T., 2014. DL-/PO-phosphatidylcholine restores restraint

stress-induced depression-related behaviors and spatial memory impairment. Behav. Pharmacol. 25, 575–581. https://doi.org/10.1097/FBP.0000000000000063.
Karege, F., Perroud, N., Burkhardt, S., Fernandez, R., Ballmann, E., La Harpe, R., et al., 2011. Alterations in phosphatidylinositol 3-kinase activity and PTEN phosphatase in the prefrontal cortex of depressed suicide victims. Neuropsychobiology 63, 224–231. https://doi.org/10.1159/000322145.
Kitagishi, Y., Kobayashi, M., Kikuta, K., Matsuda, S., 2012. Roles of PI3K/AKT/GSK3/mTOR pathway in cell signaling of mental illnesses. Depress. Res. Treat. 2012. https://doi.org/10.1155/2012/752563.
Kivity, S., Arango, M.T., Molano-González, N., Blank, M., Shoenfeld, Y., 2017. Phospholipid supplementation can attenuate vaccine-induced depressive-like behavior in mice. Immunol. Res. 65, 99–105. https://doi.org/10.1007/s12026-016-8818-6.
Küllenberg, D., Taylor, L.A., Schneider, M., Massing, U., 2012. Health effects of dietary phospholipids. Lipids Health Dis. 11, 3. https://doi.org/10.1186/1476-511X-11-3.
Kumar, V., 2005. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. J. Neurosci. 25, 11288–11299. https://doi.org/10.1523/JNEUROSCI.2284-05.2005.
Lassale, C., Batty, G.D., Baghdadli, A., Jacka, F., Sánchez-Villegas, A., Kivimäki, M., et al., 2019. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. Mol. Psychiatry 24, 965–986. https://doi.org/10.1038/s41380-018-0237-8.
Lauritzen, L., Hansen, H.S., Jorgensen, M.H., Michaelsen, K.F., 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog. Lipid Res. 40, 1–94.
Lauwers, E., Goodchild, R., Verstreken, P., 2016. Membrane lipids in presynaptic function and disease. Neuron 90, 11–25. https://doi.org/10.1016/j.neuron.2016.02.033.
Lawlor, M.A., Alessi, D.R., 2001. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J. Cell. Sci. 114, 2903–2910. https://doi.org/10.1042/bst0290001.
Lee, B., Sur, B.-J., Han, J.-J., Shim, I., Her, S., Lee, H.-J., et al., 2010. Krill phosphatidylserine improves learning and memory in Morris water maze in aged rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 1085–1093. https://doi.org/10.1016/j.pnpbp.2010.05.031.
Lefebvre, J.L., Sanes, J.R., Kay, J.N., 2015. Development of dendritic form and function. Annu. Rev. Cell Dev. Biol. 31, 741–777. https://doi.org/10.1146/annurev-cellbio-100913-013020.
Lemaire, M., Dou, S., Cahu, A., Formal, M., Le Normand, L., Romé, V., et al., 2018. Addition of dairy lipids and probiotic Lactobacillus fermentum in infant formula programs gut microbiota and entero-insular axis in adult minipigs. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-29971-w.
Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X., et al., 2015. A review on phospholipids and their main applications in drug delivery systems. Asian J. Pharm. Sci. 10, 81–98. https://doi.org/10.1016/j.ajps.2014.09.004.
Lim, S.-Y., Suzuki, H., 2008. Dietary phosphatidylcholine improves maze-learning performance in adult mice. J. Med. Food 11, 86–90. https://doi.org/10.1089/jmf.2007.060.
Liu, H., Radlowski, E.C., Conrad, M.S., Li, Y., Dilger, R.N., Johnson, R.W., 2014. Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. J. Nutr. 144, 1903–1909. https://doi.org/10.3945/jn.114.199828.phospholipids.
Lupien, S.J., De Leon, M., De, S.S., Convit, A., Tarshish, C., Nair, N.P.V., et al., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69–73.
Lupien, S.J., Maheu, F., Tu, M., Fiocco, A., Schramek, T.E., 2007. The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. Brain Cogn. 65, 209–237. https://doi.org/10.1016/j.bandc.2007.02.007.
Mather, I.H., 2011. Milk fat globule membrane. In: Fuquay, J., Fox, P., McSweeney, P. (Eds.), Encyclopedia ofDairy Sciences, Second Edition, Vol. 3. Elsevier L, San Diego, pp. 680–690.
McCabe, P.J., Green, C., 1977. The dispersion of cholesterol with phospholipids and glycolipids. Cirqnishy Andpfiysics of Lipids 20, 319–330.
McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216. https://doi.org/10.1016/0959-4388(95)80028-X.
Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al., 2011. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br. J. Nutr. 105, 755–764. https://doi.org/10.1017/s0007114510004319.
Monteleone, P., Maj, M., Beinat, L., Natale, M., Kemali, D., 1992. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. Eur. J. Clin. Pharmacol. 41, 385–388. https://doi.org/10.1007/BF02285106.
Moriyama, T., Uezu, K., Matsumoto, Y., Chung, S.-Y., Uezu, E., Miyagi, S., et al., 1996. Effects of dietary phosphatidylcholine on memory in memory deficient mice with low brain acetylcholine concentration. Life Sci. 58, 111–118.
Mudd, A.T., Alexander, L.S., Berding, K., Waworuntu, R.V., Berg, B.M., Donovan, S.M., et al., 2016. Dietary prebiotics, milk fat globule membrane, and lactoferrin affects structural neurodevelopment in the young piglet. Front. Pediatr. 4, 1–10. https://doi.org/10.3389/fped.2016.00004.
Müller, C.P., Reichel, M., Mühle, C., Rhein, C., Gulbins, E., Kornhuber, J., 2015. Brain membrane lipids in major depression and anxiety disorders. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids 1851, 1052–1065. https://doi.org/10.1016/j.bbalip.2014.12.014.
Nicoletti, F., Wroblewski, J.T., Novelli, A., Alho, H., Guidotti, A., Costa, E., 1986. The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. J. Neurosci. 6, 1905–1911.
Norris, G.H., Jiang, C., Ryan, J., Porter, C.M., Blesso, C.N., 2016. Milk sphingomyelin improves lipid metabolism and alters gut microbiota in high fat diet-fed mice. J. Nutr. Biochem. 30, 93–101. https://doi.org/10.1016/j.jnutbio.2015.12.003.
O'Mahony, S.M., McVey Neufeld, K., Waworuntu, R.V., Pusceddu, M.M., Manurung, S., Murphy, K., et al., 2019. The enduring effects of early-life stress on the microbiota–gut–brain axis are buffered by dietary supplementation with milk fat globule membrane and a prebiotic blend. Eur. J. Neurosci. https://doi.org/10.1111/ejn.14514.
O'Neill, C., 2013. PI3-kinase/Akt/mTOR signaling: impaired on/off switches in aging, cognitive decline and Alzheimer's disease. Exp. Gerontol. 48, 647–653. https://doi.org/10.1016/j.exger.2013.02.025.
Oliveira, T.G., Chan, R.B., Bravo, F.V., Miranda, A., Silva, R.R., Zhou, B., et al., 2016. The impact of chronic stress on the rat brain lipidome. Mol. Psychiatry 21, 80–88. https://doi.org/10.1038/mp.2015.14.
Park, H.-J., Soo Shim, H., Soo Kim, K., Han, J.-J., Kim, J.S., Ram Yu, A., et al., 2013. Enhanced learning and memory of normal young rats by repeated oral administration of Krill Phosphatidylserine. Nutr. Neurosci. 16, 47–53. https://doi.org/10.1179/1476830512y.0000000029.
Picciotto, M.R., Higley, M.J., Mineur, Y.S., 2012. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 76, 116–129. https://doi.org/10.1016/j.neuron.2012.08.036.
Roca, M., Vives, M., Lopez-Navarro, E., Garcia-Campayo, J., Gili, M., 2015. Cognitive impairments and depression: a critical review. Actas Esp. Psiquiatr. 43, 187–193.
Sandhu, K.V., Sherwin, E., Schellekens, H., Stanton, C., Dinan, T.G., Cryan, J.F., 2017. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. Transl. Res. 179, 223–244. https://doi.org/10.1016/j.trsl.2016.10.002.
Sastry, P.S., 1985. Lipids of nervous tissue: composition and metabolism. Prog. Lipid Res. 24, 69–176. https://doi.org/10.1016/0163-7827(85)90011-6.
Savignac, H.M., Tramullas, M., Kiely, B., Dinan, T.G., Cryan, J.F., 2015. Bifidobacteria modulate cognitive processes in an anxious mouse strain. Behav. Brain Res. 287, 59–72. https://doi.org/10.1016/j.bbr.2015.02.044.
Scali, C., Casamenti, F., Pazzagli, M., Bartolini, L., Pepeu, G., 1994. Nerve growth factor increases extracellular acetylcholine levels in the parietal cortex and hippocampus of aged rats and restores object recognition. Neurosci. Lett. 170, 117–120.
Schipper, L., van Dijk, G., Broersen, L.M., Loos, M., Bartke, N., Scheurink, A.J., et al., 2016. A postnatal diet containing phospholipids, processed to yield large, phospholipid-coated lipid droplets, affects specific cognitive behaviors in healthy male mice. J. Nutr. 146, 1155–1161. https://doi.org/10.3945/jn.115.224998.
Schubert, M., Contreras, C., Franz, N., Hellhammer, J., 2011. Milk-based phospholipids increase morning cortisol availability and improve memory in chronically stressed men. Nutr. Res. 31, 413–420. https://doi.org/10.1016/j.nutres.2011.05.012.
Scott, K.A., Ida, M., Peterson, V.L., Prenderville, J.A., Moloney, G.M., Izumo, T., et al., 2017. Revisiting Metchnikoff: age-related alterations in microbiota-gut-brain axis in the mouse. Brain Behav. Immun. 65, 20–32. https://doi.org/10.1016/j.bbi.2017.02.004.
Sherwin, E., Rea, K., Dinan, T.G., Cryan, J.F., 2016. A gut (microbiome) feeling about the brain. Curr. Opin. Gastroenterol. 32, 96–102. https://doi.org/10.1097/MOG.0000000000000244.
Söderberg, M., Edlund, C., Kristensson, K., Dallner, G., 1991. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 26, 421–425. https://doi.org/10.1007/BF02536067.
Söderberg, M., Edlund, C., Kristensson, K., Dallner, G., 1990. Lipid compositions of different regions of the human brain during aging. J. Neurochem. 54, 415–423. https://doi.org/10.1111/j.1471-4159.1990.tb01889.x.
Starks, M.A., Starks, S.L., Kingsley, M., Purpura, M., Jäger, R., 2008. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J. Int. Soc. Sports Nutr. 5. https://doi.org/10.1186/1550-2783-5-11.
Suetsugu, S., Kurisu, S., Takenawa, T., 2014. Dynamic shaping of cellular membranes by phospholipids and membrane-deforming proteins. Physiol. Rev. 94, 1219–1248. https://doi.org/10.1152/physrev.00040.2013.-All.
Sun, W., Lo, C.M., Tso, P., 2009. Intestinal lipid absorption. American Journal of Physiology-Endocrinology and Metabolism 296, E1183–E1194. https://doi.org/10.1002/9781444303254.ch18.
Svennerholm, L., Boström, K., Jungbjer, B., Olsson, L., 1994. Membrane lipids of adult human brain: lipid composition of frontal and temporal lobe in subjects of age 20 to 100 years. J. Neurochem. 63, 1802–1811. https://doi.org/10.1046/j.1471-4159.1994.63051802.x.
Tanaka, K., Hosozawa, M., Kudo, N., Yoshikawa, N., Hisata, K., Shoji, H., et al., 2013. The pilot study: sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. Brain Dev. 35, 45–62. https://doi.org/10.1016/j.braindev.2012.03.004.
Tanila, H., 2018. Testing cognitive functions in rodent disease models: present pitfalls and future perspectives. Behav. Brain Res. 352, 23–27. https://doi.org/10.1016/j.bbr.2017.05.040.
Thompson, R.S., Roller, R., Mika, A., Greenwood, B.N., Knight, R., Chichlowski, M., et al., 2017. Dietary prebiotics and bioactive milk fractions improve NREM sleep, enhance REM sleep rebound and attenuate the stress-induced decrease in diurnal temperature and gut microbial alpha diversity. Front. Behav. Neurosci. 10, 1–16. https://doi.org/10.3389/fnbeh.2016.00240.
Timby, N., Domellöf, E., Hernell, O., Lönnerdal, B., Domellöf, M., 2014. Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-proteinformula supplemented with bovine milk fat globule membranes: a randomized controlled trial. Am. J. Clin. Nutr. 99, 860–868. https://doi.org/10.3945/ajcn.113.064295.

Tracey, T.J., Steyn, F.J., Wolvetang, E.J., Ngo, S.T., 2018. Neuronal lipid metabolism: multiple pathways driving functional outcomes in health and disease. Front. Mol. Neurosci. 11, 1–25. https://doi.org/10.3389/fnmol.2018.00010.

Ueda, Y., 2014. The role of phosphoinositides in synapse function. Mol. Neurobiol. 50, 821–838. https://doi.org/10.1007/s12035-014-8768-8.

Veereman-Wauters, G., Staelens, S., Rombaut, R., Dewettinck, K., Deboutte, D., Brummer, R.J., et al., 2012. Milk fat globule membrane (INPULSE) enriched formula milk decreases febrile episodes and may improve behavioral regulation in young children. Nutrition 28, 749–752. https://doi.org/10.1016/j.nut.2011.10.011.

Verardo, V., Gómez-caravaca, A.M., Arráez-román, D., Hettinga, K., 2017. Recent advances in phospholipids from colostrum, milk and dairy by-products. Int. J. Mol. Sci. 18, 1–23. https://doi.org/10.3390/ijms18010173.

Verardo, V., Gomez-Caravaca, A.M., Gori, A., Losi, G., Caboni, M.F., 2013. Bioactive lipids in the butter production chain from Parmigiano Reggiano cheese area. J. Sci. Food Agric. 93, 3625–3633. https://doi.org/10.1002/jsfa.6152.

Vickers, M.H., Guan, J., Gustavsson, M., Krägeloh, C.U., Breier, B.H., Davison, M., et al., 2009. Supplementation with a mixture of complex lipids derived from milk to growing rats results in improvements in parameters related to growth and cognition. Nutr. Res. 29, 426–435. https://doi.org/10.1016/j.nutres.2009.06.001.

Wallace, T.L., Ballard, T.M., Glavis-Bloom, C., 2015. Animal paradigms to assess cognition with translation to humans. In: Kantak, K.M., Wettstein, J.G. (Eds.), Cognitive Enhancement. Cham: Springer International Publishing, pp. 27–57. https://doi.org/10.1007/978-3-319-16522-6_2.

Wang, D., Cheng, S.L., Fei, Q., Gu, H., Raftery, D., Cao, B., et al., 2018. Metabolic profiling identifies phospholipids as potential serum biomarkers for schizophrenia. Psychiatry Res. 272, 18–29. https://doi.org/10.1016/j.psychres.2018.12.008.

Watson, H., 2015. Biological membranes. Essays Biochem. 59, 43–70. https://doi.org/10.1063/1.2913788.

Wehrmüller, K., 2008. Impact of dietary phospholipids on human health. ALP Science.

Wilson, M.A., Fadel, J.R., 2017. Cholinergic regulation of fear learning and extinction. J. Neurosci. Res. 95, 836–852. https://doi.org/10.1002/jnr.23840.

Wirtz, P.H., Elsenbruch, S., Emini, L., Rüdisüli, K., Groessbauer, S., Ehlert, U., 2007. Perfectionism and the cortisol response to psychosocial stress in men. Psychosom. Med. 69, 249–255. https://doi.org/10.1097/PSY.0b013e318042589e.

Zeisel, S.H., Da Costa, K.-A., Franklin, P.D., Alexander, E.A., Lamont, J.T., Sheard, N.F., et al., 1991. Choline: an essential nutrient for humans. Faseb J. 5, 2093–2098. https://doi.org/10.1016/S0899-9007(00)00349-X.

Zhang, Y., Appelkvist, E.L., Kristensson, K., Dallner, G., 1996. The lipid compositions of different regions of rat brain during development and aging. Neurobiol. Aging 17, 869–875. https://doi.org/10.1016/S0197-4580(96)00076-0.

Zhao, H., Wang, C., Zhao, N., Li, W., Yang, Z., Liu, X., et al., 2018. Potential biomarkers of Parkinson's disease revealed by plasma metabolic profiling. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 101–108. https://doi.org/10.1016/j.jchromb.2018.01.025.

Zunszain, P.A., Anacker, C., Cattaneo, A., Carvalho, L.A., Pariante, C.M., 2011. Glucocorticoids, cytokines and brain abnormalities in depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 722–729. https://doi.org/10.1016/j.pnpbp.2010.04.011.