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REVIEW

Egg yolk lipids: separation, characterization, and utilization

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Abstract

Egg yolk contains very high levels of lipids, which comprise 33% of whole egg yolk. Although triglyceride is the main lipid, egg yolk is the richest source of phospholipids and cholesterol in nature. The egg yolk phospholipids have a unique composition with high levels of phosphatidylcholine followed by phosphatidylethanolamine, sphingomyelin, plasmalogen, and phosphatidylinositol. All the egg yolk lipids are embedded inside the HDL and LDL micelles or granular particles. Egg yolk lipids can be easily extracted using solvents or supercritical extraction methods but their commercial applications of egg yolk lipids are limited. Egg yolk lipids have excellent potential as a food ingredient or cosmeceutical, pharmaceutical, and nutraceutical agents because they have excellent functional and biological characteristics. This review summarizes the current knowledge on egg yolk lipids' extraction methods and functions and discusses their current and future use, which will be important to increase the use and value of the egg.

Keywords Yolk lipids · Phospholipids · Separation · Functionality · Application

Introduction

Egg yolk comprises 1/3 of the whole egg and is an emulsion-like system comprising high-density lipoprotein (HDL)- and low-density lipoprotein (LDL)-particles suspended in a protein solution (Fig. 1). Approximately 50% of egg yolk is water, and the rest are lipids, proteins, minerals, and vitamins (Ahn et al., 1997). Almost all the lipids of eggs are present in the egg yolk and are mainly consisted of triglycerides (62%), phospholipids (33%), and cholesterol (5%). The egg yolk lipids are the key constituents of HDL, LDL, and very-low-density lipoprotein (VLDL), the spherical particles of triglycerides and cholesterol ester core encapsulated by a phospholipid monolayer, cholesterol, and proteins (Anton, 2013). The HDL, LDL, and VLDL particles differ in their densities depending upon the amounts of proteins embedded in the micelle structure (Plonne, 2021). The HDL particles are larger and heavier in density than the LDL and VLDL particles. The egg yolk granules are made of multiple HDL-submicells linked by phosvitin, embedded LDL vesicles, and a hydrophobic cavity filled with neutral lipids and cholesterol esters (Fig. 2) and easily can be separate from the liquid (plasma) fraction using high-speed centrifugation (Stadelman and Cotterill, 1995; Strixner et al., 2014). The granules comprise 70% HDL, 18% phosvitin, and 12% LDL. In addition, up to 40 phospholipids are found in a single HDL molecule (Li et al., 2020, 2021; Xiao et al., 2020; Zhou et al., 2018). Therefore, the granule fraction is an excellent starting material for separating phosvitin and HDL. The upper layer after the centrifugation is plasma and is made of 78% LDL and 22% livetin (Stadelman and Cotterill, 1995). The LDL micelles also have a similar structure to the HDL micelles but with fewer proteins embedded. Because the plasma is

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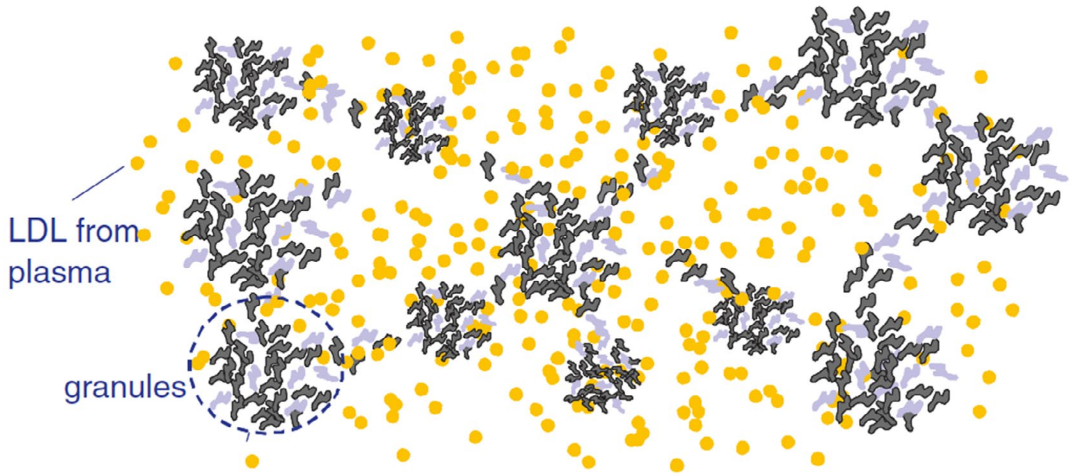
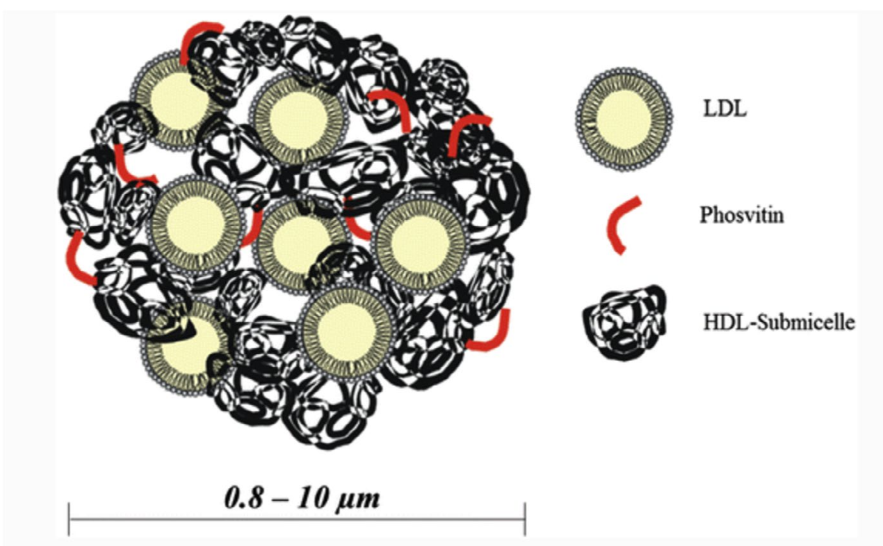


A. Schematic model of egg yolk. Egg yolk granules and LDL particles are suspended in the protein solution of egg yolk. From Anton (2013).

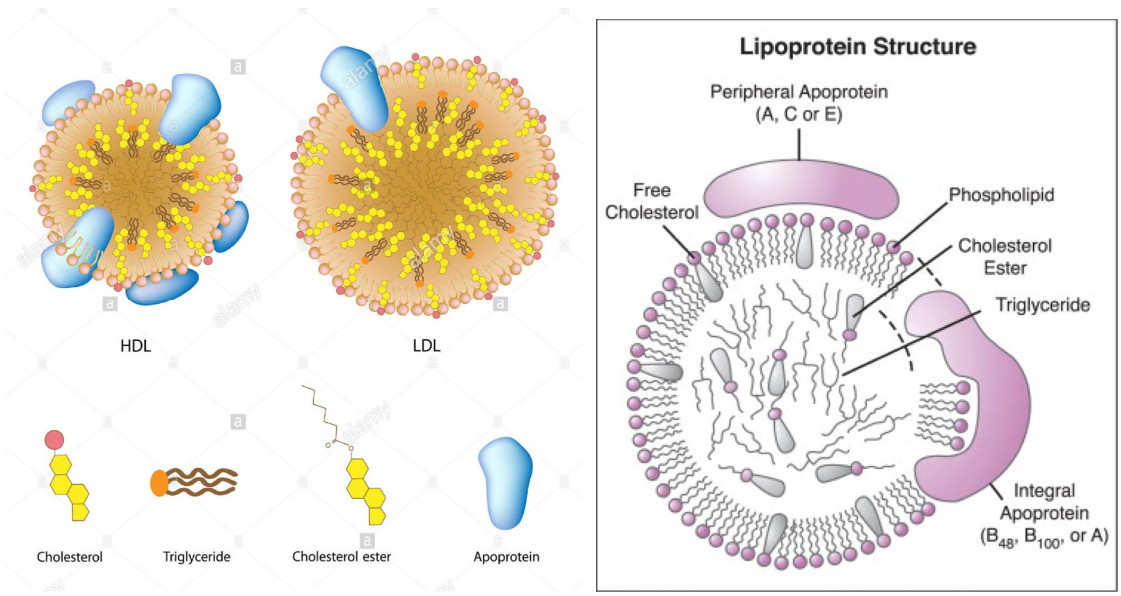


B. Schematic model of a granule from egg yolk at pH 6.5 consisting of both HDL-submicelles linked by phosvitin via calcium phosphate bridges and embedded LDL vesicles. From Strixner et al. (2014).

Fig. 1 Schematic model of egg yolk (**A**) and the structure of egg yolk granule (**B**)

mainly composed of LDL with a high emulsifying capacity, it is used in mayonnaise production or as a starting material for phospholipids and neutral lipid separation (Anton, 2006; Laca et al., 2010). All the lipoprotein micelles of egg yolk contain neutral lipids, phospholipids, and cholesterol, but their composition in each type of lipoprotein is different (Kamal and Shahidan, 2020; Wang et al., 2020a, b).

Neutral lipids are the main egg yolk lipids and can have up to three fatty acids to the glycerol backbone as monoacylglycerides (MAG), diacylglycerides (DAG), and triacylglycerides (TAG). Neutral lipids are the primary energy source for the chick embryo, providing 90% of the energy for the development, and are used as the structural components for cell membrane biosynthesis (Speake



A. The structure of HDL and LDL particles is the same, but HDL particle contain more protein embedded on the surface layer (coat) than the LDL particle. The layer (coat) is made of phospholipids, cholesterol, and proteins. From Plonne (2021).

B. Lipoprotein particles are composed of a lipid core containing cholesteryl esters and triglycerides, and a surface coat of phospholipids, unesterified cholesterol and apolipoproteins. From Engelking (2015).

Fig. 2 The Schematic models of HDL and LDL (**A**) and the structure of lipoprotein particles (**B**)

et al., 1998). They are involved in signal transduction and serve as precursors to glycerophospholipids (Wood et al., 2021). Neutral lipids separated from egg yolk can be used as cooking oil and animal feeds, and the nutritive value of neutral lipids from egg yolk is better than vegetable oils (Ahn et al., 2006).

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), plasmalogen (PL) phosphatidylinositol (PI) are the main types of phospholipids (Ali et al., 2017; Li et al., 2020; Wang et al., 2012; Xiao et al., 2020) in egg yolk. The composition of phospholipids in egg yolk is unique compared with other phospholipid sources such as soybean phospholipids. Egg yolk phospholipid is composed of 78.5% PC, 17.5% PE, 2.5% SM, 0.9% plasmalogen and 0.6% PI.

PC is known to have hepatoprotective effects, and thus egg yolk phospholipids can be used to treat nonalcoholic fatty liver disease, one of the most common chronic liver diseases worldwide, and prevent its progress (Duric et al., 2012; Maev et al., 2020; Zhang et al., 2021). Dietary PC is known to have beneficial effects on reducing insulin resistance that is directly related to type-2 diabetes, but the effects are largely related to its fatty acid composition. Only the PC with high eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in its structure ameliorated insulin resistance in mice by changing the gut microbiota composition, lowering the chronic inflammation in the adipose tissues, and improving the transduction of insulin signaling pathways (Gao et al., 2021). The yolk PC effectively improves obesity in mice fed a high-fat diet by regulating glycerophospholipid metabolism (Yu et al., 2021).

Phosphatidylethanolamine (PE) is another important phospholipid found in egg yolk. Sun et al. (2019) reported that the PE from egg yolk had high radical-scavenging activity because of its high unsaturated fatty acids content. Mainly the phosphatidylserine decarboxylase enzyme located in the inner membrane of mitochondria synthesizes PE, but non-mitochondrial PE can also produce with the cytidine diphosphate-ethanolamine pathways in the endoplasmic reticulum (Cho et al., 2021; Kumar et al., 2021).

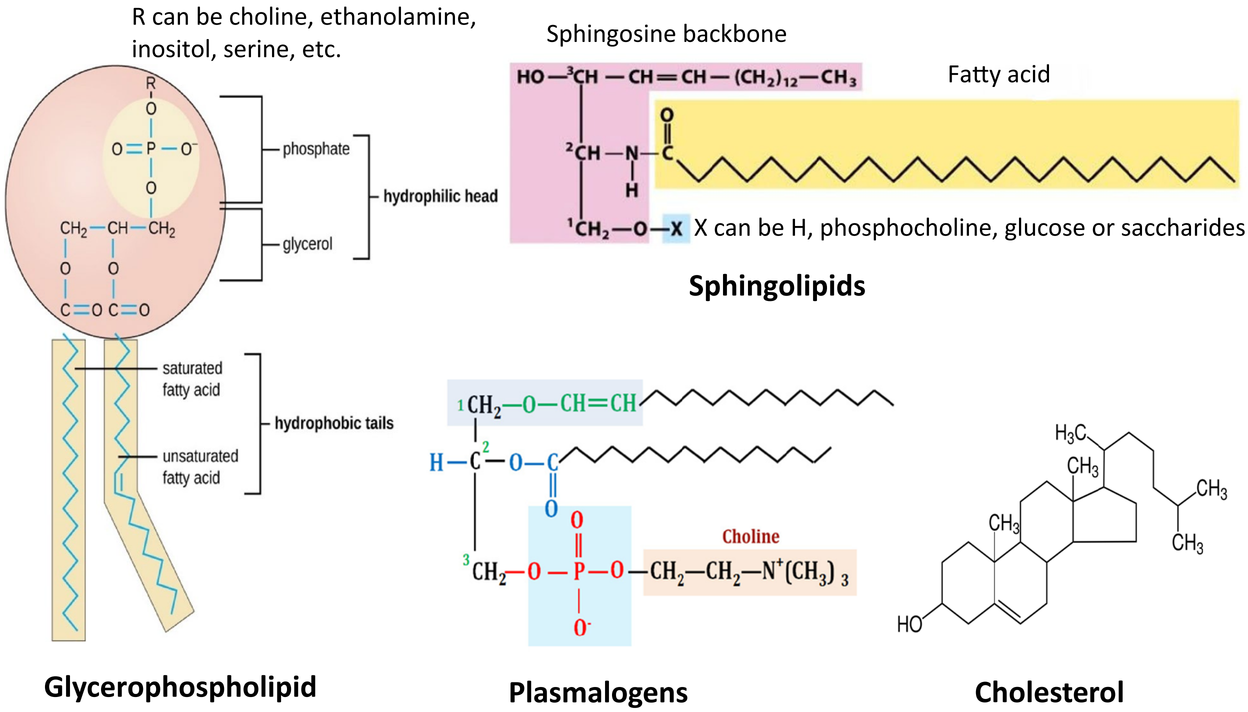


Fig. 3 The general structure of glycerophospholipids (PC, PE, PS, and PI), sphingolipids, plasmalogens, and cholesterol

Both PC and PE are important in regulating VLDL in blood plasma. The ratio between PC/PE is important for removing VLDL particles from the circulation system in an animal body, and a high PC/PE ratio in the bloodstream protects mammals against atherosclerosis.

On the other hand, the inhibition of PC biosynthesis could promote triacylglycerides and increase the lipid droplets' size, leading to many adverse conditions like promoting cardiovascular disorders in humans (van der Veen et al., 2017; Xiao et al., 2020). The production of PE and PC has several pathways in the eukaryotic cells. The cytidine 5'-diphosphate (CDP)-ethanolamine formation mainly synthesizes PE by the Kennedy pathway. Several enzymes mediate these pathways (Farine et al., 2015).

Sphingomyelin (SM) is a yolk phospholipid with significant biological importance, and egg yolk is one of the best SM sources. SM is synthesized through the esterification of phosphorylcholines with ceramide by the sphingomyelin synthase (Delgado et al., 2007; Wood et al., 2021). In the cells, ceramides and sphingosine, the structural components of SM, can also phosphorylate to the corresponding phosphate esters, ceramide-1-phosphate and sphingosine-1-phosphate that are directly involved in cell signaling (Delgado et al., 2007). SM directly involves the central nervous systems and affects brain cells' viability and signal transduction of T-cells, and regulates cholesterol absorption and inflammation in the human body (Blesso, 2015; Hussain et al., 2019). Chicken egg yolk is also an excellent source of complex lipids needed for lipid homeostasis (Wood et al., 2021).

Egg yolk lipids contain pigments such as carotenoids (mainly carotene, lutein and xanthophylls), which color the egg yolk. The amount of egg yolk pigments varies depending upon the amounts of carotenoids and xanthophylls in the diet that the hens are consuming (Karadas et al., 2006). Since the carotenoids and xanthophylls in egg yolk are in bioavailable form, they can prevent macular degeneration, act as an antioxidant compound, prevent cataracts, cardiovascular diseases, Alzheimer's, and cancers (Eisenhauer et al., 2017; Xiao et al., 2020; Zaheer, 2017). Lutein and zeaxanthin belong to xanthophylls that accumulate in the eye's retina and can prevent and slow the progression of age-related macular degeneration, the leading cause of blindness in older adults (Stahl, 2005; Zaheer, 2017).

Even though the egg yolk contains a high cholesterol level, the cholesterol present in the egg does not adversely affect human health (Kuang et al., 2018). Over 95% of the cholesterol present in the egg yolk is considered free cholesterol, which plays an important role in maintaining the structure of the lipoproteins. These cholesterols are packed between the adjacent phospholipids, which help maintain the oil and water interface (Griffin, 1992). This review discusses the status and future of egg yolk lipids, including their separation, functions, applications, and future. The general structures of glycerophospholipids, sphingolipids, plasmalogens, and cholesterol are shown in Fig. 3.

Separation of Yolk Lipids and Phospholipids

Egg yolk lipids are composed of phospholipids, neutral lipids, cholesterol, and other minor lipids, and many methods have developed to separate them separately or together from the rest of the egg components. The two most common methods of separating lipid components from the egg yolk are solvent and supercritical extraction. Organic solvents can be classified into two main groups, polar and non-polar. Among the solvents used, ethanol, methanol, dichloromethane, and ethyl acetate are considered polar, while hexane, heptane, butane, and chloroform are non-polar solvents (Kier, 1981).

Solvent extraction methods of yolk lipids

Solvent extraction can use single solvents or solvent combinations and scale up easily to industrial levels, and the separated compounds using solvents can be used in food, pharmaceutical, nutraceutical, and cosmeceutical industries (Huang and Ahn, 2019). The conventional lipid extraction method uses solvents called the Bligh and Dyer method, which uses chloroform and methanol to extract lipids from an organic source (Schrienier, 2006). The combinations of aqueous ethanol, hexane, acetone, and petroleum are commonly used to extract various lipids from the organic materials based on the polarity of the target lipids (Wang et al., 2012). The mixture of 2-propanol: hexane (30:70) was successfully used to separate lecithin (mainly phosphatidylcholine) from liquid egg yolk, and the solvent residues were easily removed by streaming nitrogen through the purified oil (Kovalcuks, 2014). Hexane tends to have a higher capacity to remove neutral lipids than cholesterol and pigments. However, combining hexane with isopropanol at a 2:1 ratio improved phospholipid extraction (Warren et al., 1988). Su et al. (2015) developed a novel process to separate phospholipids, neutral lipids, and cholesterol from the egg yolk using ethanol, low-temperature crystallization, and β-cyclodextrin. Although the phospholipids' purity was 51%, almost all the cholesterol could be removed from the fraction. However, this method suffered two major drawbacks: high residual solvents and low extraction efficiency. Another solvent combination, petroleum ether: ethanol at 35:65, extracts cholesterol easily from the yolk lipids. This combination can also remove the major component of phospholipids present in the egg yolk (Paraskevopoulou and Kiosseoglou, 1994). Maximiano et al. (2008) used petroleum ether: acetone mixture followed by several chloroform washings to separate lecithin from the egg yolk and recommended this method for the scale-up production of lecithin from the egg yolk. However, chloroform, methanol, petroleum ether, and acetone are not allowed if the products are for humans in most countries. After removing water-soluble proteins from the egg yolk, Ahn et al. (2006) developed a large-scale separation of phospholipids and neutral lipids using ethanol and n-hexane. According to the methods, > 85% ethanol was used to extract phospholipids first and then hexane to separate neutral lipids, and the extraction efficiency was excellent.

Supercritical extraction method

Supercritical carbon dioxide (SC-CO_2) is an alternative to the solvent extraction used at the industrial level. Supercritical carbon dioxide is mainly used to produce nutraceuticals or nutritionally specific fish oils and plants. Carbon dioxide (CO_2), the primary extraction fluid in the Supercritical fluid extraction (SFE), is inexpensive, poses no threat to the environment or human health, and is easily applied for scaled-up (Aro et al., 2009). SFE is a "greener technology" and reduces energy usage (Uwineza and Waśkiewicz, 2020). Supercritical fluids are neither a gas nor a liquid, but they have some phases' characteristics. Different lipid components will have identical triple points, and during the point, each lipid component can be separated (Gere et al., 1997). The SFE produces higher yield and purity products than the conventional chloroform: methanol: water extraction (Boselli and Caboni, 2000). The SFE can be done with carbon dioxide alone or with ethanol as a co-solvent to extract neutral lipids and phospholipids from the egg yolk under 45–50 MPa pressure and at an operating temperature of 70 °C (Aro et al., 2009; Navidghasemizad et al., 2014). Accordingly, carbon dioxide and ethanol combinations extracted polar lipid components from the egg yolk better than using the SFE with CO_2 alone. Cholesterol effectively separated from the egg yolk using the SFE with 306 atm at 45 °C or 374 atm at 55 °C conditions, while phospholipids were better at 163 atm at 40 °C, 238 atm at 45 °C, and 306 atm at 45 °C conditions. These results indicated that different temperature and pressure conditions help separate each lipid component selectively and efficiently using the SFE (Froning et al., 1990; Warren et al., 1991). Neutral lipids and phospholipids with > 90% purity can be easily produced from the lipid-rich IgY-separation residue either using solvents (hexane and ethanol) or the supercritical fluid extraction (SFE) method with ethanol as a co-solvent (Catchpole et al., 2018). However, SFE is effective only for extracting lipids from the dried egg yolk, and the initial capital investments for the facility are high (Aro et al., 2009). The extraction of yolk lipid components is summarized in Table 1.

The separated crude phospholipids from the egg yolk can be used as a starting material to further separate PC, PE, SM, PS, and PI or modified using enzymes to improve their functionality. Separating individual phospholipids from the egg yolk phospholipids can greatly increase the value of the egg yolk, but it is very difficult. Various methods, including alumina, silica gel, solid-phase microextraction, and ion-exchange cellulose, have been proposed (Burdge et al., 2000; Lopez-Rodriguez et al., 2019) to separate individual phospholipids from the phospholipids mix. Most of these methods use many toxic solvents, and many are tested only at laboratory scales. Therefore, developing a scalable separation method for the individual phospholipids from the crude egg yolk phospholipids is one of the important tasks for egg scientists to increase the value and use of the egg yolk in the future.

Health Beneficial Functions of Egg Yolk Lipids

Lipids are considered one of the six major nutrients required for embryonic development and survival and the biological activities of infants and adults (Xiao et al., 2020). Neutral lipids and cholesterol in the yolk are directly transferred into embryo tissues without degradation and resynthesis, but some phospholipids can be synthesized de novo in the developing chick embryo. However, most of the phospholipids in the embryo and its organs originate from the yolk phospholipids, and the transfer rate becomes faster at later stages of embryo development (Yadgary et al., 2014). Phospholipids comprise two fatty acids, a glycerol backbone and a phosphate group. Dietary glycerophospholipids can be easily absorbed from the small intestine after broken down into lysophospholipids and free fatty acids. Phospholipids are the major components in cell membranes, facilitate cell fusion, cause hemolysis, and affect the permeability of phosphatidylcholine liposomes (Balleza, 2012; Blesso, 2015; Lee, 2020). They also affect the pathways related to inflammation and the metabolism of HDL and cholesterol. Since phospholipids have good biocompatibility and amphiphilicity, they can be widely used as a drug delivery agent (Li et al., 2015).

PC is an important component of cell membranes and plays a vital role in the functions of nerve cells, cholinergic neurotransmission, and lipid transport in the liver (Buang et al., 2005; Ueland, 2011; van der Veen et al., 2017; Zeisel,

Table1 Summary of the separation methods of yolk lipids

Separation method	Principle	Common chemicals used	Advantages of the method	Disadvantages of the method	References
Solvent extraction (Bligh and Dyer method)	Use the polarity of the solvent and the fatty acid in the yolk lipid	Chloroform: methanol: water (1:2:0.8) Petroleum ether: ethylic ether: acetic acid (90:10:1) 2-Propanol: hexane (30:70) Hexane: isopropanol (2:1) Petroleum ether: acetone (35:65) 100% n-hexane 95–100% ethanol 100% acetone 100% petroleum ether	Simple technique Cost-effective Can be applied on an industrial scale Comparatively high yield Can be separated several yolk lipids using combinations of solvents	Chloroform and methanol are toxic to humans Residual components are present after separation A large quantity of solvents is needed	Ahn et al. (2006) Paraskevopoulou and Kiosseoglou (1994) Kovalcuks, (2014) Maximiano et al., (2008) Schreiner, (2006) Su et al. (2015) Wang et al. (2012) Warren et al. (1988)
Supercritical extraction	Use the triple point of the oil and convert the solid lipid into gas form, and then separate each component	Carbon dioxide gas and as ethanol a co-solvent is used at 45–50 MPa pressure and with 70 °C operating temperature	High-quality end-products Reduce the energy usage Minimum use of solvents Safe production Environment friendly	Advanced techniques are used Skilled technicians are need High cost	Aro et al. (2009) Boselli and Caboni (2000) Catchpole et al. (2018) Froning et al. (1990) Gere et al. (1997) Navidghasemizad et al. (2014) Warren et al. (1991)

2006). PC, the major egg yolk phospholipids, can suppress the growth of the hepatoma cells by inducing apoptosis. It also can reduce the Angiotensin-converting enzyme activity, oxidation, and cholesterol absorption and help the memory recovery of dementia patients by increasing the acetylcholine concentration. Hartmann et al. (2009) investigated the effect of PC in chemically induced arthritis (with carrageen) in rats and found a significantly reduced development of arthritis after PC supplementation, most likely due to an inhibition of the neutrophil leukocyte-mediated inflammatory reaction. PC is highly effective in controlling Alzheimer's disease in humans (Whiley et al., 2014; Xiao et al., 2020). In addition, PC showed an anti-inflammatory effect by inhibiting nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) and preventing cardiovascular diseases by preventing cholesterol uptake and transport (Xiao et al., 2020). The three metabolites of dietary PC (choline, trimethylamine N-oxide, and betaine) are considered predictors of cardiovascular disorders in humans. The derivatives of PC and other phospholipids include 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) showed high skin pentation-enhancing effects. The liposomes prepared with these compounds entrap drugs and enhance the skin penetration of hydrophilic and hydrophobic compounds and thus are promising compounds for supplying and delivering drugs to humans (Lee, 2020). The liposomes are produced from phospholipids derived from pH-sensitive enzymes. Therefore, it is important to maintain the pH during the delivery of drugs when these liposomes are used (Li et al., 2015; Sakdiset et al., 2018).

The egg yolk is one of the most concentrated choline sources, containing 680 mg of choline in 100 g of egg yolk because 78% of egg yolk phospholipids are PC. Choline is an essential nutrient grouped under the vitamin B-complex that has many biological functions, including synthesis of neurotransmitters (e.g., acetylcholine) and PC, cell membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) in humans (Inazu, 2019; Li and Vance, 2008; Zeisel and da Costa, 2009). Choline is also a structural component of PC, choline plasmalogen, and sphingomyelin, the essential components of membranes (Zeisel et al., 1991). It is also important for brain development in the fetus (Zeisel, 2006). However, choline, phosphatidylcholine, glycerophosphocholine, phosphocholine, and sphingomyelin are converted to betaine in the metabolic pathways and are involved in methylation reactions of membrane phosphatidylcholine synthesis (Buang et al., 2005; Zeisel et al., 2003). Therefore, it is indirectly involved in signal transduction, membrane transport, and integrity (Hamlin et al., 2013).

In humans, the dietary deficiency of choline causes fatty liver, birth defects, and neurological dysfunctions. The estrogen mediates choline requirement from the diet, single nucleotide polymorphisms (SNPs) in the specific genes of choline and folate metabolism (Corbin and Zeisel, 2012; Fischer et al., 2007; Zeisel et al., 1991). Choline increases lymphocyte apoptosis and DNA and muscle damage in humans (Zeisel, 2010), and its supplementation during pregnancy enhances hippocampal neurogenesis in the fetus, increases the size of cholinergic neurons, and enhances acetylcholine storage and release in the basal forebrain in adulthood (Zeisel and da Costa, 2009). The choline intake of 450–550 mg/day is recommended for pregnant and lactating women. Pregnant women with less choline in their diet have four times higher risk of birth to a child with a neural tube defect (Zeisel, 2010; Zeisel and da Costa, 2009). Low plasma concentrations of free choline were significantly associated with high anxiety levels, and choline supplementation in humans reduced the total homocysteine (tHcy) concentration in the plasma. Consuming over 360 mg of choline per day lowered the homocysteine level in the blood, improved vision, and reduced the risk of cardiovascular disorders in humans (Chiuve et al., 2007; Duric et al., 2012; Kuang et al., 2018; Zeisel and da Costa, 2009; Zhao et al., 2013). Therefore, supplying high phosphatidylcholine will increase plasma choline, lower cardiovascular diseases, and improve human health (Olthof et al., 2005; Santilli et al., 2016). Choline is catabolized in the digestive tract by the microbes to produce gas trimethylamine (TMA), which can be easily oxidized into TMAO in the liver in the presence of liver enzymes. Choline can also reduce several inflammatory markers such as C-reactive proteins (CRP), homocysteine, interleukin-6, and necrosis, leading to low inflammation and healing of hepatitis A and C (Schneider et al., 2010; Zeisel and da Costa, 2009). There is a positive relationship between cardiovascular diseases and the level of TMAO, suggesting that a high intake of PC from the egg yolk may influence the production of plasma TMAO in humans, which can cause adverse conditions. However, the effect of PC varies depending on the gut microflora in each human, and more research is needed to confirm the effect of egg yolk lipids on cardiovascular diseases (Blesso, 2015; Lang et al., 1998). The choline, methionine, and folate metabolic pathways intersect to form methionine from homocysteine, and altering these pathways can cause methionine deficiencies, leading to cardiovascular diseases. An abnormal folate-dependent carbon metabolism causes autism in children (Duric et al., 2012; Hamlin et al., 2013).

PE is the second most common phospholipid in egg yolk. However, only four partially redundant biochemical pathways produce PE in the eucaryotic cells (Calzada et al., 2016; Farine et al., 2015). PE showed antioxidant activities through its radical scavenging functions. In addition, PC can reduce the accumulation of oxidants, leading to redox imbalance by oxidative stress and many chronic diseases (Xiao et al., 2020). PE is mainly present in the brain and neural tissues in the spinal cord in animals and can induce relaxation and improve the animal body's cognitive function (Calzada et al., 2016; Cho et al., 2021). PE also acts as a critical anticoagulant at the luminal endothelial surface of the aortic flow dividers, the secondary aorta, and the curvature of the aortic arch (Li et al., 2011). PE of egg yolk contained a higher amount of arachidonic acid and DHA than PC, which could have significant physiological effects in vivo. Because arachidonic acid and DHA are the precursors for the biosynthesis of eicosanoids, they could be closely related to immune response and carcinogenesis in animals (Du et al., 2000). The balance between PE and PC in the skeletal muscles in humans, along with insulin, is a key determinant factor of muscle contractile function and metabolism (Newsom et al., 2016). The derivatives of PE such as 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) and 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine are responsible for interacting with cholesterol in monolayers, an important function of phospholipids (Blesso, 2015). Both PC and PE are related to insulin sensitivity and respond to acute exercise in humans. They are the key determinant of muscle contraction functions and metabolism (Newsom et al., 2016). PE is also involved in mitochondrial stability, protein biogenesis, activity, and autophagy and acts as a precursor of the other lipids synthesis in the body. PE acts as a critical substrate for two fundamental posttranslational modifications (GPI anchors and lipidation of Atg8p/LC-3). PE provides the ethanolamine group to produce the phosphoethanolamine bridge between the glycan and the C-terminal of the amino acid in the GPI-anchored proteins. GPI anchor is a glycolipid added post-translation to the C-terminal of many eukaryotic proteins in cells. They are synthesized in the ER of the cells. These compounds have several biological functions: embryogenesis, neurogenesis, immune responses, and fertility. PE act as the base unit in producing PC in cells. PC synthesis from PE is based on the CDP-choline pathway. In mammalian cells, the PE N-methyl transferase (PEMT) enzyme is responsible for the biosynthesis of the PC from the PE, while yeast contains two enzymes—PE methyltransferase (Pem1p) and phospholipid methyltransferase (Pem2p)—to convert the PE to the PC (Calzada et al., 2016; Tasseva et al., 2013).

PI is the base unit of phosphoinositides, important components of brain cells. Various PIs are synthesized by the phosphorylation of the inositol head group in the PI (Raghu et al., 2019). These phosphoinositides regulate key cellular functions through the non-PLC mediated mechanisms. PI also regulates sub-cellular processes such as membrane transport, cytoskeletal function, and plasma membrane signaling (Raghu et al., 2019; Sabogal-Guáqueta et al., 2018). Shirouch et al. (2008) reported that dietary PI prevented the development of nonalcoholic fatty liver disease in a rat model by increasing serum adiponectin and fatty acid β-oxidation and suppressing the expression of inflammatory mRNA in the liver. Animal brains, liver, and muscle are excellent sources of PI, but egg yolk is also among the best sources of PI in nature (Weihrauch and Son, 1983).

Plasmalogens are a group of glycerophospholipids with a glycerol backbone. It constitutes 20% of the total phospholipids found in humans and is particularly high in the nervous, immune, and cardiovascular systems (Braverman and Moser, 2012). Unlike other phospholipids (PC, PE, PS, and PI), they have an ether at the first position and ester at the second position. They are important in cellular signaling and the stability of the lipid rafts microdomains (Farooqui et al., 2000; Messias et al., 2018), and the reduced levels of plasmalogen are leading causes of morbidity in prematurely born babies and Alzheimer's disease. They also protect mammalian cells against reactive oxygen species effects, regulate ion transport and cholesterol efflux, act as a precursor for eicosanoid and platelet-activating factors, and support membrane fusion. Unlike many other nutrients, lipid composition can be altered through dietary changes. Studies showed that providing plasmalogens to the diet helped mitigate memory loss and improved mild Alzheimer’s disease (Fujino et al., 2017; Hossain et al., 2018). Plasmalogens are high in sea squirts, mussels, and scallops, but egg yolk is also an excellent source (Yamashita et al., 2016).

Sphingolipids are considered the building blocks of cellular membranes and are found in the myelin sheath. A recent study with mice showed that low levels of SM in colon tissue could increase inflammation-related gene expression and proinflammatory cytokines. Low levels of SM in colon tissues increase the expression of inflammation-related genes, proinflammatory cytokines, chemokines, leukocyte infiltration and MAPK signal transducer and activate transcription three activators (Ohnishi et al., 2017). The egg is an excellent source of SM (2.5% of egg yolk phospholipids) that can be used to produce “bioactive lipids” such as ceramide, ceramide-1-phosphate (C1P), and sphingosine-1-phosphate (S1P). The SM derivatives are critical mediators of cell growth, survival, and death (Ahn and Schroeder, 2010). SM is the major sphingolipid in the egg yolk and is the only sphingolipid class that belongs to phospholipid. SM has sphingosine and amino alcohol instead of a glycerol backbone and a fatty acid (Moskot et al., 2018). However, ceramides have only an N-acyl fatty acid, whereas hexosylceramides have galactosyl or glucosyl compounds at the terminal hydroxy group. Sphingosine is cytotoxic, while phosphorylation of sphingosine to S1P renders it bioprotective (Chalfant and Spiegel, 2005). S1P can attenuate apoptosis, promote cell proliferation, and regulate calcium release from the endoplasmic reticulum and calcium entry through plasma membrane channels (Törnquist, 2012). S1P treatment protected ovaries from radiation-induced damage and prevented oocyte loss in mice, suggesting that S1P treatment can be a new approach to preserving ovarian function in vivo (Morita et al., 2000). Ceramide, like S1P, also is a signaling pathway that regulates cell proliferation, apoptosis, motility, differentiation, angiogenesis, stress responses, protein synthesis, carbohydrate metabolism, lymphocyte trafficking, migration, invasion, and autoimmune demyelination (Leong and Saba 2010; Podbielska et al., 2012). Ceramide has multiple roles, such as preventing proliferation, inducing apoptosis, and playing a significant role in mitogenesis and endocytosis (Spiegel and Milstien, 2002; Young et al., 2012). Therefore, elevating cellular ceramide can arrest growth or promote apoptosis, especially for cancer cells (Kolesnick, 2002). C1P regulates cell growth and survival, apoptosis, inflammation mediation, DNA synthesis stimulation, and cell division by acting as an extracellular signaling molecule the following binding to a family of five G-protein coupled to receptors found on the cell surface (Lankalapalli et al., 2009).

Although cholesterol is known as the villain of cardiovascular diseases and atherosclerosis, it has many critical biological functions in the body. Cholesterol maintains the integrity and fluidity of cell membranes and serves as a precursor to producing several important substances such as hormones, bile acids, and vitamin D. Cholesterol in eggs acts as the main carrier of egg-derived carotenoids, which control oxidative stress and prevent cardiovascular disorders (CVD) (Mineo and Shaul, 2012; Xiao et al., 2020; Zampelas and Magriplis, 2019). Egg yolk sphingomyelin and phosphatidylcholine can control the absorbance of cholesterol through the intestine in the mammalian body (Xiao et al., 2020; Yang et al., 2018).

Current and Potential Use of Egg Yolk Lipids and Their Derivatives

Almost all the phospholipids used in the food and non-food applications at the industry level are from the soybean, and only limited amounts of egg yolk phospholipids are used. However, the amount of phospholipid in egg yolk is more than tenfold (dry-weight basis) higher than that in the soybean (Weihrauch and Son, 1983). As discussed above, many methods to separate egg yolk lipids are currently available. Thus, finding new areas for egg yolk lipids will be important to increase egg consumption.

As a food supplement

Chicken egg yolk is an excellent food emulsifier and important in many foods such as mayonnaise, salad dressings, ice cream, and bakery products. The plasma and granule fractions are not lipids, but once the proteins like IgY and phosvitin are removed from the egg yolk plasma and granules, respectively, the remaining fractions are rich in lipids (neutral lipids and phospholipids) (Shen et al., 2020). The fraction from plasma is mainly composed of LDL with high emulsifying capacity and which use for mayonnaise production or as a starting material for phospholipids or neutral lipid separation (Ahn et al., 2006; Anton, 2013; Laca et al., 2015).

When the granules were treated with PBS buffer at pH 7.4 and subjected to ultrasonic treatment, their emulsifying ability was higher than the native form. The pH increases, and adding lecithin to a solution can reduce the accumulation of yolk granules, which is important in the food industry to increase emulsification and foam properties (Marcet et al., 2022; Shen et al., 2020). The egg yolk granules have an excellent emulsifying ability, and the emulsification property in food was good at pH 4.0 when the granules were small (Gamach et al., 2019; Wang et al., 2020a, b). Yolk granules also can use to produce biofilms since they are rich in proteins and lipids. These biofilms are flexible and have good antimicrobial properties, improved water resistance, and solubilization at alkaline pH. However, with the presence of lipids, the strength of these films tends to be low (Marcet et al., 2017, 2022). The potential use of the yolk lipid granules is shown in the Fig. 4 (Marcet et al., 2022; Sk´orkowska-Telichowska et al., 2016).

The PC and PE produced from the egg yolk are excellent emulsifiers and lubricating agents in the food or cosmetic industry. They are also effective wetting agents to solubilize or increase the emulsifying activities of the proteins in food (Li et al., 2021; Su et al., 2015). Phospholipases are diverse enzyme groups with 1-acyl hydrolytic activity. These enzyme groups' mechanisms and kinetics are mainly responsible for triacylglycerol hydrolysis from phospholipids to produce lysophospholipids and fatty acids (Wang et al., 2010). The lysophospholipids produced by enzymes like phospholipase-D and phospholipase-A_2 can increase the emulsifying capacity of phospholipids and improve the stability of emulsions, important in mayonnaise and sauce production (Laca et al., 2015; Lee, 2020; Marcet et al., 2022; Maria et al., 2007). Lyso-phospholipids can increase the foaming and encapsulating abilities of the natural phospholipids, which are important in the food industry as a delivery or encapsulation agent (Chaudhary et al., 2012; Li et al., 2021). An egg-derived phospholipid-enriched diet caused a significant improvement in the endothelial vasodilatory function, a significant decrease in daytime systolic blood

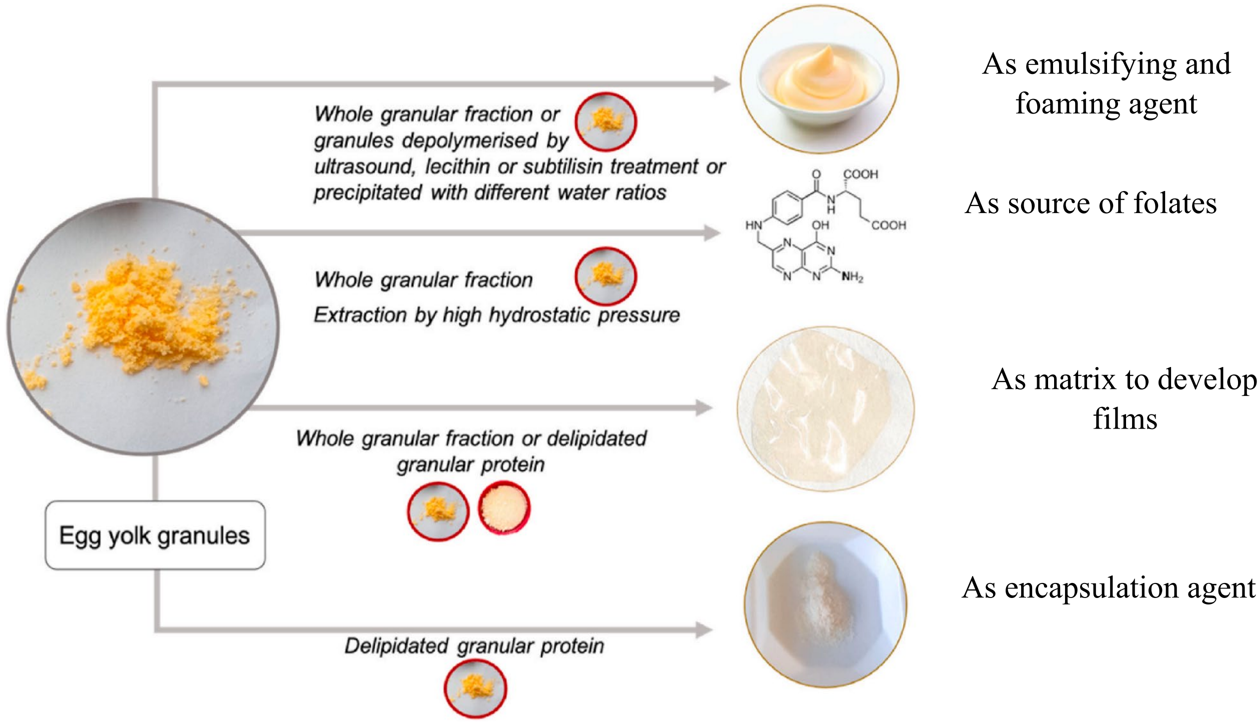


Fig. 4 Potential uses of the dried egg yolk lipid granules (Source: Marcet et al., 2022)

pressure, and waist to hip ratio in patients with metabolic syndromes (Skórkowska-Telichowska et al., 2016). Neutral lipids can also be modified using enzymes to produce diglycerides or monoglycerides and in food products with less energy. Since egg yolk lipid contains a high amount of lutein and zeaxanthin with high bioavailability, they can be used as the functional agent that can prevent age-related macular degeneration in humans (Walchuk et al., 2022) and coloring agents in the food industry to provide unique yellow color (Xiao et al., 2020; Zaheer, 2017).

As pharmaceutical and nutraceutical agents

The hydrolysis products of phospholipids (lysophospholipids) can be used as nutraceuticals and pharmaceutical agents to improve fertility, maintain pregnancy, cell proliferation, and cell growth, apoptosis, or cure/prevent various diseases, including cancers (Bieberich, 2012). Liposomes prepared with phospholipids use as a drug carriers for drug delivery systems because they can protect drugs from digestion in the stomach, improve bioavailability in the guts, reduce drug toxicity, and deliver drugs to the targeted sites (Chaudhary et al., 2012; Lee, 2020). These yolk lipid components are also useful for gene delivery to cells in culture and gene therapy in preclinical and clinical trials because cationic liposomes can easily form stable complexes with DNA. The use of liposomes facilitates the oral administration of drugs as an alternative to injection (Zhang et al., 2005). Therefore, liposomes have a high potential in advanced medical and pharmaceutical fields and other fields such as the food industry, particularly functional foods.

Since Plasmalogens and PI involve in cellular signaling, they have the potentials to use as a biomarker in detecting brain function, disorders in the nervous system, and cognitive behaviors (Farooqui et al., 2000; Messias et al., 2018; Raghu et al., 2019; Sabogal-Guáqueta et al., 2018). Choline in blood, plasma, and serum is an emerging biomarker to detect acute coronary syndromes in humans. In addition, the elevated levels of choline help predict cardiac disorders, and therefore, they can consider good agents in future medicine. Choline is popular as a “nootropic” or "smart drug" because of its role in the formation of acetylcholine (Duric et al., 2012).

Yolk granules consider a good source of folates, a vitamin mostly related to gestational problems in mothers and cardiovascular disorders. The folates are converted to 5-methyltetrahydrofolate (5-MTHF), a highly bioavailable form of folate, under high pressure (House et al., 2002; Marcet et al., 2022). Combining nanoparticles of HDL and curcumin produced an encapsulation agent. They can be coated with either chitosan or steric acid-conjugated chitosan to ensure the slower release of curcumin in the gastrointestinal tract. Therefore, in combination with other organic compounds, yolk lipids can be used as a potential encapsulation agent that can bypass the stomach and slowly release the compounds (Marcet et al., 2022; Zhou et al., 2018). The HDL in egg yolk has antioxidant, anti-inflammatory, and anti-arteriosclerosis activities, which are closely associated with the composition of PC and phosphatidylinositol. Since yolk HDL reduces oxidative stress, it can be an alternative compound against CVD. In addition, apolipoproteins and antioxidant enzymes found in HDL can use as anti-inflammatory agents (Xiao et al., 2020). Carotenoids can use as a functional food component to prevent many non-communicable diseases, including Alzheimer's, CVD, and breast cancers in women (Zaheer, 2017).

Other potential uses

The neutral lipids (triglycerides) extracted from the egg yolk can be used in shampoo, skin creams, lotions, soaps, cooking oil, and various food processing. Phospholipids separated from egg yolk can be used as a nutritional supplement, moisturizing agent in cosmeceutical products, or the starting material to produce functional lipids (Huang and Ahn, 2019; Li et al., 2021). Liquid egg yolk is better starting material than the dried ones for producing value-added functional lipids since the quality of lipids separated is better, and the functionality of separated proteins can maintain. Using the liquid egg yolk as the starting material enables the sequential separation of value-added components, and additional energy is not needed to dry the egg yolk (Marcet et al., 2022). However, transportation and storage of the liquid egg yolk are more difficult and costly than the dried ones if the separation process cannot do in the egg-breaking locations.

Summary and Future Aspects of Yolk Lipids

The separation of yolk proteins, phospholipids, and neutral lipids has been studied for several decades, but few scale-up methods are available. Even though egg yolk contains very high levels of high potential value-added lipids, the use of egg yolk lipids is limited at industrial levels. As evidenced by the successful examples of soybean and milk industries, there are very high possibilities of using egg lipids as new ingredients with excellent functionalities. Egg yolk is among the best sources of PC, cholesterol, and SM, and separating them from the composite phospholipids would provide an excellent opportunity to produce bioactive lipids. Although bioactive lipids have been studied for over 40 years, using the separated lipids in emerging fields such as nutraceutical, cosmeceutical, and pharmaceutical areas is highly limited. With a global growth rate of 7.2% for natural health and functional food products, these bioactive lipids hold excellent opportunities in the agri-food, cosmetic, and health-related industries in the future.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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