

Lipid Profiles of Five Essential Phospholipid Preparations for the Treatment of Nonalcoholic Fatty Liver Disease: A Comparative Study

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Abstract Nonalcoholic fatty liver disease (NAFLD) is associated with an imbalance in fatty acid composition and can progress from simple steatosis to steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. Essential phospholipids (EPL), which contain high levels of 1,2-dilinoleoylphosphatidylcholine, can be used to treat NAFLD. Polyenylphosphatidylcholine (PPC) preparations are external, commercially available EPL products. The lipid composition of five commercially available PPC preparations, including Essentiale Forte, Fortifikat, Hepatoprotect Regenerator, Fortifikat Forte, and Esentin Forte were compared, the outcome of which may impact physician choice in the treatment of NAFLD. Following lipid extraction, a comparative analysis of key lipid content was performed using a QTRAP6500+ triple quadrupole ion trap hybrid mass spectrometer (Sciex) in nanoelectrospray ionization mode. The glycerophospholipid composition of each PPC was determined, including levels of phosphatidylcholine (PtdCho), and phosphatidylethanolamine (PtdEtn) species, as well as PtdCho:PtdEtn ratio. Of the five preparations

analyzed, Essentiale Forte contained the highest PtdCho levels (61.9 mol%) and lowest PtdEtn levels (4.9 mol%). PtdCho 36:4 levels, a polyunsaturated species of PtdCho, were highest in Esentin Forte (39.3 mol%) and Essentiale Forte (38.3 mol%) compared with other PPCs (28.7–35.8 mol%). Levels of lysophosphatidylcholine, phosphatidylinositol, phosphatidic acid, and phosphatidylglycerol were low in all five preparations. Lipid composition was consistent between the preparations. The high PtdCho:PtdEtn ratio composition of Essentiale Forte compared with the other PPC analyzed, as well as the presence of polyunsaturated fatty acids, suggest it could be the most clinically beneficial commercially available hepatoprotective product in the treatment of NAFLD.

Keywords Essential phospholipids · NAFLD · Phosphatidylcholine · Phosphatidylethanolamine · Polyenylphosphatidylcholine

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Supporting information Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Abbreviations

DLPC	dilinoleoylphosphatidylcholine
EPL	Essential phospholipids
lysoPtdCho	lysophosphatidylcholine
NAFLD	non-alcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
PEMT	PtdEtn N-methyltransferase
PPC	polyenylphosphatidylcholine
PtdCho	phosphatidylcholine
PtdEtn	phosphatidylethanolamine
PtdGro	phosphatidylglycerol
PtdIns	phosphatidylinositol
PtdOH	phosphatidic acid

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PtdSer	phosphatidylserine
SD	standard deviation
TAG	triacylglycerol

Introduction

Phospholipids are essential structural components of biological membranes. The basic structure of a glycerophospholipid comprises two hydrophobic fatty acids and a hydrophilic phosphate-containing head group (Escriba et al., 2008). In combination with cholesterol, sphingolipids, and proteins, phospholipids form layers that make up mammalian cellular and sub-cellular membranes, as well as lipoprotein and lipid droplet surface monolayers, serous membranes of the lung and heart, and synovial membranes of the joints (Escriba et al., 2008; Gundermann et al., 2011). As well as maintaining cell integrity, phospholipids play a vital role in membrane fluidity and permeability to facilitate the transport of molecules across membranes. Phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) are the most abundant phospholipids found in biological membranes (Li et al., 2006). Additionally, phosphatidylinositol (PtdIns), phosphatidic acid (PtdOH), phosphatidylserine (PtdSer), and phosphatidylglycerol (PtdGro) are also commonly present within lipid membranes (Gundermann et al., 2016; Li et al., 2006; Yang and Chen, 2018). Furthermore, lysoPtdCho is a major plasma phospholipid and a precursor to lysophosphatidic acid, and can be found both extracellular and intracellular as a signaling mediator or membrane component, respectively (Yang and Chen, 2018). The integrity and fluidity of a biological membrane is highly dependent on the phospholipid composition, as well as the sterol and sphingolipid arrangement. In particular, the ratio of PtdCho to PtdEtn has been shown to be a key regulator of membrane integrity in animal models (Gundermann et al., 2016; Li et al., 2006). In mammalian cells, the majority of PtdCho is produced from dietary choline and is enriched in the outer leaflet of the membrane. A smaller amount of PtdCho is synthesized in the liver, by converting PtdEtn, found in the inner leaflet, *via* the PtdEtn N-methyltransferase (PEMT) pathway (Li et al., 2006).

Altered phospholipid compositions and decreased membrane fluidity can result in damage to the liver, leading to nonalcoholic fatty liver disease (NAFLD). NAFLD has a global prevalence of 25% in general adult populations (Ahmed, 2015; World Gastroenterology Organisation [WGO], 2014; Younossi et al., 2016) and is becoming an increasingly important health concern due to a strong association with obesity and insulin resistance (WGO, 2014). The prevalence of NAFLD can be as high as 90% in obese populations (Ahmed, 2015; WGO, 2014; Younossi

et al., 2016). In some patients, NAFLD can progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma. Therefore, therapeutic strategies that prevent progression or can reverse damage by facilitating hepatic regeneration are warranted (Ahmed, 2015; Arendt et al., 2013).

Disruptions in phospholipid metabolism and fatty acid composition, including a decrease in the hepatic PtdCho:PtdEtn ratio (Li et al., 2006), have been observed in the livers of patients with NAFLD and have even been linked with its development (Arendt et al., 2013; Arendt et al., 2015; Li et al., 2006). Phospholipid disruptions are more pronounced in patients who have progressed to NASH (Ma et al., 2016), while hepatic tissue concentrations of lysoPtdCho have been seen to be greater in the liver of NASH patients than in healthy controls (Han et al., 2008; Li et al., 2006). A significant proportion of patients with NAFLD and NASH have a lower hepatic PtdCho:PtdEtn ratio compared to healthy subjects, highlighting the importance of maintaining cellular PtdCho:PtdEtn ratios at the most appropriate levels to ensure liver health (Li et al., 2006). Preparations of essential phospholipids (EPL) can be used to treat these disruptions. In the liver, treatment with EPL can lead to the repair of damaged membranes, increased membrane fluidity and metabolic functioning, normalization of lipoprotein metabolism, and cytoprotective and regenerative effects (Gundermann et al., 2016). The term EPL refers to highly purified poly-enylphosphatidylcholine (PPC) extracts from soybeans (Gundermann et al., 2016). EPL supply an organism with standardized contents of 72–96% 1,2-diacyl-sn-glycero-3-phosphocholine molecules, which are nontoxic and contain a large amount of polyunsaturated fatty acids, including linoleic acid (Gundermann et al., 2016). The main active ingredient in EPL is 1,2-dilinoleoylphosphatidylcholine (DLPC), which represents up to 52% of the administered PtdCho molecules (Gundermann et al., 2011; Lieber et al., 1994). This high proportion of DLPC within the soy bean extract distinguishes EPL from other typical phospholipids, including triple, raw, and egg lecithin, as well as phospholipids that can be obtained through diet or those that are synthesized within the body (Gundermann et al., 2011). Serum levels of DLPC are low, at approximately 1.3% of the total serum PtdCho (Oette et al., 1995). Administration of external EPL preparations alters the DLPC percentage within the membranes of certain cells, including hepatocytes, blood corpuscles, and pancreatic tissue (Gundermann et al., 2011). Increased amounts of membrane DLPC increases membrane fluidity and affects key functions that are dependent on membrane function (Gundermann et al., 2011).

Limited information is currently available on the precise lipid composition of available EPL preparations,

representing a challenge for clinical decision-making when treating patients with NAFLD (Gundermann et al., 2011). The objective of this study was to conduct a comparative analysis of the lipid profiles of five PPC that are commercially available. Mass spectrometry was performed to establish the relative ratios of each lipid species within the separate PPC preparations and to determine the key differences and identify superiority between PPC preparations. Considerable differences were found between the lipid compositions of the preparations analyzed in terms of major PtdCho and PtdEtn species. A better understanding of the precise lipid compositions of each PPC could aid clinical decision-making in the treatment of NAFLD with the PPC examined.

Materials and Methods

Lipid Extraction

Capsules containing the five PPC preparations were analyzed upon opening. A biphasic lipid extraction (Bligh and Dyer, 1959) was performed in the presence of at least one standard lipid per class, using an acidic chloroform/methanol/water solution (Özbalci et al., 2013).

The five PPC preparations investigated in this study included: Esentin Forte (Sunwave Pharma SRL, Romania [Preparation A]) (Sunwave Pharma, 2019); Essentiale® Forte 300 mg (Sanofi Romania SRL [Preparation B]), which is available in 20 countries worldwide (Sanofi, 2019); Fortifikat 500 mg (Terapia, Romania [Preparation C]) (Terapia, 2019a); Fortifikat Forte 750 mg (Terapia, Romania [Preparation D]) (Terapia, 2019b); and Hepatoprotect Regenerator 712.5 mg (S.C. Biofarm S.A. Romania [Preparation E]) (BIOFARM, 2018).

Lipid Analysis

Lipid extracts were resuspended in 60 µL methanol and samples were analyzed on a QTRAP 6500+ mass spectrometer (Sciex, Canada) with chip-based (HD-D ESI Chip, Advion Biosciences, USA) nanoelectrospray infusion and ionization via a Triversa Nanomate (Advion Biosciences, Ithaca, USA) as previously described (Özbalci et al., 2013). Prior to measurement, resuspended lipid extracts were diluted 1:10 with methanol and ammonium acetate from a 50 mM stock to a solution containing 10 mM ammonium acetate in methanol in 96-well plates (Eppendorf twin tec 96, colorless, Sigma, Z651400-25A). Lipid classes were analyzed in positive ion mode applying either specific precursor ion (PtdCho, lysoPtdCho) or neutral loss (PtdEtn, PtdIns, PtdGro, PtdOH) scanning. Data evaluation was

done using LipidView (Sciex) and an in-house-developed software (ShinyLipids).

Mass spectrometry was carried out to determine the relative ratios of each lipid species within the separate PPC preparations.

Statistical Analysis

A one-way analysis of variance (ANOVA) with factor PPC was performed (six independent preparations for each of the PPCs were executed, except for Preparation E, where eight independent preparations were analyzed) for each of the three endpoints.

If the global ANOVA test was significant, a one-tailed lower Dunnett's test was performed to determine a statistically significant ($p < 0.05$) difference between the PtdCho: PtdEtn ratio and the content of 36:4 lipids between Essentiale Forte and the comparative PPC.

Results

Lipid Composition

The mean lipid composition for each of the five PPC was determined by a minimum of six repetitions, and are presented in Fig. 1 and Table 1.

Mean concentration of PtdCho was highest in Preparation B (61.9 mol%), followed by Preparation E (32.8 mol%), Preparation D (16.3 mol%), Preparation C (12.8 mol%), and Preparation A (9.6 mol%), respectively (Table 2). The differences in mean concentration of PtdCho were found to be significant ($p < 0.0001$) between all PPC, as measured by one-way ANOVA. When evaluated directly, Preparation B was found to have a significantly higher ($p < 0.0001$) mean concentration of PtdCho, compared with all other PPC preparations (Fig. 2). Conversely, Preparation B and Preparation E had the lowest concentrations of PtdEtn at 4.9 mol% and 4.7 mol%, respectively. In contrast, higher concentrations of PtdEtn were found in Preparation C (15.5 mol%), Preparation D (14.4 mol%), and Preparation A (11.8 mol%). PtdIns concentrations for Preparation D, Preparation C, and Preparation A were 8.3, 6.5, and 4.3 mol%, respectively, which is substantially more than that found in Preparation B and Preparation E (0.5 mol% for both). PtdOH was also present in higher amounts in Preparation C, Preparation D, and Preparation A at 7.4, 6.2, and 2.7 mol%, respectively, compared with Preparation B (0.4 mol%) and Preparation E (1.0 mol%). Preparation E had the highest mean concentration of lysoPtdCho (20.5 mol%) compared with 16.2 mol% for Preparation B,

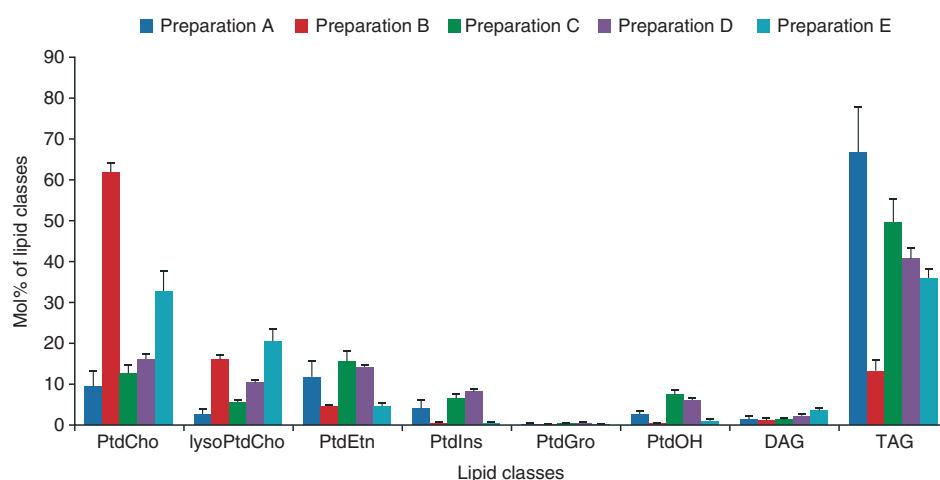


Fig. 1 Lipid profiles of hepatoprotective phospholipid preparations A, B, C, D, and E. DAG, diacylglycerol; lysoPtdCho, lysophosphatidylcholine; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdIns, phosphatidylinositol; PtdOH, phosphatidic acid; SD, standard deviation; TAG, triacylglycerol.

Table 1 Lipid profiles of hepatoprotective phospholipid preparations A, B, C, D, and E

Preparation	Lipid, mean mol/% (SD)							
	PtdCho	lysoPtdCho	PtdEtn	PtdIns	PtdGro	PtdOH	DAG	TAG
Preparation A	9.61 (3.78)	2.84 (1.08)	11.82 (3.78)	4.27 (1.82)	0.43 (0.15)	2.71 (1.02)	1.43 (0.69)	66.65 (11.00)
Preparation B	61.94 (2.23)	16.18 (1.33)	4.85 (0.48)	0.47 (0.22)	0.31 (0.04)	0.40 (0.06)	1.31 (0.38)	13.50 (2.40)
Preparation C	12.78 (1.92)	5.59 (0.59)	15.45 (2.50)	6.52 (0.93)	0.44 (0.10)	7.44 (1.08)	1.46 (0.27)	49.83 (5.52)
Preparation D	16.25 (1.01)	10.62 (0.49)	14.37 (0.49)	8.34 (0.77)	0.57 (0.10)	6.26 (0.69)	2.43 (0.59)	40.74 (2.60)
Preparation E	32.79 (5.13)	20.54 (3.38)	4.73 (0.53)	0.48 (0.12)	0.33 (0.05)	0.95 (0.47)	3.69 (0.74)	35.95 (2.23)

DAG, diacylglycerol; lysoPtdCho, lysophosphatidylcholine; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdIns, phosphatidylinositol; PtdOH, phosphatidic acid; SD, standard deviation; TAG, triacylglycerol.

Table 2 Comparison of preparations A, C, D, and E versus comparator polyenylphosphatidylcholine preparation B on mean concentration of PtdCho, PtdCho:PtdEtn ratio and mean percentage of PtdCho species 36:4, using a one-tailed lower Dunnett's test

Comparison	Difference between group means	Standard error	Statistical value	<i>p</i> value
PtdCho (mol %)				
Preparation B versus Preparation A	52.34	1.9366	27.0264	<i>p</i> < 0.0001
Preparation B versus Preparation C	49.162	1.9366	25.38523	<i>p</i> < 0.0001
Preparation B versus Preparation D	45.695	1.9366	23.59518	<i>p</i> < 0.0001
Preparation B versus Preparation E	29.157	1.8115	16.0949	<i>p</i> < 0.0001
PtdCho:PtdEtn				
Preparation B versus Preparation A	12.048	0.2772	43.4638	<i>p</i> < 0.0001
Preparation B versus Preparation C	12.007	0.2772	43.31349	<i>p</i> < 0.0001
Preparation B versus Preparation D	11.717	0.2772	42.26733	<i>p</i> < 0.0001
Preparation B versus Preparation E	5.941	0.2593	22.911	<i>p</i> < 0.0001
PtdCho species 36:4 (%)				
Preparation B versus Preparation A	−1.005	0.3251	−3.09174	<i>p</i> = 1.0000
Preparation B versus Preparation C	9.568	0.3251	29.43561	<i>p</i> < 0.0001
Preparation B versus Preparation D	3.958	0.3251	12.17725	<i>p</i> < 0.0001
Preparation B versus Preparation E	2.483	0.3041	8.165728	<i>p</i> < 0.0001

PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine.

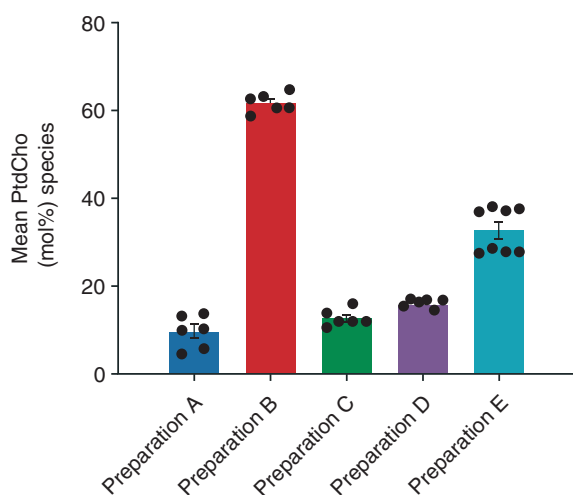


Fig. 2 Mean phosphatidylcholine (mol%) species in each hepatoprotective phospholipid preparation A, B, C, D, and E. PtdCho, phosphatidylcholine; Error bars represent standard error of the mean

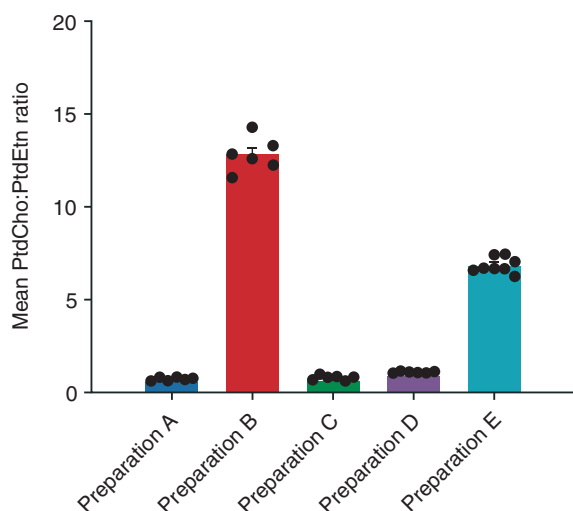


Fig. 3 Mean PtdCho:PtdEtn ratio in each hepatoprotective phospholipid preparation A, B, C, D, and E. PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine. Error bars represent standard error of the mean.

10.6 mol% for Preparation D, 5.6 mol% for Preparation C, and 2.8 mol% for Preparation A (Table 1).

PtdCho:PtdEtn

The PtdCho:PtdEtn \pm SD ratio was highest in Preparation B (12.9 ± 2.2) compared to the other PPC investigated (6.9 ± 0.5 , 1.1 ± 0.1 , 0.8 ± 0.2 , and 0.8 ± 0.1 , in Preparation E, Preparation D, Preparation C, and Preparation A, respectively) (Fig. 3). When analyzed together by one-way ANOVA, a significant difference ($p < 0.0001$) in the PtdCho:PtdEtn ratio was seen between

the PPC. When evaluated directly, Preparation B had a significantly higher PtdCho:PtdEtn ratio compared with each of the other PPC investigated ($p < 0.0001$) (Table 2).

Fatty Acid Composition

The quantitative distribution of PtdCho species was analyzed in each preparation to obtain further information regarding fatty acid composition (Table 3). PtdCho distribution was consistent across all five preparations (Fig. S1). PtdCho 36:4 was the most common species detected in all preparations and present at a range of 28.7–39.3%, followed by PtdCho 34:2 (22.8–26.9) and PtdCho 36:3 (13.1–17.4%) (Fig. 4). Distribution of PtdCho species 36:4 was significantly different between PPCs ($p < 0.0001$), as measured by one-way ANOVA. In addition, a significantly higher ($p < 0.0001$) distribution of PtdCho species 36:4 was seen with Preparation B when compared directly with each of the other PPC preparations, except Preparation A, where the value was higher ($p = 1.0000$) (Table 2).

The distribution of lysoPtdCho species across the five preparations was also similar (Fig. S2). The most common lysoPtdCho species detected were 18:2 (56.4–65.0%), 18:1 (11.9–18.4%), and 16:0 (12.0–16.3%).

As seen with PtdCho and lysoPtdCho, the distribution of PtdEtn species across the five preparations was similar (Fig. S3). The most common PtdEtn species detected were 34:2 (33.6–38.8%), 36:4 (22.1–31.7%), and 36:3 (9.6–13.5%).

PtdIns species were detected in small quantities across the five PPC preparations (Fig. S4). The most common PtdIns species detected were 34:2, 36:2, and 36:4. Preparation B contained less PtdIns 34:2 (19.8%) compared with the other PPC preparations (range 29.5–60.8%). However, as the absolute levels of PtdIns were low for all PPC investigated; this difference was not significant or considered clinically meaningful.

PtdOH and PtdGro were also detected in small quantities. The most common species detected were PtdOH 36:4, 34:2, and 36:3 (Fig. S5) and PtdGro 34:2, 36:4, and 34:1 (Fig. S6).

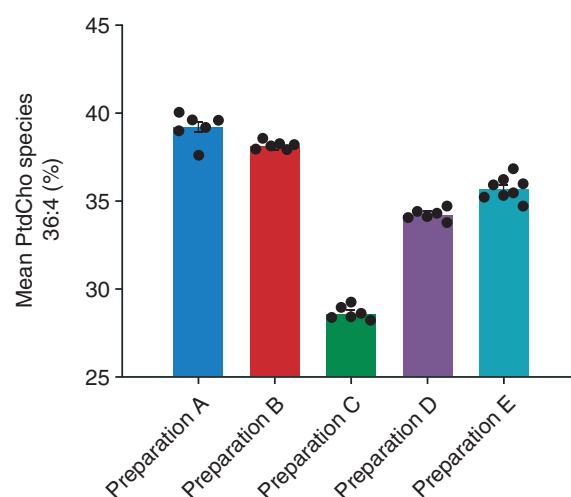
Discussion

The lipid profiles of five commercially available PPC preparations were analyzed and comparatively assessed. Interestingly, there were considerable differences between the lipid compositions of the preparations investigated, in terms of the levels of major PtdCho and PtdEtn species, whereas the fatty acid compositions of these preparations were relatively similar.

Table 3 Quantitative distribution of PtdCho species in hepatoprotective phospholipid preparations

Preparation	PtdCho species, % (SD)									
	32:0	34:3	34:2	34:1	36:6	36:5	36:4	36:3	36:2	36:1
Preparation A	0.23 (0.04)	2.61 (0.10)	23.53 (1.35)	3.17 (0.11)	0.57 (0.02)	8.07 (0.17)	39.25 (0.87)	13.13 (0.26)	6.38 (0.20)	0.69 (0.05)
Preparation B	0.35 (0.01)	2.77 (0.03)	22.75 (0.36)	3.37 (0.06)	0.65 (0.02)	8.41 (0.10)	38.25 (0.24)	13.41 (0.14)	6.51 (0.06)	0.72 (0.02)
Preparation C	0.86 (0.07)	2.35 (0.10)	26.89 (0.56)	7.30 (0.12)	0.23 (0.01)	4.27 (0.05)	28.68 (0.41)	17.40 (0.34)	7.87 (0.21)	1.20 (0.05)
Preparation D	0.98 (0.03)	2.45 (0.04)	26.59 (0.35)	5.13 (0.10)	0.28 (0.01)	5.37 (0.06)	34.29 (0.32)	14.82 (0.09)	6.58 (0.10)	0.86 (0.05)
Preparation E	0.87 (0.06)	2.36 (0.06)	26.06 (0.26)	4.88 (0.20)	0.28 (0.01)	5.46 (0.08)	35.76 (0.67)	15.47 (0.17)	5.84 (0.08)	0.72 (0.03)

PtdCho, phosphatidylcholine; SD, standard deviation.

**Fig. 4** Mean phosphatidylcholine species 36:4 (%) in each hepatoprotective phospholipid preparation A, B, C, D, and E. PtdCho, phosphatidylcholine. Error bars represent standard error of the mean.

Previous findings have indicated that PPC preparations with high molecular levels of PtdCho (containing two esterified fatty acids) and a high PtdCho:PtdEtn ratio are superior in the clinical management of NAFLD compared with PPC composed of low levels of PtdCho and a low PtdCho:PtdEtn ratio (Gundermann et al., 2016; Li et al., 2006; Ma et al., 2016). Preparation B was found to contain significantly ($p < 0.0001$) higher levels of PtdCho when compared directly with each of the PPC analyzed, in addition to having one of the lowest levels of PtdEtn. Due to the high molecular levels of PtdCho and the low levels of PtdEtn observed, Preparation B had a significantly higher PtdCho:PtdEtn ratio compared with the other PPC preparations analyzed. PtdCho:PtdEtn ratio is negatively correlated with changes in the liver that are specific to NAFLD (Ma et al., 2016). A low PtdCho:PtdEtn ratio is associated with disrupted membrane integrity, hepatocyte damage, and inflammation (Arendt et al., 2015), which are observed in the manifestation of NAFLD and other types of liver disease (Arendt et al., 2013). Thus, patients with NAFLD and NASH have been seen to have a lower hepatic PtdCho:PtdEtn ratio compared to healthy subjects (Li et al., 2006). The higher PtdCho:PtdEtn ratio observed in Preparation B, coupled with the knowledge that a higher PtdCho:PtdEtn ratio could provide substantial clinical benefit in the early treatment of hepatic disorders, and may serve as key information when selecting a PPC preparation in clinical practice. Furthermore, a review investigating the clinical effects of EPL, and in particular Preparation B, on patients with NAFLD found EPL treatment resulted in marked improvements in their pathophysiology (Gundermann et al., 2016).

In addition to PtdCho and PtdEtn, the levels of PtdIns, PtdOH, and PtdGro present within the PPC preparations were analyzed. These phospholipids were present in small

quantities across each of the preparations. PtdIns is considered to be hepatoprotective (Shirouchi et al., 2008) while high levels of PtdOH are reported to increase the rate of glucose production in the liver (Agarwal and Sankella, 2014). However, considering the overall low levels of these phospholipids, the differences between formulations are not likely to be clinically relevant.

The levels of lysoPtdCho were found to be highest in Preparation E and Preparation B. In patients with NAFLD and NASH, serum lysoPtdCho levels have been shown to be lower than those of healthy subjects, suggesting higher levels of lysoPtdCho are favorable in maintaining a healthy liver (Tiwari-Heckler et al., 2018). Thus, compounds with higher lysoPtdCho levels may provide significant clinical benefit in this patient population.

The fatty acid composition across the five products was similar, with polyunsaturated fatty acid being the most common species present. A higher concentration of polyunsaturated fatty acids is favorable as polyunsaturated fatty acids have been shown to suppress inflammatory reactions and protect against various types of experimental liver damage in animal models and in isolated hepatocytes (Karaman et al., 2003). Interestingly, Preparation A and Preparation B contained a significantly higher mean percentage of PtdCho species 36:4 compared with all other preparations (39.3% vs 38.3%, respectively). Studies have shown that fatty acids with higher levels of unsaturation are considered to have the potential to cause a more pronounced therapeutic effect compared with lower levels (Karaman et al., 2003). In addition, high levels of polyunsaturated PtdCho, similar to those observed in Preparations A and B, have been found to have a hepatoprotective role through increased regeneration of the liver and increased transport through hepatocyte membranes (Holecek et al., 1992; Karaman et al., 2003). Human and animal studies indicate that an increase in unsaturation levels in the lipid membrane is associated with a higher insulin sensitivity (Guelzim et al., 2014; Perona, 2017; Vessby et al., 2001). Furthermore, imbalances in the levels of polyunsaturated fatty acids are associated with a number of diseases, including dyslipidemia, hypertension, type 2 diabetes, inflammation, and abdominal obesity (Abbott et al., 2012; Ramsden et al., 2010; Vessby et al., 2001).

Overall, Preparation B contained the lowest amount of PtdEtn and one of the highest levels of polyunsaturated fatty acids. In addition, PtdCho 36:4 in particular, was present in Preparation B in abundance. Taken together, these findings provide evidence that this PPC, which also contains a high PtdCho:PtdEtn ratio, may have substantial clinical benefit in the treatment of NAFLD.

A limitation with this study is that no inter-batch analysis was carried out; therefore, it can only be assumed that each

sample provided consistent data with its counterpart when analyzed individually.

As reviewed by Gundermann et al. (2011), an obvious correlation exists between the effect on hepatocellular membrane function and the observed beneficial clinical impact of polyunsaturated phospholipids. The findings of the present study demonstrate that the five PPC preparations analyzed contained the optimal content of PtdCho and PtdEtn, as well as high levels of unsaturated free fatty acids. In particular, the high PtdCho:PtdEtn ratio found in the lipid composition of Preparation B, and the associated effect on increased membrane integrity and fluidity this has, could provide further evidence for its proposed clinical efficacy in the management of NAFLD.

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Author Contributions C.L., B.B., B.P. and G.F. were involved in designing and conducting the study. B.N. performed the statistical analysis. All authors made substantial contributions to the analysis and interpretation of the work, and supported in all aspects of the development of the manuscript, including providing final approval of the version to be published.

Conflict of Interest C.L., B.B. and G.F. have nothing relevant to disclose. B.N. and B.P. are employees of Sanofi-Aventis Deutschland GmbH and have the opportunity to hold shares in the company.

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