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Differential plasma postprandial lipidomic responses to krill oil and fish oil supplementations in women: A randomized crossover study

Hyunsin H. Sung Ph.D. [a], Andrew J. Sinclair Ph.D. [b,c], Kevin Huynh B.Sc. [d], Alexander T. Smith Ph.D. [d], Natalie A. Mellett B.Sc. [d], Peter J. Meikle Ph.D. [d,1], Xiao Q. Su Ph.D. [a,1,*]

[a] *Institute for Health and Sport, Victoria University, Melbourne, Victoria, Australia*
[b] *Faculty of Health, Deakin University, Geelong, Victoria, Australia*
[c] *Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, Victoria, Australia*
[d] *Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia*

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ABSTRACT

Objectives: There is no convincing evidence that krill oil (KO) consumption results in a higher incorporation of long chain ω-3 polyunsaturated fatty acids into blood lipid fractions than fish oil (FO). This study examined the postprandial plasma lipidomic responses to KO supplementation compared with FO supplementation in healthy women.

Methods: Ten women (aged 18–45 y) consumed a high-fat (15 g of olive oil) breakfast, supplemented with 5 g of KO or FO in a randomized crossover study with a minimum 7-d washout period between the supplementations. Plasma samples collected at the fasting state and at 3 and 5 h postprandially were analyzed using liquid chromatography electrospray ionization–tandem mass spectrometry.

Results: After the supplementations, 5 out of 34 lipid classes or subclasses had significantly greater concentrations from KO compared with FO. There were 27 molecular species including 5 ether-phospholipid species, out of a total of 701, which had significant differences between supplementations in the postprandial period. Eicosapentaenoic acid and docosahexaenoic acid from KO were preferentially partitioned toward phospholipid molecular species, whereas eicosapentaenoic acid and docosahexaenoic acid from FO were preferentially partitioned toward neutral lipids.

Introduction

Marine oils containing long chain ω-3 fatty acids are common supplements available over the counter. The estimated total worldwide sales of these supplements in 2016 amounted to $33 billion [1]. There are several major sources of long chain ω-3 fatty acids, including fish oil (FO), krill oil (KO), and algal oils. KO differs from FO in the major lipid classes [2]. Indeed, in FO, triacylglycerols are the predominant lipid class (>98%), whereas in KO the lipid classes include phospholipids (mostly phosphatidylcholine [PC]), triacylglycerols, and free fatty acids (FFA). Furthermore, ω-3 fatty acids are found in all three of these lipid classes in KO [3].

There have been many studies on the incorporation of ω-3 fatty acids into blood and tissue lipids from marine oils, with a number of them, but not all, concluding that there is greater incorporation of ω-3 fatty acids into blood lipids from KO compared with FO [4–11]. In fact, advertising for KO often mentions a greater bioavailability than FO, without a specific indication of whether this refers to ω-3 fatty acids or other components in the oils, such as carotenoids [12].

Four postprandial studies have investigated the incorporation of long chain ω-3 fatty acids into plasma from KO compared with FO [6–9]. Two of these studies compared KO with reesterified triacylglycerols (TG) from FO or ethyl-ester concentrates of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from FO [6,7]. The other two studies compared KO with FO [9] and krill meal [8]. The findings from these studies have not been consistent. The postprandial studies by Schuchardt et al. [7] and Yurko-Mauro et al. [6] reported that the incorporation of EPA and DHA into plasma phospholipids from KO was not significantly greater than FO. These studies also suggested that substantial variability between

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* Corresponding author: Tel.: +61-3-99192318; Fax: +61-3-99192465.
E-mail address: Xiao.su@vu.edu.au (X.Q. Su).

[1] P.J.M. and X.Q.S. are co-senior authors.

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participants may have limited their capacity to detect significant differences between the study oils. Kohler et al. [8] reported a significantly greater incorporation of ω-3 fatty acids into the plasma phospholipids from KO than that of FO. Sung et al. [9] found that a lower dose of EPA from KO (70% of that provided from FO) resulted in a similar incorporation of EPA into plasma total lipid fatty acids. Moreover, out of three longer-term studies that compared KO with FO [6,10,11], only one [11] reported a significantly higher incorporation of EPA into plasma lipids from KO than that of FO.

All the studies referred to here used gas chromatography of the fatty acid methyl esters derived from the plasma lipid classes (TG, PL, total fatty acids) to compare KO with FO. Because KO and FO contain distinctly different lipid classes, a more effective approach to compare the incorporation of the two oils is to examine a wider plasma lipidome, enabling the detection and identification of hundreds of different lipid species, including the classes of interest [13,14].

There have been no clinical studies comparing the postprandial incorporation of ω-3 fatty acids from KO and FO into different lipid molecular species; in other words, comparing the resulting postprandial plasma lipidomes. In recent years lipid analysis techniques have become increasingly available through the use of ultra-high-performance liquid chromatography, electrospray ionization–tandem mass spectrometry (UHPLC ESI-MS/MS) [15,16]. This methodology gives greater detail about temporal patterns of fatty acid uptake into molecular species of lipids and allows more detailed understanding of the relative significance of the biochemical pathways being followed by the fatty acids [17–19]. The aim of this study was to examine the postprandial incorporation of long chain ω-3 fatty acids into different molecular species of plasma lipids after the ingestion of KO compared with FO. We hypothesized that the plasma lipidome would be differently affected by intakes of KO compared with FO.

Material and methods

Study participants

Ten healthy premenopausal women aged 18 to 45 y within a body mass index range of 20 to 30 (kg/m^2) were recruited via e-mails to all Victoria University staff and students, and flyer advertisements in the Nutritional Therapy Teaching Clinic of the university, community centers, and local medical practices. Participants were screened for their suitability using a medical questionnaire and anthropometric measurements before enrolling into the study. The study was undertaken at Victoria University Metabolic Clinical Laboratory, St Albans campus. Participants were excluded if they were cigarette smokers; were pregnant or lactating; had heart, liver, kidney, or inflammatory bowel disease or diabetes; took medications interfering with lipid metabolism or lowering blood lipids; had an allergy to fish or seafood; or had consumed oily fish more than twice a week or supplements including ω-3 fatty acids in the past 4 wk before the study. The mean daily intake of long chain ω-3 fatty acids based on a 24-h recall (see later for details) was 106 ± 91 mg at baseline [9].

Study design

The details of study design have been fully described in our previous publication [9]. In brief, this study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Victoria University Human Research (HRE 14-040), and informed consent was obtained from all participants before the study. This trial was registered with the Australian and New Zealand Clinical Trial Registry (ACTRN 12615000620527).

The study was a randomized crossover intervention with a test breakfast containing different dietary oils consumed in a randomized order with a 7-d washout period between the test meals (Fig. 1) [9]. The sequence of the oil ingestion was determined by a randomization list generated by an independent person. During the study period, all participants were instructed to maintain their habitual diet and not to consume fish, seafood, or ω-3 fortified foods more than twice a week. On arrival, a fasting blood sample was collected and the participants then consumed a single test meal (breakfast), which consisted of 150 g of fresh mashed potato mixed with 15 g of olive oil, together with five capsules of KO or FO with 250 mL of water. All participants finished the test meal, including the capsules, within 15 min, and they were only allowed to drink water during the following 5 h. After the test meal consumption, postprandial blood samples (10 mL) were collected every hour for 5 h, thus making a total of six blood samples, including the sample at baseline, per participant per test meal. The lipidomic analysis was conducted on samples collected at time 0, 3 and 5 h from the KO and FO supplementations.

Dietary recording

Dietary information was collected using a 24-h recall where a nutritionist interviewed each participant before the study and each of the three supplementations. The data from 24-h recall were analyzed using FoodWorks Version 8 (Xyris Software, Brisbane, QLD, Australia). We note that disadvantages of the 24-h recall include the inability of a single day's intake to describe the typical diet. The success of the recall also depends on the memory, cooperation, and communication ability of the participant.

Each participant, in a random order, received each of the three supplementations (olive oil, olive oil/krill oil, or olive oil/fish oil). There was a 7-d washout between each supplementation.

Study oils

The KO and FO capsules (both products from Swisse Wellness Pty Ltd., Melbourne, VIC, Australia) were purchased from a local pharmacy. The fatty acid profile of these oils was analyzed using gas chromatography before the commencement of intervention and were typical of such oils, as reported in the literature, with KO containing 18% and FO containing 29% of long chain ω-3 fatty acids, respectively [9]. Five grams of KO and FO provided 907 mg and 1441 mg of EPA plus docosapentaenoic acid (DPA) plus DHA, respectively. These included 542 mg of EPA, 67 mg of DPA, and 298 mg of DHA in KO and 786 mg of EPA, 182 mg of DPA, and 473 mg of DHA in FO. It is recognized that the 5 g of KO and FO did not contain identical amounts of ω-3 polyunsaturated fatty acids (PUFA), but it was deemed that was preferable to use capsules rather than liquid oils to maintain the test oils in as blinded a fashion as possible and to minimize the participant withdrawal from the trial as a result of smell and taste considerations.

The lipid species of studied oils were analyzed using UHPLC ESI-MS/MS techniques. KO contained 28.2 nmol/μL oil as phospholipids (mainly lysophosphatidylcholine [LPC]) and PC compared with FO, which contained 0.03 nmol/μL oil as phospholipids (Table 1). The neutral lipid concentration was similar between the study oils (KO 34.3 nmol/μL compared with 36.6 nmol/μL for FO); however, the proportions of the neutral lipid species varied (KO was rich in diacylglycerol [DG], whereas FO was rich in TG). The olive oil, which acted as a carrier of the study oils to stimulate chylomicron formation [20,21], was rich in TG plus DG (71.3 nmol/μL) and almost devoid of phospholipids (0.03 nmol/μL).

Lipid extraction and lipidomics analysis

Briefly, plasma samples (10 μL) were extracted in a single-phase extraction with 20 volumes of $CHCl_3$:MeOH (2:1) and 10 μL of an internal standard mix (in $CHCl_3$:MeOH [1:1]) containing between 50 and 1000 pmol each of 23 non-physiological or stable isotope-labeled lipid standards, as previously described [13]. Lipidomic analysis was performed by UHPLC ESI-MS/MS, using an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer.

Identification of plasma lipid molecular species

A total of 701 lipid species were measured and analyzed using dynamic multiple reaction monitoring where data were collected for a retention time window specific to each lipid species. Results from the chromatographic data were analyzed using Mass Hunter Quant where relative lipid abundances were calculated by relating the area under the chromatogram for each lipid species to the corresponding internal standard. Correction factors were applied to adjust for different response factors, where these were known [22]. Although many of the measured classes had a single acyl chain, which was easily identified in a single experiment, many were esterified with two or more. DG were easily characterized through the neutral loss of a single acyl chain during collision-induced dissociation. Triacylglycerol species were determined through the neutral loss of a single acyl chain, allowing for partial structural characterization (in particular polyunsaturated species). However, glycerophospholipids do not provide acyl composition through collision-induced dissociation of the standard $[M+H]^+$ precursor ion aside from ethanolamine plasmalogens [23]. Additional characterization was conducted using either negative mode (phosphatidylethanolamine and phosphatidylinositol species) or positive mode in the presence of lithium and monitoring the lithiated adduct (PC and sphingomyelin species). Species that were chromatographically separated were labeled as such (i.e., PC [16:0_22:6] and PC [18:2_20:4], whereas species that were mixed isomers were given the standard phospholipid notation (i.e., PC [40:8] was a mixture of 20:4/20:4 and 18:2_22:6).

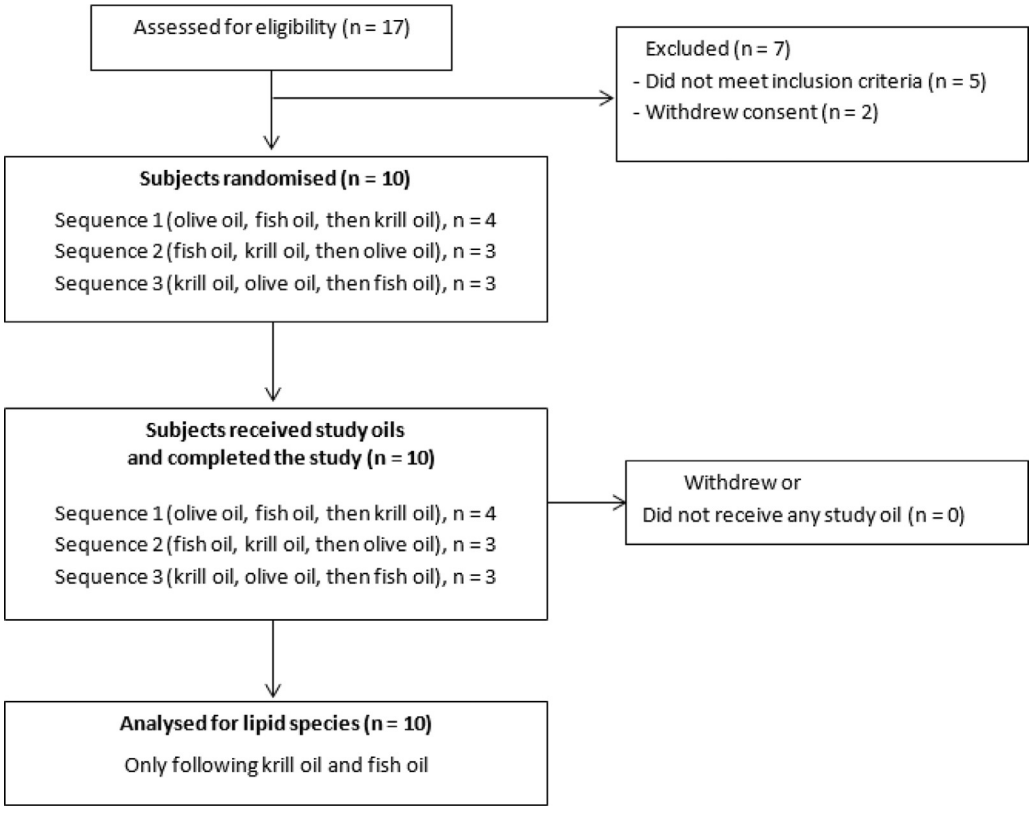

Fig. 1. Flow chart of study design.

ω-3 Lipid species analyses

Many lipid species had sufficiently resolved structures to identify those containing ω-3 components (20:5 EPA, 22:5 DPA, and 22:6 DHA). However, some measured "species" actually represented mixtures of molecular species. In these cases, we drew on our expert knowledge of the possible main molecular species that were part of the mixture to assign these lipids as ω-3 lipids or not (e.g., 40:8 can be 20:4/20:4 and/or 18:2/22:6).

Table 1
Concentrations of major lipid classes in krill oil and fish oil

	Concentration (pmol/μL)		
Lipid classes	Krill oil	Fish oil	Olive oil
Total DG	20 492	10 037	29 498
Total TG	10 797	23 935	41 777
Total COH	2451	591	0
Total CE	315	2027	43
Total ubiquinone	233	306	15
Sum of neutral lipids	34 288	36 896	71 333
Total PC	12 346	9	11
Total PC-O	1370	1	1
Total PC-P	50	1	1
Total LPC	13 196	8	8
Total LPC-O	446	0	0
Total LPC-P	2	0	0
Total PE	281	2	2
Total PE-O	68	0	0
Total PE-P	27	4	6
Total LPE	250	1	1
Total LPE-P	2	0	1
Total PG	24	3	1
Sum of polar lipids	28063	30	33

CE, cholesteryl ester; COH, cholesterol; DG, diacylglycerol; LPC, lysophosphatidylcholine; LPC-O, lysoalkylphosphatidylcholine; LPC-P, lysoalkenylphosphatidylcholine; LPE, lysophosphatidylethanolamine; LPE-P, lysoalkenylphosphatidylethanolamine; PC, phosphatidylcholine; PC-O, alkylphosphatidylcholine; PC-P, alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE-O, alkylphosphatidylethanolamine; PE-P, alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; TG, triacylglycerol.

Statistical analysis

Using an a priori power calculation based on a previous study [24], we determined that the minimum number of participants needed to detect a difference in EPA in the plasma was 6 (two-tailed *t* test at the 5% significance level for a power of 90% based on a change of 24.8 μg/mL with SD 14.3). Our 10-participant crossover study was thus adequately powered for this outcome.

For each lipid species and lipid class, we performed two types of analyses to statistically evaluate changes over time. On the one hand, we calculated the ratio (expressed as a percentage) between the area under the curve (AUC) delimited by lipid concentration across all available time points, and the AUC generated by assuming that the baseline lipid concentration remains constant (i.e., continued fasting). This "relative AUC" (relAUC) provides a single per-participant/oil estimate of the total 5-h lipid flux in plasma after supplementation. We then analyzed relAUCs between supplementations using paired *t* tests for each species and class.

On the other hand, we performed linear mixed modeling for each lipid class and species, wherein (log10) lipid concentration was modeled as being modulated by a combination of time, supplementation, and the interaction between the two, using participant as a random effect to account for participant-specific variation. Several tests were carried out on this model. To evaluate whether the overall model was capturing a non-negligible fraction of lipid concentration variation, it was evaluated against the corresponding null mixed model using analysis of variance. To identify which predictors in the model were contributing to the fit, we performed type III sums-of-squares analysis of variance on each term, thus providing *P* values for each of the predictors. Finally, to determine which categories of the predictors were contributing to the fit, we performed typical post hoc tests on the model coefficients. We selected contrasts so that the 3-h and 5-h time points were compared with the baseline (0 h) as a reference and so that FO was the reference against which KO was compared; consequently, the interaction coefficients were KO + 3 h and KO + 5 h.

The *P* value distributions across all lipids obtained for each analysis (relAUC, linear mixed model, predictors, post hoc tests, each for lipid species and each lipid class) were evaluated visually for proper test behavior, and all those reported here were deemed to perform sufficiently well (i.e., relatively flat distribution, with perhaps a peak toward zero) for use in further analysis (not shown). We then enforced a three-step strategy for multiple testing correction. Initially, model-level *P* values

were corrected for multiple testing using Storey's local false discovery rate (FDR) procedure [25] across all lipids, generating q values. For significant (q value ≤ 0.05) models only, we then corrected the predictor P values using the same procedure; others were discarded. Finally, only post hoc P values for coefficients corresponding to significant (q value ≤ 0.05) predictors were corrected using the same procedure; others were discarded. This strategy allowed us to maintain maximal power for calling lipids affected by dietary intake across the large number of lipids examined. Despite this, multiple testing corrections were very stringent for class-level P values. We thus report on nominal P values for class-level results hereafter.

We considered corrected species-level and nominal class-level P values of <0.05 as significant. Lipid species and classes with at least one significant result in either analysis (relAUC or mixed models) were examined further. Results for both of these analyses, including nominal P values and local FDR-corrected q values, are provided in Supplementary Tables 1 (for lipid classes) and 2 (for lipid species). Mean lipid species and class concentrations and mean percentage changes at 3-h and 5-h time points with respect to baseline, are also provided descriptively. We also used one-sided hypergeometric enrichment tests to evaluate whether the various lists of significantly affected lipid species were enriched in lipids containing ω-3 moieties (i.e., testing whether such moieties were found within the affected lipid lists than expected by chance); P values and fold enrichments are reported in text. All statistical analyses were carried out in R (x64, Version 3.5.0) (R Foundation for Statistical Computing, Vienna, Austria) [25].

Results

Participant characteristics and intake of dietary long chain ω-3 PUFA at baseline

Ten healthy women completed the crossover study with KO and FO supplementations. Baseline measurements (mean ± standard deviation) included participant's age (28.5 ± 9.3 y), systolic blood pressure (113.0 ± 10.9 mm Hg), diastolic blood pressure (70.8 ± 9.9 mmHg), and a body mass index of 25.8 ± 3.6 (kg/m^2). All participants completed a 24-h dietary recall on each study day, which was analyzed using FoodWorks Version 8. The daily intake of long chain ω-3 PUFA was 106 ± 91.0 mg. There was no significant difference in ω-3 fatty acid food intake for each participant between the study days.

KO-specific changes in lipid classes

Thirteen of the 34 lipid classes presented with a nominally significant supplementation-related change during the 5-h postprandial period (Supplementary Table 1). Figure 2A shows the distributions of their relAUCs after each type of supplementation. Six classes had significantly different relAUCs between KO and FO supplementations (cholesterol [COH], GM3 ganglioside [GM3], trihexosylceramide (Hex3 Cer), PE-P, sphingosine-1-phosphate, ubiquinone). Interestingly, five lipid classes (COH, GM3, Hex3 Cer, lysophosphatidylinsitol, sphingomyelin [SM]) had higher concentrations at the 3-h or 5-h time point, or both, after KO supplementation compared with FO (Fig. 2B).

Thirteen lipid classes presented at least one nominally significant change after at least one supplementation. Relative area under the curve represents total plasma lipid flux over the 5-h postprandial period. Boxplots represent the distributions of relAUC percentages across all 10 participants after KO (orange) and FO (light blue) supplementations. Asterisks (*) denote lipid classes with nominally significant differences between supplementations. The circles represent outliers.

Five lipid classes presented a nominally significant KO-specific increase in concentration during the postprandial period. Concentration trajectories in response to each supplementation (orange: KO; light blue: FO) over the time points with lipidome data (baseline, 3 and 5 h). All values are relative to baseline values. Trajectories for individual participants are shown as light lines. Per-supplementation averages are shown in bold lines with whiskers of ±1 standard deviation. Asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) represent significant terms for FO compared with a constant baseline. Hashes (#$P < 0.05$; ##$P < 0.01$; ###$P < 0.001$) represent significant terms for KO compared with FO.

Individual lipid species levels are extensively modulated during the postprandial period

A total of 701 lipid species, including many species containing ω-3 components, were detected across the 34 lipid classes. Of these, 134 had levels that were altered in one way or another after a supplementation (significantly different relAUC, significant supplementation, or significant time × supplementation interaction) (Fig. 3; Supplementary Table 2). A total of 88 of these were neutral lipids (of classes DG, TG, and ceramide), and 46 were polar lipids (acylcarnitine, GM3, Hex3 Cer, LPC, lysophosphatidylethanolamine, PC, alkylphosphatidylcholine [PC-O], PE, PE-P, PI, SM), including 39 phospholipid-related species, of which five were ether-phospholipids, including one containing an EPA and one DHA.

A total of 134 lipids presented a significant change in concentration during the postprandial period. Figure 3A represents neutral lipids species, whereas Figure 3B represents polar lipid species. Each row represents a lipid species; each column corresponds to a coefficient in the linear mixed model (3-h and 5-h FO indicate changes over the postprandial time against the baseline for FO supplementation; Tmt represents the overall change between FO and KO; 3-h and 5-h KO indicate the *additional* changes over time after KO supplementation compared with FO reference). Heat map colors indicate the amplitude and direction of changes (black to blue to cyan: increasingly negative; black to red to magenta: increasingly positive). Cells are marked with + and − signs to indicate direction and significance (+++ or − $P < 0.001$, ++ or − $P < 0.01$, + or − $P < 0.05$, [+] or [−] $P < 0.1$). The ω-3 bar on the left is colored to reflect ω-3 moiety presence (EPA: orange; DPA: dark orange; DHA: orange-red). The relAUC bar on the left indicates significant relAUC differences between FO and KO (pink $P < 0.10$, red $P < 0.05$).

KO-specific changes in plasma lipid species

Twenty-seven species, including 16 phospholipid-related species (PC, PE-P, LPC, and five ether-phospholipid species) had significant KO-related changes in the postprandial period using at least one of our analyses (Supplementary Table 2). The relAUC of 16 out of these 27 lipid species, including seven EPA- and three DHA-containing species, were significantly greater after KO supplementation compared with FO ($P < 0.05$) (Fig. 4A).

Of these 27, 12 species presented with KO-specific increases over time (Fig. 4B). Nine of these were phosphatidylcholine-related (LPC, PC, PC-O), including two ether-phospholipid species. In the neutral lipid classes (DG and TG), another five of the 27 species had levels that significantly increased after FO supplementation but that increased significantly less after KO supplementation (Fig. 4C).

Twenty-seven lipid species presented a significant KO-specific concentration change over the postprandial period. The relAUC represents total plasma lipid flux over the 5-h postprandial period. Boxplots represent the distributions of relAUC percentages across all 10 participants after KO (orange) and FO (light blue) supplementations. Asterisks (*) denote lipid classes with nominally significant differences between supplementations. The circles represent outliers.

Twelve lipid species presented a significant KO-specific concentration increase compared with FO during the postprandial period. Concentration trajectories in response to each supplementation (orange: KO; light blue: FO) over the postprandial time points are

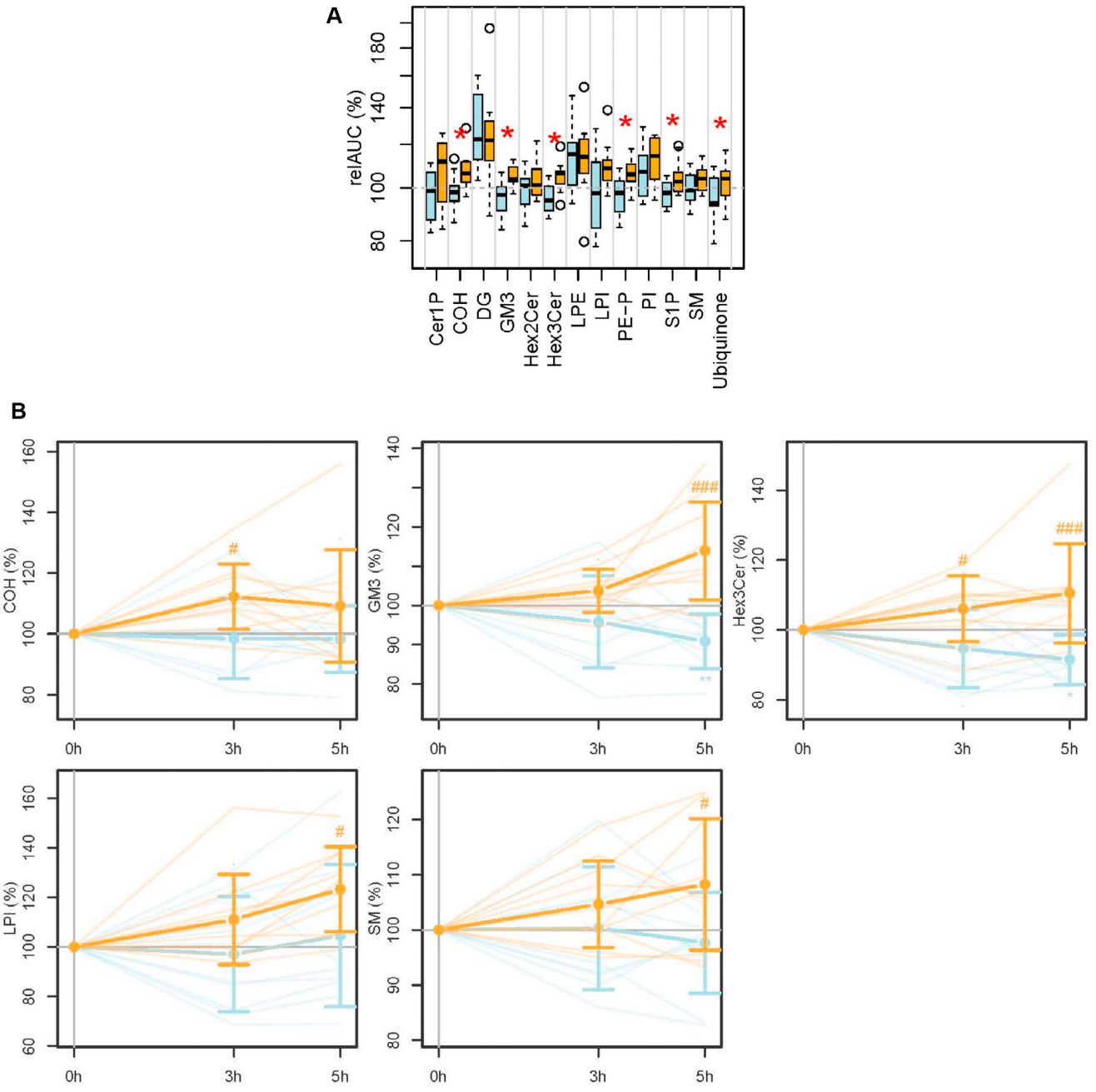

Fig. 2. (A) Relative AUCs after KO and FO supplementations for 13 lipid classes of interest. (B) Relative postprandial concentration changes after KO and FO supplementations for five lipid classes of interest. AUC, area under the curve; Cer1 P, ceramide-1-phosphate; COH, cholesterol; FO, fish oil; GM3, GM3 ganglioside; Hex2 Cer, dihexosylceramide; Hex3 Cer, trihexosylceramide; KO, krill oil; LPI, lysophosphatidylinsitol; PI, phosphatidylinositol; S1 P, sphingosine-1-phosphate; SM, sphingomyelin.

provided with lipidome data (baseline, 3 h, and 5 h). All values are relative to baseline values. Trajectories for individual participants are shown as light lines. Per-supplementation averages are shown in bold lines with whiskers of ±1 standard deviation. Asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) represent significant terms for FO compared with a constant baseline. Hashes (#$P < 0.05$; ##$P < 0.01$; ###$P < 0.001$) represent local FDR-corrected significant terms for KO compared with FO.

Five lipid species presented a significantly lower increase after KO supplementation compared with FO supplementation. Concentration trajectories in response to each supplementation (orange: KO; light blue: FO) over the postprandial time points are provided with lipidome data (baseline, 3 h, and 5 h). All values are relative to baseline values. Trajectories for individual participants are shown as light lines. Per-supplementation averages are shown in bold lines with whiskers of ±1 standard deviation. Asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) represent significant terms for FO compared with baseline. Hashes (#$P < 0.05$; ##$P < 0.01$; ###$P < 0.001$) represent significant terms for KO compared with FO.

Postprandial lipids changes are most marked in ω-3 species

As shown in Figure 3, the set of 134 lipids with concentrations significantly modulated during the postprandial period was enriched in lipids containing ω-3 PUFA (hypergeometric enrichment test, fold = 2.632, $P << 0.05$). In particular, the 27 lipids with

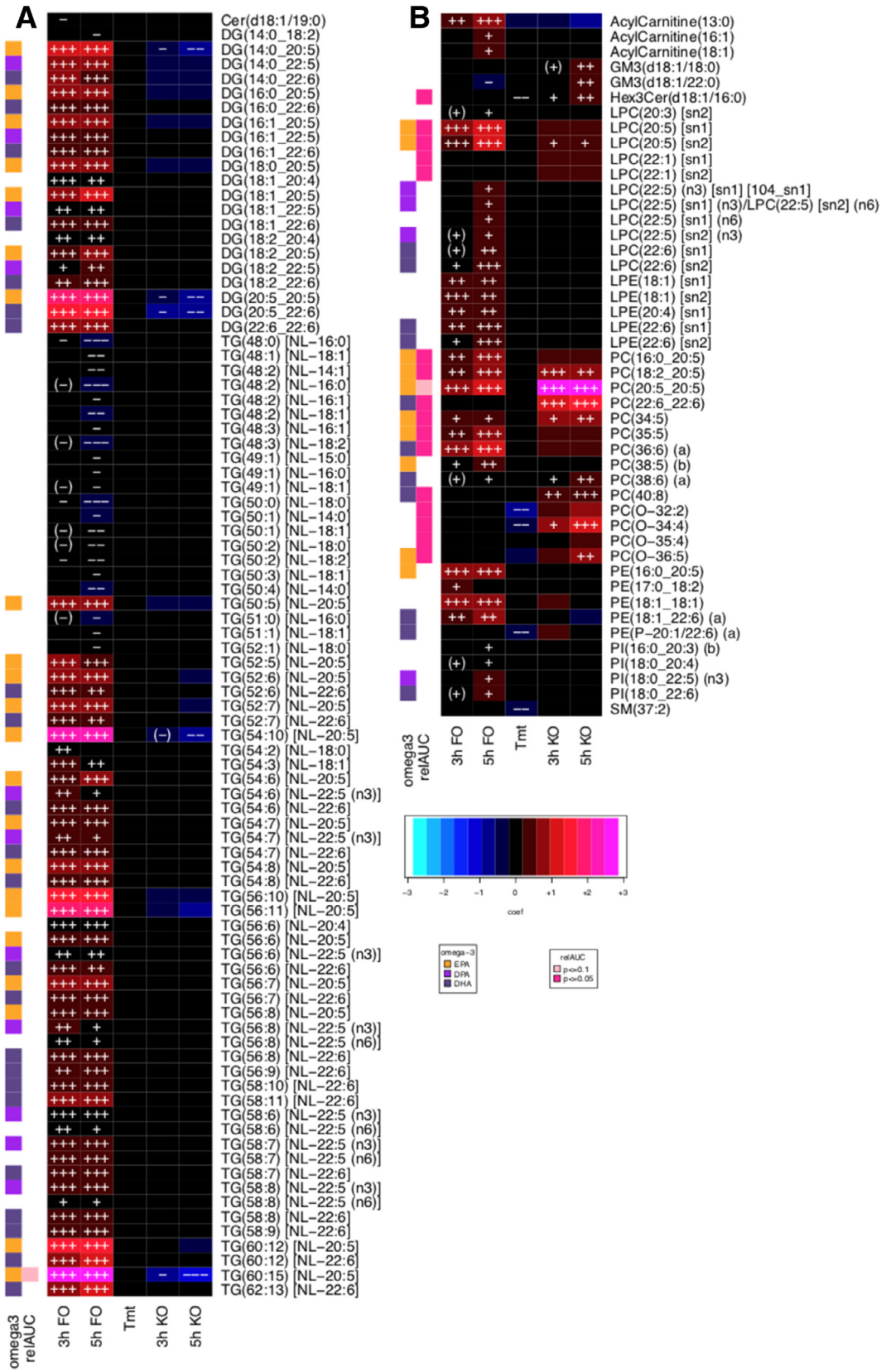

Fig. 3. Summary heat map of changes in 134 lipid species concentration during the postprandial period. COH, cholesterol; DG, diacylglycerol; FO, fish oil; GM3, GM3 ganglioside; Hex3 Cer, trihexosylceramide; KO, krill oil; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinsitol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; relAU; relative area under the curve; SM, sphingomyelin; TG, triacylglycerol.

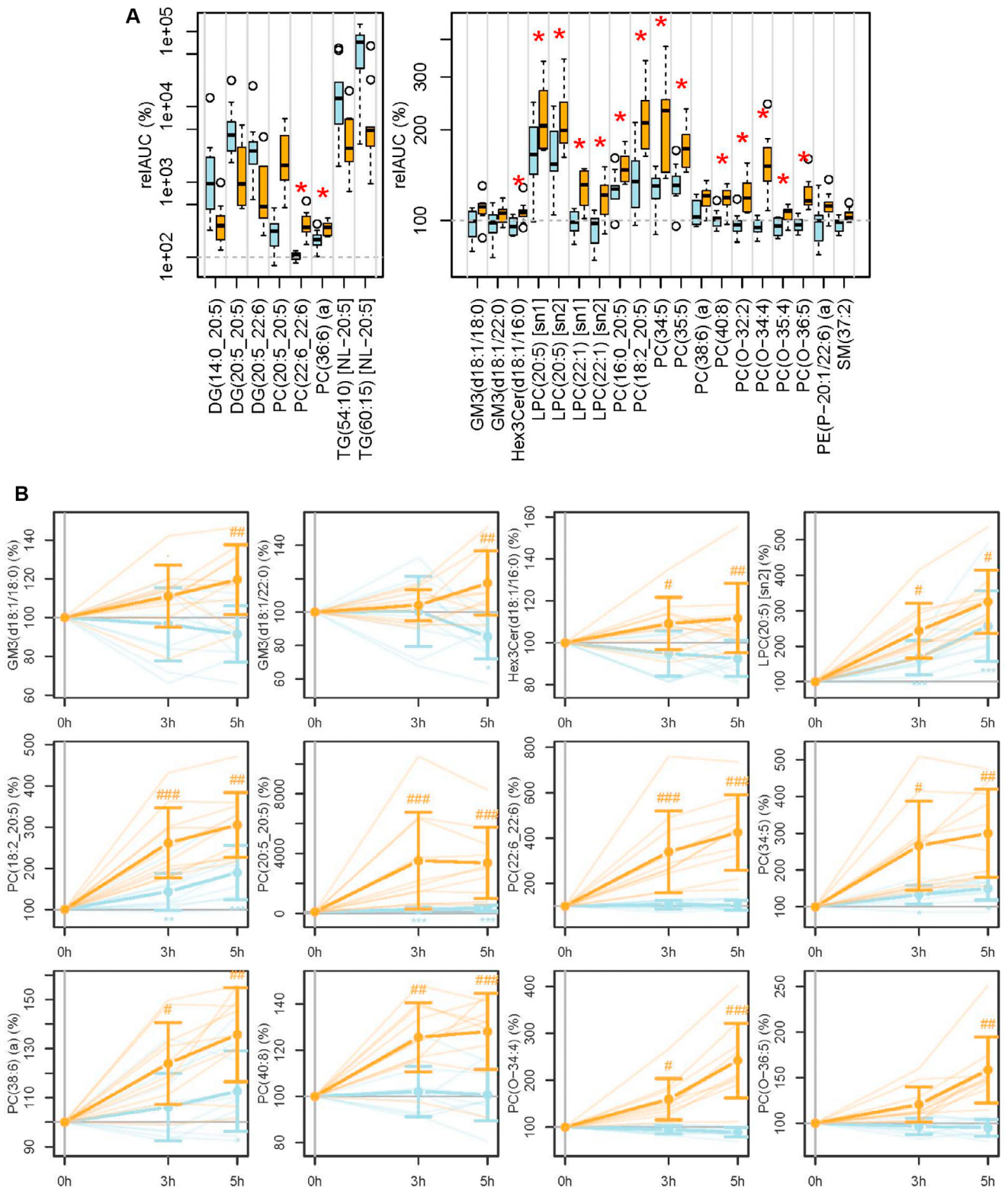

Fig. 4. (A) Relative AUCs after FO and KO supplementations for 27 lipid species of interest. (B) Relative postprandial concentration changes after FO and KO supplementations for 12 lipid species with KO-specific increases. (C) Relative concentration changes over time after FO and KO supplementations for five lipid species with FO-specific increases. DG, diacylglycerol; FO, fish oil; GM3, GM3 ganglioside; Hex3 Cer, trihexosylceramide; KO, krill oil; LPC, lysophosphatidylcholine; NL, ***; PC, phosphatidylcholine; PE, phosphatidylethanolamine; relAUC, relative area under the curve; SM, sphingomyelin; TG, triacylglycerol.

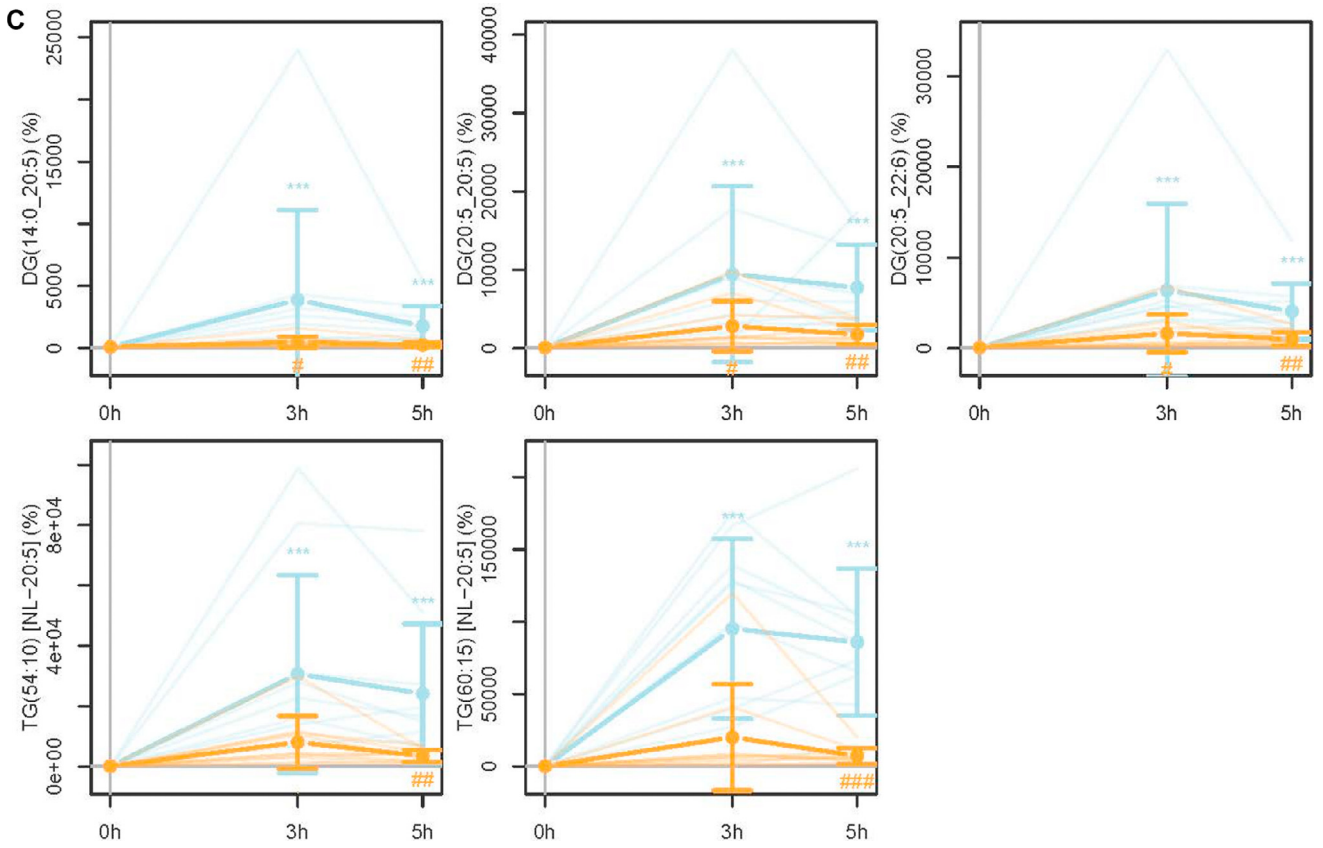

Fig. 4 Continued.

KO-specific modulations were even more enriched in ω-3 PUFA (hypergeometric enrichment test, fold = 2.903, $P << 0.05$). Furthermore, of the 88 neutral lipids significantly modulated during the postprandial period, a large fraction of ω-3 TG and all ω-3 DG were strongly and significantly increased after FO supplementation and generally had lower increases after KO supplementation (though only five were significantly less so) (Fig. 3). Conversely, a large fraction of non−ω-3 TG with significant postprandial changes were often significantly decreased at 5 h after FO supplementation (Fig. 3). Conversely, most of the TG species containing fewer carbons and double bonds were significantly decreased at 5 h after FO supplementation (Fig. 3). The 39 phospholipid species (LPC, lysophosphatidylethanolamine, PC, PC-O, PE, PE-P, PI) with significant postprandial changes were enriched in species containing ω-3 components (hypergeometric test, fold = 2.791, $P < 0.05$) and were typically increased at 3-h and 5-h time points after FO and equally or even more increased after KO supplementation (Fig. 3).

Discussion

The principal aim of this randomized crossover study was to compare the postprandial lipidomic responses to supplementation with KO and FO in healthy women over a 5-h postprandial period to understand the dynamic metabolism of the plasma lipids at the molecular species level. It is recognized that the amounts of long chain ω-3 fatty acids in the two study oils were not identical for practical reasons as stated in the Methods section (mainly blinding of participants). It is acknowledged that the impact on the results would be that FO rich in TG compared with KO would have greater effects on TG lipidomic responses, and in contrast KO rich in PL compared with FO would have greater effects on PL lipidomic responses. To overcome the impact of these differences in lipid contents of the study oils on the results, we have compared the mean percentage change from the baseline after each oil supplementation. In addition, relative AUCs were analyzed.

Most studies comparing KO with FO have examined the fatty acid composition of total plasma lipids, plasma phospholipids, or plasma TG. These fractions are very heterogeneous pools of lipid classes and molecular species, and to date such approaches have not revealed consistent significant differences in the uptake of ω-3 fatty acids from KO and FO in either short-term or long-term studies [7,8,10,11].

In terms of lipid classes, of the six that had a significantly greater relAUC (%) for KO compared with FO, only two of these lipid classes were present in the study oils (cholesterol and alkenyl phosphatidylethanolamine). Five of these six lipid classes were present in very low abundance in the plasma, compared with COH, which was one of the main plasma lipid classes. Both the oils contained COH and cholesteryl ester, albeit in different concentrations, although the total concentration of COH plus cholesteryl ester was similar between the two oils (Table 1). It can be speculated that these two sterols are digested and absorbed at different rates and have differing influence on lipoprotein turnover once incorporated into the chylomicrons (CM) and plasma lipoproteins. The KO contained a number of different ether lipids, whereas such lipids were virtually absent from the FO (Table 1). Little is known about the digestion and absorption of ether lipids, apart from assuming they would follow similar pathways to diacyl- or monoacyl-phospholipids. PE-P was one of the least abundant ether-phospholipids in KO, and it is not clear why there was a significantly greater response for this lipid class in the postprandial plasma after the KO supplementation compared with FO. We propose that after the

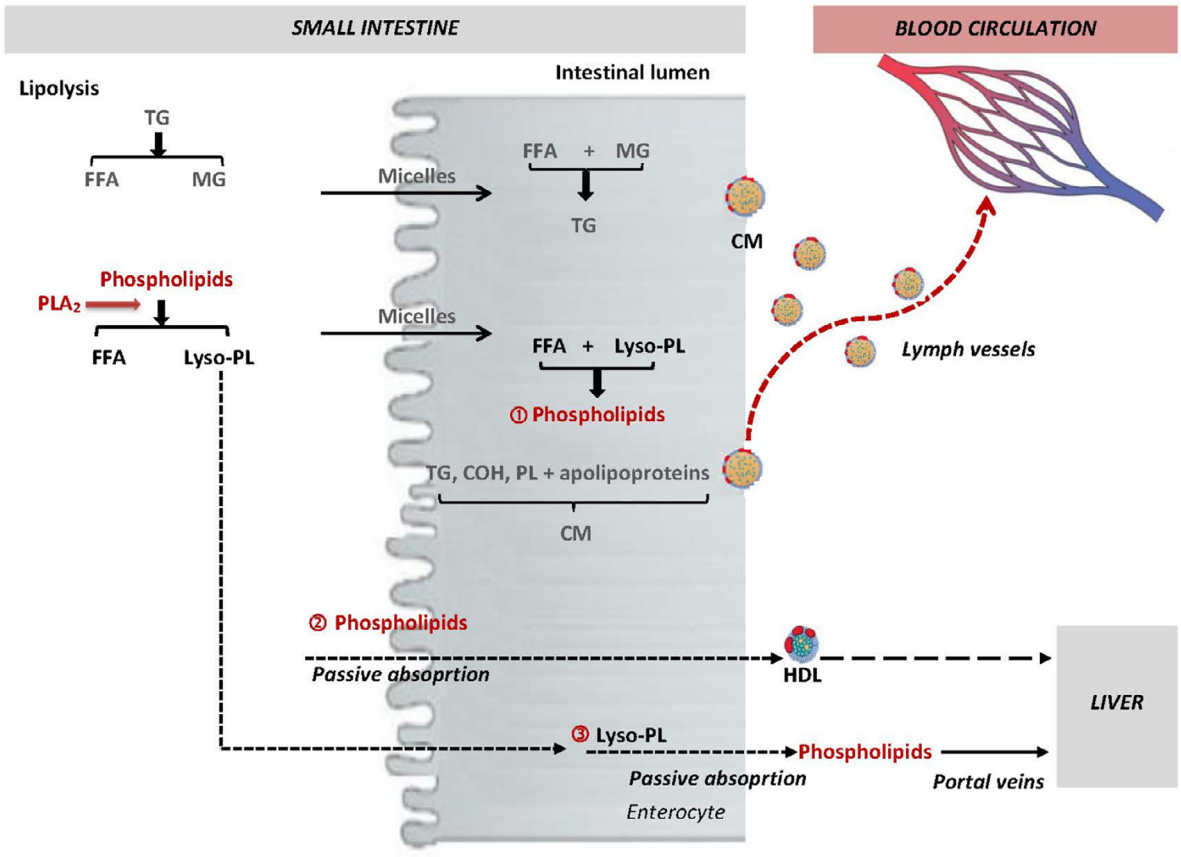

Fig. 5. Dietary phospholipids digestion, absorption, and transport. CM, chylomicron; COH, cholesterol; FFA, free fatty acids; HDL, high-density lipoproteins; LC FA, long chain fatty acid; Lyso-PL, lysophospholipid; MG, monoacylglycerol; PL, phospholipids; PLA, ***; TG, triacylglycerol. Modified from Cohn et al. [26], Küllenberg et al. [27], and Tall et al. [28].

uptake or absorption of the alkenyl phosphatidylcholine from the KO, it was converted to PE-P, likely in the liver. The origin of the other four lipid classes (GM3, trihexosylceramide, ubiquinone, and sphingosine-1-phosphate) in the postprandial plasma is unknown; thus the explanation for their levels in the postprandial plasma after KO supplementation being significantly greater than FO requires further investigation.

As shown in Supplementary Table 2 for lipid species, there were significant increases in many molecular species containing EPA, DHA, and DPA in phospholipids (the main one being PC) and in TG and DG after supplementation with both the ω-3 oils over the postprandial period. The ω-3 fatty acids from KO were preferentially partitioned toward the PC (diacyl- and ether-phospholipids), whereas the ω-3 fatty acids from FO were preferentially partitioned to neutral lipids (DG and TG). These novel data highlight the importance of being able to study changes in a postprandial study in many different lipids classes and molecular species using a sufficiently sensitive technique. It is of interest that the results from Sung et al. [9], using thin-layer chromatography, were indicative of different responses to KO versus FO for plasma phospholipids and TG. In the present study, this is the first time that it has been possible to differentiate specific responses to KO and FO consumption in terms of plasma lipids containing ω-3 fatty acids.

There are several possible explanations for these novel findings—first, the difference in TG content of KO and FO.

The KO used in this study had a lower proportion of TG (24% of total lipid classes of KO) compared with the FO, which contained 98% TG (quantitative Iatroscan thin-layer chromatography, data not shown). Therefore, based on mass of fed TG and the well-known lipid digestion and absorption of fatty acids from TG, it should be expected that there would be a lower relAUC for ω-3 fatty acids in plasma TG and DG from the KO arm than that in FO arm of the study.

Second, The KO used in this study contained 61% phospholipids (of the total lipid classes) compared with the FO, which contained 1% phospholipids (quantitative thin-layer chromatography, data not shown). Therefore it should be expected that there would be a difference in the phospholipid absorption in the postprandial period between the two oils. However, the pathways of digestion and absorption of phospholipids, for both diacyl- and ether-phospholipids, are poorly understood (Fig. 5).

The literature suggests that phospholipids in the intestine (from food or bile) are digested by pancreatic phospholipase A_2 [26,27] into FFA and lysophospholipids (Fig. 5). The lysophospholipids and FFA are then absorbed and reassembled into phospholipids in the mucosal cells and exported into the blood circulation via CM [28,27,29]. Some of the FFA from the dietary/bile phospholipids are believed to be incorporated into the TG and then exported in the CM [28]. Two studies have reported that dietary phospholipids after digestion and absorption are preferentially incorporated into high-density lipoproteins (HDL) either via preferential transfer or passive exchange [27,29]. It has also been suggested that some intestinal phospholipids may be absorbed passively without hydrolysis [29]. A final possibility, which has not been extensively studied, is that lysophospholipids may be transferred directly from the intestinal mucosa to the portal vein for direct transport to the liver [29]. Thus it might explain why PC-O from the KO could be metabolized to PE-P in the liver and

then released into plasma. Because lysophospholipids bind strongly to serum albumin, it is likely that this is how the absorbed lysophospholipids from the digestion of dietary phospholipids could be transported in the blood. Phospholipids from HDL, via the action of lecithin-cholesterol acyl transferase, can be transferred to the plasma membranes of various cells and tissues [30]. It is likely that lipoprotein phospholipid fatty acids, derived from dietary phospholipids, can be incorporated into cellular membranes, resulting in alteration of the cellular membrane composition [31,32]. Although there is uncertainty about the digestion and absorption of diacyl-PC species as described earlier, almost nothing is known about such processes for ether-phospholipid species. Perhaps these are digested and absorbed in the same way as the diacyl-phospholipid species, but this is uncertain.

There is one study in which the postprandial incorporation of 13 C-DHA-PC was followed for 20 h in volunteers [33]. The 13 C-labeled DHA was incorporated into FFA, LPC, PC, and TG. A contrasting study, where 13 C-DHA-TG was fed in a similar study design, indicated that the 13 C-label was mostly found in very low density lipoprotein TG with lesser amounts in albumin-bound FFA [34]. Therefore dietary phospholipids might be transported into the bloodstream after digestion and absorption in CM, HDL, or as lysophospholipids bound to albumin. In contrast, dietary TG are mostly transported via CM, followed by uptake in the liver and redistribution of the TG fatty acids into different lipids of exported lipoproteins (very low density). The previously discussed studies about possible metabolism of dietary phospholipids reveal that fatty acids from these phospholipids could be incorporated into plasma phospholipids, which make up lipoproteins in the postprandial phase. Thus in postprandial studies of KO compared with FO, plasma phospholipids and TG should both be examined.

A number of studies have reported negative associations between ether-phospholipids and several medical conditions [35–42]. Because this is a postprandial study, it is not known whether the increase in plasma EPA-, DHA- and DPA-containing ether-phospholipids, such as alkylphosphatidylethanolamine, after KO supplementation will be translated to long-term effects. Further longer-term studies are required to determine those effects and the potential health benefits associated with KO supplementation.

Limitations of the study

It is acknowledged that the present study had several limitations, which include lipidome data covering a short time frame (5 h), having a small number of participants ($n = 10$), with participants not totally blinded to test oils (capsule contents were different colors: KO dark and FO light oil color). It is possible that the small participant number has obscured some differences between KO and FO supplementations. Further study with a large number of participants and more samples collected from additional time points is warranted to verify the lipidomic responses to the two marine oils.

Conclusions

There were clear differences between KO and FO supplementations in the postprandial period, which were most noticeable in the changes in diacyl-phospholipids and ether-phospholipids. It is not known whether the changes identified in the short term translate to changes in the longer term. Further studies are required to validate the findings from this postprandial study through larger longer-term studies and to determine the potential health benefits associated with KO supplementation.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.03.021.

References

[1] Statista. Global omega-3 supplement market size in 2016 and 2025. Available at: www.statista.com/statistics/758383/omega-3-supplement-market-size-worldwide. Accessed 7 February 2018.
[2] Xie D, Jin J, Sun J, Liang L, Wang X, Zhang W, et al. Comparison of solvents for extraction of krill oil from krill meal: lipid yield, phospholipids content, fatty acids composition and minor components. Food Chem 2017;233:434–41.
[3] Phleger CF, Nichols PD, Virtue P. Lipids and trophodynamics of Antarctic zooplankton. Comp Biochem Physiol B 1998;120:311–23.
[4] Ghasemifard S, Turchini GM, Sinclair AJ. Omega-3 long chain fatty acid "bioavailability": a review of evidence and methodological considerations. Prog Lipid Res 2014;56:92–108.
[5] Ulven SM, Holven KB. Comparison of bioavailability of krill oil versus fish oil and health effect. Vasc Health Risk Manag 2015;11:511.
[6] Yurko-Mauro K, Kralovec J, Bailey-Hall E, Smeberg V, Stark JG, Salem N. Similar eicosapentaenoic acid and docosahexaenoic acid plasma levels achieved with fish oil or krill oil in a randomized double-blind four-week bioavailability study. Lipids Health Dis 2015;14:1–9.
[7] Schuchardt JP, Schneider I, Meyer H, Neubronner J, von Schacky C, Hahn A. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations—a comparative bioavailability study of fish oil vs. krill oil. Lipids Health Dis 2011;10:145.
[8] Kohler A, Sarkkinen E, Tapola N, Niskanen T, Bruheim I. Bioavailability of fatty acids from krill oil, krill meal and fish oil in healthy subjects-a randomized, single-dose, cross-over trial. Lipids Health Dis 2015;14:19.
[9] Sung HH, Sinclair AJ, Lewandowski PA, Su XQ. Postprandial long-chain n-3 polyunsaturated fatty acid response to krill oil and fish oil consumption in healthy women: a randomized controlled, single-dose, crossover study. Asia Pac J Clin Nutr 2018;27:148–57.
[10] Maki KC, Reeves MS, Farmer M, Griinari M, Berge K, Vik H, et al. Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women. Nutr Res 2009;29:609–15.
[11] Ramprasath VR, Eyal I, Zchut S, Jones PJ. Enhanced increase of omega-3 index in healthy individuals with response to 4-week n-3 fatty acid supplementation from krill oil versus fish oil. Lipids Health Dis 2013;12:178.
[12] Barros MP, Poppe SC, Bondan EF. Neuroprotective properties of the marine carotenoid astaxanthin and omega-3 fatty acids, and perspectives for the natural combination of both in krill oil. Nutrients 2014;6.
[13] Weir JM, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, et al. Plasma lipid profiling in a large population-based cohort. J Lipid Res 2013;54:2898–908.
[14] Kutzner L, Ostermann AI, Konrad T, Riegel D, Hellhake S, Schuchardt JP, et al. Lipid class specific quantitative analysis of n-3 polyunsaturated fatty acids in food supplements. J Agric Food Chem 2017;65:139–47.
[15] Zhao YY, Miao H, Cheng XL, Wei F. Lipidomics: novel insight into the biochemical mechanism of lipid metabolism and dysregulation-associated disease. Chem Biol Interact 2015;240:220–38.
[16] Mundra PA, Shaw JE, Meikle PJ. Lipidomic analyses in epidemiology. Int J Epidemiol 2016;45:1329–38.
[17] Kontush A, Chapman MJ. Lipidomics as a tool for the study of lipoprotein metabolism. Curr Atheroscler Rep 2010;12:194–201.
[18] Lamaziere A, Wolf C, Barbe U, Bausero P, Visioli F. Lipidomics of hepatic lipogenesis inhibition by omega 3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2013;88:149–54.
[19] Zhong H, Fang C, Fan Y, Lu Y, Wen B, Ren H, et al. Lipidomic profiling reveals distinct differences in plasma lipid composition in healthy, prediabetic and type 2 diabetic individuals. GigaScience 2017;6:1–12.
[20] Bonham MP, Linderborg KM, Dordevic A, Larsen AE, Nguo K, Weir JM, et al. Lipidomic profiling of chylomicron triacylglycerols in response to high fat meals. Lipids 2013;48:39–50.
[21] Mansbach 2nd CM, Gorelick F. Development and physiological regulation of intestinal lipid absorption. II. Dietary lipid absorption, complex lipid synthesis,

and the intracellular packaging and secretion of chylomicrons. Am J Physiol Gastrointest Liver Physiol 2007;293:G645–50.
[22] Huynh K, Barlow CK, Jayawardana KS, Weir JM, Mellett NA, Cinel M, et al. High-throughput plasma lipidomics: detailed mapping of the associations with cardiometabolic risk factors. Cell Chem Biol 2019;26:71–84.
[23] Berry KAZ, Murphy RC. Electrospray ionization tandem mass spectrometry of glycerophosphoethanolamine plasmalogen phospholipids. J Am Soc Mass Spectrom 2004;15:1499–508.
[24] Kagan ML, West AL, Zante C, Calder PC. Acute appearance of fatty acids in human plasma–a comparative study between polar-lipid rich oil from the microalgae Nannochloropsis oculata and krill oil in healthy young males. Lipids Health Dis 2013;1:102.
[25] Team RC. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017. Available at: https://www.r-project.org.
[26] Küllenberg D, Taylor LA, Schneider M, Massing U. Health effects of dietary phospholipids. Lipids Health Dis 2012;11:3.
[27] Tall AR, Blum CB, Grundy SM. Incorporation of radioactive phospholipid into subclasses of high-density lipoproteins. Am J Physiol 1983;244:E513–6.
[28] Cohn JS, Kamili A, Wat E, Chung RW, Tandy S. Dietary phospholipids and intestinal cholesterol absorption. Nutrients 2010;2:116–27.
[29] Zierenberg O, Grundy SM. Intestinal absorption of polyenephosphatidylcholine in man. J Lipid Res 1982;23:1136–42.
[30] Glomset JA. The plasma lecithin: cholesterol acyltransferase reaction. J Lipid Res 1968;9:155–67.
[31] Taylor LA, Pletschen L, Arends J, Unger C, Massing U. Marine phospholipids—a promising new dietary approach to tumor-associated weight loss. Support Care Cancer 2010;18:159–70.
[32] Küllenberg D, Taylor LA, Schneider M, Massing U. Health effects of dietary phospholipids. Lipids Health Dis 2012;11:1.
[33] Lemaitre-Delaunay D, Pachiaudi C, Laville M, Pousin J, Armstrong M, Lagarde M. Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [13 C]DHA in phosphatidylcholine. J Lipid Res 1999;40:1867–74.
[34] Brossard N, Croset M, Normand S, Pousin J, Lecerf J, Laville M, et al. Human plasma albumin transports [13 C]docosahexaenoic acid in two lipid forms to blood cells. J Lipid Res 1997;38:1571–82.
[35] Wood PL, Locke VA, Herling P, Passaro A, Vigna GB, Volpato S, et al. Targeted lipidomics distinguishes patient subgroups in mild cognitive impairment (MCI) and late onset Alzheimer's disease (LOAD). BBA Clin 2016;5:25–8.
[36] Wood PL. Lipidomics of Alzheimer's disease: current status. Alzheimers Res Ther 2012;4:1–10.
[37] Fujino T, Yamada T, Asada T, Tsuboi Y, Wakana C, Mawatari S, et al. Efficacy and blood plasmalogen changes by oral administration of plasmalogen in patients with mild Alzheimer's disease and mild cognitive impairment: a multicenter, randomized, double-blind, placebo-controlled trial. EBioMed 2017;17:199–205.
[38] Maeba R, Ueta N. A novel antioxidant action of ethanolamine plasmalogens in lowering the oxidizability of membranes. Biochem Soc Trans 2004;32:141–3.
[39] Goodenowe DB, Cook LL, Liu J, Lu Y, Jayasinghe DA, Ahiahonu PW, et al. Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. J Lipid Res 2007;48:2485–98.
[40] Maeba R, Araki A, Ishii K, Ogawa K, Tamura Y, Yasunaga M, et al. Serum ethanolamine plasmalogens improve detection of cognitive impairment among elderly with high excretion levels of urinary myo-inositol: a cross-sectional study. Clin Chim Acta 2016;453:134–40.
[41] Otoki Y, Kato S, Kimura F, Furukawa K, Yamashita S, Arai H, et al. Accurate quantitation of choline and ethanolamine plasmalogen molecular species in human plasma by liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal 2017;134:77–85.
[42] Maeba R, Maeda T, Kinoshita M, Takao K, Takenaka H, Kusano J, et al. Plasmalogens in human serum positively correlate with high-density lipoprotein and decrease with aging. J Atheroscler Thromb 2007;14:12–8.