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The effects of fatty acid-based dietary interventions on circulating bioactive lipid levels as intermediate biomarkers of health, cardiovascular disease and cardiovascular disease risk factors: A systematic review and meta-analysis of randomized clinical trials

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ABSTRACT

Context. Dietary fatty acids (FAs), primarily n-3 polyunsaturated fatty acids (PUFAs), have been associated with enrichment of the circulating bioactive lipidome and changes in the enzymatic precursor lipoprotein-associated phospholipase A2 (Lp-PLA2) mass; however, the magnitude of this effect remains unclear.

Objective. The aim of this systematic review and meta-analysis was to evaluate the effect of different dietary FAs, provided as supplements, enriched food components or diets, on the bioactive lipid profile of healthy subjects and those with cardiovascular disease (CVD) and CVD risk factors.

Data sources. PubMed, SCOPUS, and the Cochrane Library databases were searched for relevant articles published from October 2010 to May 2022.

Data extraction. Data were screened for relevance, and then retrieved in full and evaluated for eligibility by 2 reviewers independently.

Data analysis. The net difference in the bioactive lipid mean values between the endpoint and the baseline, and the corresponding SDs or SEs, were used for the qualitative synthesis. For the meta-analysis, a fixed-effects model was used.

Results. Twenty-seven randomized clinical trials (representing over 2560 subjects) were included. Over 78% of the enrolled subjects presented at least one associated CVD risk factor, whereas less than 22% were healthy. In the meta-analysis, marine n-3 supplements (0.37-1.9 g/day) significantly increased pro-inflammatory lysophosphatidylcholines (lyso-PCs) (mean (95%CI): +0.52(0.02,1.01) μM for lyso-PC(16:0) and +0.58 (0.09,1.08) μM for lyso-PC(18:0)) in obese subjects. Additionally, n-3 supplementation (1-5.56 g/day) decreased plasma Lp-PLA2 mass, a well-known inflammation marker, in healthy (-0.35 (-0.59,-0.10) ng/mL), dyslipidemic (-0.36 (-0.47,-0.25) ng/mL) and stable coronary artery disease subjects (-0.52 (-0.91,-0.12) ng/mL).

Conclusions. Daily n-3 provided as EPA+DHA supplements and consumed from 1 to 6 months, reduced plasma Lp-PLA2 mass in healthy subjects and those with CVD and CVD risk factors, suggesting an

anti-inflammatory effect. However, the saturated lyso-PC response to n-3 was impaired in obese subjects.

Keywords: glycerophospholipids; lysoglycerophospholipids; sphingolipids; lipoprotein-associated phospholipase A2; polyunsaturated fatty acids; cardiovascular disease

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1. INTRODUCTION

Cardiovascular diseases (CVDs) comprise a group of clinical disorders of the heart and blood vessels, with ischemic heart disease (IHD) and stroke being the most common cardiovascular events and the leading cause of mortality and disability worldwide.[1] CVDs can be prevented if potential CVD risk factors, such as hypertension, elevated LDL cholesterol, and a cluster of interrelated metabolic factors, are detected early and addressed.[2] In recent years, advances in lipidomic methodologies have opened a new frontier in the identification of specific lipid-based molecules able to mediate physiological and metabolic functions in the host, and they have been shown to play prominent roles as biomarkers in CVD development.[3,4] Among various lipid molecules, glycerophospholipids (PLs), such as phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and phosphatidylinositols (PIs); lysoglycerophospholipids (lyso-PLs), including those containing a choline group, namely, lysophosphatidylcholines (lyso-PCs), and lysophosphatidylethanolamines (lyso-PEs); and sphingolipids (SPs), including ceramides (Cer), dihydroceramides (diCer), glucosylceramides (gluCer), lactosylceramides (lacCer) or sphingomyelin (SM) classes, represent a large and structurally diverse group of circulating bioactive lipids generated from membrane lipids by hydrolysis.[5] Additionally, the plasma lipoprotein-associated phospholipase A2 (Lp-PLA2) enzyme plays an intermediary role in the hydrolysis of oxidized PLs in LDL-cholesterol particles, leading to the generation of plasma lyso-PLs and oxidized free fatty acids (FAs).[6]

Bioactive lipids can regulate key cell biological functions and signaling pathways, including growth regulation, cell differentiation, angiogenesis, autophagy, cell migration, and inflammatory response.[7,8] In this way, lyso-PCs, the most abundant lyso-PLs in human blood, have been widely regarded as pro-inflammatory mediators in atherosclerosis.[9] However, as recently reviewed, additional anti-inflammatory effects of lyso-PCs are documented in the vascular system, and these effects may be highly dependent on the lyso-PC acyl chain length and the degree of saturation.[10,11] Moreover, certain lyso-PLs have been postulated as non-invasive biomarkers of dyslipidemia in animal models,[12] but evidence in humans is scarce. Other circulating bioactive lipids, such as Cer, have been linked to pleiotropic actions in metabolism and are measured clinically as prognostic indicators of major adverse cardiovascular events.[13] Additionally, in a 2-week prospective study, elevated serum Lp-PLA2

concentrations, were proposed as a possible risk biomarker in IHD, especially in hospitalized patients with LDL cholesterol lower than 130 mg/dL.[14]

Overall, bioactive lipids and their mediators reflect different aspects of the development of CVDs and could provide useful tools for successful predictions. The simplicity of their chemical structures, mainly composed of a hydrophobic tail of FA residues and a hydrophilic head group, could largely determine their biological functions. However, little is known about the effect of FAs from the diet on the bioactive lipid profile in healthy subjects and those with CVD and CVD risk factors.

FAs comprise a large variety of lipid molecules derived from dietary fat breakdown and are divided into four categories: saturated (SFAs), monounsaturated (MUFAs), polyunsaturated (PUFAs), and trans (TFAs). Notably, essential FAs, including the very long-chain omega-3 (n-3) PUFAs such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids and omega-6 (n-6) PUFAs, have aroused great interest because of their beneficial properties. It has been reported that consumption of n-3 PUFAs reverses several CVD risk factors.[15] The primary sources of n-3 PUFAs in the diet of humans are marine fish, especially oily fish, and, if consumed, fish oil supplements. Additionally, vegetable oils, such as soybean, nuts and canola oils, provide alternative plant forms of n-3 known as alpha-linolenic acid (ALA).

The n-3 PUFA levels in plasma PLs show dose-dependent responses to fish oil supplementation and have been used as potential biomarkers of disease.[16,17] Recently, a limited number of human intervention studies have reported a selective enrichment of long-chain n-3 PUFAs in plasma PLs and lyso-PLs following intake of both n-3-rich oily fish [18] and supplements.[19] This enrichment effect could be explained by an alteration of the fatty acyl moiety of bioactive lipids; however, the extent to which dietary n-3 PUFAs, or other common dietary FAs, modify the plasma bioactive lipidome in healthy subjects and those with cardiovascular disease (CVD) and CVD risk factors has not been widely evaluated.

In that context, the main goal of the present systematic review and meta-analysis was to evaluate the effect of SFAs, MUFAs, PUFAs and TFAs, provided as supplements, enriched food components or diets, on the bioactive lipid profile of healthy subjects and those with CVD and CVD risk factors. Understanding the pivotal role of dietary FAs in the human lipidome will allow the development of appropriate nutritional therapies for the management and prevention of CVDs.

2. METHODS

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[20] The protocol was prospectively registered at the PROSPERO database: registration number CRD42021218335.

2.1. Search strategy and eligibility criteria

We searched PubMed (https://pubmed.ncbi.nlm.nih.gov/), SCOPUS (www. scopus.com), and Cochrane Library (https://www.cochranelibrary.com/) databases for English-language literature, published in the last 12 years in peer-reviewed journals, and indexed until May 2022. Search terms included the specific bioactive lipid classes, different FA categories, and CVD or cardiometabolic diseases or their risk factors. We also reviewed additional publications found in the references of the retrieved articles. The search strategy is detailed in ***Supplemental File 1***.

We included clinical trials involving adult humans (age 18 years or older) that reported the effects of FA-based interventions, such as supplements or enriched food components (EF-C) and enriched diets (ED), on circulating bioactive lipid levels. The selected studies included subjects who were either healthy, with CVD risk factors or with established CVD; healthy subjects had ideal levels of blood glucose, triglycerides (TG), HDL cholesterol, blood pressure and waist circumference without using medications. Subjects with CVD or cardiometabolic disease risk factors, included type 2 diabetes (T2D) obesity, overweight, dyslipidemia, metabolic syndrome (MetS) and hypertension. Most subjects with established CVD had coronary artery disease (CAD). Excluded studies involved pregnant women and less common cardiovascular or autosomal disorders, such as non-alcoholic fatty liver disease, diabetic nephropathy, obstructive sleep apnea syndrome, or Fabry's disease, among other. Additionally, we excluded studies assessing the effects of medicinal plants or pharmacological therapy and studies that did not specify bioactive lipid classes. The Population, Intervention, Comparison, Outcomes and Study design (PICOS) criteria were used to define the inclusion and exclusion criteria as listed in ***Table 1***.

2.2. Study selection and data extraction

All titles and abstracts found by the search strategy were screened for relevance, and then retrieved in full and evaluated for inclusion eligibility by LC-P and JC. Eligible results were compared and

discussed between AP and RMV. The screening process was conducted through *Covidence* web-based software (www.covidence.org), which allowed asynchronous collaboration of all members. Data were extracted into pre-specified structured tables. Missing data were entered as "not reported".

2.2.1. Summary measures

The data extracted from the selected trials were summarized in two pre-specified tables for each CVD risk factor and the health status. In the first pre-specified table, we reported the first author, the year of publication, the country, the study design, the population (including age range, sex, and main baseline features), the details of the FA-based intervention and dosage, the comparator used, the duration of exposure, the number of participants in the study, and the intention-to-treat analysis. In the second pre-specified table, we reported the bioactive lipid or enzymatic precursor data. Regarding bioactive lipids, the extracted data included the assessment method used for lipidomic characterization,[21] the identification reference (software or internal procedure used), the bioactive lipid class, i.e., the lipid subclass and molecular formula,[22] and significant and non-significant changes in the particular bioactive lipid concentrations.

The enzymatic precursor data comprised the assessment method used for the measurement of enzymatic mass or activity, the enzyme class and subclass, and significant and non-significant changes in the enzymatic precursor.

We also reported the direction of change for each bioactive lipid and enzymatic precursor as "increased" or "decreased".

2.3. Statistical analyses

The systematic review and meta-analysis were performed using Cochrane Collaboration's Review Manager (RevMan) software (v 5.4). For the qualitative synthesis, we evaluated the net difference in the bioactive lipid mean values between the endpoint and the baseline – effect before vs. after intervention – and the corresponding standard deviations (SDs) or standard errors (SEs). When necessary, we estimated the SDs or SEs of the net change from reported variance data. If the SD or SE values were missing in the original article, we contacted the corresponding author for additional data (n = 4). The studies were clustered into subjects with CVD, with CVD risk factors or the healthy subjects according to the population receiving the intervention.

For the meta-analysis, we selected randomized controlled trials (RCTs) assessing the effects of PUFA-supplemented dietary interventions (including n-3 and n-6) on bioactive lipids or Lp-PLA2 enzymatic precursor. This restriction of studies was applied because the PUFA-supplemented interventions had greater effects than EF-C or ED, and also because the mode of administration, the dose, and the expression of results were comparable across studies. A set of meta-analyses was performed by using a fixed-effects model to assign a weight to each study included (n = 10) according to the within-study variability and the between-study heterogeneity. In parallel, a sensitivity analysis was performed to explore the degree of heterogeneity between studies, expressed by the heterogeneity statistic (I^2). An I^2 between 0% and 40% represented low heterogeneity [23] and therefore poor variability in the results.

Overall, the results of the meta-analyses are expressed as standard mean differences (SMDs) that are defined as the differences between the end and baseline values and 95% confidence intervals (CIs). An exception was made in the meta-analysis for the effects of n-3 PUFAs on Lp-PLA2 mass in dyslipidemia, where the effect was estimated from the net difference of the outcome compared to placebo.

Bioactive lipid levels are reported using either molar concentration, relative abundance, relative amount, or molar percentage (%) of LDL or HDL surface lipids. The studies reporting only the peak intensities of lipid subclasses were excluded from the analysis (n = 2).

Given the limited number of studies included in the individual meta-analyses and their similarities in the supplementation method, dose, duration, and the way the effect was measured, we did not consider perform additional meta-regression.

2.4. Quality assessment

All randomized clinical trials were assessed for study quality and risk of bias. The quality assessment was conducted using RevMan software (v 5.4). The following quality items were assessed: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases, such as publication bias. Each quality item was rated as low risk/high risk/unclear risk according to the Cochrane Collaboration's tool for assessing the risk of bias in randomized trials.[24] After considering each quality

item, an overall rating was assigned to each included study, with "Low risk" indicating the least bias and valid results; "Moderate risk" indicating susceptibility to some bias but not enough to invalidate the results; and "High risk" indicating significant bias that may invalidate the results. The risk of bias judgment was performed by the first author (LC-P), and any inconsistency was resolved by a second author (JC). The overall quality classification, along with the target bioactive lipids assessed, is summarized in ***Table 2***.

3. RESULTS

3.1. Study selection

The literature search in PubMed, SCOPUS, and Cochrane databases yielded 382 articles to be retrieved and reviewed. One additional article was manually added. After duplicate removal (n = 73), we retrieved and reviewed 309 articles, of which 245 were excluded for not meeting the eligibility criteria. Finally, 64 full-text studies were assessed for eligibility, of which 27 were included in the overall systematic review, with 10 studies selected for meta-analysis, as detailed in ***Figure 1***.

3.2. Study characteristics

Of the 27 included studies, 21 were RCTs, of which 19 involved sustained interventions with parallel design,[19,25,34–42,26–33] except for one RCT that used a crossover design.[43] The remaining RCT conducted a postprandial and crossover intervention.[44] The other 6 included studies were non-controlled RTs, of which 2 were sustained interventions with a parallel [45] and crossover [18] design, 3 were postprandial test meals using parallel [46] and crossover [47,48] design, and 1 was a 4-day short-term study with a crossover design (***Tables 3-9***).[49] These studies represented approximately 2560 subjects (men and women) aged between 18 and 75 years. In one RCT, participants were restricted to postmenopausal women.[26] Over 78% of the enrolled subjects presented at least one associated CVD or cardiometabolic disease risk factor, including obesity and overweight, dyslipidemia, MetS, T2D and hypertension, or established CVD, primarily stable CAD, whereas less than 22% were healthy (***Table 2***). The intervention period ranged from 1 to 6 months in sustained studies, and from 4 to 6 hours in postprandial test meals. In the crossover studies, the washout periods varied from 4 days to 8 weeks after the first intervention. The populations studied originated from upper- to middle-income countries, including studies from

Northern America (n = 11), Northern Europe (n = 6), Southern Europe (n = 4), Eastern Europe (n = 1), Eastern Asia (n = 2), Western Asia (n = 2), and Oceania (n = 1).

3.2.1. Characteristics of the studied bioactive lipid classes

All the studies reported the effects of FA-based dietary interventions on circulating bioactive lipid levels, among other biochemical compounds, after lipidomic profiling. PL classes, including PCs, PEs and PIs, were assessed in 11 studies; lyso-PL classes, including lyso-PCs, lyso-PEs and lyso-PIs, in 14 studies; and SP classes, including Cer and SM, in 11 studies (***Table 2***). The PL, lyso-PL and SP molecular structures were diverse according to the configuration and the degree of saturation of their acyl chains (***Table 2***). Among the studied PL subclasses, those containing very long chain PUFAs (VLC-PUFAs) in the sn-2 position of the glycerol backbone were predominant. In the lyso-PLs, the subclasses containing long chain PUFAs (LC-PUFAs) were most abundant followed by MUFAs and SFAs in their acyl chains. However, among SPs, subclasses containing SFAs and MUFAs predominated (***Table 2***). In most cases, circulating bioactive lipids were extracted with added internal standards of common lipid subclasses and quantified using both targeted and untargeted mass spectrometry (MS) approaches, primarily liquid-phase separations coupled to MS (typically LC-MS). Then, they were characterized with data processing software, such as LipidView (v. 1.1), MATLAB (v. 7.2) or MZmine (v. 2.7). In addition, lipid databases, such as LipidBlast, Lipid MAPS, the Human Metabolome Database, METLIN or MassBank, were used for lipid identification.

Other intermediates, such as acylcarnitine (AC) and the enzyme Lp-PLA2, were assessed by immunoassay methods in 1 and 10 studies, respectively.

3.2.2. Characteristics of the studied fatty acid-based dietary interventions

The dietary interventions varied between studies, and FAs were administered in three different forms: as supplements (n = 12), EF-C (n = 9), or ED (n = 6) (***Table 2***).

Most interventions with supplements consisted of marine fish oil capsules providing n-3 PUFAs in the form of EPA+DHA with doses ranging from 0.6 to 5.56 g/day in the intervention groups (n = 9). Each capsule contained 105 to 465 mg EPA and 375 to 758 mg DHA. In the control groups, corn oil, soybean oil and olive oil were used as placebo. In 2 studies,[28,42] the n-3 EPA+DHA capsules were complexed

with olive oil. In addition, in 2 studies, n-3 PUFA supplementation was combined with standard statin [28] and aspirin [29] therapy. One study assessed n-3 PUFAs from flaxseed oil, providing ALA at doses of 0.57 g/day.[42] Alternatively, two studies administered high fat enteral supplements in the form of medium-chain fatty acids (MCFAs) [34] or long-chain triglyceride (LCT) fat emulsions.[46]

EF-C in the studies consisted of dietary fish (n = 2) or enriched plant oils (n = 3), spreads (n = 1), dairy products (n = 1) and cereal-based matrices (n = 2). Two studies assessed n-3 PUFA-rich oily fish, such as salmon, provided in diets ranging from 160 to 400 g per week (1.0 to 1.6 g/day of EPA+DHA).[18,27] One crossover study compared n-3 PUFA-enriched milk, providing 0.37 g/day EPA+DHA, with phytosterol-enriched milk.[43] In 6 studies, n-6 PUFAs from four different plant oils, including soybean oil,[26,41,48] sunflower oil,[45] rapeseed oil [38] and canola oil,[39] were provided as enriched oils or added into food matrices differing in their FA content, such as soy-based dairy, soy- and sunflower-based cereals, and rapeseed oil-based spread (***Table 2***).

The studies using ED included diets with controlled FA content (n = 6). In 5 studies, the dietary interventions were based on the Mediterranean diet, which is rich in fruits and vegetables (sources of fiber and polyphenol), MUFAs and PUFAs.[25,32,33,36,49] In 2 studies,[25,32] the RESMENA (Reduction of Metabolic Syndrome in Navarra) diet, a new dietary strategy for reducing MetS characterized by an increased meal frequency, high total antioxidant capacity and reinforced n-3 PUFA intake,[50] was used. Overall, the daily doses of PUFAs ranged from 0.4 to 6.8% of total energy intake (TEI) (***Table 2***). The control diets usually provided lower % TEI as PUFAs. The remaining study, instead of the Mediterranean diet, involved two postprandial test meals differing in their SFA content, including a high-saturated fat (24 g SFAs) vs. a high-carbohydrate meal (0 g SFAs).

3.3. Study quality and risk of bias

The risk-of-bias assessment of the 27 randomized clinical trials included in our analysis is shown in ***Supplemental Figure 1***. All included studies were randomized, although 14 studies did not report the methods used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups. In 15 studies, the methods used to conceal the allocation sequence were not properly described, and 3 studies did not ensure the adequate concealment of allocations before

assignment, supporting a selection bias. Blinding of both participants and personnel was performed correctly in 17 studies; however, 3 studies were susceptible, and 7 studies were at high risk of performance bias due to the open-label or single-blind design. Only 13 studies correctly blinded the outcome assessment. Incomplete outcome data were adequately addressed in 18 studies, considering overall losses to follow-up, drop-outs, and whether an "intention-to-treat" analysis was performed. No systemic differences were found in any of the included studies between planned and reported findings. However, we detected 10 studies that would be susceptible to measurement bias due to poor reliability of the diet monitoring during intervention. Overall, 12 studies were judged as "low risk", 2 studies as "high risk", and 13 studies as "unclear risk".

3.4. Results of individual studies and meta-analyses

3.4.1. Effects of dietary fatty acid-based interventions on bioactive lipid profiles in subjects with CVD and CVD risk factors

We systematically reviewed a total of 18 RCTs and 1 RT that contained data on dietary FA intake, including SFAs, MUFAs, PUFAs and TFAs, and circulating bioactive lipids, including PL, lyso-PL or SP classes and subclasses, and Lp-PLA2 enzymatic precursor, in subjects with CVD or CVD risk factors. The complete information for each study is summarized in ***Tables 3-8***. Additionally, a set of fixed-effects model meta-analyses were performed in 7 RCTs, which assessed the effects of PUFA-supplemented dietary interventions, to investigate at what point the published RCT data were sufficient to yield the same results as those found in the qualitative synthesis (***Figures 2-4***).

3.4.1.1. Overweight and obesity

Four RCTs and 1 RT, involving 248 subjects, reported the effect of FA-based dietary interventions on the bioactive lipid profile in overweight and obese individuals (***Table 3***).[19,25,26,43,45] The baseline body mass index (BMI) of the included individuals ranged from 25 to 40 kg/m^2. In 2 studies,[26,45] the effects of TFAs, SFAs and n-6 PUFAs from plant oils (partially hydrogenated soybean oil, palm oil and sunflower oil provided as EF-C in cereal-based matrices) on PC, lyso-PC, SM and Cer concentrations were reported after an overfeeding period. In detail, TFAs administered at daily doses of 15.7 g (26 g soybean oil) induced significant increases in serum PC-containing VLC-PUFAs, mainly PC (40:7), and

SM (36:3),[26] whereas the administration of SFAs, mostly in the form of palmitate, at daily doses of 21.4 g (40 g palm oil) induced significant increases in saturated- and monounsaturated-chain Cer and SM subclasses, including diCer (16:0), gluCer (16:0), and SM (16:0, 18:0, 16:1, 18:1).[45] In contrast, n-6 PUFA-rich oils at daily doses of 26.1 g (40 g sunflower oil) mediated a marked decrease in several saturated-chain serum Cer subclasses, including Cer, diCer, gluCer and lacCer (18:0, 20:0 and 24:1).[45] Both studies reported weight gain approximately 4 % after the overfeeding period, and it was preceded by a diet-based weight loss program.

In one RCT,[25] an overall decrease in serum concentrations of lyso-PC, lyso-PE and lyso-PI subclasses, which was statistically significant for lyso-PCs (14:0, 15:0, 16:1, 18:4, 20:4) (-0.10 to -5.5 μM, $P<0.05$) and lyso-PE (22:6) (-0.24 μM, $P=0.05$), was reported after an ED intervention with the RESMENA diet. In addition, positive relationships were established between decreased lyso-PCs and weight loss.

In two RCTs,[19,43] the effects of n-3 PUFA-supplemented dietary interventions were analyzed. n-3 PUFAs were administered as marine fish oil capsules or added to a milk matrix. The fixed-effects model meta-analysis found a significant increase in saturated lyso-PCs (16:0 and 18:0) [SMD (95% CI); 0.52 (0.02, 1.01) and 0.58 (0.09, 1.08) μM, respectively] with n-3 PUFAs supplemented at daily doses ranging from 0.37 to 1.9 g/day EPA+DHA (***Figure 2***). The heterogeneity between two RCTs was low ($I^2=0\%$, $P<0.05$). Given the lack of similarity between the lipid subclasses studied in overweight and obesity, it was not possible to include the rest of the subclasses in the meta-analysis, therefore, they were systematically reviewed (***Table 3***).

3.4.1.2. Dyslipidemia

Three RCTs involving 248 subjects and 6 experimental arms evaluated the effect of n-3 PUFA EF-C provided in a milk matrix, a plant oil-based spread or as oily fish, on the molar concentrations or percentage (%) of EPA and DHA-containing lyso-PLs on the serum LDL surface or in plasma lyso-PLs in dyslipidemia (***Table 4***).[27,38,43] Dyslipidemia was characterized by moderate to high serum LDL cholesterol (between 135 and 196 mg/dL) or TG (between 150 and 2000 mg/dL) and low levels of HDL cholesterol (between 33 and 65 mg/dL) at baseline. None of the subjects were under lipid-lowering therapy. The results showed a significant increase in the LDL molar concentrations of saturated-chain

lyso-PC(18:0) (+11 µg/100 mL, P=0.04) after 0.375 g/day EPA+DHA intake from enriched milk.[43] In addition, the molar % of LC- and VLC-PUFA-containing PC and lyso-PC subclasses, including lyso-PCs (18:2, 20:3, 20:4 and 20:5), were significantly increased (+0.01 to +0.17%, P<0.05 or less) after the intake of 20 g/day plant stanol ester-enriched rapeseed oil spread (STAEST) which provided 3.3 g n-6 LA and 1.3 g n-3 ALA.[38] In addition, LC- and VLC-PUFA-containing lyso-PCs were negatively associated with LDL aggregation susceptibility, especially in lean individuals.[38] Remarkably, the plasma % of VLC lyso-PCs (20:5 and 22:6), containing n-3 EPA and DHA, was significantly increased after oily fish interventions, including salmon, herring and pompano, at doses of 80 g, 5 days a week (1 to 1.6 g/day EPA+DHA) (+0.9 to +1.5%, P≤0.05 or less).[27] However, the plasma % of lyso-PCs (18:2 and 20:4), both containing n-6, was significantly decreased after oily fish interventions (-1.2 and -2.9%; P≤0.05, respectively). In two of the studies,[27,43] a variation in lipid profile, mainly a decrease in TG levels (from -16 to -52.2 mg/dL; P≤0.05), was reported after dietary interventions, especially in the oily fish experimental groups. Given the lack of similarity between interventions and the studied lipid subclasses, it was not possible to perform a meta-analysis for dyslipidemia and circulating bioactive lipids; thus, they were systematically reviewed (***Table 4***).

Additionally, in 4 RCTs involving 1015 dyslipidemic subjects and 7 experimental arms, the effects of n-3 PUFA supplements on Lp-PLA2 mass were analyzed (***Table 4***).[28–31] The n-3 PUFAs were provided in capsules as highly bioavailable forms of carboxylic acids (CA), acylglycerols (AG) or ethyl esters (EE). Across the studies, the fixed-effects model meta-analysis revealed a significant decrease in Lp-PLA2 plasma mass [SMD (95% CI); -0.36 (-0.47, -0.25) ng/mL] with daily doses ranging from 1 to 5.56 g of EPA+DHA (***Figure 3***). The heterogeneity between the 4 studies was low (I^2=0.2%, P<0.00001); therefore, the duration of the intervention (between 1 and 3 months) was not expected to influence the treatment effects. Also, a significant increase in LDL and a decrease in HDL particle sizes were reported after n-3 PUFA interventions compared to placebo.[28] Furthermore, a significant reduction (by -10.9%; P=0.01) in oxidized LDL cholesterol (ox-LDL) [29] and in non-fasting plasma TG levels (between -21.5 and -28%; P<0.05 or less) was documented in the n-3 PUFA treatments versus placebo.[30,31]

3.4.1.3. Metabolic syndrome

Three RCTs and 1 RT involving 237 subjects reported the effect of n-3 PUFA ED [25,32,33] or EF-C [48] on the circulating bioactive lipid profile in MetS (***Table 5***). The MetS features were visceral obesity, dyslipidemia, hyperglycemia and hypertension according to the International Diabetes Federation criteria.[51] In 3 RCTs,[25,32,33] the effects of n-3 PUFA-rich Mediterranean-based dietary interventions on PC, PE, lyso-PC and lyso-PE subclasses were tested. In detail, a short-term significant increase in the plasma levels of LC- and VLC-PUFA-containing bioactive lipids, including PC (38:4, 40:4, 40:6) ($P \leq 0.001$), PE (38:6, 40:4) ($P < 0.0005$), and lyso-PC (20:5) ($P < 0.0005$) subclasses, was observed after 2 months of n-3 PUFA ED providing 4.2 to 6.2% TEI as n-3. In particular, PC(38:4) was revealed as a discriminative marker between the RESMENA diet and the control diet at 2 months and was positively associated with a reduction in inflammation markers.[32] In the long term, the plasma levels of most of the saturated and unsaturated-chain PC, PE, lyso-PC and lyso-PE subclasses were drastically reduced after 6 months of intervention, except for some subclasses, such as lyso-PCs (16:0, 18:1, 20:5 and 22:6), the levels of which significantly increased ($P \leq 0.020$). In 2 studies, notable changes in MetS features were reported at the end of n-3 PUFA ED.[25,33] In particular, there was a significant reduction in fasting plasma TG levels (-15 to -38 mg/dL; $P < 0.05$ or less).

On the other hand, one acute postprandial RT reported opposite changes in PC, lyso-PC and SM subclasses, especially in those containing saturated chains, 4 hours after a dietary intervention based on high-fat breakfast meals providing dairy- and soy oil-based foods differing in their FA content (***Table 5***).[48] In detail, a significant decrease was shown in PC (28:0, 29:0, 30:0, 31:0, 32:0), lyso-PC (14:0) and SM (32:0, 34:0) levels 4 hours after soy oil-based meal (-7.1 to -15% median change, $P < 0.01$), while a significant increase was noted 4 hours after dairy-based meal (+4.6 to +43% median change, $P < 0.01$). In addition, mono- and polyunsaturated-chain PC, PE, PI and lyso-PE subclasses were significantly increased after the high saturated fat dairy-based meal ($P < 0.001$). Given the lack of similarity between the lipid subclasses studied in MetS, it was not possible to perform meta-analysis; thus, they were systematically reviewed (***Table 5***).

3.4.1.4. Type 2 diabetes

Two RCTs involving 86 subjects reported the effect of MCFA [34] and alpha lipoic acid [35] supplements on circulating bioactive lipid profiles and plasma Lp-PLA2 mass in T2D (***Table 6***). In both

384 studies the participants were non-insulin-dependent and met the diagnostic criteria for T2D according
385 to the American Diabetes Association.[52] The baseline fasting blood glucose (FBG) levels ranged from
386 121 to 165 mg/dL, and the HbA1c was between 6.2 and 8.3%. In the first RCT,[34] a significant decrease
387 was reported in monounsaturated and saturated-chain SM, Cer and AC subclasses (-0.01 to -0.3 μM,
388 P<0.01 or less) after the administration of an MCFA-rich diet (providing 33-34% SFAs and only 1-2%
389 PUFAs) compared to an LCFA-rich diet. No lipid subclasses showed difference after the LCFA diet.
390 Moreover, the decreases in several SM subclasses, including SM(14:0, 15:0, 16:0, 20:0, 21:0, 23:1),
391 were positively correlated with lower fasting plasma insulin and homeostatic model assessment-insulin
392 resistance (HOMA-IR) (P<0.05 or less).

393 In the second RCT,[35] a significant decrease in plasma Lp-PLA2 mass was shown (-16.52 ng/mL,
394 P=0.001) after a daily dose of 1200 mg alpha lipoic acid (***Table 6***). This decrease was related to a
395 significant reduction in the % of ApoB-associated Lp-PLA2 (P=0.001) and a significant increase in the
396 % of HDL-associated Lp-PLA2 (P=0.03) compared to placebo. Additionally, there was a positive
397 correlation between the reduction in the ox-LDL level and total plasma Lp-PLA2 mass in the alpha
398 lipoic acid experimental group. Given the lack of similarity between the lipid subclasses and enzymatic
399 precursors studied in T2D, it was not possible to perform a meta-analysis; thus, they were systematically
400 reviewed (***Table 6***).

401 ***3.4.1.5. Hypertension***

402 Two RCTs involving 391 subjects evaluated the effect of the Dietary Approaches to Stop
403 Hypertension (DASH) diet, which is widely recommended for CVD risk reduction,[36] and n-3 PUFA
404 supplements [37] on bioactive lipid concentrations and plasma Lp-PLA2 mass in hypertension (***Table 7***).
405 In both studies, the subjects presented grade 1 hypertension with baseline systolic BP mean values
406 ranging from 130 to 133 mm Hg and diastolic BP from 81 to 84 mm Hg. Between 52 and 85% of the
407 participants used antihypertensive medication. In the first RCT,[36] the DASH diet consisted of a high
408 intake of fruit, vegetables, and low-fat dairy products, providing a high amount of fiber and protein and
409 low amounts of saturated and monounsaturated fats (7.4% TEI as SFAs and 10.5% as MUFAs)
410 compared to the control diet (14.4% TEI as SFAs and 12.6% as MUFAs). The results showed the change
411 in a broad array of serum bioactive lipids significantly associated with the DASH diet, mainly MUFA-

and LC-PUFA-containing Cer, SM, PC, PE, lyso-PCs and lyso-PEs. Most of the lipid subclasses had negative correlation coefficients (β^2) ranging from -0.06 to -0.67, representing lower serum concentrations with the DASH diet compared to the control diet. Conversely, 3 bioactive lipids had positive correlation coefficients, and statistically significant differences were observed for VLC-PUFA-containing PC(40:6) and PE(40:6) ($\beta^2 > 0.15$, $P < 0.00001$), representing higher concentrations.

In the second RCT,[37] a decrease in plasma Lp-PLA2 mass was documented after n-3 PUFA supplementation at a daily dose of 3.36 g EPA+DHA; however, the change was not significant compared to placebo (-18.1 ng/mL, P=0.08) (***Table 7***). Furthermore, no significant changes in BP or arterial stiffness were reported. Given the lack of similarity between the lipid subclasses and enzymatic precursors studied in hypertension, it was not possible to perform a meta-analysis; thus, they were systematically reviewed (***Table 7***).

3.4.1.6. Stable coronary artery disease

Two RCTs involving 96 subjects assessed the effect of supplementation with n-3 and n-6 PUFAs [29,39] on the plasma Lp-PLA2 mass in stable CAD (***Table 8***). In the 2 RCTs, the participants were middle-aged and older adults with poor dietary habits. Subjects who were referred for coronary angiography or underwent percutaneous coronary intervention (PCI) were included. The majority of subjects received standard statin therapy. All participants received dietary advice on a heart-healthy diet at baseline. Significant changes in Lp-PLA2 mass were reported in subjects with stable CAD after n-3 PUFA [29] and n-6-rich canola oil [39] supplementation (***Table 8***). The fixed-effects model meta-analysis found a significant decrease in Lp-PLA2 mass [SMD (95% CI); -0.52 (-0.91, -0.12) ng/mL] after 4 to 6 weeks of n-3 PUFAs at daily doses of 1 g (0.84 g EPA+DHA) and n-6 PUFA-rich canola oil at daily doses of 25 mL (providing 7.3 g PUFAs) (***Figure 4***). The heterogeneity between the 2 studies was moderate (I_2=41%, P=0.01), which could be explained by the different administration forms. Moreover, treatment with n-3 PUFAs was suggested as an independent predictor of plasma Lp-PLA2 mass changes.[29]

3.4.2. Effects of dietary fatty acid-based interventions on bioactive lipid profiles in healthy subjects and comparison with subjects with CVD and with CVD risk factors

We systematically reviewed a total of 5 RCTs and 4 RTs that contained data on dietary FA intake, circulating bioactive lipids, and Lp-PLA2 enzymatic precursor in healthy subjects (***Table 9***) and, if possible, compared the changes with those found in subjects at risk for CVD and with established CVD. Additionally, a set of fixed-effects model meta-analyses were performed in 4 RCTs, which assessed the effects of n-3 PUFA-supplemented dietary interventions (***Figures 5; Supplemental Figure 2***).

3.4.2.1. Effects of enriched diets

Two crossover RTs,[47,49] involving 25 healthy individuals, reported the effect of short-term (4-day) [49] or postprandial (6-hour) [47] exposure to high saturated fat (HSF) meals compared to low saturated fat meals on lipemia (***Table 9***). The HSF meals were composed primarily of SFA-rich foods, such as heavy whipping cream and fast-food components (hamburgers, fries, frosted pop tarts), providing 24 to 44.7 g SFAs. Consumption of the HSF meals led to plasma HDL enrichment in PLs, particularly LC-PUFA-containing PC and PE subclasses, including PCs (32:2, 33:2, 34:2, 35:2, 35:3, 36:2, 36:3, 36:4, 38:4, 38:5) and PE (34:2, 36:2, 38:4), which were significantly increased in abundance (P from <0.05 to <0.0001). In contrast, PC-containing VLC-PUFAs, including PCs (40:6 and 40:7), tended to increase only after low saturated fat meals (P<0.05 and P=0.001, respectively). Additionally, the abundance of saturated-chain SM(14:0) was significantly elevated after HSF meals (P=0.013), whereas the abundance of polyunsaturated-chain SM (34:2 and 42:3) was reduced (P<0.05). These multiple changes in the plasma HDL lipidome of healthy subjects indicate that bioactive lipid FA composition, which is highly affected by dietary fats, is able to drastically remodel the composition of plasma HDL particles in the short term.

Overall, PL results, particularly the increased LC-PUFA-containing PCs in healthy subjects, went in the same direction as those previously reported in MetS subjects after a postprandial alteration induced by a high-fat, dairy-based breakfast meal.[48] Interestingly, PC (38:4), which was pointed out as a discriminative marker of a 6-month n-3 PUFA-enriched dietary intervention in MetS subjects,[32] was also significantly elevated in healthy subjects after HSF test meals (P≤0.001).

3.4.2.2. Effects of enriched food-components

Two studies,[18,41] including 159 healthy individuals, assessed the effect of PUFA EF-C, provided as n-3 PUFA-rich oily fish [18] and n-6 PUFA-rich soy oil,[41] on circulating bioactive lipids and Lp-PLA2 activity (***Table 9***). In the first study,[18] a crossover-design RT was used to assign two different doses of Atlantic salmon (90 g and 180 g) twice weekly. The results showed significant selective increases of plasma VLC-PUFAs-containing PC, PE and lyso-PE subclasses, including PCs (36:5, 38:5, 38:6, 40:6), PE (38:6, 40:6, 40:7), and lyso-PE (22:6) (+0.20 to +18.5 μM, P between <0.05 and <0.0001) after 8-week 180 g salmon intervention (6.15 g/day EPA+DHA). Moreover, PCs (38:6 and 40:6) were elevated in a dose-dependent manner in the same way reported in MetS subjects after a diet rich in LC-n-3 PUFAs.[33] Remarkably, for the n-3 EPA and DHA-containing PCs, 16:0, 18:0 and 18:1 were observed as the major paired complementary FAs (e.g., the forms of PCs (18:1_20:5, 16:0_22:6 and 18:0_22:6)). Compared to subjects at risk for CVD, especially dyslipidemic subjects,[27] healthy individuals did not show significant changes in VLC-PUFA-containing lyso-PCs (20:5 and 22:6) after inclusion of dietary salmon. However, lyso-PE (22:6) showed opposite results from obese subjects after an n-3 PUFA ED.[25]

In the second RCT,[41] a significant increase in Lp-PLA2 activity (+1 nmol/mL/min, P<0.01) was evident after the intake of high n-6 linoleic acid (LA)-enriched soy oil at daily doses of 9.9 g (54.2% LA). Moreover, changes in plasma LA positively correlated with changes in Lp-PLA2 activity. In parallel, the high LA experimental group showed higher levels of serum ApoB and plasma ox-LDL.

3.4.2.3 Effects of dietary supplements

Five studies involving 283 healthy adults evaluated the effect of a postprandial test meal [46] and marine [19,40,42,44] or plant-derived [42] n-3 PUFA supplements on circulating bioactive lipid profiles and plasma Lp-PLA2 mass (***Table 9***).

One RT [46] identified specific PE, lyso-PE and Cer subclasses that were modulated by a postprandial test meal that consisted of a 5-hour oral lipid tolerance test (OLTT) based on an LCT emulsion providing 96% of TEI as fat (31 g MUFAs and 16 g PUFAs). In particular, PE (36:2, 36:3), lyso-PEs (18:1, 18:2) and Cer (16:1) showed a fold change > 1.5 at 2 hours (P<0.05) and remained elevated for the duration of the OLTT. In addition, saturated lyso-PCs (16:0 and 18:0) had a negative fold change at 5 hours, while the unsaturated lyso-PCs (18:1, 18:2 and 20:4) had a positive fold change at the final time point

(***Table 9***). These results indicated that the dynamic changes in lyso-PCs highly depended on the saturation level of these lyso forms. Similarly, in a previous study in obese and normal-weight subjects,[19] a depletion in the plasma concentrations of saturated lyso-PCs (16:0 and 18:0) in response to a high-fat meal challenge (providing 85.8 g fat) was observed only in normal-weight subjects who had previously chronically consumed n-3 PUFAs through fish oil.

Two RCTs,[19,44] involving 3 experimental arms of n-3 PUFA marine fish oil supplements, analyzed acute [44] and sustained [19] changes in lyso-PC and lyso-PE concentrations (***Table 9***). The fixed-effects model meta-analysis demonstrated a marked decrease in saturated lyso-PCs (16:0 and 18:0) [SMD (95% CI); -0.19 (-0.53, 0.16) and -0.17 (-0.51, 0.18) μM, respectively] and unsaturated lyso-PCs (18:1, 18:2 and 20:4) [SMD (95%); -0.13 (-0.47, 0.21), -0.14 (-0.48, 0.20) and -0.18 (-0.53, 0.16) μM, respectively] with EPA+DHA doses ranging from 1.9 to 4 g/day, although the model did not show statistically significant differences in results (***Supplemental Figure 2***). Despite this, in the qualitative synthesis (***Table 9***), a significant increase was observed in EPA-containing lyso-PC (20:5) after acute EPA+DHA supplementation (+2.20 μM, P=0.002).[44] Additionally, DHA-containing lyso-PE (22:6) was significantly increased after n-6 PUFA-rich corn oil was provided as placebo at daily doses of 3 g (+0.10 μM, P<0.01).[19] Compared to subjects at risk for CVD, opposite results were found in saturated lyso-PCs (16:0 and 18:0). Whereas these lyso forms tended to decrease in healthy subjects, a significant increase was reported in subjects at risk for CVD, including subjects with obesity,[19,43] dyslipidemia [43] and MetS,[32] after receiving n-3 PUFA EF-C, ED and supplements for 1 to 6 months. Nevertheless, VLC-PUFA-containing lyso-PCs, particularly lyso-PCs (20:5 and 22:6), remained elevated in both subjects at risk for CVD and healthy subjects after both n-3 and n-6 PUFA dietary interventions.

In the other 2 RCTs,[40,42] with 5 experimental arms of n-3 PUFA-enriched marine or plant oil supplements, a significant decrease was found in plasma Lp-PLA2 mass when fixed-effects model meta-analysis was performed [SMD (95% CI); -0.35 (-0.59, -0.10) ng/mL] (***Figure 5***). The effects were noted with daily doses ranging from 0.6 to 2 g of marine EPA and DHA, in combination or by themselves, or ALA from flaxseed oil at daily doses of 0.57 g, for a period of 1.5 to 2 months. The heterogeneity between the 2 studies was low (I^2=19%, P=0.006). In addition, a dose-concordant effect on plasma Lp-PLA2 mass was demonstrated with EPA administered alone (600 to 1800 mg).[40] Also, increases in ox-

519 LDL were positively associated with increases in Lp-PLA2 mass (P<0.01).[42] These effects of n-3 PUFAs
520 on plasma Lp-PLA2 mass were concordant with those in dyslipidemic subjects.

521 **4. DISCUSSION**

522 The extent to which the dietary intake of FAs modulates the circulating bioactive lipidome through
523 an enrichment effect and whether circulating bioactive lipids could be mediators of health, CVD or CVD
524 risk factors remain a gap in knowledge. Our systematic review and meta-analysis have collected a large
525 body of evidence about the effects of major dietary FAs, encompassing different food sources and
526 administration forms, on the circulating bioactive lipid profile of healthy subjects and those with CVD
527 and CVD risk factors. Overall, we analyzed 21 RCTs and 6 RTs which covered 11 bioactive lipid classes
528 representing more than 150 lipid subclasses differing in their fatty acyl chains, including LC-PUFA-,
529 VLC-PUFA-, MUFA-, SFA- and TFA-containing bioactive lipids. The results showed great versatility
530 in biochemical structures of bioactive lipids according to the type of FA intervention, which reflects a
531 highly dynamic lipidome with both concordant and discordant findings between healthy subjects and
532 those with CVD or CVD risk factors.

533 The most direct effects on circulating bioactive lipids were attributed to the increased dietary intake
534 of n-3 PUFAs, provided as marine fish or plant oil supplements; EF-C, including oily fish; and n-3
535 enriched Mediterranean-based diets. To a lesser extent, other interventions included n-6 PUFAs as EF-
536 Cs in the form of plant oils, as well as SFAs and TFAs, which predominated in HSF test meals.

537 Our set of meta-analyses of RCTs found consistent changes, especially in subjects with CVD risk
538 factors. Notably, we documented in obese subjects an approximately 0.50 μM net increase in plasma
539 concentrations of saturated-chain lyso-PCs (16:0 and 18:0) with n-3 PUFA supplementation at daily
540 doses ranging from 0.37 to 1.9 g EPA+DHA.[19,43] These increases were also reported in subjects with
541 dyslipidemia and MetS. Nevertheless, the results were opposite in healthy subjects, showing a
542 decreasing trend. Both lyso-PCs (16:0 and 18:0), which contain palmitic and stearic acid, have been
543 recognized as major components of ox-LDL with potent pro-inflammatory activities, such as eosinophil
544 adhesion, neutrophil priming, and cytokine secretion.[43,53] Their increased levels in obesity could be
545 mediated by the state of low-grade chronic inflammation [54]; but could also reflect a decrease in lyso-PC
546 catabolism under altered cardiometabolic conditions. As previously reported,[19] obesity may impair the

response of lyso-PL metabolism to n-3 PUFAs. Unlike the healthy state, where free n-3 PUFAs from enriched diets can remodel the fatty acyl moieties of saturated PCs and lyso-PCs by incorporation of EPA and DHA into lipid pools,[16,55] this transformation process may not operate in obesity. However, there are still certain discrepancies regarding the pro-inflammatory or anti-inflammatory effects of saturated lyso-PCs, as reported in a recent review.[10] Thus, we cannot draw firm conclusions about the effect of n-3 PUFAs on these outcomes.

Concomitant with increased saturated lyso-PCs, in subjects with CVD risk factors, particularly in dyslipidemia and MetS, high n-3 PUFA EF-C, including oily fish [27] and ALA-rich foods,[38] resulted in long-term (2 to 6 months) increases in molar % on the serum LDL surface or the % of EPA- and DHA-containing lyso-PCs (20:5 and 22:6) in the plasma. Greater effects were observed in dyslipidemic subjects when they consumed oily fish at doses of at least 400 g per week (providing 1 to 1.6 g EPA+DHA),[27] constituting approximately one to twice as much as the mean recommended intake for fish in European countries.[56] In parallel, n-3 PUFA ED decreased serum TG levels, among other MetS features, and reduced LDL aggregation susceptibility. In agreement with these results, previous studies suggested that fish oil supplementation could alter the lipid metabolism of lyso-PLs, increasing the proportion of VLC-PUFA-containing lyso-PLs.[57] Moreover, polyunsaturated lyso-PCs promote anti-inflammatory and anti-atherogenic actions against saturated lyso-PC-induced effects.[53] Thus, the dietary inclusion of oily fish at high doses improves the FA profile of lyso-PCs on the serum LDL surface and in the plasma. Likewise, EPA- and DHA-containing lyso-PCs constitute promising bioactive lipids in the management of subjects at risk of dyslipidemia, and the changes in the LDL lipidome indicate reduced atherogenic effects.

The beneficial effects of n-3 PUFAs on the bioactive lipid profile may be related in part to the lowering of the Lp-PLA2 enzymatic precursor. Concordant decreases in plasma Lp-PLA2 mass of approximately -0.35 ng/mL were found in both dyslipidemic and healthy subjects in our meta-analysis. Additionally, in stable CAD, the meta-analysis showed more pronounced effects, reaching decreases of -0.52 ng/mL. Although these effects were typically found with daily n-3 PUFA doses ranging from 1 to 5.56 g EPA+DHA, in two studies, the highest effects were specific for EPA supplementation,[31,40] which indicated an independent effect. In contrast, n-6 LA provided as EF-C increased Lp-PLA2 activity in healthy subjects,[41] which indicated a detrimental effect compared to n-3 supplements. In agreement with

these results, a recent meta-analysis of 15 prospective studies showed that a higher plasma Lp-PLA2 mass was independently associated with an increased risk of CVD events in patients with stable CAD.[58] Despite this, the effects of n-3 PUFAs on plasma Lp-PLA2 mass have not been fully reviewed to date. n-3 PUFA treatment is the only lipid-altering therapy identified thus far that lowers plasma Lp-PLA2 mass without lowering LDL cholesterol; however, the mechanisms by which n-3 PUFAs decrease Lp-PLA2 mass are unclear. Since Lp-PLA2 is a known vascular-specific inflammation marker,[14] it is quite likely that the greatest benefit of dietary n-3 against CVD risk may be related to its effects on decreasing the body's inflammatory responses, including the reduction of pro-inflammatory lyso-PCs. Additionally, the capacity of n-3 PUFAs to lower ox-LDL might reduce the substrate for Lp-PLA2, which is another potential mechanism. In this regard, the reported decreases in Lp-PLA2 mass after alpha lipoic acid supplementation in T2D subjects might result from its capacity to lower ox-LDL serum levels.[35] As previously reported, alpha lipoic acid, an essential cofactor of dehydrogenase enzymes, has antioxidant characteristics and easily reaches to the cardiovascular system.[59]

Among the bioactive lipid categories identified in our qualitative synthesis, PLs and SPs, particularly PC, SM and Cer classes, were the most highly represented, with 34, 41 and 29 different subclasses, respectively. Importantly, the major changes found in these lipid classes were in the postprandial period after HSF test meals. Throughout the day, people are primarily in a postprandial state; thus, fasting bioactive lipid levels may not always reflect the risk of CVDs. In this sense, the postprandial response of circulating bioactive lipids after test meals may reveal more accurate information on the effects of dietary FAs than the fasting state in sustained interventions.[60] From our results, there was a wide range of net effects found in circulating PC subclasses, particularly those containing LC- and VLC-PUFAs and fewer double bonds, such as PCs (36:2, 36:3, 36:4, 36:5, 38:4, 38:5, 38:6, 40:6, 40:7). These effects highly depended on the dietary source of the FAs and were concordant among healthy subjects and those with CVD risk factors. Whereas a significant increase was found in unsaturated PCs after SFA- and TFA-rich sources, including partially hydrogenated soybean oil,[26] whole dairy-based meals,[48] and fast food-based meals,[47,49] a generalized decrease was reported after high-n-3 PUFA ED,[32,33,49] with the exception of PCs(38:4, 38:6, 40:4 and 40:6), which remained increased after both n-3 PUFA and oily fish interventions, especially in the long term (1 to 6 months). These effects were especially noticeable with postprandial (4- to 6-hours) or short-term (4-day) doses

of SFAs ranging from 24 to 67 g; daily doses of TFAs of 15.7 g; and PUFAs representing from 4.2 to 6.2% TEI in EDs. In addition, significant effects were noted in the plasma HDL PC content, as HDL particles usually have the highest concentrations of PLs. n-3 LC-PUFA levels in plasma PLs have been suggested as potential biomarkers of disease.[18] In agreement with a previous dose-response study,[16] our PL results reported here indicate that the PL-FA content exhibits increased unsaturation following SFAs and TFAs compared to n-3 PUFA dietary interventions. These effects could be explained by a general upregulation in the formation of LC- and VLC-PUFA-containing PLs through a preferential integration of SFAs and TFAs into the sn-1 position of PCs, all containing PUFAs in the sn-2 position.[26] This structural reconfiguration of PCs after SFAs and TFAs leads to shorter and fewer double bonded forms than n-3 PUFAs, which comprise longer and double bonded forms. Therefore, the inclusion of an SFA at the sn-1 position confers a more condensed shape to the PC molecule, which may influence the distribution of n-3 PUFAs in lipid rafts.[61] These effects may contribute to the regulation of cholesterol homeostasis, mainly LDL cholesterol increases, as PL-containing TFAs and SFAs behave similarly to free SFAs.[26,62] Conversely, the selective increases of DHA-containing PCs (38:6 and 40:6) with n-3 PUFA ED may support a protective role against CVDs, as the negative associations have shown in large prospective studies.[63] Additionally, higher PC (38:4), which contains arachidonic acid (ARA), was positively associated with a reduction in inflammation markers in MetS subjects following n-3 PUFA ED.[32] Thus, these findings suggest that high n-3 PUFA ED could mediate a protective response against CVD risk by increasing select VLC-PUFA-containing PCs. Despite this, more studies are needed to resolve discrepancies over the divergent effects of the different dietary FAs on PLs and to determine whether their changes could be influenced by the duration of the intervention.

The effects of SFAs on circulating SM and Cer classes were, for the most part, consistent with those reported for PCs, especially in subjects with CVD risk factors. The most notable changes were found in saturated- and monounsaturated-chain SM and Cer subclasses, most frequently in SM (14:0, 15:0, 16:0, 18:0, 32:0, 34:0, 16:1, 18:1) and Cer (16:0, 18:0, 20:0, 24:1), after receiving HSF postprandial test meals with over 40 g SFAs.[45,48] Interestingly, significant increases were reported following SFA EF-C, while a decrease was evident after n-6 PUFA EF-C. Both bioactive lipids, Cer and SM, are important signaling molecules linked with inflammation, which plays critical roles in the pathogenesis of CVDs.[64] As recently reviewed, serum Cer levels are accurate biomarkers of adverse

CVD outcomes.[64] Our reported effects are similar to those found in a recent large-scale lipidomic analysis where higher saturated fat intake, in contrast to a diet rich in polyunsaturated fat, was associated with higher concentrations of saturated- and monounsaturated-chain SPs.[65] On the other hand, in with T2D, the reported decreases in both saturated SM and Cer following an MCFA-rich diet support a positive effect against the progression of insulin resistance.[34] Nonetheless, plasma SP concentrations were not affected in any of the n-3 PUFA dietary interventions.

Among the less frequent classes of circulating bioactive lipids in our qualitative synthesis, lyso-PE, lyso-PI, PE and PI showed the most characteristic behaviors. Specifically, lyso-PEs and lyso-PIs, which encompass a smaller head group than lyso-PCs, were present at lower levels in circulation. Plasma levels of VLC-PUFA-containing lyso-PE subclasses, including lyso-PEs (20:4 and 22:6), have been elevated approximately threefold in subjects with acute coronary syndrome.[66] However, as reported in lyso-PCs, they have also been shown to attenuate the inflammatory response.[67] Our findings documented discordant effects of n-3 PUFAs, n-6 PUFAs and SFAs on lyso-PEs (20:4 and 22:6) among subjects with CVD risk and healthy subjects. Whereas lyso-PE (22:6) significantly increased in healthy subjects following n-6 PUFAs and n-3 PUFAs-rich oily fish interventions,[18,19] it was decreased in obese subjects after n-3 PUFA ED.[25] However, lyso-PE (20:4) was only increased after n-6 PUFAs in obese subjects. These findings provide further insights into the versatile responses of bioactive lyso-PLs to dietary FAs according to the cardiometabolic state.

The assessment of HDL and LDL lipidome could be an effective approach to identify and characterize new biomarkers related to lipid metabolism. In 3 and 2 of our reviewed studies, bioactive lipid concentrations were determined on the HDL and LDL particle surfaces, respectively, after different FA interventions. In particular, significant increases were reported in unsaturated PCs on the plasma HDL surface following HSF meals. As recently reported in hypercholesterolemic subjects, the inclusion of dietary FAs, mainly MUFAs, modulates HDL function through dysregulations of unsaturated bioactive lipid levels, including increased PCs.[68] Similarly, increases in HDL unsaturated PCs in our report indicate that SFAs may remodel the composition and function of HDL particles. In this context, although similar patterns of unsaturated PCs were reported in the total plasma and HDL fraction in one of our reviewed studies,[33] we cannot rule out a reporting bias in studies measuring changes only in the HDL fraction lipidome.

Importantly, in some of the FA-supplementation studies, n-3 PUFAs were complexed with CA.[28,29] Unlike EE forms (e.g., Lovaza), n-3-CA contains free FAs and does not require hydrolysis by lipase before they can be absorbed.[69] Thus, these formulations are expected to improve the systematic bioavailability of n-3 PUFAs. Moreover, the combination of n-3-CA with 2 g olive oil appeared to be more effective in reducing Lp-PLA2 mass in dyslipidemic subjects. This could be explained by a beneficial synergistic effect of n-3 and olive oil on lipid metabolism and oxidative stress, as previously reported in MetS.[70] Despite these improved formulations, as foods are mixtures of different nutrients and other components, it is worth noting that dietary interventions involving FA EF-C and ED might be influenced by other compounds capable of interfering with n-3 absorption from food sources. For instance, in one of our reviewed RCTs,[38] the inclusion of a plant stanol-enriched rapeseed oil might have exerted a substantial matrix effect mediated by phytosterols rather than n-6 and n-3 PUFAs. Hence, although our results highlight the clear effects of different sources of FAs on bioactive lipid metabolism, the lack of adjustment for multiple testing of the included studies may engender an overestimation of the observed effects. Therefore, the results concerning single bioactive lipid subclasses should be interpreted with caution.

To our knowledge, the circulating lipidome has not been extensively studied in relation to bioactive lipid classes and subclasses. Overall, the results from our systematic review and meta-analysis offer for the first time an overview of the effects of dietary FAs on individual bioactive lipids. Notably, marine n-3 PUFAs, provided either as supplements or as oily fish, at doses between 0.30 and 3.4 g/day EPA+DHA induced the most marked changes in circulating bioactive lipid profile in subjects with CVD risk factors. Additionally, EPA+DHA in doses between 0.6 and 5.56 g/day decreased plasma Lp-PLA2 mass in healthy subjects, those with CVD and those with CVD risk factors. These EPA+DHA doses are greater than the last recommended doses of the European Food Safety Authority (EFSA) in 2012 based on cardiovascular risk considerations for European adults, which range from 250 to 500 mg/day of combined EPA+DHA.[71] According to the 2000 AHA Dietary Guidelines, although the ideal amount of EPA+DHA intake is not firmly established, evidence from prospective secondary intervention studies suggests that intakes of EPA+DHA ranging from 0.5 to 1.8 g/day (either as oily fish or supplements) significantly reduce the number of deaths from heart disease and all causes.[72]

The application of targeted and untargeted high-throughput lipidomics analysis by quantitative MS approaches, such as separation or direct infusion-MS, has allowed the characterization of a wide set of bioactive lipid moieties. Furthermore, the inclusion of the molecular formula provides information on the degree of complexity of the fatty acyl chains. Although our review focuses on the most prevalent bioactive lipid categories, future research should include other emergent bioactive lipid intermediates, such as lysophosphatidic acid, that have been shown to play an important role in the initiation and progression of CVDs.[73]

Our review and the studies included in our analysis had several limitations that need to be highlighted. First, several RCTs had small sample sizes and varied significantly in terms of design, duration, FA administration forms and dosages, which made it difficult to pool all results in a meta-analysis. Second, the smaller number of studies included for certain CVD risk factors, such as T2D and hypertension, limited the establishment of rigorous conclusions. Third, the significant estimated effects could be influenced by potential confounders such as sex or ethnicity, since these factors could produce between-subject variations in circulating bioactive lipids.[74] Fourth, the choice of olive oil as a placebo in some studies may have had non-neutral effects on bioactive lipids. Fifth, regarding CVD risk factors, a large number of the study subjects were receiving statin therapy, which could limit the ability to discern a treatment effect.

5. CONCLUSIONS

The results from the present systematic review and meta-analysis support the conclusion that marine n-3 PUFA supplementation, provided as EPA+DHA at doses between 1 and 5.56 g/day and consumed for 1 to 6 months, reduce plasma Lp-PLA2 mass in healthy subjects and subjects with CVD and CVD risk factors, suggesting an anti-inflammatory effect. Decrease in Lp-PLA2 mass was more prominent in healthy, dyslipidemic and stable CAD subjects, which may signify improved circulating bioactive lipid profiles by lowering pro-inflammatory lyso-PC synthesis. However, further investigation is required to elucidate the exact mechanisms by which n-3 PUFAs decrease Lp-PLA2.

From the meta-analysis of RCTs, the most consistent effects are on plasma pro-inflammatory lyso-PC (16:0 and 18:0) which is increased in obese subjects after supplementation of 0.37 to 1.9 g/day EPA+DHA, and this observation suggests an impaired saturated lyso-PCs response to n-3 PUFAs. From

the systematic review, we found that the dietary intake of n-3 PUFA-rich oily fish at weekly doses of at least 400 g increased plasma EPA- and DHA-containing lyso-PC (20:5 and 22:6) in dyslipidemic subjects, reinforcing the promising role of unsaturated lyso-PCs in the management of subjects at risk for dyslipidemia.

Larger meta-analyses with high-quality RCTs are required to confirm the results and further determine the optimal dose of n-3 PUFAs and the length of treatment duration. Lastly, whether the identified bioactive lipid moieties are involved in biological pathways of metabolic risk is unclear and warrants further lipidomic research.

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Author contributions

The author's responsibilities during the research were as follows –LC-P, RMV and AP: Conceptualization, Methodology; LC-P and JC: Investigation; LC-P: Data curation, Formal analysis, Writing original draft, Visualization; RMV, AP, JC and RS: Review, Editing the original draft; RMV, AP and RS: Supervision. All authors have read and approved the submitted version of the manuscript and agree to be personally accounted for by the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated, solved, and documented. The NFOC-Salut group is a consolidated research group of Generalitat de Catalunya, Spain (2017 SGR522).

Declaration of interests

The authors report there are no competing interests to declare.

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743 **Supporting information**

744 **Supplemental File 1 |** Search strategy.

745 **Supplemental File 2 |** PRISMA 2020 Checklist.

746 **Supplemental Figure 1 |** Risk of bias summary: review authors' judgements about each risk of bias
747 item for each included study.

748 **Supplemental Figure 2 |** Meta-analysis of RCTs of the effect of n-3 PUFA supplemented dietary
749 interventions on saturated and unsaturated Lyso-PC levels in healthy subjects.

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Figure legends

Figure 1 | PRISMA 2020 flow diagram of the systematic review and meta-analysis. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

Figure 2 | Meta-analysis of RCTs of the effect of n-3 PUFA-supplemented dietary interventions on saturated lyso-PC levels in overweight or obese subjects. The squares and bars estimate the net standard mean difference (SMD) (difference between the end and baseline concentrations of bioactive lipid subclasses) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. Lyso-PC, lysophosphatidylcholine; n-3, omega-3 polyunsaturated fatty acids.

Figure 3 | Meta-analysis of RCTSs of the effect of n-3 PUFA supplemented dietary interventions on Lp-PLA2 mass in subjects with dyslipidemia. The squares and bars estimate the net standard mean difference (SMD) (difference in Lp-PLA2 concentrations compared to placebo) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. n-3, omega-3 polyunsaturated fatty acids.

Figure 4 | Meta-analysis of RCTs of the effect of PUFA supplemented dietary interventions (n-3 and n-6) on Lp-PLA2 mass in subjects with stable CAD. The squares and bars estimate of the net standard mean difference (SMD) (difference between the end and baseline concentrations of Lp-PLA2) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. n-3, omega-3 polyunsaturated fatty acids; n-6-, omega-6 polyunsaturated fatty acids.

Figure 5 | Meta-analysis of RCTs of the effect of n-3 PUFA supplemented dietary interventions on Lp-PLA2 mass in healthy subjects. The squares and bars estimate the net standard mean difference (SMD) (difference between the end and baseline concentrations of Lp-PLA2) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3, omega-3 polyunsaturated fatty acids.

Table legends

Table 1 | PICOS criteria for inclusion and exclusion of studies.

Table 2 | Summary of evaluated evidence for the effect of fatty acid-based dietary interventions on circulating bioactive lipid levels.

Table 3 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in overweight or obese subjects.

1014 **Table 4** | Description of included studies assessing the effects of fatty acid-based interventions on
1015 bioactive lipid levels in subjects with dyslipidemia.
1016 **Table 5** | Description of included studies assessing the effects of fatty acid-based interventions on
1017 bioactive lipid levels in subjects with metabolic syndrome.
1018 **Table 6** | Description of included studies assessing the effects of fatty acid-based interventions on
1019 bioactive lipid levels in subjects with type 2 diabetes.
1020 **Table 7** | Description of included studies assessing the effects of fatty acid-based interventions on
1021 bioactive lipid levels in subjects with hypertension.
1022 **Table 8** | Description of included studies assessing the effects of fatty acid-based interventions on
1023 bioactive lipid levels in subjects with stable coronary artery disease.
1024 **Table 9** | Description of included studies assessing the effects of fatty acid-based interventions on
1025 bioactive lipid levels in healthy subjects.

TABLE 1 | PICOS criteria for inclusion and exclusion of studies

Criteria	Inclusion reason	Exclusion reason
Population	- Adult humans (age 18 years or older) - All sexes and races - Healthy subjects, or subjects with associated cardiovascular or cardiometabolic risk factors (type 2 diabetes, obesity, overweight, dyslipidemia, metabolic syndrome, hypertension), or with established CVD (stable CAD)	- Pregnant women - Bariatric surgery - Acute coronary syndrome - Obstructive sleep apnea syndrome - Non-alcoholic fatty liver disease - Chronic kidney disease - Renal disease - Prediabetes - Diabetic nephropathy - Prehypertension - Fabry's disease - Alzheimer's disease
Intervention	- Studies involving fatty acid-based dietary interventions. - Any fatty acid type is allowed (SFA, PUFA, MUFA, TFA) - Fatty acids administered as supplements (capsule), or enriched food components (dairy- and cereal-based or oily matrixes) and enriched diets - Sustained, postprandial or short term interventions	- Studies not involving fatty acid-based dietary interventions - Studies assessing the effects of pharmacological treatment only. E.g., statin, insulin sensitizers, Lp-PLA2 inhibitors, ACE inhibitors - Pharmacological and medicinal plant interventions
Comparison	- Placebo capsule - Control arms with dietary components, meals or diets	
Outcomes	- Differences in bioactive lipid levels in terms of lipid class (PC, PE, PI, lyso-PC, lyso-PE, lyso-PI, Cer, diCer, gluCer, lacCer, SM, AC), in terms of lipid subclass (particular bioactive lipids conformed by monounsaturated, polyunsaturated or saturated acyl chains), or in terms of enzymatic precursor, after exposure to dietary fatty acids in healthy subjects or subjects with CVD or different cardiovascular/cardiometabolic risk factors	- Studies that not specify bioactive lipids class or subclass
Study design	- Randomized clinical trials involving controlled and not controlled interventions (RCTs and RTs) - Parallel or crossover design	- Non-randomized clinical trials - Systematic reviews - Meta-analysis - Case-control studies - Cohort studies
Meta-analysis	- RCTs providing the net difference (molar concentration or abundance) of circulating bioactive lipid or enzymatic precursors after fatty acid intervention compared to placebo or controlled group - Supplemented fatty acid interventions - At least 2 studies for each bioactive lipid or enzymatic precursor in the different clustered cardiovascular/ cardiometabolic risk factors or in healthy subjects*	- RCTs or RTs not providing the mean difference, SD or SE values - RCTs or RTs involving interventions with hypocaloric or fatty acid-enriched diets

Abbreviations: ACE, angiotensin-converting enzyme; AC, acylcarnitines; CAD, coronary artery disease; Cer, ceramides; CVD, cardiovascular disease; diCer, dihydroceramides; gluCer, glucosylceramides; lacCer, lactosylceramides; Lp-PLA2, lipoprotein-associated phospholipase A2; lyso-PC, lysophosphatidylcholines; lyso-PE, lysophosphatidylethanolamines; lyso-PI, lysophosphatidylinositols; MUFA, monounsaturated fatty acids; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PI, phosphatidylinositols; PUFA, polyunsaturated fatty acids; RCTs, randomized controlled trials; RTs, randomized trials; SD, standard deviation; SE, standard error; SFA, saturated fatty acids; SM, sphingomyelin; TFA, trans fatty acids. *Meta-analysis could be performed minimally with two RCTs whenever those two can be meaningfully pooled and provided their results as sufficiently similar.

TABLE 2 | Summary of evaluated evidence for the effect of fatty acid-based dietary interventions on circulating bioactive lipid levels

Outcome	No. of randomized trials	Fatty acid intervention (n)			Dose range according to fatty acid intervention	Study subjects, n *(%)*		Overall study quality (n)		
		S	EF-C	ED		Healthy	CVD risk factors	Low	High	Unclear
Phosphatidylcholines, PCs										
All subclasses	11	1	6	4	**S:** SFA, 7 g/d; MUFA, 3 g/d; PUFA, 16 g/d **EF-C:** TFA, 15.7 g/d; n-3 EPA+DHA, 0.3-0.6 g/d; n-6 LA, 3.3 g/d; n-3 ALA, 1.3 g/d **ED:** PUFA, 3.3-7.6% TEI; 1.5-24g/d MUFA, 10.5 % TEI; 1.8-40 g/d SFA, 7.4 % TEI; 13-67 g/d	74 *(9.9%)*	674 *(90.1%)*	4	1	6
[a] *Containing VLC-PUFA*	*11*									
[c] *Containing MUFA*	*6*									
[d] *Containing SFA*	*4*									
Phosphatidylethanolamines, PEs										
All subclasses	7	1	2	4	**S:** SFA, 7 g/d; MUFA, 3 g/d; PUFA, 16 g/d **EF-C:** PUFA, 5-24 g/d; MUFA, 23-40 g/d; SFA, 37-67 g/d; n-3 EPA+DHA, 0.30-0.61 g/d **ED:** PUFA, 3.3-7.6 % TEI; 3.2-15.6 g/d; MUFA, 10.5 % TEI; 1.8-41 g/d; SFA, 7.4 % TEI; 13-44.6 g/d	59 *(10.5%)*	500 *(89.5%)*	1	1	5
[a] *Containing VLC-PUFA*	*6*									
[c] *Containing MUFA*	*3*									
[d] *Containing SFA*	*1*									
Phosphatidylinositols, PIs										
All subclasses	1	0	1	0	**EF-C:** PUFA, 5-24 g/d; MUFA, 23-40 g/d; SFA, 37-67 g/d	0 *(0%)*	21 *(100%)*	0	0	1
[a] *Containing VLC-PUFA*	*1*									
[c] *Containing MUFA*	*1*									
Lysophosphatidylcholines, Lyso-PCs										
All subclasses	14	3	6	5	**S:** n-3 EPA+DHA, 1.9-3.4 g/d; SFA, 7 g/d; MUFA, 31 g/d;	165 *(16.7%)*	823 *(83.3%)*	5	1	8
[a] *Containing VLC-PUFA*	*3*									

[b] *Containing LC-PUFA*	*11*				PUFA, 16 g/d **EF-C:** TFA, 15.7 g/d; n-3 EPA+DHA, 0.3-0.6 g/d; PUFA, 17.6-24 g/d; n-3 PUFA, 2.1-3.2 g/d MUFA, 23-40 g/d; SFA, 37-67 g/d; n-6 LA, 3.3 g/d; n-3 ALA, 1.3 g/d **ED:** PUFA, 0.4-7.6 % TEI; MUFA, 10.5 % TEI; SFA, 7.4 % TEI					
[c] *Containing MUFA*	*11*									
[d] *Containing SFA*	*11*									
Lysophosphatidylethanolamines, Lyso-PEs										
All subclasses	7	2	2	3	**S:** n-3 EPA+DHA, 1.9 g/d **EF-C:** n-3 EPA+DHA, 1-1.6 g/d; PUFA, 5-24 g/d; MUFA, 23-40 g/d; SFA, 37-67 g/d **ED:** PUFA, 0.4-7.6 % TEI; MUFA, 10.5 % TEI; SFA, 7.4 % TEI	49 *(8.5%)*	526 *(91.5%)*	2	1	4
[a] *Containing VLC-PUFA*	*5*									
[b] *Containing LC-PUFA*	*5*									
[c] *Containing MUFA*	*4*									
[d] *Containing SFA*	*6*									
Lysophosphatidylinositols, Lyso-PIs										
All subclasses	2	1	0	1	**S:** n-3 EPA+DHA, 1.9 g/d **ED:** PUFA, 0.4-6.2 % TEI	0 *(0%)*	104 *(100%)*	1	0	1
[c] *Containing MUFA*	*2*									
[d] *Containing SFA*	*2*									
Ceramide, Cer										
All subclasses	7	2	2	3	**S:** PUFA, 16 g/d; MUFA, 31 g/d; SFA, 7 g/d; SFA, 33-34 % as MCFA **EF-C:** n-6 PUFA, 8.9-64.5 % of FC; PUFA, 5-24 g/d; MUFA, 23-40 g/d; SFA, 37-67 g/d **ED:** PUFA, 1.5-15.6 g/d; 7.6 % TEI; MUFA, 1.8-41 g/d; 10.5 % TEI; SFA, 13-44.6 g/d; 7.4 % TEI	65 *(13.2%)*	426 *(86.8%)*	2	0	5
[a] *Containing VLC-PUFA*	*1*									
[b] *Containing LC-PUFA*	*3*									
[c] *Containing MUFA*	*4*									
[d] *Containing SFA*	*6*									
Sphingomyelin, SM										
All subclasses	11	2	5	4	**S:** PUFA, 16 g/d; MUFA, 31 g/d; SFA, 7 g/d;	74 *(12.7%)*	510 *(87.3%)*	5	0	6
[b] *Containing LC-PUFA*	*3*									

[c] *Containing MUFA*	*8*				SFA, 33-34 % as MCFA **EF-C:** TFA, 15.7 g/d; n-3 EPA+DHA, 0.3-0.6 g/d; n-6 PUFA, 8.9-64.5 % of FC; PUFA, 5-24 g/d; MUFA, 23-40 g/d; SFA, 37-67 g/d; n-6 LA, 3.3 g/d; n-3 ALA, 1.3 g/d **ED:** PUFA, 1.5-15.6 g/d; 7.6 % TEI; MUFA, 1.8-41 g/d; 10.5 % TEI; SFA, 13-44 g/d; 7.4 % TEI					
[d] *Containing SFA*	*9*									
Acylcarnitine, AC										
All subclasses	1	1	0	0	**S:** SFAs, 33-34 % as MCFAs	0 *(0%)*	16 *(100%)*	0	0	1
[b] *Containing LC-PUFA*	*1*									
[d] *Containing SFA*	*1*									
Lipoprotein-associated phospholipase A2, Lp-PLA2										
	10	8	2	0	**S:** n-3 EPA+DHA, 0.6-5.56 g/d; n-3 ALA, 0.57 g/d Alpha lipoic acid, 1.2 g/d **EF-C:** n-6 LA, 54.2 % of EF-C; PUFA, 29.2 g/d; MUFA, 61.3 g/d; SFA, 6.4 g/d	330 *(21.7%)*	1189 *(78.3%)*	6	1	3

Abbreviations: ALA, alpha-linolenic acid; CVD, cardiovascular disease; DHA, docosahexaenoic acid; ED, enriched diet; EF-C, enriched food component; EPA, eicosapentaenoic acid; LA, linoleic acid; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; S, supplement; SFA saturated fatty acids; TEI, total energy intake; TFA, trans-fatty acids.

n: absolute number of studies

a, b, c, d: Fatty acid contained in the sn-2 position of the acyl chain of bioactive lipids: VLC-PUFA, very long chain polyunsaturated fatty acids; LC-PUFA, long chain polyunsaturated fatty acids.

□ The reported dose range depends on the type of intervention and on the units the results are expressed: grams/day, % total energy intake, % of EF-C.

TABLE 3 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in overweight or obese subjects

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Cantero et al. 2018 [25]	Spain	Randomized, open-label, controlled trial. Two sequential periods: 2-month nutritional-learning and 4-month dietary self-control	35-70 y men and women *BMI:* >27 kg/m^2	RESMENA diet based on the Mediterranean dietary pattern reinforcing high n-3 PUFA. 7 meals/day providing 6.2±0.4 % TEI as PUFA	Control diet based on the American Heart Association (AHA) guidelines. 3 to 5 meals/day providing 5±0.2 % TEI as PUFA	6 months	66 (33/33)	No
Del Bas et al. 2016 [19]	United Kingdom	Randomized, double-blind, placebo-controlled parallel trial	18-65 y men and women *BMI:* 30-40 kg/m^2	Marine n-3 PUFA capsule providing 1.1:0.8 g of EPA:DHA. Daily dose: 1.9 g	Placebo corn-oil capsule Daily dose: 3 g	3 months	38 (19/19)	No
Gürdeniz et al.2013 [26]	Denmark	Randomized, double-blind, controlled trial	45-70 y postmenopausal women *BMI:* 25-32 kg/m^2	Partially hydrogenated soybean oil provided into frozen bread rolls containing 15.7 g of trans fatty acids (TFA, *trans* 18:1) Daily dose: 26 g oil	Control oil provided intro frozen bread rolls containing a mix of palm oil and high oleic sunflower oil	4 months	52 (25/27)	No
Padro et al. 2015 [43]	Spain	Randomized, double-blind, two-arm cross-over trial. 4-week washout period	25-70 y men and women *BMI:* 25-35 Kg/m^2	• *Intervention sequence 1:* Phytosterol (phyS)-enriched milk + n-3 PUFA enriched milk Daily dose: 0.37 g EPA +DHA/250 mL • *Intervention sequence 2:* n-3 PUFA enriched milk + PhyS-enriched milk Daily dose: 0.37 g EPA +DHA/250 mL	Run-in period. Plain low-fat milk free of PhyS and n-3 PUFA Daily dose: 250 mL	Two 28 days study periods	32	No
Rosqvist et al. 2019 [45]	Sweden	Randomized, double-blind, parallel-assignment trial	20-55 y men and women *BMI:* 25-32 kg/m^2	• *Group 1:* Muffins containing sunflower oil providing 65.4 % of 18:2 n-6 PUFA Daily dose: 40 g oil • *Group 2:* Muffins containing palm oil providing 53.5 % of 16:0 SFA (palmitate) and only 8.9 % of n-6 PUFA Daily dose: 40 g oil	Not controlled	2 months	60 (30/30)	No

Table 3. continued

	Bioactive lipid						
	Assessment method	**Identification reference**	**Class**	**Subclass[$]**	**Molecular formula**	**Changes in concentration attributed to intervention and direction of change**	
Cantero et al. 2018 [25]	Targeted LC-MS (Agilent 6550 Accurate-Mass Quadrupole-Time of Flight (Q-TOF) mass spectrometer (MS))	Calibration curves and internal standards	Lyso-PC	14:0	$C_{22}H_{46}NO_7P$	B: 1.70 (0.1); F: 1.20 (0.1)*, P=0.008	Decreased
				15:0	$C_{23}H_{48}NO_7P$	B: 0.80 (0.05); F: 0.70 (0.04)*, P=0.024	
				16:0	$C_{24}H_{50}NO_7P$	B: 147 (6); F: 136 (8)*, P=0.231	
				16:1	$C_{24}H_{48}NO_7P$	B: 3.70 (0.2); F: 3.0 (0.19)*, P=0.009	
				18:0	$C_{26}H_{54}NO_7P$	B: 78.6 (4.8); F: 65.5 (5)*, P=0.060	
				18:1	$C_{26}H_{52}NO_7P$	B: 55.8 (4.1); F: 49.8 (3.3)*, P=0.189	
				18:2	$C_{26}H_{50}NO_7P$	B: 56.8 (3.2); F: 57.0 (4)*, P=0.856	Decreased
				18:4	$C_{26}H_{46}NO_7P$	B: 1.10 (0.06); F: 0.89 (0.05)*, P=0.006	
				20:1	$C_{28}H_{56}NO_7P$	B: 0.84 (0.06); F: 0.74 (0.04)*, P=0.124	
				20:2	$C_{28}H_{54}NO_7P$	B: 0.83 (0.06); F: 0.70 (0.04)*, P=0.090	
				20:3	$C_{28}H_{52}NO_7P$	B: 9.94 (0.6); F: 8.77 (0.56)*, P=0.214	
				20:4	$C_{28}H_{50}NO_7P$	B: 36.5 (2); F: 31.0 (1.7)*, P=0.026	
			Lyso-PE	18:0	$C_{23}H_{48}NO_7P$	B: 25.7 (1.7); F: 23.0 (1.5)*, P=0.230	
				18:1	$C_{23}H_{46}NO_7P$	B: 3.80 (0.36); F: 3.57 (0.3)*, P=0.627	
				18:2	$C_{23}H_{44}NO_7P$	B: 4.10 (0.3); F: 3.90 (0.4)*, P=0.803	
				20:2	$C_{27}H_{44}NO_7P$	B: 5.20 (0.39); F: 5.10 (0.4)*, P=0.911	Decreased
				20:4	$C_{25}H_{50}NO_7P$	B: 3.37 (0.16); F: 3.15 (0.21)*, P=0.427	
				20:5	$C_{25}H_{42}NO_7P$	B: 0.75 (0.06); F: 0.77 (0.16)*, P=0.917	
				22:5	$C_{27}H_{46}NO_7P$	B: 0.27 (0.0); F: 0.27 (0.0)*, P=0.931	
				22:6	$C_{25}H_{48}NO_7P$	B: 1.90 (0.13); F: 1.66 (0.1)*, P=0.054	
			Lyso-PI	18:0	$C_{27}H_{53}O_{12}P$	B: 2.50 (0.2); F: 2.20 (0.23)*, P=0.367	Decreased
				18:1	$C_{27}H_{51}O_{12}P$	B: 0.61 (0.06); F: 0.50 (0.03)*, P=0.105	
Del Bas et al. 2016 [19]	Targeted LC-MS (Agilent 6550 Accurate-Mass Quadrupole-Time of Flight (Q-TOF) mass spectrometer (MS))	Calibration curves and internal standards	Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 125.7; F: 131.9 (6.74)*, P<0.05	Increased
				18:0	$C_{26}H_{54}NO_7P$	B: 22.11; F: 25.50 (4.41)*, `P<0.05	
				18:1	$C_{26}H_{52}NO_7P$	B: 11.88 ; F: 19.90 (9.76)*, P>0.05	
						B: 13.12; F: 18.00 (8.71)**, P<0.05	
				18:2	$C_{26}H_{50}NO_7P$	B: 0.770; F: 1.790 (0.82)*, P>0.05	
						B: 1.070; F: 1.760 (0.82)**, P<0.05	
				20:1	$C_{28}H_{56}NO_7P$	B: 0.177; F: 0.186 (0.07)*, P<0.05	
				20:2	$C_{28}H_{54}NO_7P$	B: 0.470; F: 0.524 (0.11)*, P>0.05	
						B: 0.525; F: 0.550 (0.10)**, P<0.05	
				20:3	$C_{28}H_{52}NO_7P$	B: 1.446; F: 1.500 (0.52)*, P>0.05	
						B: 1.260; F: 1.760 (0.56)**, P<0.05	
				20:4	$C_{28}H_{50}NO_7P$	B: 2.870; F: 4.070 (1.52)*, P>0.05	
			Lyso-PE	18:0	$C_{23}H_{48}NO_7P$	B: 0.056; F: 0.089 (0.08)*, P>0.05	Increased
				18:1	$C_{23}H_{46}NO_7P$	B: 0.277; F: 0.421 (0.11)*, P>0.05	
				18:2	$C_{23}H_{44}NO_7P$	B: 0.394; F: 0.802 (0.19)*, P>0.05	
				20:2	$C_{27}H_{44}NO_7P$	B: 0.766; F: 1.130 (0.21)*, P>0.05	
				20:4	$C_{25}H_{50}NO_7P$	B: 0.277; F: 0.421 (0.05)*, P>0.05	
						B: 0.357; F: 0.467 (0.10)**, P<0.05	
					$C_{25}H_{42}NO_7P$	B: 0.118; F: 0.135 (0.08)*, P>0.05	

				20:5	$C_{27}H_{46}NO_7P$	B: 0.084; F: 0.097 (0.009)*, P>0.05	Increased
				22:5	$C_{25}H_{48}NO_7P$	B: 0.355; F: 0.379 (0.04)*, P>0.05	
				22:6			
					$C_{27}H_{53}O_{12}P$	B: 0.101; F: 0.109 (0.02)*, P>0.05	
			Lyso-PI	18:0	$C_{27}H_{51}O_{12}P$	B: 0.081; F: 0.109 (0.01)*, P>0.05	Increased
				18:1			
Gürdeniz et al.2013 [26]	Untargeted UPLC-MS (coupled to Quadrupole-Time of Flight (Q-TOF) mass spectrometer (MS)) combined with NMR analysis	MZmine (version 2.7) MATLAB (version 7.2)	PC	40:7	$C_{48}H_{82}NO_8P$	Not reported	
				40:6	$C_{48}H_{84}NO_8P$		
				44:9	$C_{52}H_{86}NO_8P$		
				38:4	$C_{46}H_{84}NO_8P$		
				38:5	$C_{46}H_{82}NO_8P$		Increased
				trans18:1/20:3	$C_{46}H_{84}NO_8P$		
				trans18:1/22:4	$C_{48}H_{86}NO_8P$		
				trans18:1/22:5	$C_{48}H_{84}NO_8P$		
				trans18:1/22:6	$C_{48}H_{82}NO_8P$		
			SM	36:3 (d18:2/18:1)	$C_{41}H_{79}N_2O_6P$		Increased
			Lyso-PC	18:1	$C_{26}H_{52}NO_7P$		Increased
Padro et al. 2015 [43]	Targeted LC/MS/MS (Agilent 1200) coupled to a AB Sciex 3200 Qtrap triple quadrupole mass spectrometer. On isolated LDL fraction	LipidView (version 1.1)	Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 39.40; F: 47.20 (32.06)#, P=0.346	Increased
				18:0	$C_{26}H_{56}NO_6P$	B: 12.50; F: 23.50 (19.56)#, P=0.040	
			PC	36:5	$C_{44}H_{78}NO_8P$	B: 52.3; F: 90.5 (49.34)#, P=0.008	Increased
			SM	38:1	$C_{43}H_{87}N_2O_6P$	B: 43.4; F: 72.0 (39.45)#, P=0.011	Increased
Rosqvist et al. 2019 [45]	Targeted UPLC-MS	Not described	Cer	18:0	$C_{36}H_{71}NO_3$	-3.27 (28.31)¥, p<0.05 (n-6 PUFA)	
				20:0	$C_{38}H_{75}NO_3$	-9.25 (11.02)¥, P<0.05 (n-6 PUFA)	Decreased
				24:1	$C_{42}H_{81}NO_3$	-213.14 (232.9)¥, P<0.01 (n-6 PUFA)	
			DiCer	16:0	$C_{34}H_{69}NO_3$	+1.96 (5.50)¥, P<0.05 (SFA)	Increased
						-1.54 (8.43) ¥,P<0.05 (n-6 PUFA)	
				18:0	$C_{36}H_{73}NO_3$	-1.80 (5.19)¥, P<0.05 (n-6 PUFA)	Decreased
				20:0	$C_{38}H_{77}NO_3$	-1.40 (4.81)¥, P<0.05 (n-6 PUFA)	
				24:1	$C_{42}H_{83}NO_3$	-18.70 (23.12)¥, P<0.01 (n-6 PUFA)	
			GluCer	16:0	$C_{44}H_{87}NO_3$	+72.22 (62.68)¥, P<0.05 (SFA)	Increased
						-16.94 (57.05)¥, P<0.05 (n-6 PUFA)	
				18:0	$C_{42}H_{81}NO_8$	-5.34 (5.48)¥ ,P<0.05 (n-6 PUFA)	Decreased
				20:0	$C_{44}H_{85}NO_8$	-9.28 (5.84)¥, P<0.05 (n-6 PUFA)	
				24:0	$C_{48}H_{93}NO_8$	+48.47 (135.84)¥, P<0.05 (SFA)	Increased
						-55.44 (86.13)¥, P<0.05 (n-6 PUFA)	Decreased
				24:1	$C_{48}H_{91}NO_8$	-118.7 (65.92)¥, P<0.01 (n-6 PUFA)	
			LacCer	18:0	$C_{48}H_{91}NO_{13}$	-2.20 (2.80)¥, P<0.05 (n-6 PUFA)	
				20:0	$C_{50}H_{95}NO_{13}$	-0.93 (1.14)¥, P<0.05 (n-6 PUFA)	Decreased
				24:1	$C_{54}H_{101}NO_{13}$	-87.50 (142.5)¥, P<0.01 (n-6PUFA)	

	SM	16:0	$C_{39}H_{79}N_2O_6P$	+10.68 (12.23)¥, P<0.05 (SFA)	Increased
		16:1	$C_{21}H_{43}N_2O_6P$	+1.31 (1.64)¥, P<0.05 (SFA)	
		18:0	$C_{41}H_{83}N_2O_6P$	+0.44 (2.76) ¥, P<0.05 (SFA)	
		18:1		+0.30 (1.25)¥, P<0.05 (SFA)	

$ Only significant subclasses or those that are comparable among the included studies are shown. * Mean expressed as µM (SD), effect after n-3 PUFA intervention or ** after corn oil intervention. # Mean of LDL lipid-specimens expressed as µg/100mL (SD), effect after n-3 PUFA-enriched milk intervention. ¥ Mean expressed as nM (SD), reported change after-before n-6 PUFAs or SFA intervention. Abbreviations: B, baseline value; BMI, body mass index; C, control group; Cer, ceramide; DHA, docosahexaenoic acid; DiCer, dihydroceramide; EPA, eicosapentaenoic acid; F; final value; GluCer; glucosylceramide; I, intervention group; LacCer, lactosylceramide; Lyso-PC, lysophosphatidylcholine; Lyso-PE, lysophosphatidylethanolamine; Lyso-PI, lysophosphatidylinositol; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin; TEI, total energy intake; TFA, trans fatty acids.

TABLE 4 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in subjects with dyslipidemia

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Padro et al. 2015 [43]	Spain	Randomized, double-blind, two-arm cross-over trial. 4-week washout period	25-70 y men and women *High-LDL group:* 160.4 (4.7) mg//dL *Low-LDL group:* 113.3 (4.2) mg/dL *AT-DYSL group:* TG: 150 - 290 mg/dL HDL: <40 in male or <50 in female	• *Intervention sequence 1:* Phytosterol (phyS)-enriched milk + n-3 PUFA enriched milk Daily dose: 0.37 g EPA +DHA/250 mL • *Intervention sequence 2:* n-3 PUFA enriched milk + PhyS-enriched milk Daily dose: 0.37 g EPA +DHA/250 mL	Run-in period. Plain low-fat milk free of PhyS and n-3 PUFA Daily dose: 250 mL	Two 28 days study periods	32	No
Ruuth et al. 2020 [38]	Finland	Randomized, double-blind, placebo-controlled parallel trial	24-66 y men and women *LDL aggregate size:* 1490 (3000) nm *LDL particle concentration:* 1355 (1939) nmol/L *LDL:* 135 (31) mg/dL *HDL:* 69.6 (19) mg/dL	Plant stanol ester-enriched rapeseed oil-based spread (STAEST) *(Raisio Nutrition Ltd)* Daily dose: 20 g (3 g plant stanols + 3.3 g n-6 LA + 1.3 g n-3 ALA)	Control spread without added plant stanols Daily dose: 20 g	6 months	90 (44/16)	No
Zhang et al. 2012 [27]	China	Randomized, open-label, parallel-group controlled dietary intervention study	35-70 y women *TG:* 200 - 216 mg/dL *LDL:*148 - 154 mg/dL *HDL:* 48 - 52 mg/dL	• *Group 1:* Experimental lunch containing Norwegian salmon (SM), provided in servings of 80 g, 5 d/wk Daily dose: 17.6 g PUFA (1.6 g EPA+DHA) • *Group 2:* Experimental lunch containing herring (HR), provided in servings of 80 g, 5 d/wk Daily dose: 17.5 g PUFA (1.6 g EPA+DHA) • *Group 3:* Experimental lunch containing local farmed pompano (PP), provided in servings of 80 g, 5 d/wk Daily dose: 17.6 g PUFA (1 g EPA+DHA)	Commonly eaten control meals mix containing pork/chicken/beef/lean fish, provided in servings of 80 g, 5 d/wk Daily dose: 16.6 g PUFA (1.1 g n-3)	2 months	126 (32/39/33/32)	No
Dunbar et al. 2015 [28]	Philadelphia, USA	Randomized, double-blind, placebo-controlled trial	≥18 y men and women *TG:* 200 - 500 mg/dL	• *Group 1:* n-3 PUFA carboxylic acids plus olive oil capsule (OM3-CA+OO) combined with usual statin therapy	Placebo olive oil capsule combined with usual statin therapy Daily dose: 4 g	6 weeks	627 (209/207/211)	Yes

				Daily dose: 4 g (2 g OM3+2 g OO) • *Group 2:* n-3 PUFA capsule (OM3-CA) combined with usual statin therapy Daily dose: 4 g				
Gajos et al. 2012 [29]	Poland	Randomized, double-blind, placebo-controlled study	30-80 y men and women *TG:* ≥ 150 mg/dL *LDL:* ≥ 140 mg/dL *HDL:* < 40 mg/dL	n-3 PUFA carboxylic acids capsule providing 460:380 mg of EPA:DHA *(Omacor; Pronova Biocare)* combined with usual Aspirin and Clopidogrel therapy Daily dose: 1 g	Placebo soybean oil capsule combined with usual Aspirin and Clopidogrel therapy Daily dose: 1 g	1 month	54 (30/24)	No
Hedengran et al. 2014 [30]	Denmark	Randomized, double-blind, placebo-controlled parallel-arm interventional trial	≥18 y men and women *TG:* 264 (97) mg/dL *HDL:* 44 (11) mg/dL	• *Group 1:* AG n-3 PUFA soft capsule providing 767:1930 mg of EPA:DHA *(Lipomar®, Marine Ingredients, Mount Bethel)* Daily dose: 5.56 g • *Group 2:* EE n-3 PUFA soft capsule providing 1702:1382 mg of EPA:DHA *(Omacor®/Lovaza®,Pronova BioPharma)* Daily dose: 4 g	Placebo olive oil capsule Daily dose: 4.6 g	2 months	119 (39/40/40)	Yes
Mosca et al. 2016 [31]	New York, USA	Randomized, double-blind, placebo-controlled trial	49-73 y women *HDL:* 18 - 65 mg/dL *LDL:* 55 - 196 mg/dL *High TG group:* 200 - 500 mg/dL *Very high TG group:* 500 - 2000 mg/dL	n-3 EPA-EE capsule *(AMR101)* containing ≥96 % EPA ethyl ester and no DHA Daily dose: 4 g	Placebo capsule containing light liquid paraffin Daily dose: 2 g	3 months	215 (109/106)	Yes

Table 4. continued

	Bioactive lipids						
	Assessment method	**Identification reference**	**Class**	**Subclass[$]**	**Molecular formula**	**Changes in concentration attributed to intervention and direction of change**	
Padro et al. 2015 [43]	Targeted LC/MS/MS (Agilent 1200) coupled to an AB Sciex 3200 Qtrap mass spectrometer. On isolated LDL fraction	LipidView (version 1.1)	Lyso-PC	16:0 18:0	$C_{24}H_{50}NO_7P$ $C_{26}H_{56}NO_6P$	B: 39.40; F: 47.20 (32.06 □, P=0.346 B: 12.50; F: 23.50 (19.56)□, P=0.040	Increased
			PC	36:5	$C_{44}H_{78}NO_8P$	B: 52.3; F: 90.5 (49.34)□, P=0.008	Increased
			SM	38:1	$C_{43}H_{87}N_2O_6P$	B: 43.4; F: 72.0 (39.45)□, P=0.011	Increased

Ruuth et al. 2020 [38]	Targeted LC/MS/MS combined with electrospray ionization (Agilent 6410 Triple Quad LC/MS; Agilent Technologies). On isolated LDL particles	Lipid Mass Spectrum Analysis software and internal standards to quantify the molar % of H surface lipids	SM	14:0	$C_{37}H_{75}N_2O_6P$	B: 2.11 (0.46); F: 2.16 (0.57)*, P<0.05	
				15:0	$C_{38}H_{77}N_2O_6P$	B: 0.80 (0.30); F: 0.89 (0.29)*, P<0.01	
				16:0	$C_{39}H_{79}N_2O_6P$	B: 29.83 (1.94); F: 29.94 (2.28)*,P<0.01	Increased
				16:1	$C_{39}H_{77}N_2O_6P$	B: 2.79 (0.46); F: 2.89 (0.48)*, P<0.01	
				18:0	$C_{41}H_{83}N_2O_6P$	B: 6.02 (0.89); F: 6.05 (0.98)*, P<0.05	
				18:1	$C_{41}H_{83}N_2O_6P$	B: 2.04 (0.41); F: 2.00 (0.46)*, P<0.01	Decreased
				23:0	$C_{46}H_{95}N_2O_6P$	B: 8.04 (1.28); F: 8.20 (1.43)*, P<0.05	Increased
				23:1	$C_{46}H_{93}N_2O_6P$	B: 6.06 (1.01); F: 5.97 (0.86)*, P<0.05	Decreased
				24:0	$C_{47}H_{95}N_2O_6P$	B: 7.75 (1.47); F: 7.82 (1.74)*, P<0.01	Increased
				24:1	$C_{47}H_{93}N_2O_6P$	B: 25.85 (2.12); F: 25.88 (2.47)*,P<0.01	
			PC	24:2	$C_{47}H_{91}N_2O_6P$	B: 8.02 (2.07); F: 7.97 (2.17)*, P<0.01	
				34:2	$C_{42}H_{80}NO_8P$	B: 7.20 (1.32); F: 7.36 (1.44)*, P<0.01	
				34:3	$C_{42}H_{78}NO_8P$	B: 3.26 (1.42); F: 3.55 (1.33)*, P<0.01	Increased
				36:3	$C_{44}H_{82}NO_8P$	B: 3.94 (0.79); F: 4.14 (0.87)*, P<0.01	
				36:5	$C_{44}H_{78}NO_8P$	B: 4.19 (1.36); F: 4.36 (1.38)*, P<0.01	
				38:5	$C_{46}H_{82}NO_8P$	B: 8.11 (1.54); F: 8.57 (2.00)*, P<0.01	
				38:6	$C_{46}H_{80}NO_8P$	B: 3.64 (1.57); F: 3.63 (1.40)*, P<0.01	Decreased
				40:7	$C_{48}H_{82}NO_8P$	B: 0.23 (0.10); F: 0.22 (0.07)*, P<0.05	
			Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 55.00 (5.27); F: 55.09 (5.73)*,P<0.05	Increased
				18:0	$C_{26}H_{54}NO_7P$	B: 25.22 (3.67); F: 24.84 (4.42)*,P<0.05	Decreased
				18:1	$C_{26}H_{52}NO_7P$	B: 8.50 (2.69); F: 8.30 (2.03)*, P<0.05	
				18:2	$C_{26}H_{50}NO_7P$	B: 9.78 (2.85); F: 10.09 (3.92)*, P<0.05	
				20:3	$C_{28}H_{52}NO_7P$	B: 0.07 (0.19); F: 0.08 (0.22)*, P<0.01	Increased
				20:4	$C_{28}H_{50}NO_7P$	B: 1.40 (2.00); F: 1.57 (2.14)*, P<0.05	
				20:5	$C_{28}H_{48}NO_7P$	B: 0.03 (0.09); F: 0.04 (0.10)*, P<0.05	
Zhang et al. 2012 [27]	TLC silica gel plates spraying with a methanolic solution and visualized under UV light. Combined with GC (Shimadzu GC 14B) fitted with a capillary column (CP-SIL 88 (VARIAN)) to separate fatty acid methyl esters.	Comparison with retention times of commercially available fatty acid standards, and quantification of peak areas with a computer data system (CBM-101 workstation; Shimadzu).	Lyso-PC	18:2	$C_{26}H_{50}NO_7P$	B: 24.5 (0.4); F: 21.6 (0.4)**, P=0.05 after SM B: 24.1 (0.7); F: 22.3 (0.4))**,P=0.05 after HR B: 24.9 (0.5); F: 22.4 (0.6)**, P=0.05 after PP	
				20:4	$C_{28}H_{50}NO_7P$	B: 9.9 (0.4); F: 8.7 (0.3)**, P<0.05 after SM B: 8.9 (0.4); F: 8.1 (0.4)**, P=0.05 after HR B: 10.0 (0.4); F: 9.0 (0.3)**, P=0.05 after PP	Decreased
				20:5	$C_{28}H_{48}NO_7P$	B: 2.6 (0.2); F: 3.6 (0.3)**, P<0.05 after SM B: 2.4 (0.2); F: 3.6 (0.3)**, P<0.01 after HR B: 2.0 (0.1); F: 2.9 (0.2)**, P=0.05 after PP	Increased
				22:6	$C_{30}H_{50}NO_7P$	B: 2.3 (0.2); F: 3.8 (0.2)**, P<0.01 after SM B: 2.2 (0.2); F: 3.5 (0.3)**, P<0.01 after HR B: 2.4 (0.1); F: 3.6 (0.2)**, P<0.01 after PP	Increased

Table 4. continued

Bioactive lipid enzymatic precursors			
Assessment method	**Class**	**Subclass**	**Changes in mass attributed to intervention and direction of change**

Dunbar et al. 2015 [28]	Latex particle-enhanced turbidimetric immunoassay on a Roche-P modular analyzer, PLAC™ test (diaDexus, Inc. South San Francisco, CA, USA)	Platelet-activating factor	Lipoprotein-associated phospholipase A2 enzyme	Lp-PLA2	B: 218 (54.7); F: 205 (52.5)[#], P<0.001 after OM3-CA+OO B: 216 (50.1); F: 194 (51.4)[#], P<0.001 after OM3-CA	Decreased
Gajos et al. 2012 [29]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)				B: 260 (68); F: 240 (64)[†], P=0.026 and P=0.041	Decreased
Hedengran et al. 2014 [30]	Colorimetric activity method, CAM assay (diaDexus, San Francisco, CA, USA)				B: 260 (102); F: 248 (104)[‡], P=0.563 after AG n-3 PUFA B: 247 (101); F: 228 (109)[‡], P<0.001 after EE n-3 PUFA	Decreased
Mosca et al. 2016 [31]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)				B: 177 (53); F: 154 (44)□, P<0.0001 ANCHOR experimental group B: 234 (177); F: 201 (88)□, P=0.652 MARINE experimental group	Decreased

[$] Only significant subclasses or those that are comparable among the included studies are shown. □ Mean of LDL lipid-specimens expressed as µg/100mL (SD), effect after n-3 PUFA-enriched milk intervention. [*] Mean expressed as molar % of LDL surface lipid subclasses (SD), effect after STAEST intervention [**] Mean of plasma Lyso-PC subclasses expressed as % of fatty acids 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3 (SEM), effect after the three oily fish interventions. [#] Mean expressed as ng/mL (SD), effect of OM3-CA 2g and 4g compared to placebo. [†] Mean expressed as ng/mL (SD), effect after n-3 PUFAs intervention and effect compared to placebo. [‡] Mean expressed as nmol/mL (SD), effect of AG and EE n-3 PUFA compared to placebo. □ Mean expressed as ng/mL (SD), effect of n-3 eicosapentaenoic EE intervention compared to placebo. Abbreviations: AG, acylglycerol; AT-DYSL, atherogenic dyslipidemia; B: baseline value; C, control group; DHA; docosahexaenoic acid; EE, ethyl esters; EPA, eicosapentaenoic acid; F, final value; HDL, high-density lipoprotein cholesterol; I, intervention group; LDL; low-density lipoprotein cholesterol; Lyso-PC, lysophosphatidylcholine; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acids; SM, sphingomyelin; TG, triglyceride. Baseline dyslipidemia features are described in the Population column (mean ± SD or range of mean values).

TABLE 5 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in subjects with metabolic syndrome

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Bondia-Pons et al. 2014 [33]	Italy	Randomized, open-label, parallel-group controlled trial. Three different isoenergetic diets. Preceded by a 3-week run-in stabilization period with the habitual diet	35-75 y men and women *BMI:* 27 - 35 Kg/m^2 *WC:* 101 - 105 cm *TG:* 120 - 138 mg/dL *HDL:* 41 - 44 mg/dL *FBG:* 100 – 104 mg/dL *SBP:* 119 - 126 mmHg *DBP:* 73 - 76 mmHg	• *Group 1:* Diet rich in LC-n-3 PUFA and low in polyphenols providing 4.2±0.0 % TEI as PUFA (0.43/0.58 % TEI as EPA/DHA) • *Group 2:* Diet rich in polyphenols and low in LC-n-3 PUFA providing 3.3±0.2 % TEI as PUFA (0.04/0.01 % TEI as EPA/DHA) • *Group 3:* Diet rich in LC-n-3 PUFA and polyphenols providing 4.2±0.1 % TEI as PUFA (0.47/0.60 % TEI as EPA/DHA)	Diet low in LC-n-3 PUFA and polyphenols providing 3.6±0.1 % TEI as PUFA (0.02/0.01 % TEI as EPA/DHA)	2 months	78 (19/20/19/20)	Yes
Bondia-Pons et al. 2015 [32]	Spain	Randomized, open-label, controlled trial. Two sequential periods: 2-month nutritional-learning and 4-month dietary self-control	35-70 y men and women *BMI:* 35.4 (4.4) Kg/m^2 *WC:* 110.9 (12) cm *TG:* 194 (124) mg/dL *HDL:* 43 (10) mg/dL *FBG:* 124 (38) mg/dL *SBP:* 147.2 (20.7) mmHg *DBP:* 84.2 (9) mmHg	RESMENA diet based on the Mediterranean dietary pattern reinforcing high n-3 PUFA. 7 meals/day providing 6.2±0.4 % TEI as PUFA	Control diet based on the American Heart Association (AHA) guidelines. 3 to 5 meals/day providing 5±0.2 % TEI as PUFA	6 months	66 (33/33)	No
Cantero et al. 2018 [25]	Spain	Randomized, open-label, controlled trial. Two sequential periods: 2-month nutritional-learning and 4-month dietary self-control	35-70 y men and women *BMI:* 34.8 (4) Kg/m^2 *WC:* 110 (13) cm *TG:* 198.6 (120) mg/dL *HDL:* 46.5 (10) mg/dL *FBG:* 121.5 (37) mg/dL *SBP:* 153.3 (21) mmHg *DBP:* 84.4 (9) mmHg	RESMENA diet based on the Mediterranean dietary pattern reinforcing high n-3 PUFA. 7 meals/day providing 6.2±0.4 % TEI as PUFA	Control diet based on the American Heart Association (AHA) guidelines. 3 to 5 meals/day providing 5±0.2 % TEI as PUFA	6 months	66 (33/33)	No
Meikle et al. 2015 [48]	Australia	Randomized, postprandial, cross-over trial 4 to 6-week washout period	40-60 y men *BMI:* 30.1 (5.7) Kg/m^2 *WC:*104 (13) cm *TG:* 1.6 (0.5) mg/dL *HDL:* 1.2 (0.2) mg/dL *FBG:* 97 (11) mg/dL	• *Intervention sequence 1:* High-fat breakfast meal consisting on dairy-based foods (cheddar cheese, butter, extra creamy milk) Daily dose: 67 g:23 g:5 g of SFA:MUFA:PUFA • *Intervention sequence 2:* High-fat breakfast meal consisting on soy oil-based foods	Not controlled	Two 4-hour postprandial test meals	21	No

				(cheddar flavoured soy cheese, soy beverage, non-dairy spread) Daily dose: 37 g:40 g:24 g of SFA:MUFA:PUFA			
Table 5. continued	**Bioactive lipid**						
	Assessment method	**Identification reference**	**Class**	**Subclass[$]**	**Molecular formula**	**Changes in concentration attributed to intervention and direction of change**	
Bondia-Pons et al. 2014 [33]	Untargeted UPLC-MS (coupled to Quadrupole-Time of Flight mass spectrometer (Q-TOF/MS)). On plasma and isolated HDL fraction	MZmine 2 combined with an internal spectral library	PC	32:2	$C_{40}H_{76}NO_8P$	-0.10-0.45*, P<0.005 after control diet	Decreased
				36:4	$C_{44}H_{80}NO_8P$		
				38:3	$C_{46}H_{86}NO_8P$		
				40:4	$C_{48}H_{88}NO_8P$	+0.10-0.70*, P<0.0005 after high LCn-3 PUFA diets	Increased
				40:6	$C_{48}H_{84}NO_8P$		
			PE	36:2	$C_{41}H_{80}NO_7P$	-0.10-0.45*, P<0.005 after control diet	Decreased
				40:3	$C_{45}H_{84}NO_8P$		
				38:6	$C_{43}H_{84}NO_8P$	+0.10-0.70*, P<0.0005 after high LCn-3 PUFA diets	Increased
				40:4	$C_{45}H_{84}NO_7P$		
			LysoPC	18:0	$C_{26}H_{56}NO_6P$	-0.10-0.45*, P<0.005 after control diet	Decreased
				20:4	$C_{28}H_{50}NO_7P$		
				20:5	$C_{28}H_{48}NO_7P$	+0.70*, P<0.0005 after high LCn-3 PUFA diets	Increased
Bondia-Pons et al. 2015 [32]	Untargeted UPLC-MS (coupled to Quadrupole-Time of Flight mass spectrometer (Q-TOF/MS), Agilent 1290/6540)	Human Metabolome Database METLIN Metabolite database Mass-Bank database	PC	34:4	$C_{42}H_{76}NO_8P$	P=0.024**after 2- and 6-month	
				32:2	$C_{40}H_{76}NO_8P$	P<0.0001**after 2- and 6-month	
				32:1	$C_{40}H_{78}NO_8P$	P=0.0001**after 2- and 6-month	
				36:4	$C_{44}H_{80}NO_8P$	P=0.036**after 2-month	Decreased
				34:2	$C_{42}H_{80}NO_8P$	P=0.028** after 2- and 6-month	
				38:3	$C_{46}H_{36}NO_8P$	P=0.0001**after 2-month	
				36:2	$C_{44}H_{84}NO_8P$	P=0.040**after 2- and 6-month	
				38:4	$C_{46}H_{84}NO_7P$	P=0.001**after 2-month	Increased
			PE	32:2	$C_{37}H_{74}NO_8P$	P=0.021** after 2- and 6-month	Decreased
			LysoPC	14:0	$C_{22}H_{46}NO_7P$	P<0.0001**after 2-month	
				15:0	$C_{23}H_{48}NO_7P$	P<0.0001**after 6-month	
				16:1	$C_{24}H_{48}NO_7P$	P=0.0003**after 2-month	Decreased
				20:3	$C_{28}H_{52}NO_7P$	P=0.002**after 2-month	
				20:4	$C_{28}H_{50}NO_7P$	P=0.002**after 2-month	
				16:0	$C_{24}H_{50}NO_7P$	P=0.023**after 6-month	
				18:0	$C_{26}H_{54}NO_7P$	P=0.027**after 6-month	
				18:1	$C_{26}H_{52}NO_7P$	P=0.003**after 6-month	Increased
				20:5	$C_{28}H_{48}NO_7P$	P=0.026**after 2- and 6-month	
				22:6	$C_{30}H_{50}NO_7P$	P=0.009**after 6-month	
			LysoPE	18:1	$C_{23}H_{46}NO_7P$	P=0.0004**after 2-month	Decreased
				18:2	$C_{23}H_{44}NO_7P$	P<0.0001**after 2-month	

Cantero et al. 2018 [25]	Targeted LC-MS (Agilent 6550 Accurate-Mass Quadrupole-Time of Flight (Q-TOF) mass spectrometer (MS))	Calibration curves and internal standards	LysoPC	14:0	$C_{22}H_{46}NO_7P$	B: 1.70 (0.1); F: 1.20 (0.1)[#], P=0.008	
				15:0	$C_{23}H_{48}NO_7P$	B: 0.80 (0.05); F: 0.70 (0.04)[#], P=0.024	
				16:0	$C_{24}H_{50}NO_7P$	B: 147 (6.00); F: 136 (8)[#], P=0.231	
				16:1	$C_{24}H_{48}NO_7P$	B: 3.70 (0.20); F: 3.00 (0.19)[#], P=0.009	Decreased
				18:0	$C_{26}H_{54}NO_7P$	B: 78.6 (4.80); F: 65.5 (5)[#], P=0.060	
				18:1	$C_{26}H_{52}NO_7P$	B: 55.8 (4.10); F: 49.8 (3.3)[#], P=0.189	
				18:2	$C_{26}H_{50}NO_7P$	B: 56.8 (3.20); F: 57.0 (4)[#], P=0.856	
				18:4	$C_{26}H_{46}NO_7P$	B: 1.10 (0.06); F: 0.89 (0.05)[#], P=0.006	
				20:1	$C_{28}H_{56}NO_7P$	B: 0.84 (0.06); F: 0.74 (0.04)[#], P=0.124	
				20:2	$C_{28}H_{54}NO_7P$	B: 0.83 (0.06); F: 0.70 (0.04)[#], P=0.090	
				20:3	$C_{28}H_{52}NO_7P$	B: 9.94 (0.6); F: 8.77 (0.56)[#], P=0.214	Decreased
				20:4	$C_{28}H_{50}NO_7P$	B: 36.5 (2.00); F: 31.0 (1.70)[#], P=0.026	
				22:5	$C_{30}H_{52}NO_7P$	B: 1 (0.07); F: 0.98 (0.05)[#], P=0.256	
				22:6	$C_{30}H_{50}NO_7P$	B: 7.8 (0.50); F: 6.6 (0.50)[#], P=0.093	
			LysoPE	18:0	$C_{23}H_{48}NO_7P$	B: 25.7 (1.7); F: 23.0 (1.5)[#], P=0.230	
				18:1	$C_{23}H_{46}NO_7P$	B: 3.80 (0.36); F:.57 (0.3)[#], P=0.627	
				18:2	$C_{23}H_{44}NO_7P$	B: 4.10 (0.3); F: 3.90 (0.4)[#], P=0.803	Decreased
				20:2	$C_{27}H_{44}NO_7P$	B: 5.20 (0.39); F: 5.10 (0.4)[#], P=0.911	
				20:4	$C_{25}H_{50}NO_7P$	B: 3.37 (0.16); F: 3.15 (0.21)[#], P=0.427	
				20:5	$C_{25}H_{42}NO_7P$	B: 0.75 (0.06); F: 0.77 (0.16)[#], P=0.917	
				22:5	$C_{27}H_{46}NO_7P$	B: 0.27 (0.0); F: 0.27 (0.0)[#], P=0.931	Increased
				22:6	$C_{25}H_{48}NO_7P$	B: 1.90 (0.13); F:1.66 (0.1)[#], P=0.054	Decreased
Meikle et al. 2015 [48]	Targeted LC/MS (Agilent 1200 LC system combined with an Applied Biosystems API 4000 Q/TRAP mass spectrometer) Semi-quantitative	Analyst (version 1.5) LIPID MAPS library	Cer	18:0	$C_{36}H_{71}NO_3$	-16 (-27, -3.6)[†], P=0.070 after soy	Decreased
			DiCer	18:0	$C_{36}H_{73}NO_3$	-20 (-36, 2.9)[†], P=0.030 after soy	
			SM	32:0	$C_{37}H_{77}N_2O_6P$	-12 (-19, -5.5)[†], P=0.007 after soy +8.1 (-3.4, 18)[†], P<0.001 after dairy	
				32:1	$C_{37}H_{75}N_2O_6P$	-3.6 (-12, 4.4)[†], P=0.022 after soy +5.4 (-0.4, 13)[†], P<0.001 after dairy	
				32:2	$C_{37}H_{73}N_2O_6P$	-5.7 (-13, 1.4)[†], P=0.016 after soy +3.4 (-2, 10)[†], P<0.001 after dairy	
				34:0	$C_9H_{19}N_2O_6P$	-15 (-21, -4.8)[†], P=0.007 after soy +14 (-0.9, 30)[†], P<0.001 after dairy	Decreased/ Increased
				35:1	$C_{40}H_{81}N_2O_6P$	-8.5 (-16, 2.4)[†], P=0.05 after soy +2.5 (-4.7, 11)[†], P<0.001 after dairy	
				36:2	$C_{41}H_{81}N_2O_6P$	-6.1 (-16, 2)[†], P=0.021 after soy +6.9 (-5.7, 14)[†], P<0.001 after dairy	
				38:1	$C_{43}H_{87}N_2O_6P$	-9.3 (-20, 0)[†], P=0.030 after soy +1.3 (-5.7, 12)[†], P<0.001 after dairy	
			PC	28:0	$C_{36}H_{72}NO_8P$	-13 (-22, -3.1)[†], P=0.007 after soy +43 (13, 94)[†], P<0.001 after dairy	Decreased/ Increased
				29:0	$C_{37}H_{74}NO_8P$	-14 (-25, 4.6)[†], P=0.010 after soy +21 (0.0, 69)[†], P<0.001 after dairy	
				30:0	$C_{38}H_{76}NO_8P$	-12 (-24, -4.4)[†], P=0.007 after soy +18 (0.5, 32)[†], P<0.001 after dairy	

		31:0	$C_{39}H_{78}NO_8P$	-7.1 (-16, 1.9)†, P=0.022 after soy +7.3 (-3.2, 23)†, P<0.001 after dairy	Decreased/ Increased
		32:0	$C_{40}H_{80}NO_8P$	-9.7 (-21, 4.1)†, P=0.012 after soy +10 (-20.4, 23)†, P<0.001after dairy	
		32:1	$C_{40}H_{78}NO_8P$	+11 (1.0, 22)†, P<0.001 after dairy	
		36:3	$C_{44}H_{82}NO_8P$	+13 (2.9, 20)†, P<0.001 after dairy	
		36:5	$C_{44}H_{78}NO_8P$	+14 (3.1, 24)†, P<0.001 after dairy	
		38:3	$C_{46}H_{86}NO_8P$	+16 (2.3, 22)†, P<0.001 after dairy	Increased
		38:5	$C_{46}H_{82}NO_8P$	+9.3 (2.0, 15)†, P<0.001 after dairy	
		38:6	$C_{46}H_{80}NO_8P$	+7.1 (-1.6, 13)†, P<0.001 after dairy	
		40:5	$C_{48}H_{86}NO_8P$	+4.7 (-1.8, 12)†, P<0.001 after dairy	
		40:6	$C_{48}H_{84}NO_8P$	+6.3 (0.3, 12)†, P<0.001 after dairy	
	PE	35:1	$C_{40}H_{78}NO_8P$	+0.6 (0.0, 58)†, P<0.001 after dairy	
		36:5	$C_{41}H_{72}NO_8P$	+38 (8.0, 83)†, P<0.001 after dairy	
		38:3	$C_{43}H_{80}NO_8P$	+37 (11, 65)†, P<0.001 after dairy	Increased
		38:6	$C_{43}H_{74}NO_8P$	+10 (-1.4, 26)†, P<0.001 after dairy	
		40:6	$C_{45}H_{78}NO_8P$	+14 (3.1, 25)†, P<0.001 after dairy	
	PI	34:1	$C_{43}H_{81}O_{13}P$	+22 (8, 30)†, P<0.001 after dairy	
		36:1	$C_{45}H_{85}O_{13}P$	+14 (4.2, 25)†, P<0.001 after dairy	
		36:2	$C_{45}H_{83}O_{13}P$	+18 (8.5, 27)†, P<0.001 after dairy	Increased
		36:4	$C_{45}H_{79}O_{13}P$	+23 811, 38)†, P<0.001 after dairy	
		38:3	$C_{47}H_{85}O_{13}P$	+17 (0.7, 37)†, P<0.001 after dairy	
		38:4	$C_{47}H_{83}O_{13}P$	+18 (2.9, 28)†, P<0.001 after dairy	
	LysoPC	14:0	$C_{22}H_{46}NO_7P$	-11 (-17, 0.1)†, P=0.016 after soy +4.6 (-1.6, 11)†, P<0.001 after dairy	Decreased Increased
		15:0	$C_{23}H_{48}NO_7P$	-8.2 (-14, -0.8)†, P=0.042 after soy	
		16:0	$C_{24}H_{50}NO_7P$	-4.1 (-16, 4.1)†, P=0.240 after soy	
		16:1	$C_{24}H_{48}NO_7P$	-9.6 (-17, -0.3)†, P=0.150 after soy	Decreased
		17:0	$C_{25}H_{52}NO_7P$	-10 (-17, 1.3)†, P=0.070 after soy	
		18:0	$C_{26}H_{54}NO_7P$	-11 (-17, 0.1) †, P=0.080 after soy	
		18:1	$C_{26}H_{52}NO_7P$	-9.3 (-19, -3.5)†, P=0.210 after soy	
		18:2	$C_{26}H_{50}NO_7P$	+7.3 (-7.0, 21)†, P=0.409 after soy	Increased
		20:1	$C_{28}H_{56}NO_7P$	-22 (-30, -1.0)†, P=0.100 after soy	
		20:2	$C_{28}H_{54}NO_7P$	-17 (-26, -6.1)†, P=0.049 after soy	
		20:4	$C_{28}H_{50}NO_7P$	-10 (-17, 0.7)†, P=0.290 after soy	Decreased
		22:5	$C_{30}H_{52}NO_7P$	-8.8 (-20, 8.3)†, P=0.820 after soy	
		22:6	$C_{30}H_{50}NO_7P$	-9.2 (-16, 4.2)†, P=0.140 after soy	
	LysoPE	18:0	$C_{23}H_{48}NO_7P$	+5.1 (-6.6, 18)†, P<0.001 after dairy	
		18:1	$C_{23}H_{46}NO_7P$	+35 (11, 66)†, P<0.001 after dairy	
		18:2	$C_{23}H_{44}NO_7P$	+40 (22, 65)†, P<0.001 after dairy	Increased
		20:4	$C_{25}H_{50}NO_7P$	+13 (-5.4, 38)†, P<0.001 after dairy	
		22:6	$C_{25}H_{48}NO_7P$	+3.3 (-8.4, 18)†, P<0.001 after dairy	

$ Only significant subclasses or those that are comparable among the included studies are shown. * Mean levels expressed as average lipid concentration (LDL amount), reported change intervals after LC-n-3 PUFA and control interventions.
** P for changes in detected mass at 2-month vs baseline, and at 6-month vs baseline in RESMENA diet intervention. # Mean expressed as μM (SD), effect after n-3 PUFA intervention.† Mean expressed as Median % change (25th, 75th

percentile) from baseline, reported change after 4-hour dairy and soy oil-based meals. Abbreviations: B, baseline value; BMI; body mass index; C, control group; Cer, ceramide; DBP; diastolic blood pressure; DHA; docosahexaenoic acid; DiCer, dihydroceramide; EPA, eicosapentaenoic acid; F, final value; FBG, fasting blood glucose; GluCer, glucosylceramide; HDL; high-density lipoprotein cholesterol; I, intervention group; LC, long chain; LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine; LysoPI, lysophosphatidylinositol; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; SFA, saturated fatty acids; SM, sphingomyelin; TEI: total energy intake; TG; triglyceride. Baseline MetS features are described in the Population column (mean ± SD or range of mean values).

TABLE 6 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in subjects with type 2 diabetes

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Airhart et al. 2016 [34]	Missouri, USA	Randomized, double-blind, controlled feeding pilot study	37-65 y men and women *FBG:* 121 - 165 mg/dL *Insulin:* 17 - 27 µU/mL *HbA1c:* 7.1 - 8.3 %	Medium-chain fatty acids (MCFA)-rich diet *(Delios S, Cognis Corporation)* Daily dose: 33-34 % SFA, 1-2 % MUFA, 1-2 % PUFA	Long-chain fatty acids (LCFA)-rich diet administered as vegetable oil Daily dose: 18-21 % SFA, 7-11 % MUFA, 3-8 % PUFA	2 weeks	16 (9/7)	No
Baziar et al. 2020 [35]	Iran	Randomized, double-blind, placebo-controlled trial	40-60 y men and women *FBG:* 122.5 - 145.6 mg/dL *Insulin:* 9.3 - 12.4 mU/L *HbA1c:* 6.2 - 7 % *HOMA-IR:* 3 - 4.1	Alpha lipoic acid capsule Daily dose: 1200 mg	Placebo capsule containing maltodextrin Daily dose: 1200 mg	2 months	70 (35/35)	Yes

Table 6. continued

Bioactive lipids

Assessment method	Identification reference	Class	Subclass[$]	Molecular formula	Changes in concentration attributed to intervention and direction of change	
Airhart et al. 2016 [34] Targeted LC-MS	Analyst (version 1.5)	SM	14:0	$C_{37}H_{75}N_2O_6P$	B: 0.083 (0.002); F: 0.071 (0.003)*, P<0.01	Decreased
			15:0	$C_{38}H_{77}N_2O_6P$	B: 0.257 (0.002); F: 0.250 (0.002)*, P<0.01	
			16:0	$C_{39}H_{79}N_2O_6P$	B: 1.169 (0.040); F: 1.066 (0.039)*, P<0.01	
			16:1	$C_{39}H_{77}N_2O_6P$	B: 0.157 (0.006); F: 0.141 (0.006)*, P<0.01	
			20:0	$C_{43}H_{87}N_2O_6P$	B: 0.194 (0.005); F: 0.165 (0.009)*, P<0.01	
			21:0	$C_{44}H_{89}N_2O_6P$	B: 0.071 (0.001); F: 0.058 (0.002)*,P<0.005	
			22:0	$C_{45}H_{91}N_2O_6P$	B: 0.324 (0.019); F: 0.267 (0.018)*, P<0.01	
			22:1	$C_{45}H_{89}N_2O_6P$	B: 0.214 (0.006); F: 0.191 (0.008)*, P<0.01	
			23:0	$C_{46}H_{93}N_2O_6P$	B: 0.139 (0.005); F: 0.117 (0.004)*, P<0.01	
			23:1	$C_{46}H_{91}N_2O_6P$	B: 0.081 (0.002); F: 0.072 (0.002)*, P<0.01	
		Cer	23:0	$C_{41}H_{81}NO_3$	B: 0.047 (0.007); F: 0.345 (0.005) *, P<0.01	Decreased
		AC	18:0	$C_{25}H_{49}NO_4$	B: 0.175 (0.013); F: 0.127 (0.013)*, P<0.01	Decreased
			18:3	$C_9H_{18}NO_4$	B: 0.040 (0.003); F: 0.029 (0.002)*, P<0.05	
			20:0	$C_{27}H_{53}NO_4$	B: 0.013 (0.001); F: 0.009 (0.001)*,P<0.001	

Table 6. continued

Bioactive lipid enzymatic precursors

Assessment method	Class	Subclass		Changes in mass attributed to intervention and direction of change	
Baziar et al. 2020 [35] PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)	Platelet-activating factor acetylhydrolases	Lipoprotein-associated phospholipase A2 enzyme	Lp-PLA2	B: 194.30 (1.60); F: 177.18 (1.56)**, P=0.001	Decreased

[$] Only significant subclasses or those that are comparable among the included studies are shown. *Mean expressed as µM (SD), effect after MCFA intervention.**Mean expressed as ng/mL (SD), effect after alpha lipoic acid intervention. Abbreviations: AC, acylcarnitine; B, baseline value; C, control group; Cer, ceramide; F, final value; FBG, fasting blood glucose; HbA1C, glycated haemoglobin; HOMA-IR, homeostasis model of assessment index; I, intervention group; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin. Baseline T2D features are described in the Population column (range of mean values).

TABLE 7 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in subjects with hypertension

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Rebholz et al. 2018 [36]	Baltimore, USA	Multicenter, randomized, single-blind, controlled feeding trial	≥ 22 y men and women *SBP:* 130.1 (12.6) mmHg *DBP:* 84.4 (6.9) mmHg	• *Group 1:* DASH diet consisting on high intake of fruits, vegetables and low-fat dairy providing 7.4 % of TEI as SFA; 10.5 % as MUFA; and 7.6 % as PUFA • *Group 2:* Diet rich in fruits and vegetables providing 13 % of TEI as SFA; 14 % as MUFA; and 6.9 % as PUFA	Control diet low in fruits, vegetables and dairy products providing 14.4 % of TEI as SFA; 12.6 % as MUFA; and 7.1 % as PUFA	2 months	329 (110/111/108)	Yes
Krantz et al. 2015 [37]	Denver, USA	Randomized, double-blind, placebo-controlled pilot study	≥ 18 y men and women *SBP:* 133 (16) mmHg *DBP:* 81 (10) mmHg *PWV:* 1652 (330) cm/s	n-3 PUFA capsules providing 465:375 mg of EPA:DHA *(Lovaza™ Glaxo Smith Kline)* Daily dose: 3.36 g	Matched corn-oil placebo capsule Daily dose: 3.36 g	3 months	62 (27/35)	No

Table 7. continued

	Bioactive lipids						
	Assessment method	**Identification reference**	**Class**	**Subclass[$]**	**Molecular formula**	**Changes in concentration attributed to intervention and direction of change**	
Rebholz et al. 2018 [36]	Untargeted GC-MS and LC-MS (Thermo Scientific Orbitrap mass spectrometers)	Characterization in an extensive chemical library Validation with reference standards	Cer	34:1	$C^{34}H^{67}NO^{3}$	-0,142 (0.029)	Decreased
				35:1	$C_{35}H_{69}NO_3$	-0.349 (0.059)	
				36:1	$C_{36}H_{71}NO_3$	-0.323 (0.039)*	
				38:1	$C_{50}H_{95}NO_{13}$	-0.211 (0.037)*	
				40:2	$C_{40}H_{77}NO_3$	-0.230 (0.040)*	
				41:2	$C_{41}H_{79}NO_3$	-0.107 (0.020)*	
			SM	32:0	$C_{37}H_{77}N_2O_6P$	-0.213 (0.034)*	Decreased
				32:1	$C_{37}H_{75}N_2O_6P$	-0.112 (0.023)	
				32:2	$C_{37}H_{73}N_2O_6P$	-0.207 (0.038)*	
				33:1	$C_{38}H_{77}N_2O_6P$	-0.148 (0.025)*	
				33:2	$C_{38}H_{75}N_2O_6P$	-0.179 (0.038)	
				34:1	$C_{39}H_{79}N_2O_6P$	-0.067 (0.016)*	
				34:2	$C_{39}H_{77}N_2O_6P$	-0.095 (0.018)*	
				35:1	$C_{40}H_{81}N_2O_6P$	-0.160 (0.029)*	
				36:0	$C_{41}H_{85}N_2O_6P$	-0.679 (0.107)*	
				36:1	$C_{41}H_{83}N_2O_6P$	-0.133 (0.029)*	
				36.2	$C_{41}H_{81}N_2O_6P$	-0.182 (0.028)*	
				36:3	$C_{41}H_{79}N_2O_6P$	-0.258 (0.039)*	
				37:1	$C_{42}H_{85}N_2O_6P$	-0.170 (0.035)*	
				38:0	$C_{43}H_{89}N_2O_6P$	-0.306 (0.067)*	

			41:1	$C_{46}H_{93}N_2O_6P$	-0.169 (0.030)*	Decreased
			41:2	$C_{46}H_{91}N_2O_6P$	-0.171 (0.029)*	
		Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	-0.167 (0.034)*	Decreased
			18:1	$C_{26}H_{52}NO_7P$	-0.117 (0.021)	
		Lyso-PE	18:0	$C_{23}H_{48}NO_7P$	-0.239 (0.049)	Decreased
			20:4	$C_{25}H_{50}NO_7P$	-0.135 (0.030)	
		PC	30:0	$C_{38}H_{76}NO_8P$	-0.254 (0.059)	
			34:0	$C_{42}H_{84}NO_8P$	-0.120 (0.024)	Decreased
			34:1	$C_{42}H_{82}NO_8P$	-0.103 (0.022)	
			36:1	$C_{44}H_{86}NO_8P$	-0.225 (0.030)	
			40:6	$C_{48}H_{84}NO_8P$	+0.151 (0.031)*	Increased
		PE	40:6	$C_{45}H_{76}NO_8P$	+0.301 (0.062)*	Increased
			40:7	$C_{45}H_{78}NO_8P$	+0.567 (0.112)	

Table 7. continued

	Bioactive lipid enzymatic precursors				
	Assessment method	**Class**	**Subclass**		**Changes in mass/activity attributed to intervention and direction of change**
Krantz et al. 2015 [37]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)	Platelet-activating factor acetylhydrolases	Lipoprotein-associated phospholipase A2 enzyme	Lp-PLA2	B: 252.0 (62); F: 233.9 (41.1)[#], P>0.05 B: -18.1 (41.1); F: -6.1 (31.7)[†], P=0.08 — Decreased

[$] Only significant subclasses or those that are comparable among the included studies are shown. *Significant change in β_2 correlation coefficient and standard error (SE) among those assigned to the DASH diet compared with those assigned to the control diet (P<0.00001). Negative/Positive β_2-coefficients indicate that the lipid metabolite was lower/higher among those randomly assigned to the DASH diet compared with those assigned to the control diet. [#] Mean expressed as ng/mL (SD), effect after n-3 PUFA intervention, and [†] effect compared to the placebo group. Abbreviations: B, baseline value; C, control group; Cer, ceramide; DBP, diastolic blood pressure; DHA; docosahexaenoic acid; EPA, eicosapentaenoic acid; F, final value; I, intervention group; Lyso-PC, lysophosphatidylcholine; Lyso-PE, lysophosphatidylethanolamine; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; PWV; pulse wave velocity; SBP; systolic blood ressure; SFA, saturated fatty acids; SM, sphingomyelin. Baseline Hypertension features are described in the Population column (mean ± SD).

TABLE 8 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in subjects with stable coronary artery disease

Author, year (reference)	Country	Study design	Population	Fatty acid intervention and daily dose	Comparator	Duration of exposure	Participants *N* (I/C)	Intention-to-treat
Gajos et al. 2012 [29]	Poland	Prospective, randomized, double-blind, placebo-controlled study	30-80 y men and women *Ox-LDL:* 83 (141) µg/L *MPO:* 245.6 (13.5) ng/mL *IL-6:* 23.2 (9) pg/mL	n-3 PUFA carboxylic acids capsule providing 460:380 mg of EPA:DHA *(Omacor; Pronova Biocare)* combined with usual Aspirin and Clopidogrel therapy Daily dose: 1 g	Placebo soybean oil capsule combined with usual Aspirin and Clopidogrel therapy Daily dose: 1 g	1 month	54 (30/24)	No
Khandouzi et al. 2020 [39]	Iran	Randomized, open-label, controlled parallel-arm trial	51-64 y men and postmenopausal women *IL-6:* 19.5 (10.5) pg/mL *LDL:* 42.9 (6.5) mg/dL *HDL:* 74.6 (30.5) mg/dL	Canola oil *(CanaPlus, British Columbia)* providing 6.40 g SFA, 61.37 g MUFA, 29.20 g PUFA (mainly n-6 LA, and n-3 ALA) per 100 g Daily dose: 25 mL	Refined olive oil providing 17.87 g SFA, 66.68 g MUFA, 11.75 g PUFA per 100 g Daily dose: 25 mL	6 weeks	42 (20/22)	No

Table 8. continued

	Bioactive lipid enzymatic precursors					
	Assessment method	**Class**	**Subclass**		**Changes in mass attributed to intervention and direction of change**	
Gajos et al. 2012 [29]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)	Platelet-activating factor acetylhydrolases	Lipoprotein-associated phospholipase A2 enzyme	Lp-PLA2	B: 260 (68); F: 240 (64)**, P=0.026	Decreased
Khandouzi et al. 2020 [39]	ELISA kit (ZellBio GmbH, Ulm, Germany)				B: 3.96 (2.45); F: 2.99 (1.65)#, P=0.008	Decreased

$ Only significant subclasses or those that are comparable among the included studies are shown.** Mean expressed as ng/mL (SD), effect after n-3 PUFA intervention.# Mean expressed as ng/mL (SD), effect after canola oil intervention. Abbreviations: ALA, alpha linoleic acid; B, baseline value; C, control group; DHA; docosahexaenoic acid; EPA, eicosapentaenoic acid; F, final value; HDL; high-density lipoprotein; I, intervention group; IL-6, interleukine-6; LA, linoleic acid; LDL, low-density lipoprotein; LysoPC, lysophosphatidylcholine; MPO, myeloperoxidase; MUFA, monounsaturated fatty acids; Ox-LDL, oxidized LDL; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin. Baseline stable CAD features are described in the Population column (mean ± SD).

TABLE 9 | Description of included studies assessing the effects of fatty acid-based interventions on bioactive lipid levels in healthy subjects

Author, year (reference)	**Country**	**Study design**	**Population**	**Fatty acid intervention and daily dose**	**Comparator**	**Duration of exposure**	**Participants** *N*, (I/C)	**Intention-to-treat**
Averill et al. 2019 [47]	Washington, USA	Randomized, postprandial, cross-over trial. ≥1-week washout period	19-49 y men and women	• *Intervention sequence 1:* High-saturated fat meal (HSF) providing 20 % TEI, and 80 % fat: 24 g SFA, 4 g MUFA and 2 g PUFA • *Intervention sequence 2:* High-carbohydrate meal (HC) providing 20 % TEI, and 10 % fat: 0 g SFA, 3 g MUFA and 1.5 g PUFA	Not controlled	Two 6-hour postprandial test meals	15	No
Block et al. 2012 [44]	New York, USA	Randomized, double-blind, placebo-controlled, postprandial cross-over trial. 1-month washout period	18-50 y men and women	• *Intervention sequence 1:* Marine n-3 PUFA capsule *(Lovaza®, Reliant Pharmaceuticals)* Daily dose: 4 g (3.4 g EPA+DHA) • *Intervention sequence 2:* Aspirin (ASA) tablet Daily dose: 81 mg • *Intervention sequence 3:* Marine n3 PUFA capsule + ASA tablet Daily dose: 4 g + 81 mg	Placebo and 30-day run-in period free of fish oil before each visit	Four 4-hour postprandial sessions	25	No
Del Bas et al. 2016 [19]	United Kingdom	Randomized, double-blind, placebo-controlled parallel trial	18-65 y men and women	Marine n-3 PUFA capsule providing 1.1:0.8 g of EPA:DHA Daily dose: 1.9 g	Placebo corn-oil capsule Daily dose: 3g	3 months	38 (19/19)	No
Morris et al. 2015 [46]	Ireland (The Metabolic Challenge Study (MECHE))	Randomized, open-label, postprandial parallel trial	21-49 y men and women	• *Group 1:* Oral lipid tolerance test (OLTT), 100 mL Calogen *(Nutricia)* combined with 50mL Liquid Duocal *(SHS Nutrition)* providing 7 g SFA, 31 g MUFA and 16 g PUFA • *Group 2:* Oral glucose tolerance test (OGTT), 75 mL anhydrous glucose plus 100 mLwater combined with a Physical tests session	Not controlled	5-hour postprandial test	40	No

Žáček et al. 2018 [18]	Grand Forks, USA (The Salmo Salar study)	Randomized, open-label, cross-over trial. 4 to 8-week washout period	48-52 y men and women	• *Intervention sequence 1:* n-3 PUFA-rich farmed Atlantic salmon provided in servings of 90 g, 2 d/wk Daily dose: 0.30 g EPA+DHA • *Intervention sequence 2:* n-3 PUFA-rich farmed Atlantic salmon provided in servings of 180 g, 2 d/wk Daily dose: 0.61 g EPA+DHA	Not controlled	Two 4-week study periods	9	No
Zhu et al. 2019 [49]	California, USA	Randomized, open-label, cross-over trial. 4-day washout period	18-25 y men and women	• *Intervention sequence 1:* Fast food or western diet (FF) enriched in red meat, simple sugars, cholesterol and SFA, providing 44.67 g SFA (29.16 % TEI), 1.89 g MUFA and 3.24 g PUFA • *Intervention sequence 2:* Mediterranean diet (Med) enriched in fruits, vegetables, fiber, MUFA and PUFA providing 13.24 g SFA (5.27 % TEI), 41.28 g MUFA and 15.63 g PUFA	Not controlled	Two 4-day study periods	10	No
Asztalos et al. 2016 [40]	Boston, USA	Randomized, double-blind, parallel-assignment, placebo-controlled trial	21-70 y men and women	• *Group 1:* n-3 EPA-enriched oil capsule providing 627 mg EPA Daily dose: 0.60 g EPA • *Group 2:* n-3 EPA-enriched oil capsule providing 1689:5 mg of EPA:DHA Daily dose: 1.8 g EPA • *Group 3:* n-3 DHA-enriched oil capsule providing 1.6:758 mg of EPA:DHA. Daily dose: 0.60 g DHA	Placebo olive oil capsule providing 3954mg of oleic acid Daily dose: 6 g	6 weeks	121 (30/31/30/30)	Yes
Kim et al. 2017 [41]	Korea	Randomized, open-label, controlled trial	30-65 y men and women	• *Group 1:* Low linoleic acid (LA); replacement of 10 mL/day soy oil with one apple • *Group 2:* High LA; reduction of 1/3 cup of cooked refined rice and addition of 9.9 g/d of soy oil as a supplement (54.2 % LA)	Medium LA control; maintenance of the usual food intake	2 months	150 (50/50/50)	No

Nelson et al. 2011 [42]	Fort Collins, USA	Randomized, open-label, controlled trial	≥50 y men and women	• *Group 1:* Isocaloric diet supplemented with fish oil capsule providing 180:120 mg of EPA:DHA plus olive oil Daily dose: 2 g EPA+DHA + 9 g OO • *Group 2:* Isocaloric diet supplemented with flaxseed oil capsule Daily dose: 11 g (0.57 g ALA)	Isocaloric diet supplemented with olive oil capsule providing Daily dose: 11 g (800 mg MUFA from oleic acid)	2 months	59 (20/20/19)	No

Table 9. continued

	Bioactive lipids						
	Assessment method	**Identification reference**	**Class**	**Subclass[$]**	**Molecular formula**	**Changes in concentration attributed to intervention and direction of change**	
Averill et al. 2019 [47]	Untargeted LC-MS on isolated HDL particles	Not reported	Cer	40:1	$C_{40}H_{79}NO_3$	-0.052 (0.06)[*], P=0.005	Decreased
				42:1	$C_{42}H_{83}NO_3$	+0.163 (0.18)[*], P=0.004	Increased
				42:2	$C_{42}H_{81}NO_3$	+0.191 (0.22)[*], P=0.005	
			LacCer	34:1	$C_{34}H_{67}NO_3$	+0.194 (0.65)[*], P=0.023	Increased
			PC	32:2	$C_{40}H_{76}NO_8P$	+0.147 (0.08)[*], P<0.0001	
				34:1	$C_{42}H_{82}NO_8P$	+0.124 (0.08)[*], P=0.0001	
				34:2	$C_{42}H_{80}NO_8P$	+0.079 (0.07)[*], P=0.001	
				36:2	$C_{44}H_{84}NO_8P$	+0.081 (0.05)[*], P=0.0001	Increased
				36:3	$C_{44}H_{82}NO_8P$	+0.083 (0.07)[*], P=0.001	
				36:4	$C_{44}H_{80}NO_8P$	+0.115 (0.06)[*], P<0.0001	
				38:4	$C_{46}H_{84}NO_8P$	+0.086 (0.06)[*], P=0.0002	
				38:5	$C_{46}H_{82}NO_8P$	+0.099 (0.07)[*], P=0.0002	
			SM	14:0	$C_{37}H_{75}N_2O_6P$	+0.108 (0.43)[*], P=0.013	Increased
Block et al. 2012 [44]	Direct infusion electrospray mass spectrometry (QTrap 2000 MS/MS using an Advion Inc. (Ithaca, NY) Nanomate nanospray (ESI) source)	Calibration curves and internal standards	Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	-2.10 (63.3)[**], P=0.390 after n-3 -2.70 (72.3)[**], P=0.840 after n-3+ASA	
				18:0	$C_{26}H_{56}NO_6P$	+3.59 (25.3)[**], P=0.390 after n-3 +0.32 (21.4)[**], P=0.810 after n-3+ASA	
				18:1	$C_{26}H_{52}NO_7P$	-7.50 (25.0)[**], P=0.750 after n-3 -11.40 (32.3)[**], P=0.270 after n-3+ASA	Decreased/
				18:2	$C_{26}H_{50}NO_7P$	-16.0 (69.9)[**], P=0.540 after n-3 -34.6 (117.0)[**], P=0.700 after n-3+ASA	Increased
				20:4	$C_{28}H_{50}NO_7P$	+2.40 (67.4)[**], P=0.310 after n-3 -13.20 (95.3)[**], P=0.910 after n-3+ASA	
				20:5	$C_{28}H_{48}NO_7P$	+2.20 (5.2)[**], P=0.002 after n-3 +0.86 (7.7)[**], P=0.130 after n-3+ASA	
Del Bas et al. 2016 [19]	Targeted LC-MS (Agilent 6550	Calibration curves and internal standards	Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 266.50; F: 199.20 (74.95)[†], P<0.01	Decreased
				18:0	$C_{26}H_{54}NO_7P$	B: 84.00; F: 60.20 (35.30)[†], P<0.01	
				18:1	$C_{26}H_{52}NO_7P$	B: 45.53; F: 51.70 (24.84)[†], P>0.05	Increased

	Accurate-Mass Quadrupole-Time of Flight (Q-TOF) mass spectrometer (MS))			18:2	$C_{26}H_{50}NO_7P$	B: 49.70; F: 72.30 (30.20)‡, P<0.05 B: 3.25; F: 4.28 (2.04)†, P<0.05 B: 3.38; F: 5.89 (2.09)‡, P<0.01	Increased
				20:4	$C_{28}H_{50}NO_7P$	B: 6.86; F: 6.55 (2.70)†, P>0.05 B: 7.80; F: 10.51 (4.41)‡, P<0.05	Decreased Increased
			Lyso-PE	16:0	$C_{21}H_{44}NO_7P$	B: 0.097; F: 0.103 (0.022)†, P>0.05	
				18:0	$C_{23}H_{48}NO_7P$	B: 0.374; F: 0.393 (0.131)†, P>0.05 B: 0.344; F: 0.433 (0.151)‡, P<0.05	
				18:1	$C_{23}H_{46}NO_7P$	B: 0.259; F: 0.634 (0.248)†, P<0.0001 B: 0.320; F: 0.723 (0.248)‡, P<0.0001	
				18:2	$C_{23}H_{44}NO_7P$	B: 0.497; F: 1.277 (0.641)†, P<0.0001 B: 0.648; F: 1.528 (0.538)‡, P<0.0001	Increased
				20:4	$C_{25}H_{44}NO_7P$	B: 0.299; F: 0.517 (0.144)†, P<0.0001 B: 0.394; F: 0.740 (0.267)‡, P<0.0001	
				22:6	$C_{27}H_{44}NO_7P$	B: 0.414; F: 0.455 (0.148)†, P>0.05 B: 0.307; F: 0.408 (0.120)‡, P<0.01	
Morris et al. 2015 [46]	Untargeted high throughput flow injection ESI-MS/MS (4000 QTrap® tandem mass spectrometer, AB SCIEX)	Internal standards and BIOCRATES in-house software MetIDQ™	Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	Fold change < 1.5#, P<0.05 at 300 min	Decreased
				18:0	$C_{26}H_{54}NO_7P$		
				18:1	$C_{26}H_{52}NO_7P$	Fold change > 1.5#, P<0.05 at 300 min	
				18:2	$C_{26}H_{50}NO_7P$		Increased
				20:4	$C_{28}H_{50}NO_7P$		
			Lyso-PE	16:0	$C_{21}H_{44}NO_7P$	Fold change > 1.5#, P<0.05 at 120 min	
				18:0	$C_{23}H_{48}NO_7P$		
				18:1	$C_{23}H_{46}NO_7P$		
				18:2	$C_{23}H_{44}NO_7P$		Increased
				20:4	$C_{25}H_{44}NO_7P$		
				22:6	$C_{27}H_{44}NO_7P$		
			PC	34:1	$C_{42}H_{82}NO_8P$	Fold change > 1.5#, P<0.05 at 180 min	
				34:2	$C_{42}H_{80}NO_8P$	AND	
				36:2	$C_{44}H_{84}NO_8P$	Fold change < 1.5#, P<0.05 at 300 min	
				36:3	$C_{44}H_{82}NO_8P$		
				36:4	$C_{44}H_{80}NO_8P$		Increased/ Decreased
				38:4	$C_{46}H_{84}NO_8P$		
				38:5	$C_{46}H_{82}NO_8P$		
				38:6	$C_{46}H_{80}NO_8P$		
				40:5	$C_{48}H_{86}NO_8P$		
				40:6	$C_{48}H_{84}NO_8P$		
			PE	36:2	$C_{41}H_{78}NO_8P$	Fold change > 1.5#, P<0.05 at 300 min	Increased
				36:3	$C_{41}H_{76}NO_8P$		
			Cer	16:1	$C_{34}H_{67}NO_3$	Fold change > 1.5#, P<0.05 at 300 min Fold change > 1.5#, P<0.05 at 180 min	Increased

			DiCer	24:0	$C_{42}H_{85}NO_3$	Fold change > 1.5[#], P<0.05 at 60 min	Increased
				26:0	$C_{44}H_{89}NO_3$		
			SM	16:0	$C_{39}H_{79}N_2O_6P$	Fold change >1.5[#], P<0.05 at 180 min	
				16:1	$C_{39}H_{77}N_2O_6P$		
				18:0	$C_{41}H_{83}N_2O_6P$		
				18:1	$C_{41}H_{83}N_2O_6P$		
				20:0	$C_{43}H_{87}N_2O_6P$		Increased
				20:1	$C_{43}H_{85}N_2O_6P$		
				20:2	$C_{43}H_{83}N_2O_6P$		
				21:0	$C_{44}H_{89}N_2O_6P$		
				22:0	$C_{45}H_{91}N_2O_6P$		
				23:0	$C_{46}H_{93}N_2O_6P$		
				24:2	$C_{47}H_{89}N_2O_6P$		
Žáček et al. 2018 [18]	Targeted LC-MS by using an AB Sciex 5500 QTRAP hybrid quadrupole ion-trap mass spectrometer with a Turbo Spray source (AB Sciex) operating at unit mass resolution	LIPID MAPS MS internal standards LipidView™ software	PC	36:5	$C_{44}H_{78}NO_8P$	B: 12.0 (4.1); F: 30.5 (18.8)□, P=0.027	Increased
				38:5	$C_{46}H_{82}NO_8P$	B: 7.6 (2.6); F: 18.3 (9.0)□, P=0.006	
				38:6	$C_{46}H_{80}NO_8P$	B: 2.0 (0.7); F: 4.2 (2.3)□, P=0.049	
				40:6	$C_{48}H_{84}NO_8P$	B: 22.5 (4.8); F: 39.9 (10.3)□, P<0.001	
				40:7	$C_{48}H_{82}NO_8P$	B: 6.7 (1.0); F: 9.2 (1.7)□, P=0.057	
			PE	36:0	$C_{41}H_{82}NO_8P$	B: 0.39 (0.09); F: 0.67 (0.10)□, P<0.001	
				38:0	$C_{41}H_{82}NO_7P$	B: 0.64 (0.14); F: 1.01 (0.25), P=0.0003	
				38:1	$C_{43}H_{86}NO_7P$	B: 0.27 (0.05); F: 0.42 (0.04)□,P<0.0001	
				38:6	$C_{43}H_{76}NO_7P$	B: 3.97 (2.17); F: 6.12 (3.17)□, P=0.001	Increased
				40:0	$C_{45}H_{90}NO_8P$	B: 0.04 (0.04); F: 0.10 (0.01)□, P=0.001	
				40:6	$C_{45}H_{80}NO_7P$	B: 2.57 (1.35); F: 3.94 (1.89)□,P=0.0003	
				40:7	$C_{45}H_{76}NO_8P$	B: 0.67 (0.35); F: 0.90 (0.50)□, P=0.007	
			Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 100.7 (10.7); F: 102.9 (21.9)□, P=0.850	
				18:0	$C_{26}H_{54}NO_7P$	B: 36.4 (4.2); F: 37.5 (8.0)□, P=0.850	
				18:1	$C_{26}H_{52}NO_7P$	B: 20.9 (4.2); F: 19.0 (4.2)□, P=0.850	Increased
				18:2	$C_{26}H_{50}NO_7P$	B: 37.9 (10.0); F: 41.3 (18.4)□, P=0.140	
				20:4	$C_{28}H_{50}NO_7P$	B: 5.1 (3.6); F: 5.1 (4.0)□, P=0.440	
			Lyso-PE	18:0	$C_{23}H_{48}NO_7P$	B: 0.06 (0.01); F: 0.08 (0.02)□, P=0.042	
				22:6	$C_{27}H_{44}NO_7P$	B: 0.33 (0.10); F: 0.53 (0.17)□, P=0.0003	
				34:1	$C_{39}H_{79}N_2O_6P$	B: 120.1 (11.1); F: 129.5 (16.5)□, P=0.580	Increased
				34:2	$C_{39}H_{77}N_2O_6P$	B: 17.5 (3.1); F: 18.2 (2.6)□, P=0.780	
				36:1	$C_{41}H_{83}N_2O_6P$	B: 25.0 (3.7); F: 27.4 (3.3)□, P=0.410	
				36:2	$C_{41}H_{81}N_2O_6P$	B: 13.5 (2.5); F: 14.5 (1.9)□,P=0.370	
			SM	40:1	$C_{45}H_{91}N_2O_6P$	B: 41.1 (5.5); F: 43.8 (11.8)□, P=0.810	
				42:1	$C_{47}H_{95}N_2O_6P$	B: 39.0 (4.9); F: 40.6 (9.5)□, P=0.890	Increased
				42:2	$C_{47}H_{93}N_2O_6P$	B: 78.9 (11.1); F: 90.3 (13.1)□, P=0.130	
Zhu et al. 2019 [49]	Untargeted LC-MS using an Agilent 6530 QTOF with positive	Internal standards and calibration curves	PC	30:0	$C_{41}H_{82}NO_8P$	B: 6.1e-04; F: 6.5e-04□, P<0.05 after FF	Increased
						B: 9.9e-04; F: 5.3e-04□, P<0.05 after MED	Decreased
				32:2	$C_{40}H_{76}NO_8P$	B: 5.1e-04; F: 5.7e-04□, P<0.05 after FF	Increased

mode, and an Agilent 6550 QTOF with negative mode. On isolated HDL fractions	In house rt-mz library and in silico LipidBlast library				B: 6.6e-04; F: 4.1e-04□, P<0.05 after MED	Decreased
			33:0	$C_{41}H_{82}NO_8P$	B: 8.1e-05; F: 4.9e-05□P<0.05 after MED	Decreased
			33:1	$C_{41}H_{80}NO_8P$	B: 3.1e-04; F: 3.6e-04□, P<0.05 after FF	Increased
					B: 3.9e-04; F: 2.7e-04□P<0.05 after MED	Decreased
			33:2	$C_{41}H_{78}NO_8P$	B: 5.1e-04; F: 6.5e-04□, P<0.0001 after FF	Increased
					B: 5.4e-04; F: 4.6e-04□, P<0.0001 after MED	Decreased
			34:1	$C_{42}H_{82}NO_8P$	B: 7.7e-04; F: 0.001□, P<0.0001 after FF	Increased
					B: 8.5e-04; F: 6.4e-04□, P<0.0001 after MED	Decreased
			34:2	$C_{42}H_{80}NO_8P$	B: 0.001; F: 0.002□, P<0.0001 after FF	Increased
			35:1	$C_{43}H_{84}NO_8P$	B: 2.9e-04; F: 3.5e-04□, P=0.001 after FF	Increased
					B: 3.2e-04; F: 2.5e-04□, P=0.001 after MED	Decreased
			35:2	$C_{43}H_{82}NO_8P$	B: 9.9e-04; F: 0.002□, P<0.0001 after FF	Increased
					B: 0.001; F: 9.4e-04□, P<0.0001 after MED	Decreased
			35:3	$C_{43}H_{80}NO_8P$	B: 2.9e-04; F: 4.2e-04□, P<0.0001 after FF	Increased
					B: 3.2e-04; F: 2.8e-04□, P<0.0001 after MED	Decreased
			36:2	$C_{44}H_{84}NO_8P$	B: 4.2e-04; F: 5.1e-04□, P<0.0001 after FF	Increased
					B: 4.1e-04; F: 3.6e-04□, P<0.0001 after MED	Decreased
			36:4	$C_{44}H_{80}NO_8P$	B: 0.004; F: 0.006□, P<0.0001 after FF	Increased
					B: 0.005; F: 0.004□, P<0.0001 after MED	Decreased
			38:4	$C_{46}H_{84}NO_8P$	B: 3.3e-04; F: 4.7e-04□, P=0.001 after FF	Increased
					B: 3.4e-04; F: 3.1e-04□, P=0.001 after MED	Decreased
			40:6	$C_{48}H_{84}NO_8P$	B: 0.002; F: 0.002□, P<0.05 after MED	Unchanged
			40:7	$C_{48}H_{82}NO_8P$	B: 0.002; F: 0.002□, P=0.001 after MED	
		PE	34:2	$C_{39}H_{74}NO_7P$	B: 9.0e-04; F: 0.001□, P<0.0001 after FF	Increased
					B: 9.5e-04; F: 6.5e-04□, P<0.0001 after MED	Decreased
			36:2	$C_{41}H_{78}NO_8P$	B: 0.001; F: 0.002□, P<0.0001 after FF	Increased
					B: 0.001; F: 8.0e-04□, P<0.0001 after MED	Decreased
			38:4	$C_{43}H_{78}NO_8P$	B: 0.003; F: 0.005□, P<0.0001 after FF	Increased
					B: 0.04; F: 0.002□, P<0.0001 after MED	Decreased
		Lyso-PC	16:0	$C_{24}H_{50}NO_7P$	B: 0.001; F: 0.001□,P>0.05 after FF and MED	Unchanged
			18:0	$C_{26}H_{54}NO_7P$	B: 6.7e-04; F: 6.4e-04□, P>0.05 after FF	Decreased
					B: 6.5e-04; F: 5.7e-04□, P>0.05 after MED	
			18:1	$C_{26}H_{52}NO_7P$	B: 4.0e-04; F: 3.3e-04□, P<0.05 after FF	Decreased
					B: 3.8e-04; F: 4.1e-04□, P<0.05 after MED	Increased
			18:2	$C_{26}H_{50}NO_7P$	B: 5.5e-04; F: 6.3e-04□, P>0.05 after FF	Increased
					B: 5.1e-04; F: 5.2e-04□, P>0.05 after MED	
			20:4	$C_{28}H_{50}NO_7P$	B: 7.9e-05; F: 6.7e-05□, P>0.05 after FF	Decreased
					B: 8.5e-05; F: 7.0e-05□, P>0.05 after MED	
		SM	34:0	$C_{39}H_{81}N_2O_6P$	B: 5.2e-04; F: 4.9e-04□, P<0.05 after FF	Decreased
					B: 5.0e-04; F: 5.4e-04□, P<0.05 after MED	Increased
			34:2	$C_{39}H_{77}N_2O_6P$	B: 0.002; F: 0.001□,P<0.05 after FF and MED	Decreased
			40:2	$C_{45}H_{89}N_2O_6P$	B: 0.004; F: 0.005□, P<0.05 after MED	Increased
			42:3	$C_{47}H_{91}N_2O_6P$	B: 0.003; F: 0.002□, P<0.0001 after FF	Decreased
					B: 0.002; F: 0.003□, P<0.0001 after MED	Increased

		Cer	40:1	$C_{40}H_{79}NO_3$	B: 2.0e-05; F: 2.1e-05□, P>0.05 after FF	Increased
					B: 1.9e-05; F: 1.9e-05□, P>0.05 after MED	Decreased
			42:1	$C_{42}H_{83}NO_3$	B: 1.1e-04; F: 1.1e-04□, P>0.05 after FF	Increased
					B: 1.1e-04; F: 1.1e-04□, P>0.05 after MED	Decreased
			42:2	$C_{42}H_{81}NO_3$	B: 2.3e-05; F: 2.3e-05□, P>0.05 after FF	Increased
					B: 1.7e-05; F: 1.3e-05□, P>0.05 after MED	Decreased
Table 9. continued	**Bioactive lipid enzymatic precursors**					
	Assessment method	**Class**	**Subclass**		**Changes in mass/activity attributed to intervention and direction of change**	
Asztalos et al. 2016 [40]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)	Platelet-activating factor acetylhydrolases	Lipoprotein-associated phospholipase A2 enzyme	Lp-PLA2	B: 170 (51.7); F: 157 (59.7)□, P=0.004 after EPA 600mg	
					B: 145.5 (29.9); F: 124.1 (33.7)□, P=0.003 after EPA 1800mg	Decreased
					B: 167.5 (41.3); F: 167.2 (49.9)□, P=0.970 after DHA 600mg	
Kim et al. 2017 [41]	PAF-AH activity assay kit (Biovision, Milpitas, CA)				B: 15.2 (4.24); F: 14.9 (3.53)□, P=0.587 after low LA	Decreased
					B: 14.7 (3.53); F: 15.7 (2.82)□, P<0.01 after high LA	Increased
Nelson et al. 2011 [42]	PLAC ELISA kit (diaDexus, Inc. South San Francisco, CA, USA)				B: 221.8 (62.1); F: 216.1 (63.5)□, P=0.910 after EPA+DHA	Decreased
					B: 223.3 (63.5); F: 213.8 (64.4)□, P=0.910 after ALA	Decreased

$ Only significant subclasses or those that are comparable among the included studies are shown. * Average relative abundance (%) change (SD) in HDL-associated lipid subclasses relative to baseline, reported change after 6-hour HSF meal test. ** Mean change expressed as µM (SD), reported change after 4-hour n-3 PUFA or n-3 PUFA + ASA intervention. † Mean expressed as µM (SD), effect after n-3 PUFA intervention or ‡ after corn oil intervention. # Fold change of the lipid subclasses which change significantly from baseline during the different timepoints of the 5-hour OLTT. □ Mean expressed as µmol/L (SD), effect after 180g Atlantic salmon intake. □ Relative abundance (mg %) of lipid subclasses after FF and MED interventions. □ Mean expressed as ng/mL (SD), effect after EPA and DHA interventions compared to placebo. □ Mean expressed as nmol/mL/min (SD), effect after LA interventions. □ Mean expressed as ng/mL (SD), effect within groups after fish oil and flaxseed oil interventions. Abbreviations: ALA, alpha-linolenic acid; B, baseline value; C, control group; Cer, ceramide; DHA, docosahexaenoic acid; DiCer, dihydroceramide; EPA, eicosapentaenoic acid; F, final value; I, intervention group; LA, linoleic acid; LacCer, lactosylceramide; Lyso-PC, lysophosphatidylcholine; Lyso-PE, lysophosphatidylethanolamine; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin; TEI, total energy intake.

Supplemental File 1 | Search strategy

We searched in PubMed, SCOPUS, and Cochrane Library, for eligible indexed articles published in English from October 2010 to May 2022. The following search strategy was applied:

#1. (lysophospholipids OR glycerophospholipids OR lysoglycerophospholipids OR sphingolipids OR lysophosphatidic acid OR lipoprotein-associated phospholipase A2 OR Lp-PLA2)

#2. (cardiovascular disease OR cardiometabolic disease OR cardiovascular risk NOT Fabry's disease)

#3. (fatty acid OR omega-3 fatty acids OR omega-6 fatty acids OR polyunsaturated fatty acids OR monounsaturated fatty acids OR saturated fatty acids)

#4. (RCT OR randomized clinical trial OR randomized control* trial NOT case-control NOT cohort NOT review)

PubMed

#A. #1 and #2 and #4

#B. #1 and #2 and #3 and #4

Additional limits:

#C. Limit to humans

#D. Limit to English language

#E. Limit to Controlled Clinical Trial; Randomized Controlled trial

SCOPUS

#A. (TITLE-ABS-KEY (#1)) AND (TITLE-ABS-KEY(#2)) AND (TITLE-ABS-KEY(#4)) AND (LANGUAGE (english)) AND (LIMIT-TO (humans))

#B. (TITLE-ABS-KEY (#1)) AND (TITLE-ABS-KEY(#2)) AND (TITLE-ABS-KEY(#3)) AND (TITLE-ABS-KEY(#4)) AND (LANGUAGE (english)) AND (LIMIT-TO (humans))

Cochrane Library

#A. #1 AND #2 Refined by: DOCUMENT TYPE: (TRIALS) AND LANGUAGES (ENGLISH)

#B. #1 AND #2 AND #3 Refined by: DOCUMENT TYPE: (TRIALS) AND LANGUAGES (ENGLISH)

Supplemental material

Supplemental File 2 | PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	p. 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	p. 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	p. 4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	p. 5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	p. 6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p. 6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	p. 6 + Supporting file 1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p. 6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p. 6 - 7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	p. 7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	p. 7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	p. 8
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	p. 7 - 8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	p. 7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	p. 8
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	p. 7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	p. 8

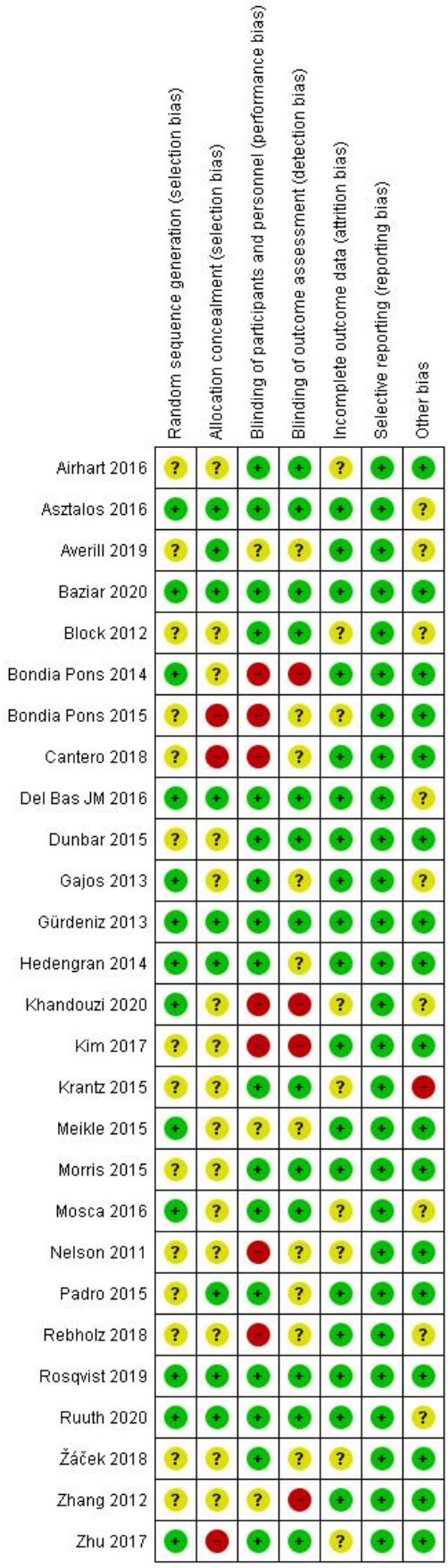
Supplemental material

Section and Topic	Item #	Checklist item	Location where item is reported
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	p. 8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	p. 8
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	p. 8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	p. 8
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p. 9
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	p. 9
Study characteristics	17	Cite each included study and present its characteristics.	p. 9 - 11
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	p. 11
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	p. 12 - 20
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	p. 12 - 20
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	p. 12 - 20
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p. 12 - 20
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	p. 12 - 20
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	p. 11 - 12
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	-
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	p. 21
	23b	Discuss any limitations of the evidence included in the review.	p. 27
	23c	Discuss any limitations of the review processes used.	p. 27
	23d	Discuss implications of the results for practice, policy, and future research.	p. 26 - 27
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p. 6
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	p. 6
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	-

Section and Topic	Item #	Checklist item	Location where item is reported
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p. 28
Competing interests	26	Declare any competing interests of review authors.	p. 28
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	p. 28

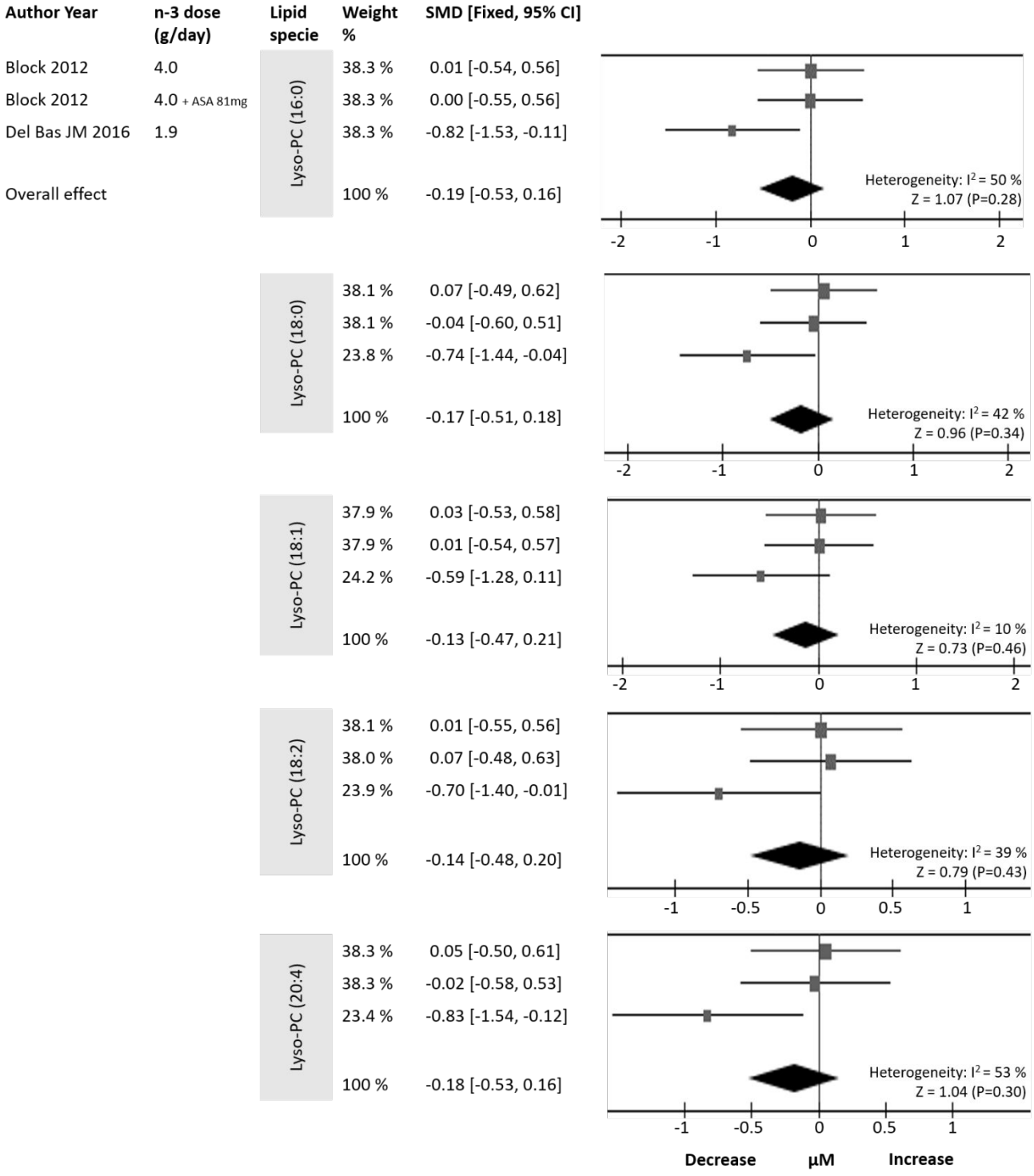
From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

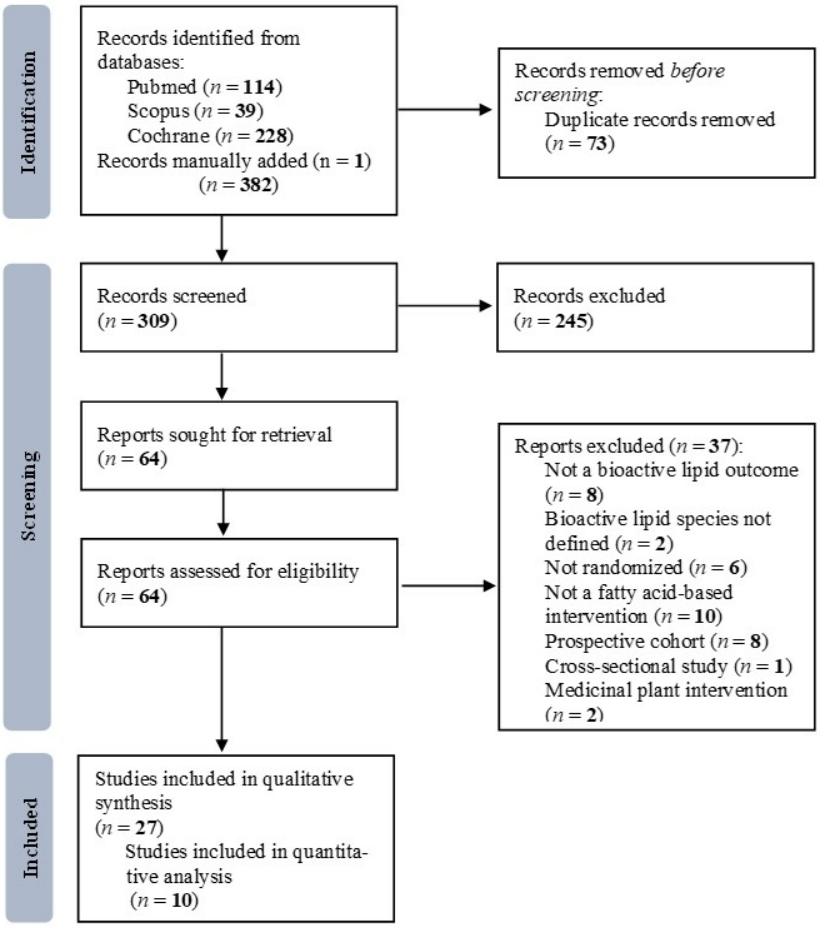
Supplemental Figure 1 | Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Supplemental material

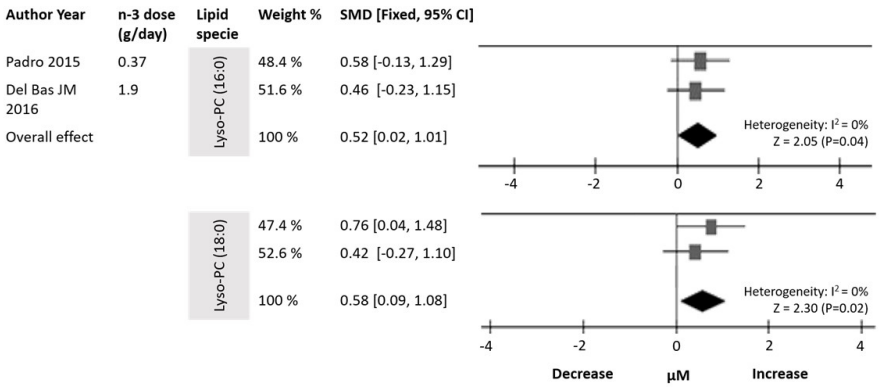
Supplemental Figure 2 | Meta-analysis of RCTs of the effect of n-3 PUFA supplemented dietary interventions on saturated and unsaturated Lyso-PC levels in healthy subjects. The squares and bars estimate of the net standard mean difference (SMD) (difference between the end and baseline concentrations of bioactive lipid species) and the corresponding 95% CI for individual studies. The fixed effects model overall results are indicated by a rhombus symbol near the bottom of each graph. Lyso-PC, lysophosphatidylcholine; n-3, omega-3 polyunsaturated fatty acids.





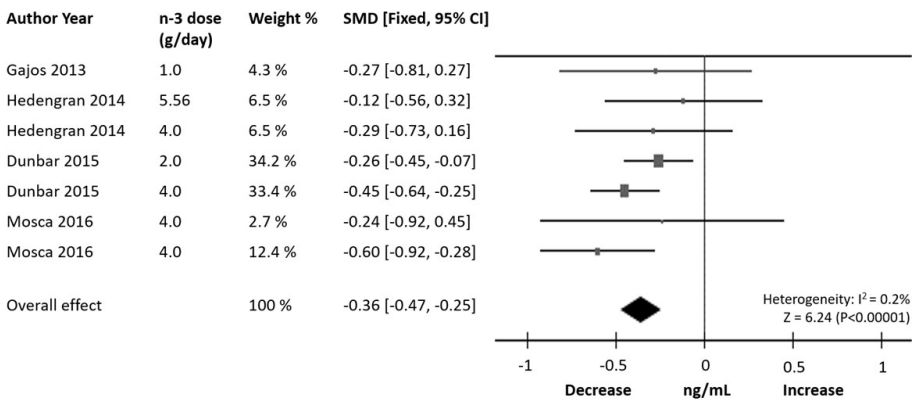
PRISMA 2020 flow diagram of the systematic review and meta-analysis. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

190x254mm (96 x 96 DPI)



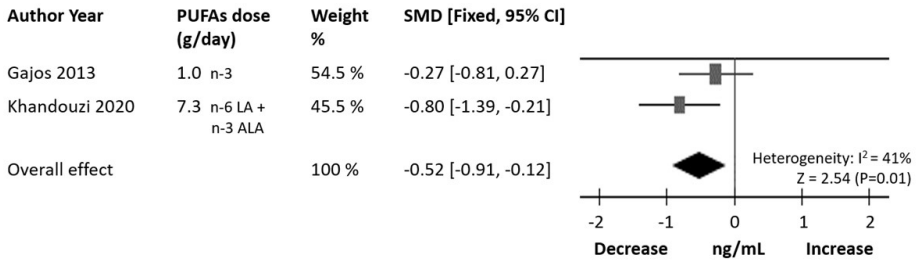
Meta-analysis of RCTs of the effect of n-3 PUFA-supplemented dietary interventions on saturated lyso-PC levels in overweight or obese subjects. The squares and bars estimate the net standard mean difference (SMD) (difference between the end and baseline concentrations of bioactive lipid subclasses) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. Lyso-PC, lysophosphatidylcholine; n-3, omega-3 polyunsaturated fatty acids.

338x254mm (96 x 96 DPI)



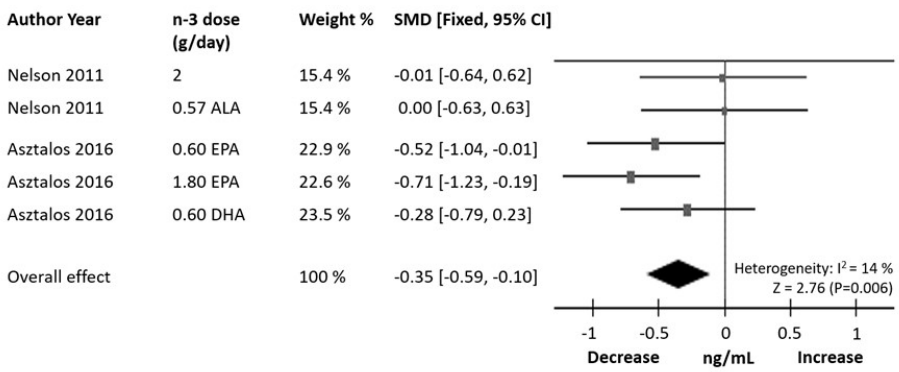
Meta-analysis of RCTSs of the effect of n-3 PUFA supplemented dietary interventions on Lp-PLA2 mass in subjects with dyslipidemia. The squares and bars estimate the net standard mean difference (SMD) (difference in Lp-PLA2 concentrations compared to placebo) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. n-3, omega-3 polyunsaturated fatty acids.

338x254mm (96 x 96 DPI)



Meta-analysis of RCTs of the effect of PUFA supplemented dietary interventions (n-3 and n-6) on Lp-PLA2 mass in subjects with stable CAD. The squares and bars estimate of the net standard mean difference (SMD) (difference between the end and baseline concentrations of Lp-PLA2) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. n-3, omega-3 polyunsaturated fatty acids; n-6-, omega-6 polyunsaturated fatty acids.

338x254mm (96 x 96 DPI)



Meta-analysis of RCTs of the effect of n-3 PUFA supplemented dietary interventions on Lp-PLA2 mass in healthy subjects. The squares and bars estimate the net standard mean difference (SMD) (difference between the end and baseline concentrations of Lp-PLA2) and the corresponding 95% CI for individual studies. The overall results of the fixed effects model are indicated by a rhombus near the bottom of each graph. ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3, omega-3 polyunsaturated fatty acids.

254x190mm (96 x 96 DPI)