Impairment of lysophospholipid metabolism in obesity: altered plasma profile and desensitization to the modulatory properties of n–3 polyunsaturated fatty acids in a randomized controlled trial

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Abstract

Background: Plasma lysophospholipids have emerged as signaling molecules with important effects on inflammation, insulin resistance, and fatty liver disease, each of which is linked closely to obesity. Dietary n–3 (ω-3) polyunsaturated fatty acids (PUFAs) may be able to improve these conditions.

Objective: The objective of this study was to assess the response of plasma lysophospholipids to obesity, n–3 PUFA consumption, and a high-fat meal challenge to better understand the role of lysophospholipid metabolism in the progression of obesity-related disorders.

Design: We determined the concentrations of 8 lysophosphatidylcholines, 11 lysophosphatidylethanolamines, and 7 lysophosphatidylinositols in the plasma of 34 normal-weight and 38 obese subjects randomly assigned to consume corn oil (control) or n–3 PUFA–rich fish oil (3 g/d; n = 15–19/group) for 90 d. Blood samples were collected on the last day of the study under fasting conditions and 6 h after a high-fat meal (1135 kcal, 86 g fat) challenge. The profile of secreted lysophospholipids was studied in HepG2 cells under palmitate-induced steatosis.

Results: Obese and normal-weight subjects had different profiles of plasma lysophospholipids. A multivariate combination of the 26 lysophospholipids could discriminate between normal-weight and obese subjects with an accuracy of 98%. The high-fat meal challenge altered the concentration of plasma lysophosphatidylcholines in an oil treatment–dependent manner in normal-weight but not obese subjects, suggesting that obesity impairs the sensitivity of lysophospholipid metabolism to n–3 PUFAs. Noncytotoxic steatosis in HepG2 cells affected the secretion pattern of lysophospholipids, partially resembling the changes observed in the plasma of obese subjects.

Conclusions: Obesity has a substantial impact on lysophospholipid metabolism, altering the plasma lysophospholipid profile and abolishing its sensitivity to dietary n–3 PUFAs. These effects could contribute to the onset or progression of alterations associated with obesity, such as inflammation, insulin

resistance, and fatty liver disease. This trial was registered at www.controlled-trials.com as ISRCTN96712688.

Key words: lysophospholipid metabolism, lysophosphatidylcholine, lysophosphatidylethanolamine, obesity, insulin resistance, inflammation, fatty liver disease, polyunsaturated fatty acids, omega-3, fish oil

Abbreviations

- HFM: high-fat meal
- MetS: metabolic syndrome
- NAFLD: nonalcoholic fatty liver disease
- PCA: principal component analysis
- PLS-DA: partial least squares discriminant analysis
- ROC: receiver operating characteristic

Introduction

Obesity is linked to chronic low-grade inflammation, insulin resistance, nonalcoholic fatty liver disease (NAFLD), metabolic syndrome (MetS), and cardiovascular disease, among other conditions (1, 2). Recent advances in the field of metabolomics have allowed untargeted exploration of the metabolic changes induced by obesity or obesity-related complications. Through the use of this approach, different lysophospholipid species, mainly lysophosphatidylcholines, have been identified as being differentially changed in the plasma of subjects with obesity (3, 4, 5, 6), nonalcoholic steatohepatitis (7, 8, 9), or NAFLD (10), and as accompanying the amelioration of different features associated with obesity and MetS (11, 12). These findings suggest that obesity and obesity-related conditions are linked to altered plasma lysophospholipids. Lysophospholipids act as signaling molecules, modulating processes such as inflammation, insulin production, and insulin sensitivity through their interaction with G protein–coupled receptors (13). Thus, lysophospholipids may be important molecules in obesity and its related disorders. Nevertheless, to our knowledge, the effects of obesity on lysophospholipid metabolism and the changes that the obese phenotype can induce in the plasma lysophospholipid profile remain unexplored.

There is increasing evidence that an increased intake of n–3 PUFAs can partly ameliorate some obesity-associated conditions. Thus, different meta-analyses show that n–3 PUFAs have beneficial effects on obesity and insulin resistance (14, 15, 16, 17), and exert anti-inflammatory actions at the local and systemic level (14, 18, 19). These effects are proposed to take place through different mechanisms, such as modulation of the activity of the peroxisome proliferator–activated receptor family of nuclear receptors or of the production of eicosanoids and other lipid mediators (16). Little is known about the actions of n–3 PUFAs on the metabolism of lysophospholipids, although it has been shown that lysophosphatidylcholine-containing n–3 polyunsaturated acyl chains (e.g., DHA and EPA) exert anti-inflammatory actions in vitro and in mice by affecting prostaglandin formation (20, 21). Therefore, incorporation into glycerophospholipids represents a mechanism by which n–3 PUFAs can interplay with

lysophospholipid metabolism.

Because fatty acid handling and hepatic lipid metabolism are dysregulated in obesity, we hypothesized that lysophospholipid metabolism, which is highly interconnected with these processes, is also altered, resulting in measurable changes in the concentrations of plasma lysophospholipid species that have been related with the progression of alterations linked to obesity. Therefore, our objective was to assess the effects of obesity on lysophospholipid metabolism and how different factors that either ameliorate this condition, such as n–3 PUFA consumption, or induce metabolic stress, such as a high-fat meal (HFM) challenge, can modulate the plasma lysophospholipid profile. Because of the emerging role of lysophospholipids as metabolic signals, our aim is to provide new evidence to support the role of lysophospholipid metabolism as a key factor in the onset and progression of obesity-related diseases.

Methods

Subjects and intervention

All procedures involving human subjects were approved by the National Research Ethics Service South Central–Berkshire Research Ethics Committee (submission no. 11/SC/0384). Normal-weight (n = 50) and obese (n = 50) male and female subjects were recruited from February 2012 to October 2013 at the University of Southampton. The primary aim of the study (registered at www.controlled-trials.com as ISRCTN96712688) was to assess the effect of n–3 PUFA consumption on blood inflammatory markers in normal-weight and obese subjects in the fasting state and in response to a standard HFM challenge. Secondary outcomes included the examination of the effects of obesity, n–3 PUFAs, and the HFM on blood lipids and related metabolites, which was the focus of the present study. Subjects were eligible for enrollment in the study if they were men or women aged 18–65 y, had a BMI (in kg/m2) of 18.5–25 (normal weight) or a BMI of 30–40 with a waist circumference >94 cm for men or >80 cm for women (obese), did not eat >1 oily fish meal/wk, and provided written informed consent. Subjects were excluded

if they met any of the following criteria: were diagnosed with diabetes; used prescribed medicine to control inflammation, hypertension, or dyslipidemia; used fish oil or other oil supplements; had chronic gastrointestinal problems; were pregnant or planning to become pregnant within the study period; were participating in another clinical trial.

The trial was designed to have 2 separate phases. The first phase was a crossover study designed to determine the effects of including n–3 PUFAs or corn oil (as control) with a single HFM on postprandial changes in metabolites and inflammatory markers in both normal-weight and obese subjects. The HFM consisted of 2 croissants served with 28 g butter and 18 g jam; three 1-g capsules containing either corn oil or n–3 PUFA–containing fish oil; and a milkshake made with 250 mL low-fat milk, 32 g Nesquik powder, and 75 g double cream. The HFM provided 85.8 g fat, 76.9 g carbohydrate, 18.6 g protein, and 1134 kcal. An abdominal adipose tissue biopsy was collected during the first phase of the trial. The current work is based on the second phase of the trial, which was focused on the effects of daily intake of n–3 PUFAs or corn oil (as control) for 90 d on blood lipids and other metabolites and inflammatory markers. Sample size for the trial was calculated based on anticipated findings from the second phase of the trial: a 20% reduction in plasma concentration of IL-6 with the 90-d n–3 PUFA treatment was anticipated. Based on means ± SDs for plasma IL-6 concentrations from previous studies, it was calculated that 20 subjects/group [i.e., the corn oil and n–3 PUFA (fish oil) groups] would be needed to detect a 20% difference with 80% power at the 5% significance level ($P < 0.05$). Thus, to allow for a 20% drop out rate, 50 normal-weight and 50 obese subjects needed to be recruited. Fifty normal-weight and 50 obese subjects initially were recruited and participated in the first phase of the trial. Before this second phase was started, 16 normal-weight and 12 obese subjects withdrew from the study; this was mainly because of the requirement to provide a second abdominal adipose tissue biopsy at the end of the second phase. The remaining cohort (34 normal-weight and 38 obese subjects) was randomly allocated, in a double-blinded fashion, to 3 g corn oil/d as control or 3 g EPAX6000 TG fish oil—a source of EPA (1.1 g/d) and DHA (0.8 g/d)—per day, for 90 d. Random allocation of subject study code to treatment (blinded

as A or B) was performed with the use of an online random number generator. Corn oil and fish oil were provided as 1-g gelatin-coated capsules. Capsules were provided to subjects in sealed containers. The appearance of the capsules and containers and the labeling on containers were identical for the 2 capsule types. Subjects and all researchers were blinded to allocation until after statistical analysis was complete. After 90 d, subjects attended the National Institute for Health Research Wellcome Trust Clinical Research Facility at Southampton General Hospital in the morning after an overnight fast (>10 h without food or drink except water). A blood sample was collected into tubes containing heparin as an anticoagulant, and then subjects consumed the same HFM described in phase 1 with placebo capsules. All subjects consumed the same meal and placebo capsules. Blood was collected into tubes containing heparin as an anticoagulant 1, 2, 3, 4, and 6 h after finishing the meal. Plasma was prepared by centrifugation (1900 × g, 10 min, room temperature) and stored at −80°C until analysis. Data for the fasting and 6-h plasma samples are presented here. There were no subject withdrawals in the second phase of the trial, so data are available for all subjects.

Determination of plasma lipid, glucose and insulin concentrations

Plasma triglyceride, cholesterol, HDL cholesterol, nonesterified fatty acid, and glucose concentrations were measured with the use of an iLAB 600 clinical chemistry analyzer and software (Instrumentation Laboratories) and enzyme-based kits provided by Wako and Instrumentation Laboratories. LDL cholesterol concentrations were estimated by using the Friedwald equation. Plasma insulin concentrations were measured with the use of a quantitative sandwich ELISA kit from Dako. HOMA-IR was calculated with the use of the following formula: {[glucose (in mmol/L)] × [insulin (in μU/L)]}/22.5.

Sample preparation for determination of lysophospholipids

For the extraction of metabolites, 100 μL human plasma was added to 900 μL methanol:water (8:1, vol:vol) containing 0.5 mg lysophosphatidylcholine/L (13:0) and 0.1 mg deuterated taurocholic acid-D5/L as internal standard. The mixture was homogenized by ultrasonication (30 s) and vortexing (20 s). After

that, samples were incubated on ice for 10 min and then centrifuged (16,000 × g, 5 min, 4°C). The supernatant was dried under nitrogen flow to eliminate the solvent. Finally, it was redissolved in 200 μL methanol:water (1:1, vol:vol) to obtain a 50% dilution of the initial plasma concentration.

Separation of lysophospholipids by reverse-phase liquid chromatography

Lysophospholipids were separated by reverse-phase liquid chromatography performed with the use of an Agilent ZORBAX C18 SB-Aq 2.1-mm × 50-mm, 1.8-μm particle analytic column (Agilent Technologies). An Agilent ZORBAX C-8 2.1-mm × 30-mm, 3.5-μm particle guard column was placed in series in front of the analytic column. An Agilent 1290 Infinity HPLC system with a binary pump and degasser, thermostated well plate autosampler, and column compartment were used. The autosampler temperature was 4°C, the injection volume was 2 μL, the column temperature was 60°C, and the flow rate was 0.6 mL/min. A 2–98% linear gradient of solvent A (0.2% acetic acid in water) to B [0.2% acetic acid in methanol (Honeywell)] was used over 16 min followed by a solvent B hold of 2 min and a 5 min post-time for both positive and negative ion polarity analysis.

Identification of lysophospholipid species by mass spectrometry

Lysophospholipid species were identified by mass spectrometry. An Agilent 6550 Accurate-Mass Quadrupole–Time of Flight mass spectrometer was operated in ESI+ and ESI− modes. Dynamic mass axis calibration was achieved by continuous infusion of a reference mass solution (121.050873 and 922.009798 for positive polarity, and 119.03632 and 980.016375 for negative polarity). Scanning conditions were as follows: drying gas temperature of 325°C and flow rate of 10 L/min; vaporizer temperature of 350°C; nebulizer pressure of 45 psi; capillary voltage 4000 V. Mass spectrometry data acquired in positive mode were used for quantitative analysis of lysophosphatidylcholine species and negative-mode mass spectrometry data were used to quantify lysophosphatidylethanolamine and lysophosphatidylinositol species. Quantitative analysis was performed as described previously (22).

Calibration curves were constructed with the use of 1–1500 μg lysophosphatidylcholine/L (16:0), lysophosphatidylcholine (18:0), lysophosphatidylcholine (20:0), lysophosphatidylethanolamine (18:1), and lysophosphatidylinositol (18:1) as standards. A lysophosphatidylcholine (16:0) calibration curve was used to quantify lysophosphatidylcholine (14:0), lysophosphatidylcholine (16:0), and lysophosphatidylcholine (16:1); a lysophosphatidylcholine (18:0) calibration curve was used to quantify lysophosphatidylcholine (18:0), lysophosphatidylcholine (18:1), lysophosphatidylcholine (18:2), lysophosphatidylcholine (18:3), and lysophosphatidylcholine (18:4); and a lysophosphatidylcholine (20:0) calibration curve was used to quantify lysophosphatidylcholine (20:0), lysophosphatidylcholine (20:1), lysophosphatidylcholine (20:2), lysophosphatidylcholine (20:3), lysophosphatidylcholine (20:4), and lysophosphatidylcholine (22:5). All lysophosphatidylethanolamine and lysophosphatidylinositol species were quantified with the use of the calibration curves of lysophosphatidylethanolamine (18:1) and lysophosphatidylinositol (18:1). The limit of detection was 0.04 μmol/L.

Cell culture experiments

HepG2 cells were maintained in complete cell culture medium obtained by supplementing DMEM (Lonza Ibérica) with 1% L-glutamine (Lonza Ibérica), 1% penicillin/streptomycin–EDTA (Sigma), 1% nonessential amino acids (Sigma), and 10% fetal bovine serum (Sigma). Cells were seeded in either 12 or 48 well plates at a concentration of 120,000 cells/mL in complete cell culture medium. Twelve hours after seeding, media were replaced with complete serum-free media containing 1% bovine serum albumin (Sigma) bound to sodium palmitate (Sigma) at 0.2, 0.5, and 0.75 mmol/L or without sodium palmitate as control for 24 h. Treatment media preparation has been described previously (23). After 24-h treatment, cell media were collected, centrifuged at 1000 × g for 10 min at 4°C to discard cell debris, and stored at −80°C until extraction and quantification of lysophospholipids. For neutral lipid staining, cells were washed extensively with PBS, fixed with 4% paraformaldehyde, and subsequently incubated with Oil Red O (Sigma). After microscopic analysis and micrography (Nikon Eclipse Ti-S; Izasa), Oil Red O was eluted with isopropyl alcohol (Sigma) and the absorbance read at 510 nm. For cytotoxicity assays, after

24 h of treatment, media were replaced with complete serum-free media containing a 1% bovine serum albumin and thiazolyl blue tetrazolium bromide (Sigma) as previously described (23). After a 4.5-h incubation, cells were washed with PBS and micrographed, and intracellular thiazolyl blue tetrazolium bromide was extracted with DMSO (Sigma) and quantified at 570 and 660 nm. Cell viability was quantified with the following formula: Viability (%) = [A570 – A660 (palmitate)/A570 − A660 (control)] × 100.

Statistical analyses

Data were available for 15 normal-weight subjects in the control group, 19 obese subjects in the control group, 19 normal-weight subjects in the n–3 PUFA group (fish oil) and 19 obese subjects in the n–3 PUFA group. Data are expressed as means ± SEMs. Differences in baseline characteristics between subject groups were analyzed by ANCOVA, in which treatment group assignment was used as a fixed factor and the variables age and sex were included as covariates. An ANCOVA model was also used to analyze the data at the endpoint, in both fasting and postprandial conditions. The circulating concentrations of lysophospholipids or their Δ values (changes between the pre- and post-HFM challenge) and the Δ values of anthropometric and biochemical markers (changes from baseline) were used as dependent variables, obesity and n–3 PUFA treatment were included as fixed factors, and the variables age and sex were included as covariates. When the interaction between obesity and treatment was statistically significant under the ANCOVA, a Bonferroni post hoc test was used to compute pairwise comparisons between groups (i.e., the effect of treatment within obesity groups and the effect of obesity within treatment groups). Grubbs' test was used to detect outliers, which were discarded for subsequent analyses. All statistical analyses were performed with SPSS Statistics 18, with the statistical significance level set at bilateral 5%. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), multivariate biomarker validation with the use of receiver operating characteristic (ROC) curves, and hierarchical clustering analyses were performed after data normalization and autoscaling with the use of the software MetaboAnalyst 3.0 (24).

Results

Subject characteristics

Data were collected between February 2012 and October 2013 from 34 normal-weight subjects who consumed daily 3 g of either corn oil (n = 15) or fish oil (n = 19) for 90 d and from 38 obese subjects who also consumed daily 3 g of either corn oil (n = 19) or fish oil (n = 19). At baseline, the obese subjects were significantly older than the normal-weight subjects and had higher body weight, BMI, waist circumference, and body fat mass (Table 1). Obese subjects had higher plasma concentrations of total cholesterol, LDL cholesterol, triglycerides, and nonesterified fatty acids than did normal-weight subjects (Table 1), although the mean concentrations of these variables in the obese subjects were typically within the acceptable concentration range, according to the American Association of Clinical Endocrinologists' Medical Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis (25). Obese subjects also had higher plasma glucose and insulin concentrations (Table 1), and a mean HOMA-IR value >2.5 indicates insulin resistance (26).

There was a significant effect of treatment on fasting HDL cholesterol and triglyceride concentrations (Table 1). n–3 PUFAs lowered triglycerides and elevated HDL cholesterol concentrations compared with corn oil (control) (Table 1). No significant changes were found in other anthropometric and biochemical markers analyzed (Table 1).

Obesity and n–3 PUFAs induce changes in plasma lysophospholipid concentrations in the fasting state

Obesity was associated with significant differences in plasma lysophospholipid concentrations in the fasting state compared with concentrations seen in normal-weight individuals (Table 2). Obese subjects, independent of treatment group, had lower concentrations of most of the identified lysophosphatidylcholines, with the exception of lysophosphatidylcholine 20:2, which was higher in the

obese subjects (Table 2). Obese subjects had lower concentrations of 5 of 11 lysophosphatidylethanolamines (lysophosphatidylethanolamine 14:1, lysophosphatidylethanolamine 18:1, lysophosphatidylethanolamine 18:2, lysophosphatidylethanolamine 20:0, and lysophosphatidylethanolamine 20:2) than did normal-weight subjects (Table 2). The interaction between obesity and treatment was significant for the plasma concentration of lysophosphatidylethanolamine 18:0, which was lower in the obese subjects who received the n–3 PUFAs than in the normal-weight participants submitted to the same treatment (Table 2). Treatment with n–3 PUFAs affected the concentration of 8 of 26 lysophospholipids. Thus, the n–3 PUFA groups had lower concentrations of lysophosphatidylcholine 18:2, lysophosphatidylcholine 20:3, lysophosphatidylethanolamine 18:1, lysophosphatidylethanolamine 18:2, and lysophosphatidylethanolamine 20:4, and higher concentrations of lysophosphatidylethanolamine 20:5, lysophosphatidylethanolamine 22:6, and lysophosphatidylinositol 18:0.

PCA including all the lysophospholipids revealed a clear phenotype-dependent clustering of the subjects when the scores of the first 3 principal components, explaining ~56% of the variance, were represented (Figure 1A). Consistent with the ANCOVA and post hoc analyses, when the individual scores were colored based on both the phenotype and the oil treatment, no clear treatment-dependent clusters were identified (Figure 1B).

The 26 lysophospholipids were used to set up a PLS-DA–predictive model for discriminating between normal-weight and obese subjects (Figure 1C). The quality parameters associated with the model were excellent. The degree of fit of the model to the data, represented by R2, was 0.86. The quality assessment statistic, which reports the result of cross-validation of the model, was 0.78, whereas a threshold of >0.4 has been proposed to be an acceptable value for a biological model (27). The prediction accuracy of the cross-validation process was 97.7%. The cross-validation revealed that the accuracy of the model was maximal (97.7%) when all 26 lysophospholipids were used, presenting an ROC AUC of 0.996, with a narrow CI ranging from 0.982 to 1 (Figure 1D). These results suggest that the profile of lysophospholipid

abundance clearly differs between normal-weight and obese subjects, and that the more lysophospholipid species used to characterize the subject, the better the discrimination between the 2 phenotypes.

An HFM challenge magnifies the differences in plasma lysophospholipids between normal-weight and obese subjects and modifies the long-term effects of n–3 PUFA treatment

The abundance of the 26 lysophospholipids was determined in the same subjects 6 h after intake of an HFM (Table 3). The HFM challenge accentuated the differences between normal-weight and obese groups, producing the same differences in the plasma concentrations of lysophosphatidylcholines and lysophosphatidylethanolamines reported in the fasting state and revealing additional differences in the concentrations of lysophosphatidylethanolamines (18:0, 20:4, 22:5, and 22:6) and lysophosphatidylinositols (18:1 and 22:6) (Table 3). These results suggest that the obesity-related differences in lysophosphatidylcholine profile are robust regardless of the absorptive state of the subject, whereas the profile of lysophosphatidylethanolamine and lysophosphatidylinositol species is sensitive to short-term high-fat loads. By contrast, the HFM challenge produced, in general, changes in the circulating concentrations of lysophospholipids that were different from those seen in the fasting state in the subjects chronically treated with n–3 PUFAs. Thus, significant interactions between obesity and treatment were found after the HFM intake for plasma concentrations of lysophosphatidylcholines (18:0, 20:1, and 20:3), which were significantly lower in the normal-weight subjects who consumed fish oil than in the normal-weight participants who consumed corn oil (Table 3). In addition, although the treatment effect was maintained for lysophosphatidylethanolamines (18:2, 20:4, and 22:6), it was abolished for lysophosphatidylcholines (18:2 and 20:3), lysophosphatidylethanolamines (18:1 and 20:5), and lysophosphatidylinositol 18:0 (Table 3).

The post-HFM challenge concentrations of the 26 lysophospholipids were used for unsupervised classification with the use of the PLS-DA model obtained in the fasted state. As a result, 67 of 72 subjects

were correctly classified (93% accuracy) (Figure 2A), obtaining an ROC AUC of 0.991 for the post-HFM challenge values (Figure 2B). Because the plasma lysophospholipid profile 6 h after the HFM challenge could be used to discriminate between normal-weight and obese subjects by applying a model that was obtained with the plasma lysophospholipid profile in fasting conditions, these results reinforce the conclusion that the changes in plasma lysophospholipid profile induced by obesity are robust and maintained even after refeeding.

The response of lysophosphatidylcholine metabolism to an HFM challenge is modulated by n–3 PUFAs in normal-weight but not in obese subjects

Different studies have shown that the obese state is associated with a decreased sensitivity to fasting and refeeding conditions in terms of regulation of genes and proteins involved in key metabolic processes (28, 29). To further characterize the effects of long-term n–3 PUFA intake on the postprandial response to an HFM challenge, we tested whether the plasma lysophospholipid profile is also sensitive to refeeding conditions. When the differences between the pre- and post-HFM challenge plasma concentrations of lysophospholipids were analyzed, an interaction between the oil treatment and the obese phenotype was found for all the lysophosphatidylcholine species, revealing that n–3 PUFA treatment affected the response of lysophosphatidylcholines to the HFM challenge only in normal-weight subjects (Figure 3 and Supplemental Table 1). Thus, in normal-weight subjects, the concentrations of lysophosphatidylcholine 16:0, lysophosphatidylcholine 18:0, and lysophosphatidylcholine 20:1 decreased in response to the HFM in normal-weight subjects who consumed n–3 PUFAs through fish oil compared with the normal-weight subjects who consumed corn oil. In addition, normal-weight subjects who consumed corn oil showed increased circulating concentrations of lysophosphatidylcholine 18:1, lysophosphatidylcholine 18:2, lysophosphatidylcholine 20:2, lysophosphatidylcholine 20:3, and lysophosphatidylcholine 20:4 after the HFM challenge compared with the normal-weight subjects who consumed fish oil (Figure 3 and Supplemental Table 1). These treatment-dependent responses found in normal-weight subjects were not

observed in obese subjects (Figure 3 and Supplemental Table 1). Treatment- and phenotype-dependent changes were not observed in lysophosphatidylethanolamine or lysophosphatidylinositol species, with the exception of lysophosphatidylethanolamine 20:4, which significantly increased in response to the HFM in the normal-weight subjects who consumed fish oil, but not in the obese subjects who consumed fish oil (Supplemental Table 1). These results indicate an insensitivity to the effects of a high-fat diet on lysophosphatidylcholine species in obese subjects.

An in vitro model of steatosis partially reproduces the plasma lysophospholipid profile of obese subjects

Because of the key contribution of the liver to the pool of plasma lysophospholipids (30), we hypothesized that the changes in the profile of lysophospholipids associated with obesity could be at least in part a response of hepatocytes to the increasing load of fatty acids that occurs in obesity (1). To test this hypothesis, we established a model of mild steatosis in HepG2 cells. Cells cultured in serum-depleted media were exposed to 1 of 3 concentrations of palmitate. All 3 concentrations resulted in statistically significant intracellular neutral lipid accumulation (Figure 4A and B). Nevertheless, only 0.2 mmol palmitate/L maintained full viability of the cells, whereas 0.5 mmol palmitate/L and 0.75 mmol palmitate/L significantly decreased cell viability by 50% and 60%, respectively (Figure 4C and D). The concentrations of all the lysophospholipids that could be detected (Table 4) were determined in the preconditioned media of HepG2 cells. PCA (80.9% of the variance explained by principal components 1, 2, and 3; Figure 4E) and hierarchical clustering analysis (Figure 4F) with the use of all the detected lysophospholipids revealed that the 3 doses of palmitate resulted in very different lysophospholipid profiles in the media. Thus, the cluster of cells treated with 0.2 mmol palmitate/L was clearly differentiated from the cluster of cells cultured without palmitate and from cells treated with 0.5 or 0.75 mmol palmitate/L. In turn, the clusters formed by the cells treated with 0.5 and 0.75 mmol palmitate/L overlapped and were differentiated clearly from the other 2 treatments. These results suggest that both

steatosis and cell viability highly affect the profile of lysophospholipids produced by HepG2 cells.

Analysis of individual lysophospholipids (Table 4) revealed exacerbated changes for cells treated with 0.5 or 0.75 mmol palmitate/L, likely because of the release of intracellular contents into the medium on apoptosis of the cells. Nevertheless, comparison of the cells treated with the vehicle with cells treated with the 0.2 mmol palmitate/L revealed differences similar to those found in human plasma. Thus, 6 of 9 lysophosphatidylcholines were changed. Although most of the lysophosphatidylcholine species detected in HepG2 media were different from those detected in human plasma, 2 of the common species (lysophosphatidylcholine 18:1 and lysophosphatidylcholine 20:2) changed in the same direction in HepG2 cell media and human plasma. The exception to this was lysophosphatidylcholine 16:0, which contains palmitic acid, the fatty acid used to establish the model. Lysophosphatidylethanolamines and lysophosphatidylinositols followed a similar pattern in HepG2 media and human plasma, with 6 of 7 common forms unchanged and only one, lysophosphatidylethanolamine 18:1, differing in both models. These results suggest that, despite the evident differences between the HepG2 and human models, induction of noncytotoxic steatosis in HepG2 cells partially reproduces the changes of lysophospholipid profiles found in human plasma when obese subjects are compared with normal-weight subjects.

Discussion

Our results agree with previous research showing decreased concentrations of different species of lysophosphatidylcholines in obesity or insulin resistance in humans and mice (3, 4, 10, 31) that persist even after weight loss (3). In contrast, increased concentrations of lysophosphatidylcholine species associated with the onset of type 1 diabetes, insulin resistance, and obesity have been described as well (5, 6, 32, 33, 34, 35), suggesting that the onset of these alterations might be associated with transient increases of lysophosphatidylcholine. Besides lysophosphatidylcholine species, plasma lysophosphatidylethanolamines have been shown to correlate negatively with BMI (36) and increase as obesity is ameliorated by diet (11). In agreement with these results, our analyses performed on plasma

collected in fasting conditions revealed lower circulating concentrations of some lysophosphatidylethanolamines in obese subjects than in normal-weight subjects. Beyond the changes found in individual lysophospholipid species, it is remarkable that, with our data, the performance of the PLS-DA predictive model was maximal when all the analyzed lysophospholipid species were used. These results highlight the fact that the impact of obesity on plasma lysophospholipids is not limited to a discrete number of species but to the whole profile of plasma lysophospholipids, including lysophosphatidylcholines, lysophosphatidylethanolamines, and lysophosphatidylinositols, suggesting that obesity widely affects the metabolism of lysophospholipids.

We have found that the metabolism of plasma lysophosphatidylcholines is sensitive to long-term intake of n–3 PUFAs. Thus, after the HFM challenge, concentrations of saturated lysophosphatidylcholines were sharply decreased and those of unsaturated lysophosphatidylcholines remained unchanged in normal-weight subjects treated with n–3 PUFAs. Circulating lysophosphatidylcholines have been related to inflammation, although whether they exert pro- or anti-inflammatory actions is still under debate. In fact, the role of lysophosphatidylcholines in inflammation might be dependent on their fatty acyl chain (13, 37). Thus, unsaturated lysophosphatidylcholines (20:4 and 22:6) counteract the proinflammatory actions of saturated (16:0) lysophosphatidylcholines (20, 21). Therefore, it could be suggested that the changes exerted by the n–3 PUFA–rich oil are positive, reflecting a lower inflammatory state after the HFM. This interpretation is consistent with the anti-inflammatory properties attributed to n–3 PUFAs (14, 15, 38, 39). In contrast, long-term intake of corn oil, rich in the n–6 PUFA linoleic acid (18:2n–6), had no effects on the response of saturated lysophosphatidylcholines to the HFM challenge, although the concentration of lysophosphatidylcholine 20:4 was increased. It is believed that the postprandial state is a period of transient acute inflammation that contributes to increasing cardiovascular disease risk (40). Therefore, it could be hypothesized that, together with other mechanisms (19, 38), n–3 PUFAs might attenuate postprandial inflammation by modulating the metabolism of lysophospholipids. Furthermore, in contrast to the findings in normal-weight subjects, obesity impaired the sensitivity of lysophosphatidylcholine

metabolism to the HFM. Recently, Kardinaal et al. (41) showed that the response of plasma inflammatory markers such as IL-6 and different proinflammatory lipids to a high-fat challenge was blunted in obese subjects with MetS compared with in healthy subjects. These differences in the response to a high-fat challenge were attributed to the lack of phenotypic flexibility that might lead to MetS. Our results support this explanation. Nevertheless, it has to be considered that most of the lysophosphatidylcholines, including the proinflammatory lysophosphatidylcholine 16:0, were lower in obese subjects than in normal-weight subjects in the fasting state. These findings might seem paradoxical at first, given that obesity is associated with chronic low-grade inflammation (42). However, it is possible that the impaired sensitivity of lysophosphatidylcholine metabolism to proinflammatory cues in obese subjects is responsible for this finding. Other possible causes for these differences, such as age differences between groups, need further research.

Although our results highlight the clear effects of obesity and, to a lesser extent, n–3 PUFAs on lysophospholipid metabolism, a potential limitation of the present study is that the lack of adjustment for multiple testing might have increased the number of false positives. Therefore, results concerning individual lysophospholipid species should be interpreted with caution, although these represent a starting point to assess the effects of obesity and n–3 PUFA consumption on individual lysophospholipids.

The changes observed in the profile of lysophospholipids could be related to other processes that also are affected by obesity. For example, it has been shown that lysophosphatidylcholines might have an important role in insulin sensitivity. Thus, lysophosphatidylcholine 12:0, 14:0, and 16:0, but not the lysophosphatidylcholines of 18 and 20 carbons, induce the uptake of glucose by cultured adipocytes (43), lysophosphatidylcholine 18:1 induces pancreatic insulin release (44), and circulating concentrations of lysophosphatidylcholine 16:0 are lower in insulin-resistant subjects than in insulin-sensitive subjects with NAFLD (10). In fact, lysophosphatidic acid, a product of lysophosphatidylcholine hydrolysis, has been suggested as being a promising agent to treat insulin resistance (13). Therefore, the lower plasma concentrations of these metabolites observed in obese subjects could be related to their lower insulin

sensitivity compared with that in normal-weight subjects, as suggested by the differences found in HOMA-IR indexes. This possibility is reinforced by the greater decrease in lysophosphatidylcholine 16:0 observed in normal-weight subjects treated with n–3 PUFAs than in the other groups after the HFM challenge, which might indicate transient adverse effects on insulin sensitivity. Indeed, a recent systematic review of meta-analyses revealed that n–3 PUFAs have unfavorable effects on type 2 diabetes in Caucasians (14). Therefore, the interactions of obesity with lysophospholipid metabolism could also contribute to or result from the onset of obesity-associated insulin resistance.

The differences observed in plasma lysophospholipids between normal-weight and obese subjects prompted us to explore further the origin of these changes. Plasma lysophospholipids might be formed in blood by the actions of lecithin cholesterol acyltransferase and secretory phospholipases, but a direct hepatic origin also has been demonstrated in rats and proposed as a quantitatively relevant source of lysophosphatidylcholines (30). In fact, it has been shown that hepatic alterations such as NAFLD and nonalcoholic steatohepatitis alter the metabolism of phospholipids and lysophospholipids in mice and humans (7, 10). Overall, the effects of NAFLD on circulating concentrations of lysophosphatidylcholines are very similar to those described by us and others in obese subjects. In view of this evidence, it is plausible to hypothesize that these common changes have a common origin. It is well known that increasing BMI is associated with an increased risk of NAFLD (45), and adipocyte cell size is associated with liver injury (46). The main underlying mechanism relates to the increased flux of fatty acids from the adipose tissue to the liver, together with altered hepatic metabolism of fatty acids and lipoproteins (1, 45). Therefore, we hypothesized that accumulation of lipids in hepatocytes might alter the profile of secreted lysophospholipids. Our experiment in the in vitro model of steatosis obtained by challenging HepG2 with palmitate supports this hypothesis. What is more, despite the obvious differences between the in vitro model and human subjects, the changes induced in the profile of lysophospholipids in HepG2 media by noncytotoxic steatosis and in plasma by obesity are remarkably similar. Therefore, it could be hypothesized that the changes induced by obesity in the lysophospholipid profile partially are due to the

metabolic stress that obesity induces in the liver, and place these molecules as promising biomarkers for studying alterations of liver homeostasis. More research is needed still to confirm this hypothesis.

Overall, our results suggest that obesity has a profound impact on the metabolism of lysophospholipids, modifying the profile of plasma lysophospholipids in the long term and affecting the sensitivity of these metabolites to dietary fatty acids. These effects could be mediated, at least partially, by the influence of obesity in the metabolism of hepatocytes. Because of the role of lysophospholipids as signaling molecules in processes that usually are altered in obesity, these findings provide more evidence in understanding the mechanisms that favor the progression of alterations such as insulin resistance, inflammation, NAFLD, or MetS. More research is needed to understand better the exact role of plasma lysophospholipids in disease progression and, therefore, to assess whether lysophospholipid metabolism represents a promising target for the prevention and treatment of obesity-associated diseases, as well as being a source of biomarkers for the early and noninvasive detection of metabolic alterations.

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Figure Legends

FIGURE 1. Multivariate analyses of 26 lysophospholipids detected in the plasma of 34 normal-weight and 38 obese subjects. Score plot of PC1, PC2, and PC3 after a principal component analysis for normal-weight and obese subjects (A). Same plot shown in panel A, showing the four 90-d diet groups (B). Score plot of PC1, PC2, and PC3 after PLS-DA with the use of phenotype, i.e., normal weight and obese, as the discriminant factor (C). ROC curve analysis of the PLS-DA model with the use of the 2, 3, 5, 10, 20, and 26 lysophospholipids, with the highest variable importance in the projection score (D). The ROC AUCs and the corresponding 95% CIs are reported in the inset. N-CO, normal-weight subjects who consumed corn oil; N-FO, normal-weight subjects who consumed fish oil; O-CO, obese subjects who consumed corn oil; O-FO, obese subjects who consumed fish oil; PC, principal component; PLS-DA, partial least squares discriminant analysis; ROC, receiver operating characteristic; Var., number of variables (lysophospholipids).

FIGURE 2. Projection in the PLS-DA model shown in Figure 1 of lysophospholipid profiles measured 6 h after a high-fat meal challenge in normal-weight and obese subjects. Classification of subjects by their lysophospholipid profile as normal weight or obese during the CV of the PLS-DA model set-up (training group) and classification of subjects by their lysophospholipid profile measured 6 h after the challenge (validation group) (A). ROC curves calculated during the cross-validation of the PLS-DA model (darker line and shadowed 95% CI of 0.982–1) and ROC curve obtained with the lysophospholipid profile determined 6 h after the challenge as a holdout group validation (lighter line) (B). The ROC AUCs for the CV and the holdout groups are reported in the inset. CV, cross-validation; PLS-DA, partial least squares discriminant analysis; ROC, receiver operating characteristic; t0, basal conditions before the challenge; t6, 6 h after the challenge.

FIGURE 3. Differences in LPC concentrations between fasting conditions and 6 h after a high-fat meal challenge in normal-weight and obese subjects treated with corn oil or n–3 PUFA–rich oil for 90 d (n =

15–19). Data are means ± SEMs. The effects of treatment, obesity, and their interaction were evaluated by ANCOVA. Δ values (changes between the pre- and post-high–fat challenge) of lysophospholipid concentrations in plasma were used as dependent variables, obesity and n–3 PUFA treatment were included as fixed factors, and the variables age and sex were included as covariates. O: significant effect of obesity; T: significant effect of treatment; O × T, significant interaction between obesity and treatment ($P < 0.05$). *Effect of obesity within fish oil groups; #effect of obesity within corn oil groups; $effect of treatment within normal-weight groups (Bonferroni post hoc comparison, $P < 0.05$). LPC, lysophosphatidylcholine; N-CO, normal-weight subjects who consumed corn oil; N-FO, normal-weight subjects who consumed fish oil; O-CO, obese subjects who consumed corn oil; O-FO, obese subjects who consumed fish oil.

FIGURE 4. Changes in the profiles of secreted lysophospholipids induced by steatosis in HepG2 cells. Cells were cultured with vehicle or the indicated doses of palmitate for 24 h. Intracellular accumulation of neutral lipids was evaluated by ORO staining (A). Spectrophotometric quantification after ORO elution from cells (B). Cell viability was determined by incubating cells with MTT after 24 h of treatment with a vehicle or palmitate at different doses (C). Reduced MTT was eluted and quantified spectrophotometrically, and cell viability was determined as a percentage of the vehicle signal (D). The concentrations of 17 lysophospholipids detected in the media of the vehicle- or palmitate-treated HepG2 cells were subjected to principal component analysis; scores for PC1, PC2, and PC3 are represented for each replicate (E). Clustering trends of the different experimental groups were confirmed further by hierarchical clustering analysis (F). Data in columns are means ± SEMs of 2 independent experiments run in triplicate. **$P < 0.01$ with respect to the vehicle group by Student's t test. ABS, absorbance; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; MTT, methyl thiazolyl tetrazolium; ORO, Oil Red O; PC, principal component; % respect the vehicle, percentage with respect to the vehicle.

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Tables

TABLE 1. Anthropometric and biochemical characteristics of normal-weight and obese subjects at baseline and changes from baseline after treatment with 3 g corn oil/d or 3 g n–3 PUFA–rich oil/d for 90 d

	Before treatment (absolute values)				**After treatment (Δ values)**				
Participants	**N-FO**	**O-FO**	**N-CO**	**O-CO**	**ANCOVA**	**N-FO**	**O-FO**	**N-CO**	**O-CO**
n	19	19	15	19					
Males, n (%)	8 (42.1)	5 (26.3)	2 (13.3)	5 (26.3)					
Age, y	30.8 ± 3.5	47.6 ± 2.7	31.9 ± 3.5	40.5 ± 2.9	O				

Weight, kg	63.2 ± 2.1	94.9 ± 2.7	61.3 ± 2.4	101.7 ± 3.2	O	0.38 ± 0.37	0.86 ± 0.68	0.25 ± 0.46	0.74 ± 0.53
BMI, kg/m2	22.2 ± 0.4	34.4 ± 0.5	22.5 ± 0.5	35.5 ± 0.7	O	0.14 ± 0.13	0.34 ± 0.26	0.09 ± 0.16	0.24 ± 0.19
Waist, cm	75.6 ± 1.2	106.8 ± 2.5	75.3 ± 1.7	108.9 ± 2.9	O	0.04 ± 0.70	−0.01 ± 1.35	−0.92 ± 0.44	−0.57 ± 0.75
Fat mass, kg	12.4 ± 1.1	38.0 ± 1.4	14.4 ± 1.1	41.6 ± 1.7	O	0.20 ± 0.30	0.47 ± 0.38	0.14 ± 0.35	0.82 ± 0.46
TC, mmol/L	4.38 ± 0.21	5.56 ± 0.27	4.37 ± 0.22	4.87 ± 0.14	O	0.01 ± 0.11	−0.07 ± 0.15	0.03 ± 0.11	0.07 ± 0.08
HDL-C, mmol/L	1.50 ± 0.08	1.51 ± 0.07	1.58 ± 0.08	1.41 ± 0.07		0.12 ± 0.06	0.05 ± 0.05	−0.06 ± 0.04	−0.01 ± 0.03
LDL-C, mmol/L	2.64 ± 0.18	3.69 ± 0.22	2.63 ± 0.21	3.23 ± 0.12	O	−0.09 ± 0.09	−0.15 ± 0.12	0.09 ± 0.09	0.04 ± 0.05
TG,	0.85 ±	1.43 ±	0.79 ±	1.15 ±	O	−0.16	−0.10	−0.03	0.17 ±

mmol/L	0.08	0.19	0.06	0.12		± 0.08	± 0.08	± 0.04	0.13
NEFAs, μmol/L	423 ± 45	630 ± 37	486 ± 56	575 ± 46	O	46.4 ± 57.2	−95.3 ± 50.5	37.6 ± 67.1	−26.9 ± 32.3
Glucose, mmol/L	4.79 ± 0.09	5.49 ± 0.18	4.73 ± 0.07	5.48 ± 0.07	O	0.00 ± 0.10	0.16 ± 0.16	0.14 ± 0.11	−0.04 ± 0.10
Insulin, μIU/mL	5.19 ± 0.61	12.7 ± 1.6	6.00 ± 0.56	16.0 ± 1.9	O	−0.63 ± 0.80	0.68 ± 1.21	0.44 ± 0.59	−0.41 ± 1.26
HOMA-IR	1.11 ± 0.12	3.33 ± 0.48	1.28 ± 0.12	3.74 ± 0.47	O	−0.12 ± 0.18	0.54 ± 0.32	0.12 ± 0.14	−0.01 ± 0.31

Values are means ± SEMs, unless otherwise indicated. n = 15–19. Blood was collected after overnight fasting at study entry and on the last day of the 90-d intervention. Differences in anthropometric and biochemical characteristics (dependent variables) between groups were evaluated by ANCOVA, in which the variables age and sex were included as covariates. At baseline, treatment group assignment was used as a fixed factor, whereas at the endpoint obesity and n–3 PUFA treatment were included as fixed factors. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NEFA, nonesterified fatty acid; N-CO, normal-

weight subjects who consumed corn oil; N-FO, normal-weight subjects who consumed fish oil; O, significant effect of obesity ($P < 0.05$); O-CO, obese subjects who consumed corn oil; O-FO, obese subjects who consumed fish oil; T, significant effect of treatment ($P < 0.05$); TC, total cholesterol; TG, triglyceride.

TABLE 2. Lysophospholipid concentrations in the plasma of normal-weight and obese subjects treated with 3 g corn oil/d or 3 g n–3 PUFA–rich oil/d for 90 d and then submitted to overnight fasting

Metabolite	**N-FO**	**O-FO**	**N-CO**	**O-CO**	**ANCOVA**
LPC					
16:0	266.5 ± 23.6	125.7 ± 10.2	253.3 ± 23.9	131.3 ± 16.6	O
18:0	84.0 ± 12.0	22.2 ± 3.1	85.0 ± 10.1	24.8 ± 3.8	O
18:1	45.6 ± 5.2	11.9 ± 1.3	49.7 ± 6.9	14.4 ± 1.9	O
18:2	3.25 ± 0.39	0.771 ± 0.09	3.73 ± 0.56	1.06 ± 0.11	O, T
20:1	0.409 ± 0.039	0.177 ± 0.010	0.409 ± 0.038	0.169 ± 0.015	O
20:2	0.297 ± 0.021	0.469 ± 0.036	0.299 ± 0.027	0.524 ± 0.038	O
20:3	2.15 ± 0.23	0.952 ± 0.07	2.95 ± 0.28	1.24 ± 0.15	O, T
20:4	6.32 ± 0.62	2.87 ± 0.26	7.01 ± 0.82	3.40 ± 0.41	O

LPE					
14:1	0.854 ± 0.028	0.791 ± 0.013	0.858 ± 0.023	0.786 ± 0.014	O
16:0	0.096 ± 0.004	0.093 ± 0.005	0.096 ± 0.006	0.088 ± 0.003	
18:0	0.374 ± 0.025	0.310 ± 0.021*	0.316 ± 0.014	0.322 ± 0.021	O, O × T
18:1	0.241 ± 0.014	0.212 ± 0.014	0.292 ± 0.029	0.256 ± 0.017	O, T
18:2	0.496 ± 0.038	0.394 ± 0.035	0.585 ± 0.051	0.516 ± 0.046	O, T
20:0	0.468 ± 0.034	0.433 ± 0.021	0.443 ± 0.026	0.423 ± 0.031	O
20:2	1.014 ± 0.060	0.766 ± 0.042	1.118 ± 0.117	0.853 ± 0.047	O
20:4	0.298 ± 0.016	0.293 ± 0.023	0.393 ± 0.028	0.356 ± 0.027	T
20:5	0.097 ± 0.019	0.109 ± 0.009	0.065 ± 0.005	0.086 ± 0.011	T
22:5	0.085 ±	0.087 ±	0.080 ±	0.090 ±	

	0.004	0.004	0.005	0.006	
22:6	0.410 ± 0.028	0.381 ± 0.023	0.305 ± 0.016	0.275 ± 0.017	T
LPI					
16:1	0.431 ± 0.014	0.405 ± 0.005	0.428 ± 0.013	0.403 ± 0.006	O
18:0	0.097 ± 0.005	0.105 ± 0.005	0.085 ± 0.005	0.094 ± 0.004	T
18:1	0.076 ± 0.004	0.081 ± 0.004	0.077 ± 0.003	0.078 ± 0.004	
18:2	0.091 ± 0.006	0.091 ± 0.004	0.091 ± 0.006	0.094 ± 0.005	
20:3	0.062 ± 0.002	0.064 ± 0.002	0.063 ± 0.002	0.065 ± 0.002	
20:4	0.149 ± 0.008	0.157 ± 0.006	0.150 ± 0.012	0.159 ± 0.010	
22:6	0.081 ± 0.004	0.086 ± 0.004	0.082 ± 0.004	0.086 ± 0.005	

652 *Values are means ± SEMs (μmol/L). n = 15–19. Blood was collected after overnight fasting on the last
653 day of the 90-d intervention. Lysophospholipid concentrations in plasma were used as dependent

variables, obesity and n–3 PUFA treatment were included as fixed factors, and the variables age and sex were included as covariates. *Effect of obesity within fish oil groups (Bonferroni post hoc comparisons, $P < 0.05$). LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; N-CO, normal-weight subjects who consumed corn oil; N-FO, normal-weight subjects who consumed fish oil; O, significant effect of obesity ($P < 0.05$); O-CO, obese subjects who consumed corn oil; O-FO, obese subjects who consumed fish oil; O × T, significant interaction between obesity and treatment ($P < 0.05$); T, significant effect of treatment ($P < 0.05$).*

TABLE 3. Lysophospholipid concentrations in plasma of normal-weight and obese subjects treated with 3 g corn oil/d or 3 g n–3 PUFA–rich oil/d for 90 d and then submitted to a high-fat meal challenge

Metabolite	**N-FO**	**O-FO**	**N-CO**	**O-CO**	**ANCOVA**
LPC					
16:0	199.2 ± 17.2	131.9 ± 17.9	244.2 ± 15.9	113.3 ± 15.5	O
18:0	60.2 ± 8.1$	25.5 ± 4.4*	88.9 ± 9.8	21.3 ± 4.3#	O, T, O × T
18:1	51.7 ± 5.7	19.9 ± 2.8	72.3 ± 7.8	18.0 ± 2.7	O
18:2	4.28 ± 0.47	1.79 ± 0.21	5.89 ± 0.54	1.76 ± 0.21	O
20:1	0.319 ± 0.028$	0.186 ± 0.019*	0.411 ± 0.031	0.166 ± 0.015#	O, O × T
20:2	0.315 ± 0.025	0.524 ± 0.048	0.437 ± 0.036	0.550 ± 0.045	O, T

20:3	2.66 ± 0.28$	1.50 ± 0.16*	4.61 ± 0.30	1.76 ± 0.20#	O, T, O × T
20:4	6.55 ± 0.62	4.07 ± 0.46	10.51 ± 1.14	4.19 ± 0.52	O, T
LPE					
14:1	0.854 ± 0.005	0.787 ± 0.012	0.899 ± 0.026	0.807 ± 0.009	O
16:0	0.103 ± 0.005	0.089 ± 0.004	0.098 ± 0.005	0.098 ± 0.004	
18:0	0.393 ± 0.030	0.342 ± 0.024	0.433 ± 0.039	0.308 ± 0.018	O
18:1	0.634 ± 0.057	0.421 ± 0.027	0.723 ± 0.064	0.402 ± 0.029	O
18:2	1.277 ± 0.147	0.802 ± 0.054	1.528 ± 0.139	0.866 ± 0.065	O, T
20:0	0.454 ± 0.041	0.434 ± 0.029	0.547 ± 0.053	0.413 ± 0.028	O
20:2	1.64 ± 0.15	1.13 ± 0.07	1.83 ± 0.15	1.12 ± 0.05	O
20:4	0.517 ± 0.033	0.421 ± 0.022	0.740 ± 0.069	0.467 ± 0.037	O, T
20:5	0.205 ±	0.135 ±	0.164 ±	0.134 ±	

	0.037	0.015	0.032	0.019	
22:5	0.108 ± 0.004	0.097 ± 0.005	0.119 ± 0.011	0.099 ± 0.006	O
22:6	0.455 ± 0.034	0.379 ± 0.019	0.408 ± 0.031	0.292 ± 0.013	O, T
LPI					
16:1	0.442 ± 0.018	0.399 ± 0.006	0.437 ± 0.013	0.410 ± 0.008	O
18:0	0.105 ± 0.005	0.109 ± 0.003	0.104 ± 0.005	0.103 ± 0.005	
18:1	0.133 ± 0.010	0.109 ± 0.004	0.133 ± 0.012	0.109 ± 0.005	O
18:2	0.142 ± 0.013	0.129 ± 0.005	0.138 ± 0.012	0.123 ± 0.008	
20:3	0.068 ± 0.003	0.068 ± 0.002	0.072 ± 0.004	0.068 ± 0.002	
20:4	0.234 ± 0.020	0.224 ± 0.010	0.243 ± 0.024	0.220 ± 0.013	
22:6	0.141 ± 0.011	0.116 ± 0.004	0.141 ± 0.012	0.116 ± 0.005	O

*Values are means ± SEMs (μmol/L). n = 15–19. Blood was collected 6 h after the administration of a high-fat meal (1135 kcal and 86 g fat, of which 59 g was saturated fat). Lysophospholipid concentrations in plasma were used as dependent variables, obesity and n–3 PUFA treatment were included as fixed factors, and the variables age and sex were included as covariates. *Effect of obesity within fish oil groups; #effect of obesity within corn oil groups; $effect of treatment within normal-weight groups (Bonferroni post hoc comparisons, P < 0.05). LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; N-CO, normal-weight subjects who consumed corn oil; N-FO, normal-weight subjects who consumed fish oil; O, significant effect of obesity (P < 0.05); O-CO, obese subjects who consumed corn oil; O-FO, obese subjects who consumed fish oil; O × T, significant interaction between obesity and treatment (P < 0.05); T, significant effect of treatment (P < 0.05).*

TABLE 4. Concentrations of lysophospholipids in the media of HepG2 cells treated with vehicle or with different concentrations of palmitate for 24 h to induce steatosis

Metabolite	**Vehicle**	**0.2 mmol/L**	**Change in obese humans**	**0.5 mmol/L**	**0.75 mmol/L**	**ANOVA**
LPC						
14:0	9 ± 1a	0.3 ± 0.2b*	ND	15 ± 2a	26 ± 4c	**
16:0	172 ± 7a	173 ± 10a	Decreased	1017 ± 127b	1455 ± 159c	**
16:1	1876 ± 30	1841 ± 34	ND	1862 ± 20	1976 ± 48	

18:1	185 ± 8a	146 ± 8b*	Decreased	245 ± 17c	330 ± 31d	**
18:3	246 ± 8a	250 ± 12a	ND	1220 ± 304b	2042 ± 336c	**
18:4	54 ± 3a	71 ± 6b*	ND	137 ± 7c	186 ± 16d	**
20:0	6 ± 1a	10 ± 2a,b*	ND	21 ± 6b	26 ± 3b	**
20:2	130 ± 5a	170 ± 8b*	Increased	210 ± 4c	200 ± 14c	**
22:5	306 ± 9	266 ± 11*	ND	328 ± 46	269 ± 61	
LPE						
18:0	11 ± 1a	12 ± 1a	Unchanged in corn oil group	29 ± 3b	36 ± 3b	**
18:1	61 ± 3a	58 ± 6a	Decreased	98 ± 6b	133 ± 9c	**
18:4	1.0 ± 0.1a	3.2 ± 0.1b*	ND	14 ± 0.4c	15 ± 1c	**
20:4	9 ± 1a	9 ± 1a	Unchanged	22 ± 2b	33 ± 3c	**
LPI						
18:1	43 ± 2	40 ± 5	Unchanged	60 ± 3	84 ± 5	

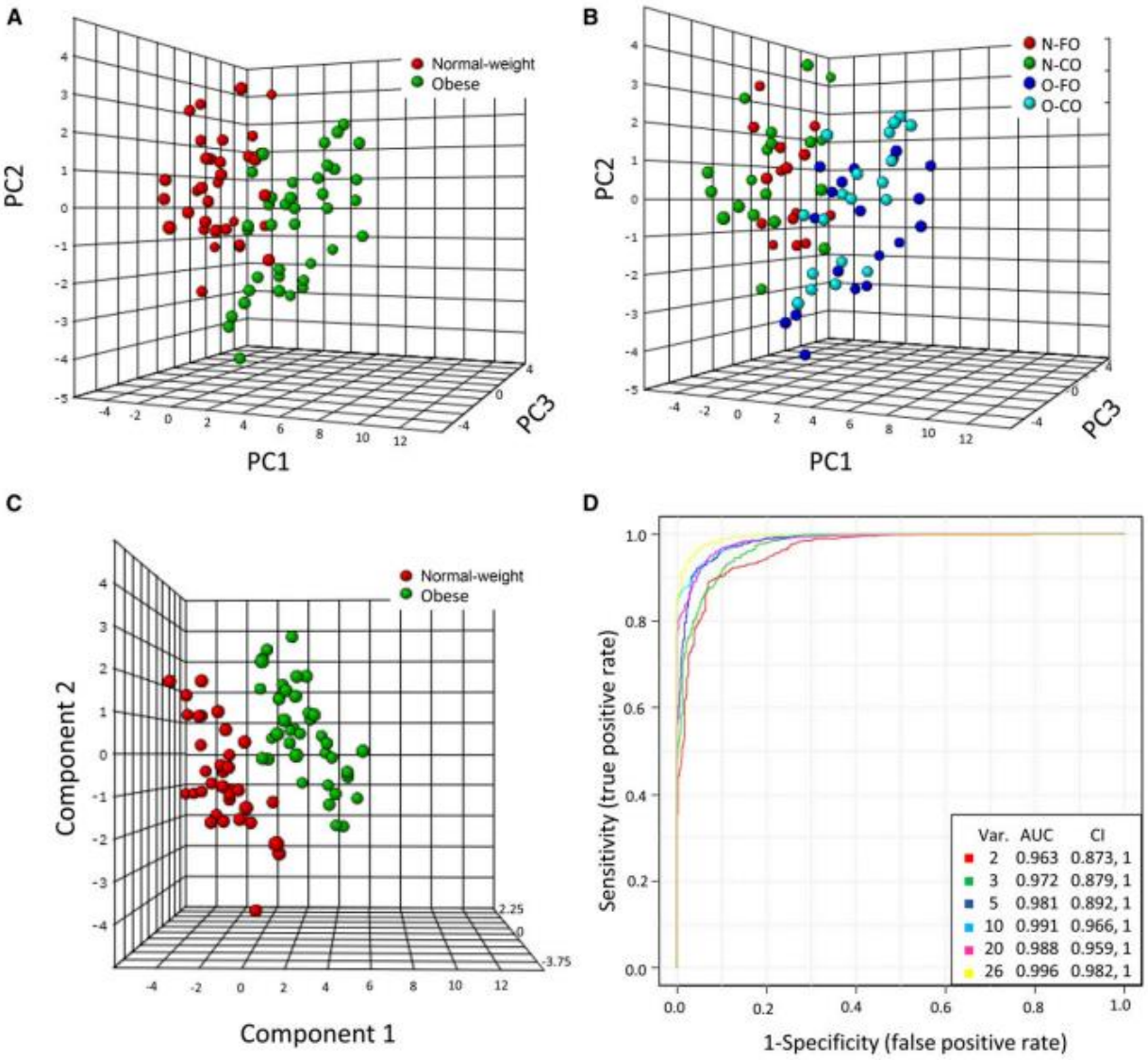
18:2	12 ± 1	13 ± 2	Unchanged	12 ± 1	16 ± 1	
20:3	32 ± 2a	27 ± 2b,c	Unchanged	24 ± 1b	30 ± 1a,c	**
20:4	48 ± 3	44 ± 4	Unchanged	41 ± 1	54 ± 4	

675 *Values are means ± SEMs. Cells were maintained for 24 h with vehicle (media supplemented with
676 bovine serum albumin) or increasing concentrations of bovine serum albumin–bound palmitate (0.2, 0.5,
677 and 0.75 mmol/L). Mean ± SEM of culture medium LPCs, LPEs, and LPIs of 2 different experiments run
678 in duplicate. *Significantly different from vehicle, $P < 0.05$ (Student's t test). **P < 0.05 for 1-factor*
679 *ANOVA. Superscript letters denote significantly different groups as identified by Fisher's least-significant*
680 *difference post hoc test. LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI,*
681 *lysophosphatidylinositol; ND, metabolite not detected in human plasma. Observed change in overweight*
682 *subjects compared with normal-weight subjects as reported in Table 2.*

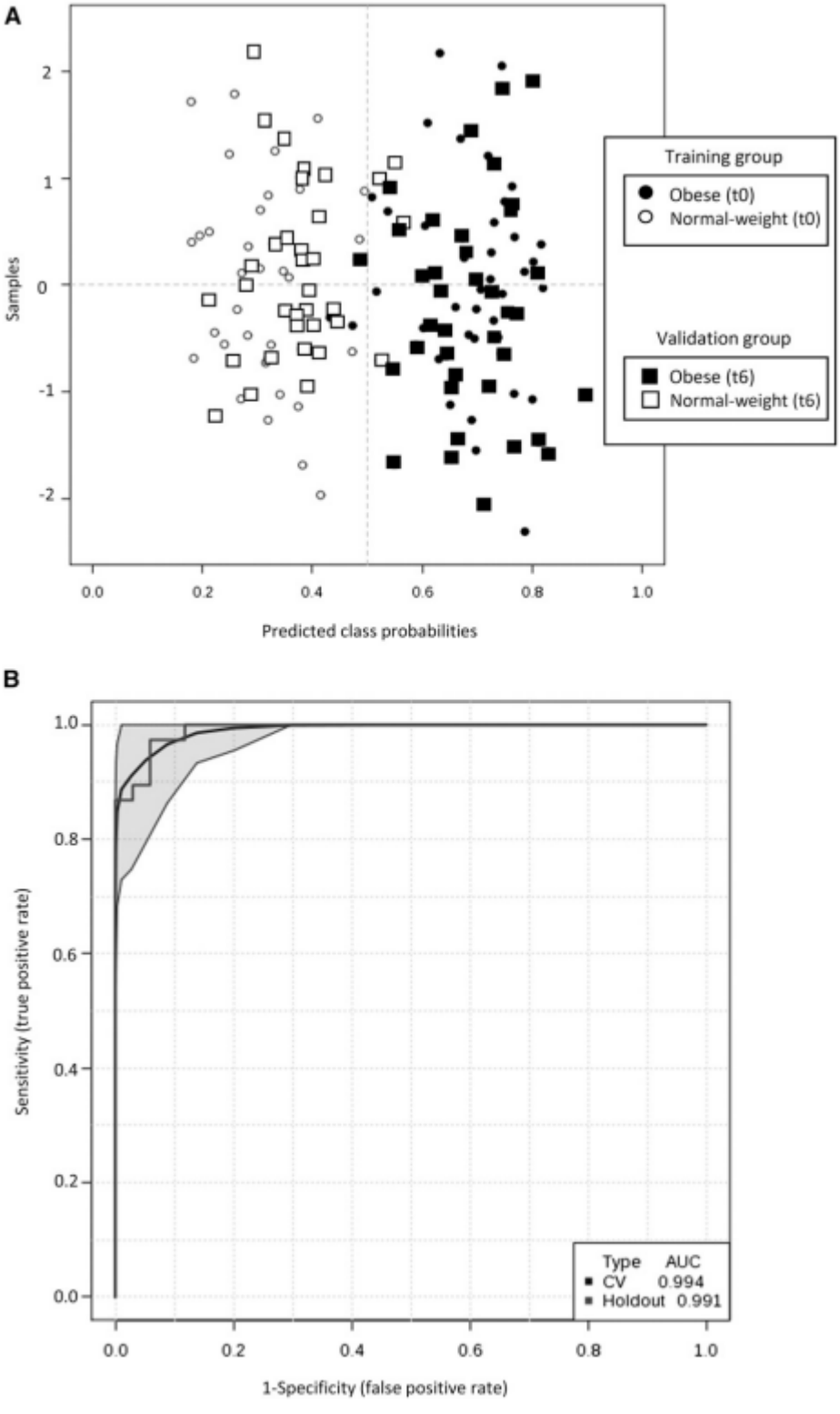
683

Figures

FIGURE 1



688 FIGURE 2



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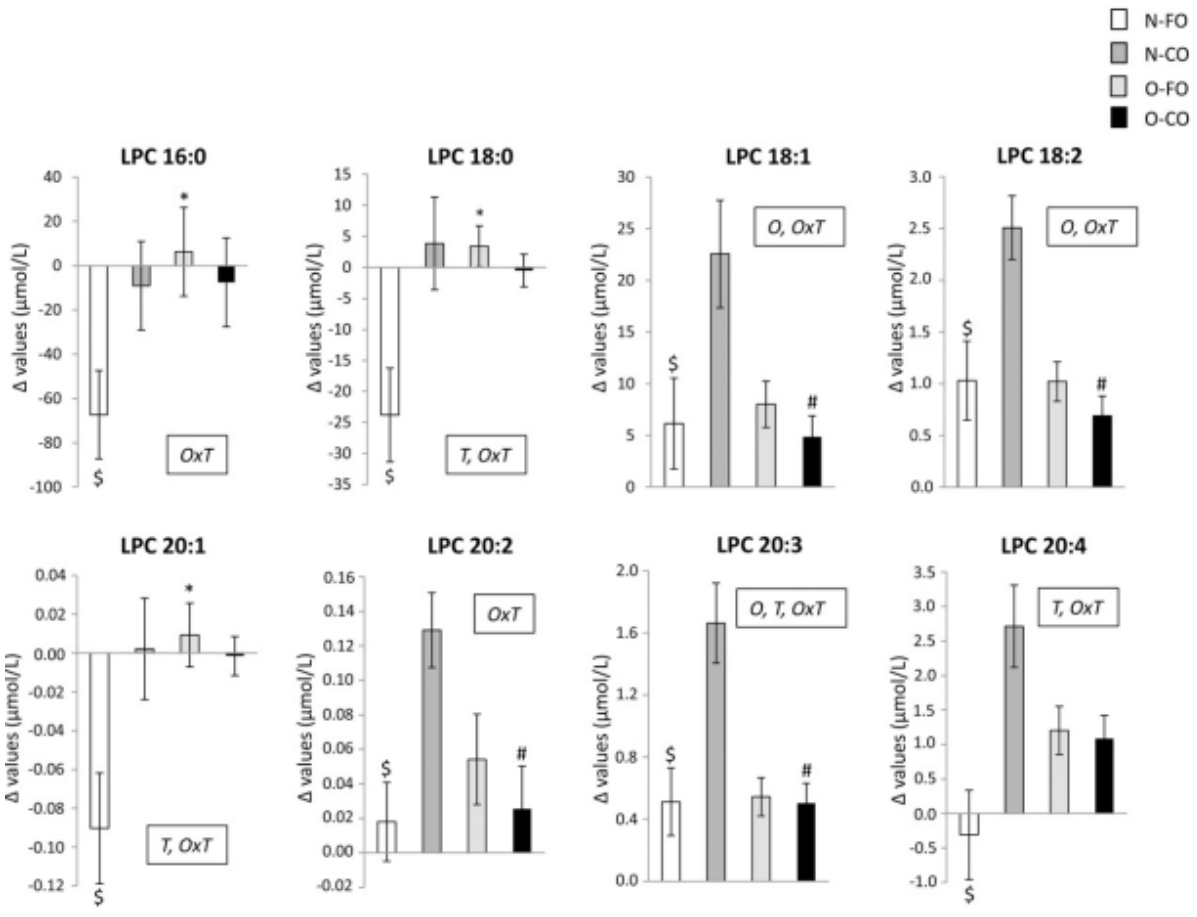
690 FIGURE 3



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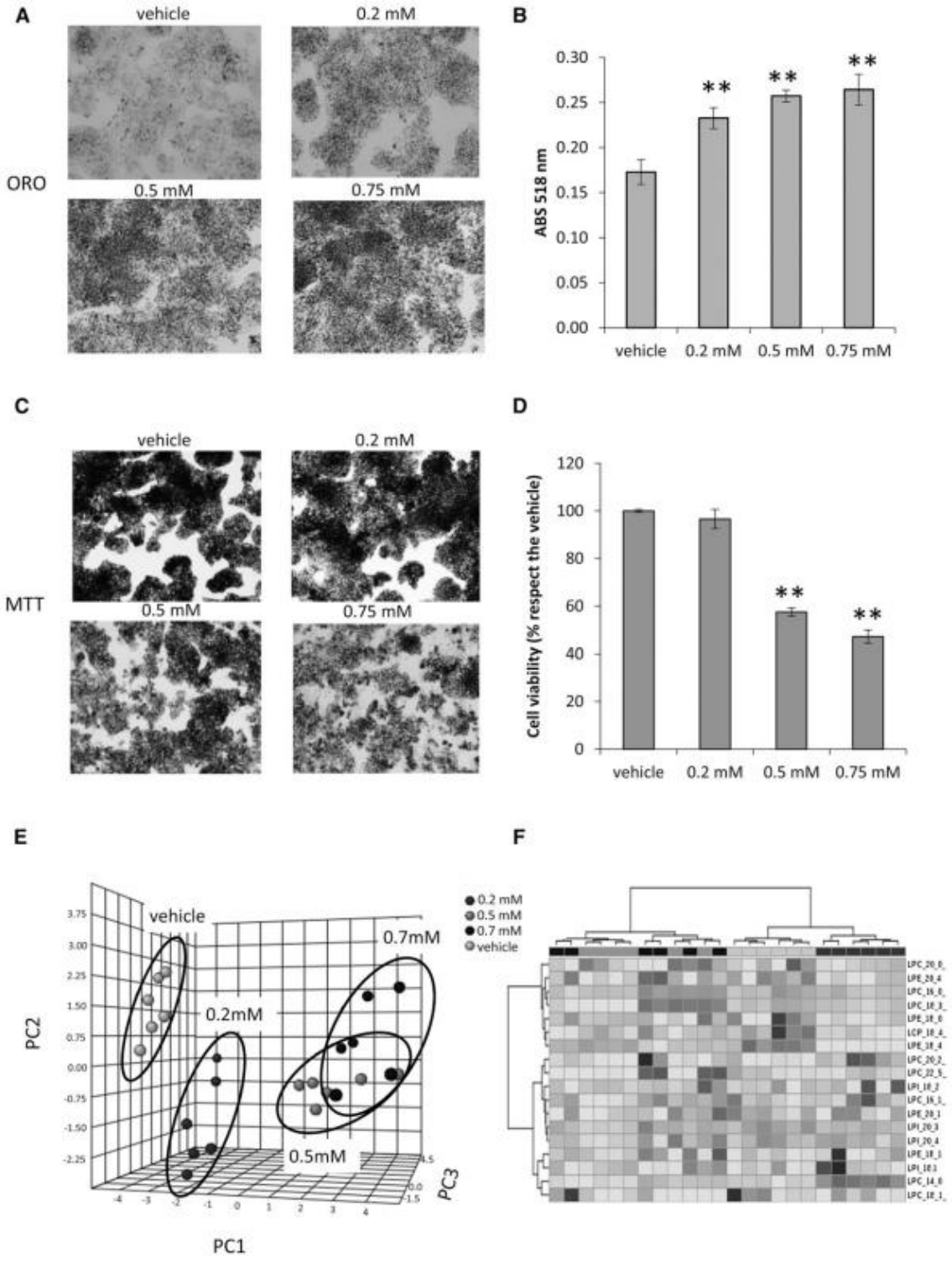
693 FIGURE 4



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Online Supplemental Material

Supplementary Table 1. Changes from baseline in plasma lysophospholipids in normoweight and obese subjects treated with 3 grams of corn oil or fish oil for 90 days and submitted to a lipid-rich meal challenge of six hours

Metabolite (Δ t6-t0)	N-FO	O-FO	N-CO	O-CO	ANCOVA
LPC					
LPC 16:0	-67.3 ± 17.0$	6.24 ± 15.0*	-9.10 ± 17.7	-7.58 ± 8.87	*OxT*
LPC 18:0	-23.8 ± 7.57$	3.39 ± 3.33*	3.85 ± 7.48	-0.511 ± 2.63	*T, OxT*
LPC 18:1	6.17 ± 4.39$	8.02 ± 2.24	22.6 ± 5.2	4.88 ± 2.00#	*O, OxT*
LPC 18:2	1.03 ± 0.38$	1.02 ± 0.19	2.51 ± 0.31	0.69 ± 0.19#	*O, OxT*
LPC 20:1	-0.090 ± 0.029$	0.009 ± 0.016*	0.002 ± 0.026	-0.002 ± 0.010	*T, OxT*
LPC 20:2	0.018 ± 0.023$	0.054 ± 0.026	0.129 ± 0.022	0.025 ± 0.025#	*OxT*
LPC 20:3	0.51 ± 0.22$	0.54 ± 0.12	1.66 ± 0.26	0.50 ± 0.13#	*O, T, OxT*
LPC 20:4	-0.31 ± 0.65$	1.20 ± 0.35	2.71 ± 0.60	1.09 ± 0.34	*T, OxT*
LPE					
LPE 14:1	-0.031 ± 0.028	-0.003 ± 0.016	0.041 ± 0.035	0.021 ± 0.016	
LPE 16:0	0.006 ± 0.006	-0.004 ± 0.005	0.007 ± 0.008	0.010 ± 0.006	
LPE 18:0	0.019 ± 0.025	0.033 ± 0.019	0.089 ± 0.046	0.004 ± 0.018	
LPE 18:1	0.375 ± 0.060	0.194 ± 0.027	0.403 ± 0.077	0.143 ± 0.022	*O*
LPE 18:2	0.780 ± 0.132	0.408 ± 0.045	0.880 ± 0.150	0.347 ± 0.041	*O*
LPE 20:0	-0.010 ± 0.032	0.001 ± 0.024	0.070 ± 0.035	-0.010 ± 0.029	
LPE 20:2	0.640 ± 0.127	0.364 ± 0.050	0.722 ± 0.167	0.271 ± 0.042	*O*
LPE 20:4	0.218 ± 0.033	0.144 ± 0.012	0.346 ± 0.060	0.110 ± 0.024#	*O, OxT*
LPE 20:5	0.144 ± 0.056	0.017 ± 0.019	0.099± 0.034	0.035 ± 0.023	
LPE 22:5	0.021 ± 0.005	0.013 ± 0.002	0.031 ± 0.007	0.008 ± 0.005	*O*
LPE 22:6	0.041 ± 0.027	0.024 ± 0.010	0.101 ± 0.028	0.017 ± 0.015	*O*
LPI					
LPI 16:1	0.010 ± 0.017	-0.006 ± 0.008	0.007 ± 0.017	0.007 ± 0.008	
LPI 18:0	0.008 ± 0.005	0.008 ± 0.005	0.020 ± 0.004	0.009 ± 0.005	
LPI 18:1	0.054 ± 0.009	0.028 ± 0.004	0.051 ± 0.014	0.029 ± 0.005	*O*
LPI 18:2	0.051 ± 0.010	0.035 ± 0.007	0.047 ± 0.012	0.021 ± 0.007	*O*
LPI 20:3	0.005 ± 0.003	0.004 ± 0.002	0.010 ± 0.005	0.003 ± 0.003	
LPI 20:4	0.072 ± 0.012	0.062 ± 0.009	0.077 ± 0.016	0.051 ± 0.012	
LPI 22:6	0.057 ± 0.009	0.030 ± 0.004	0.054 ± 0.015	0.031 ± 0.005	*O*

Blood was collected after overnight fasting and again 6 hours after a high fat meal challenge. The data are given as the mean ± SEM difference (n = 15-19). The effects of treatment, obesity and their interaction were evaluated by ANCOVA. Changes from

baseline in plasma lysophospholipids were used as dependent variables, obesity (O) and n-3 PUFA treatment (T) were included as fixed factors and the variables age and sex were included as covariates. *O*: the effect of obesity; *T*: the effect of treatment; *O*x*T*, the interaction between obesity and treatment (ANCOVA, $p<0.05$). * Effect of obesity within FO groups; # effect of obesity within CO groups; $ effect of treatment within normoweight groups (Bonferroni *post hoc* comparison, $p<0.05$). N-FO: normoweight subjects treated with fish oil; O-FO: obese subjects treated with fish oil. N-CO: normoweight subjects treated with corn oil; O-CO: obese subjects treated with corn oil.

Online Supplemental Material

Supplementary Figure 1: Schematic of study design