Evaluation of Two Highly Effective Lipid-Lowering Therapies in Subjects With Acute Myocardial Infarction: A Lipidomic Analysis

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Research Article

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Abstract

Introduction For cardiovascular disease prevention, statins alone or combined with ezetimibe have been recommended to achieve low density lipoprotein - cholesterol targets, but their effects on other lipids are less reported.

Objective To examine lipid changes in subjects with ST-segment elevation myocardial infarction (STEMI) after two highly effective lipid-lowering therapies.

Methods Twenty patients with STEMI were randomized to be treated with rosuvastatin 20 mg qd or simvastatin 40 mg combined with ezetimibe 10 mg qd during 30 days. Fasting blood samples were collected in the first day (D1) and after 30 days (D30). Lipidomic analysis was performed using the Lipidyzer platform. Selected reaction monitoring (SRM) was used in positive and negative ionization modes with and without differential mobility spectrometry (DMS). Univariate and multivariate analyses were performed.

Results Comparable classic lipid profile was observed in both groups of lipid-lowering therapies at D1 and after treatments. However, differences in other lipids were observed between groups at these time points. After treatments, main differences between groups were for lysophosphatidylcholine and triacylglycerides.

Conclusion Despite similar changes in the classic lipid profile, differences in lipidomic analysis were found before and after exposure to highly effective lipid-lowering therapies in subjects after STEMI.

Trial registration: ClinicalTrials.gov, NCT02428374, registered on 28/09/2014.

Introduction

Therapies aiming to reduce LDL-C substantially changed the natural history of cardiovascular disease (CVD), especially coronary heart disease (CHD) \(^1\). However, despite the achievement of very low levels of LDL-C, recurrent events after acute coronary syndromes are still observed suggesting that other lipid components, including FFAs may contribute to the atherothrombotic disease \(^2\), \(^3\), \(^4\). Interestingly, a prospective cohort of individuals with peripheral artery disease showed that the occurrence of myocardial infarction was inversely related to baseline serum concentrations of phosphatidylcholine (PC) species \(^5\). In addition, decreased plasma levels of plasmalogens were reported in subjects with CHD \(^6\). In fact, their role in atherosclerosis is still poorly understood, involving possibly antioxidant properties that impact the movement of molecules in and out of the cells, as plasmalogens are components of cell membranes\(^7\).

High free fat acids (FFA) concentrations can induce activation of NLRP3 inflammasome, triggering a pro-inflammatory response related to atherosclerosis, and lipid-lowering therapies combinations, such as statin combined with the inhibitor of intestinal cholesterol absorption (simvastatin plus ezetimibe), can partially revert many inflammatory biomarkers\(^8\), \(^9\), \(^10\). Following rosuvastatin treatment, a lipidomic study revealed significant decrease in sphingomyelin (SM), triglycerides (Tg), phosphatidylinositol (PI) and
phosphatidylethanolamines (PE) levels, but for lysophosphatidylcholines (LPC) and phosphatidylycerolines (PC), no significant changes were reported\textsuperscript{11}. Despite effective achievement of LDL-C and non HDL-C targets by the use of less potent statin combined with ezetimibe, their effects in other lipids are less reported. Therefore, this study aimed to compare lipid composition in the plasma of subjects with very high cardiovascular risk with STEMI at baseline and after 30 days of exposure to two highly effective lipid-lowering therapies (rosuvastatin alone or simvastatin combined with ezetimibe).

### Methods

6.1 Reagents and standards

Methanol, 1-propanol and dichloromethane in HPLC grade were purchased from JTBAKER (Avantor Performance Materials, Mexico, Mexico). Water was purified by the Milli Q system (Millipore Waters, Darmstadt, Germany). Ammonium acetate was obtained from Sigma-Aldrich (Saint Louis, MO, USA). The Lipidyzer isotope labeled internal standards mixture kit consisting of 54 isotopes from 13 lipid classes (LPC, lysophosphatidylethanolamines (LPE), PC, PE, Sphingomyelin (SM), diacylglycerols (DAG), TAG, FFA, Cholesterol ester (CE), Ceramide (CER); dihydroceramides (DCER), hexosylceramides (HCER), lactosylceramides (LCER) was purchased from Sciex (Framingham, MA, USA).

6.2 Study design

This prospective, randomized, open label study was delineated to evaluate differences in the composition of lipids in patients with STEMI at D1 and at D30 after implementing the two lipid-lowering therapies (rosuvastatin 20 mg [Crestor®, AstraZeneca] or simvastatin 40 mg combined with ezetimibe10 mg [Vytorin, MSD]). Patients were randomized using a central computerized system. Plasma of patients were categorized in four groups: patients in the rosuvastatin group at D1 (G1) and at D30 (G2); patients treated with simvastatin plus ezetimibe at D1 (G3) and at D30 (G4). Investigators involved in the biochemical and lipidomic analyses were unaware of the lipid-lowering treatment.

6.3 Cohort

The study included mainly middle-aged males, approximately half of them with type 2 diabetes. Table S1 shows main characteristics of study population. The cohort is part of the B And T Types of Lymphocytes Evaluation in Acute Myocardial Infarction (BATTLE-AMI) study\textsuperscript{39} (ClinicalTrials.gov, NCT02428374, registered on 28/09/2014). They had no prior MI and were naive for lipid-lowering treatment. All patients were submitted to pharmacological thrombolysis with tenecteplase in the first 6 h of STEMI, followed by coronary angiogram and percutaneous intervention when needed in the first 24 h of STEMI (pharmacoinvasive strategy). Key exclusion criteria included hemodynamic instability, autoimune disease, known malignancy, pregnancy and signs of active infections. The same sample size of the BATTLE-AMI trial was previsouly published\textsuperscript{39} and, for this sub analysis, a convenient sample was used. The study protocol was approved by the local ethics committee (Escola Paulista de Medicina – UNIFESP IRB 0297/2014; CAAE: 71652417.3.0000.5505), which follows the Declaration of Helsinki, and written
informed consent was provided by all subjects before their inclusion. Fasting blood samples were collected, in the morning at D1 and at D30 after lipid-lowering therapy, in tubes containing EDTA, followed by centrifugation at 1300 g for 15 min, at room temperature and storage at -80 °C before analysis.

6.4 Clinical measurements

All samples for general biochemical tests, including the classic lipid profile were performed in the Central Laboratory of the University Hospital and the LDL-C was estimated by the Friedewald equation.

6.5 Sample preparation

Exactly 100 μL of plasma were transferred to a borosilicate glass culture tube (16 × 100 mm). Next, 900 μL water, 2 mL methanol, and 900 μL dichloromethane were added to all samples and the mixture was vortexed for 5 s. Samples were left to incubate at room temperature for 30 min. Next, another 1 mL water and 900 μL dichloromethane were added to the tube, followed by gentle vortexing for 5 s, and centrifugation at 2500 g at 15 °C for 10 min. The bottom organic layer was transferred to a new tube and 1.8 mL dichloromethane were added to the original tube for a second extraction. The combined extracts were concentrated under nitrogen. Exactly 100 μL of the isotope labeled internal standards mixture were added to the dried extract and another 30 min incubation was allowed until equilibrium is reached. Finally, 250 μL mobile phase solution (10 mmol L⁻¹ ammonium acetate in 50:50 methanol:dichloromethane) were added.

6.6 Sample analysis

Quantitative lipidomics was performed with the Sciex Lipidyzer platform configured by an ExionLC AD instrument (Sciex) coupled to a QTRAP® 5500 mass spectrometer (Sciex) equipped with SelexION® for differential mobility spectrometry (DMS). The solvent 1-propanol was used as the chemical modifier for the DMS. Samples were introduced to the mass spectrometer by flow injection at 8 μL min⁻¹. Each sample was injected twice, with the DMS on (PC/PE/LPC/LPE/SM) and off (CE/CER/DAG/DCER/FFA/HCER/LCER/TAG). Lipid molecular species were analysed by selected reaction monitoring (SRM) in both electrospray ionization modes using positive/negative polarity switching. Positive ion mode was used to detect the lipid classes SM/DAG/CE/CER/DCER/HCER/DCER/TAG and the negative ion mode to detect the lipid classes LPE/LPC/PC/PE/FFA. All data obtained from the Lipidyzer Platform were automatically processed in the Lipidomics Workflow Manager (LWM). Signals of all lipids and fatty acids obtained for each sample were quantified with their appropriate internal standards using the Lipidyzer™ platform. A detailed description of the quantitation process can be found in previous references 40,41,42,43,44.

Quality control (QC) consisted of a standard plasma sample was obtained from the Lipidyzer kit. The reconstituted lyophilized plasma was extracted following the procedure described previously. The QC
sample was injected five times at the beginning of the randomized sample batch, every 10 injections and, at the end of the sample batch.

6.7 Data treatment

Box-plot was performed using Minitab 17.0 (Minitab® Statistical Software; https://www.minitab.com/pt-br/). Microsoft Excel 2013 and R 3.5.2 (The R Project for Statistical Computing; https://www.r-project.org/) were used for univariate analysis (S2). Multivariate analysis was performed by using SIMCA 16 (Statistical Software Package, Umetrics, Sweden; http://umetrics.com/product/simca). Correlation graphics were performed by using the followed softwares: MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/), Microsoft Excel 2016 e Phyton 3.6.5 (https://www.python.org/downloads/release/python-365/).

From the original generated table compiling the lipids and FFAs identified, only the ones that had presented concentrations (µmol L\(^{-1}\)) different of zero were used for data treatment (S2). The discriminant lipids of the four groups were obtained from multivariate analysis by OPLS-DA loadings plot (UV- scaling and CV-ANOVA evaluation), by variable importance in projection (VIP) evaluation, and/or univariate analysis by \(t\)-test and \(q\)-value (FPR\(^45,46\) with 95% confidence interval.

Results

2.1 Classic lipid parameters

Prior to the lipidomic study, a classic lipid profile was obtained. Fig. 1 shows the box plots of measurements of total cholesterol (TC), high-density lipoprotein - cholesterol (HDL-C), low-density lipoprotein – cholesterol (LDL-C) and triglycerides (Tg) in each group of samples. As expected mainly changes were observed for TC and LDL-C.

2.2 Lipids and FFAs evaluation

Fig. 2 shows the plasma concentrations of the analyzed lipids, categorized by classes (CE, CER, FFA, LPC, LPE, PC, PE, SM, and TAG) (Fig. 2a) and by groups (G1, G2, G3 and G4) (Fig. 2b). Comparing the mean values in each group, it is possible to observe that all metabolites were decreased in both groups after the exposure to the treatments at D30, except for LPC, LPE and TAG.

Considering rosuvastatin administration at D30, there was a decrease for CE (40%), CER (40%) and SM (36%), PC (31%), PE (26%), and FFA (20%). However, LPC was the only lipid increased (23%) in G2. Following simvastatin plus ezetimibe therapy at D30, there was a decrease in PE (36%), SM (25%), PC (23%), CE (21%), CER (10%) and FFA (11%). LPC, TAG and LPE were almost inalterd in G4. Comparing the two arms of treatment at D1 (G1 and G3), it is possible to observe similar lipid and FFA level reduction in G3, except for CE and CER; CER was 29% less abundant in G3, followed by CE (20% less), SM (14% less), LPE (11% less), FFA (10% less), and PC (7% less). Regarding the effects of treatments at D30 (G2
and G4), LPC and PE were 13% less abundant in the simvastatin plus ezetimibe arm (G4), while TAG was 32% more abundant.

2.3 Correlation studies

Levels of LDL-C, HDL-C, TC, and Tg were correlated with levels of lipid classes (CE, CER, FFA, LPC, LPE, PC, PE, SM, and TAG) for all studied patient groups (Fig. 3). Fig. 3a presents the correlations between the two therapies at D1 of STEMI. Opposite correlations between the clinical parameters and some lipids (PE, TAG, LPE, and CE) were observed.

Considering rosuvastatin administration, PE was negatively correlated with CT, HDL-C and LDL-C and positively correlated with Tg; TAG was negatively correlated with HDL-C, but positively correlated with Tg; LPE was negatively correlated with LDL-C and positively correlated with HDL-C; and CE was positively correlated with Tg. Fig. 3b depicts correlations between the two therapies at D30. Opposite correlations between the clinical parameters and some lipid classes were also observed. However, besides PE, TAG, LPE, and CE, PC, SM, and CER also presented contrasting correlations with the clinical parameters at D30.

Considering simvastatin plus ezetimibe administration, PE was negatively correlated with CT and HDL-C; TAG was negatively correlated with TC and HDL-C, but positively correlated with Tg; LPE was negatively correlated with LDL-C; CE was positively correlated with TC and LDL-C and Tg, but positively correlated with Tg; PC was negatively correlated with CT and HDL-C; SM was negatively correlated with TC, HDL-C and LDL-C; and CER was positively correlated with Tg.

Figure 3 shows different behaviors among some clinical parameters with lipids at D30. For instance, alterations in LDL-C and Tg levels related to PE were not observed at D30 in both lipid-lowering therapies and the same was observed for LDL-C levels regarding LPE.

With rosuvastatin, HDL-C levels related to LPE, which were positively correlated at D1, became negatively correlated at D30. In addition, TC and HDL-C levels related to PC and SM became negatively correlated with CE, as well as LDL-C levels related to SM, whereas, Tg levels related to CT, LDL-C, and LPE became positively correlated. With simvastatin plus ezetimibe at D30, Tg levels related to TC, LDL-C, LPE, PC and SM became positively correlated and with rosuvastatin at D30.

2.4 Identification of statistically significant metabolites

2.4.1 Univariate and multivariate analyses

Data quality was carried out by inspecting the repeatability of lipids and FFAs in the QC plasma sample, analysed throughout data acquisition. More than 80% of the quantified metabolites in the QCs have acceptable coefficient of variation percentages (% CV) for peak areas; in this work, < 20% CV was used as a criterion to retain that particular component in the dataset for further evaluation, which were in agreement to the recomendation\textsuperscript{12,13}.
The OPLS-DA scores plot (Fig. 4) shows that patients of G1, G2, G3 and G4 are clustered and they have distinct lipid profiles from each other ($R^2 = 0.231; Q^2 = 0.246; p = 0.032$). The discriminant variables were obtained by multivariate (VIP > 1) and/or univariate evaluation ($p$-value and $q$-value < 0.05) with 95% confidence interval. These findings with their respective increasing or decreasing percentage related to each studied group are better visualized in Fig 5 and S2. Patients of G1 are clustered due LPC, CE, CER, FFA, PC, PE, and SM alteration levels. LPC(20:4/0:0) presented the largest contribution, with 80% and 74% decrease related to G2 and G4, followed by PE(16:0/18:2), with 57% and 55% increase related to G2 and G4, respectively; SM (d18:1/20:0) with 50% increase related to G2, and 29% and 55% related to G3 and G4, respectively; PC(16:0/18:2) with 48% increase related to G2 and 46% related to G4; PE(18:0/18:2) with 47% increase related to G2 and 45% related to G4 and 45% increased related to G2 and G4 and PC(18:1/20:4) with 41% and 45% decreased related to G2 and G4. The others, PC, PE, CER, SM and FFA presented a range from 7% up to 44% increasing. However, patients of G2 are clustered mainly, because LPC was the metabolite that presented the highest increased levels, being LPC (20:4/0:0) 80% increased related to G1 and 32% related to G3; LPC (17:0/0:0) 56% increased related to G1, 34% related to G3 and 20% related to G4; LPC (16:1/0:0) 52% increased related to G1 and 43% related to G3, and LPC (18:1/0:0) was increased 50% related to G1 and 33% related to G4. Lauric acid (12:0) level slightly increased in G2: 18% related to G1, 22% and 14% related to G3 and G4, respectively, and PC(18:1/20:4) was increased 41% related to G1 and 13% related to G3.

In addition, Figure 4 shows that patients of G3 are clustered due the increased levels in TAG, CE, SM, PC and FFA, being the higher increasing in TAG50:2-FA18:2 and CE (22:5), with increase of 113% and 65% related to G2, respectively. SM (d18:1/16:0) and oleic acid (18:1) presented almost the same contribution, with an increasing of 50% and 47% related to G2, and 34% and 30% related to G4, respectively. The other FFAs and PCs presented an increase range from 2% up to 34%. However, the major increased levels in G4 were observed in TAG and LPC, being the major and similar increased levels in TAG56:5-FA20:4, TAG56:4-FA20:3 and LPC(20:4/0:0), with increase of almost 75%, followed by TAG56:5-FA20:3 and TAG56:5-FA18:0 with an increase of 68% and 64% related to G1, respectively. The other TAGs and PC presented an increase range from 1% up to 57%. SM (d18:1/16:0) decreased, with 30%, 1% and 34%, respectively, related to G1, G2 and G3. Fig. 6 presents the discriminant metabolites and their similarity for each group. LPC(20:4/0:0) and PC(18:1/20:4) levels were similar in G1, G2 and G4. However, SM (d18:1/16:0) levels was similar to G1, G3 and G4.

**Discussion**

This study revealed differences in lipid composition in subjects with STEMI despite similar classic lipid profiles. Even after highly effective lipid-lowering therapies, examining the effects of rosuvastatin or simvastatin plus ezetimibe, promoting similar changes in the classic lipid profile, and substantial alterations in the lipid composition, differences in the some lipid were still observed between these lipid-lowering therapies. These differences were specially noted for LPC, TAG, and LPE. These findings seems
relevant, due to the involvement reported for free fat acids (FFA) in crucial mechanisms of atherosclerosis, such as inflammation and oxidative stress. 

A recent review article addressing CVD with lipidomic analysis showed that CER and PCs containing saturated (SAFA) and monounsaturated (MUFA) fatty acids were related to CVD events, while those containing polyunsaturated fatty acids (PUFA) were inversely associated with cardiovascular outcomes. This observation also suggests a potential benefit, once the increase in FFA concentrations are related to the activation of inflammatory pathways and insulin resistance. Inflammation has been considered as a part of the pathophysiology of acute coronary syndromes and their recurrences. Different FFA can be metabolized into different pro- and anti-inflammatory signaling molecules, in particular, a few n-6 FFA (first and foremost arachidonic acid) are precursors to pro-inflammatory molecules (primarily prostaglandins). SAFAs act as major inducers of inflammation through several mechanisms, one of these mechanisms involves the activation of toll-like receptor-4 (TLR4), which activates the production of inflammatory cytokines (IL-1β; IL-6). FFA and their esters are the major sources of energy for the heart muscle. However, an excess of FFA has profound effects on the heart causing an enhanced susceptibility to oxidative stress and ischemic damage (fibrosis and hypertrophy). Endothelial dysfunction due to the NF-kappa B activation are also induced by SAFA resulting in increased superoxide production, while NLRP3 inflammation activation increases endothelial permeability.

In our study, in the two arms of therapy, higher concentrations of SAFA, MUFA, PUFA, and PC levels were observed at D1. However, PC and FFA levels were decreased after treatment, in G2 and G4. These results suggest a potential beneficial effect on CVD. Interestingly, our findings demonstrated that FFAs were positively correlated to PC in all groups, but less positively correlated in G2. However, the reasons for different implications of PCs according to the types of FA (MUFA, SAFA or PUFA) on CVD are still poorly understood, the same for their effects on the metabolism of lipoproteins.

The literature reports that LPC, formed by hydrolysis of PC, is related to atherosclerosis development. In our study, LPC(20:4/0:0) and PC(18:1/20:4) were significantly altered after treatment (G2 and G4). Choi et al. reported that LPC and PC presented large differences in drug response. The hydrolysis of PC to LPC is promoted by phospholipase A2 and rosuvastatin may affect the A2 activity. Thus, the levels of PC and LPC seems related to drug response. The same work reports that LPC, ether-linked, and plasmalogens species of PC were negatively associated with stable coronary heart disease. In patients with stable coronary heart disease, rosuvastatin treatment promoted an increase of PCs, while atorvastatin a decrease.

In our study, interesting results were related to TAG findings. The most discriminant metabolites in G4 were TAGs (S2). Simvastatin plus ezetimibe treatment resulted in an increment of TAG species enriched with long chain FFAs, mainly MUFA and PUFA. These can be due to targeted effects of statin action on lipid metabolism. Differences between rosuvastatin and simvastatin effects on TAG can be explained by the shorter half-life of simvastatin. When TAGs are cleaved, the resulting FFAs are not always directly
taken up by nearby cells. In such cases, these spill-over FFAs bind to serum albumin and are transported through the circulation to other cells \(^2^6\). Another source of plasma FFA is their endogenous synthesis from excess of carbohydrates uptake, in a process termed lipogenesis. After synthesis, FFA are converted in TAG and released by the liver as VLDL particles.

In addition, our study showed that both lipid-lowering therapies decreased SM concentrations at D30. Sphingomyelin was measured in the large Multi-Ethnic Study of Atherosclerosis (MESA), and the authors reported a modest negative association with incident CVDs, after adjustment for lipoproteins and full adjustment for other risk factors \(^2^7\). Higher decrease with rosuvastatin compared with atorvastatin treatments was found in the SM/SM+PC ratio \(^2^8\). Improvement in the SM/PC ratio by statins suggested reduced susceptibility of SM to hydrolysis by sphingomyelinase within the vessel wall. These results corroborate Choi et al. findings, that reported a significant decrease in SM, TAG and PE, especially in PE \((18:0/18:2)\) levels, and an increase in LPC \((20:4/0:0)\) and LPC \((18:1)\) \(^1^1\). In 2018, Lee et al. hypothesized that these increases may be due to pleiotropic effects of statins, since phospholipase A2 is inhibited in response to rosuvastatin \(^2^9\).

Considering the correlation between lipids, FFA, and clinical parameters, a previous study reported that LPC are predominantly found in HDL, CER in LDL, and PC are present in both lipoproteins (HDL and LDL) \(^1^5\). Thus, FFA composition may also be important for the lipoprotein function and metabolism. Studies examining CVD with lipidomics found that after adjusting for HDL-C and LDL-C levels, only PC remained associated with cardiovascular events\(^1^5,3^0,3^1,3^2,3^3,3^4,3^5\). Kolovou et al. observed a correlation of SM, PC, PE, phosphatidylinositol, CEs and Tg in subjects with obesity, and a positive correlation between SM and LDL-C, and between LPC and VLDL-C, among subjects with familial hypercholesterolemia. In addition, the same lipidomic study demonstrated that every lipoprotein class was associated with a particular arrangement of lipids (LDL-C with CER and SM; HDL-C with PC, PE, and PE based plasmalogens)\(^2^4\). Some findings in this work were in agreement with previous reports; PC and LPC were positively correlated to HDL-C at for both lipid-lowering therapies, respectively, at D1 and D30. SM and CER were positively correlated to LDL-C, but CER only at D1 in G1. FFA was positively correlated to HDL-C, LDL-C and CT for both lowering therapies. Depending on the therapy, the correlations between PC and SM with HDL-C were different at D30. Same results were found for CE, PC, SM and TAG with CT.

Finally, regarding to the changes in the classic lipid profile, our results are in agreement with previous studies\(^3^6,2^9,3^7,1^1,3^8\). There are no direct comparisons between rosuvastatin and simvastatin plus ezetimibe in cardiovascular outcomes.

**Limitations**

This study compared two highly effective lipid-lowering therapies in the acute phase of myocardial infarction. However, we are unable to estimate the effects of the acute myocardial infarction per se on lipids and FA composition. It is expected some decrease in lipids due to the healing process involving the necrotic and ischemic myocardium, but these changes have been reported as insignificant in the
following days after the acute coronary event. Our sample size is relatively small, but the patients included in the study were all submitted to same treatment strategy (pharmacoinvasive) with similar characteristics at baseline. All subjects included in this study were fully compliant to the study drug and no harm related to the lipid-lowering therapy was observed. There were no follow-up losses.

**Conclusions**

In spite of comparable classic lipid profile at baseline and after the exposure to the treatments, significant differences in the lipid composition were found following between two highly effective lipid-lowering therapies. The main differences were higher levels of TAG with simvastatin plus ezetimibe therapy and increased levels of LPC following rosvuastatin therapy.

**Declarations**

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**Author contributions** AK conducted data treatment, prepared and revised the manuscript; AS analyzed data and revised the manuscript; ECSC conducted correlation data treatment; FAHF designed study, prepared and revised the manuscript and, acquired funding for It; MCI prepared and revised the manuscript; AMFN helped with the research design, revised the manuscript and provided financial support; MFMT analyzed data and revised the manuscript; PBMCD designed study, conducted experiments and revised the manuscript; CESF designed study; RTB helps with data treatment; SCF helps with data treatment; ATF helps with data treatment; ASL analyzed data and revised the manuscript.

**Competing Interests**

The authors declare that they have no competing interest.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Data availability statement**
We state that all the data generated or analysed during this study are included in this published article and in its Supplementary Information files (S2).

References

1. Baigent, C. et al. Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170 000 participants in 26 randomised trials. *Lancet* (2010) doi:10.1016/S0140-6736(10)61350-5.

2. Schwartz, G. G. et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N. Engl. J. Med.* (2018) doi:10.1056/NEJMo1801174.

3. Schwartz, G. G. Effects of Atorvastatin on Early Recurrent Ischemic Events in Acute Coronary Syndromes <SUBTITLE>The MIRACL Study: A Randomized Controlled Trial</SUBTITLE>. *JAMA* (2001) doi:10.1001/jama.285.13.1711.

4. Cannon, C. P. et al. Ezetimibe added to statin therapy after acute coronary syndromes. *N. Engl. J. Med.* 372, (2015).

5. Moxon, J. V. et al. Baseline serum phosphatidylcholine plasmalogen concentrations are inversely associated with incident myocardial infarction in patients with mixed peripheral artery disease presentations. *Atherosclerosis* 263, 301–308 (2017).

6. Sutter, I. et al. Decreased phosphatidylcholine plasmalogens - A putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction. *Atherosclerosis* (2016) doi:10.1016/j.atherosclerosis.2016.01.003.

7. Koivuniemi, A. The biophysical properties of plasmalogens originating from their unique molecular architecture. *FEBS Letters* (2017) doi:10.1002/1873-3468.12754.

8. Wang, X., Huang, H., Su, C., Zhong, Q. & Wu, G. Cilostazol ameliorates high free fatty acid (FFA)- induced activation of NLRP3 inflammasome in human vascular endothelial cells. *Artif. Cells, Nanomedicine Biotechnol.* 47, 3704–3710 (2019).

9. Duewell, P. et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* (2010) doi:10.1038/nature08938.

10. Ghanim, H. et al. Ezetimibe and simvastatin combination inhibits and reverses the pro-inflammatory and pro-atherogenic effects of cream in obese patients. *Atherosclerosis* (2017) doi:10.1016/j.atherosclerosis.2017.06.010.

11. Choi, J. M., Kim, T. E., Cho, J. Y., Lee, H. J. & Jung, B. H. Development of lipidomic platform and phosphatidylcholine retention time index for lipid profiling of rosuvastatin treated human plasma. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 944, 157–165 (2014).

12. Gika, H. G., Macpherson, E., Theodoridis, G. A. & Wilson, I. D. Evaluation of the repeatability of ultra-performance liquid chromatography-TOF-MS for global metabolic profiling of human urine samples. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* (2008) doi:10.1016/j.jchromb.2008.05.048.
13. U.S. Department of Health and Human Services. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. FDA (2001).

14. Jiao, P. et al. FFA-induced adipocyte inflammation and insulin resistance: Involvement of ER stress and IKKβ pathways. Obesity (2011) doi:10.1038/oby.2010.200.

15. Ding, M. & Rexrode, K. M. A review of lipidomics of cardiovascular disease highlights the importance of isolating lipoproteins. Metabolites (2020) doi:10.3390/metabo10040163.

16. Sobczak, A. I. S., Blindauer, C. A. & Stewart, A. J. Changes in plasma free fatty acids associated with type-2 diabetes. Nutrients vol. 11 (2019).

17. Libby, P. Mechanisms of acute coronary syndromes and their implications for therapy. N. Engl. J. Med. (2013) doi:10.1056/NEJMa1216063.

18. Ridker, P. M. et al. Modulation of the interleukin-6 signalling pathway and incidence rates of atherosclerotic events and all-cause mortality: Analyses from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). Eur. Heart J. (2018) doi:10.1093/eurheartj/ehy310.

19. Rogero, M. M. & Calder, P. C. Obesity, inflammation, toll-like receptor 4 and fatty acids. Nutrients 10, 1–19 (2018).

20. Ghosh, A., Gao, L., Thakur, A., Siu, P. M. & Lai, C. W. K. Role of free fatty acids in endothelial dysfunction. Journal of Biomedical Science (2017) doi:10.1186/s12929-017-0357-5.

21. Carpentier, A. C. Abnormal Myocardial Dietary Fatty Acid Metabolism and Diabetic Cardiomyopathy. Canadian Journal of Cardiology (2018) doi:10.1016/j.cjca.2017.12.029.

22. Matsumoto, T., Kobayashi, T. & Kamata, K. Role of Lysophosphatidylcholine (LPC) in Atherosclerosis. Curr. Med. Chem. (2007) doi:10.2174/092986707782793899.

23. Tanaka, H. et al. Lysophosphatidylcholine Acyltransferase-3 Expression Is Associated with Atherosclerosis Progression. J. Vasc. Res. (2017) doi:10.1159/000473879.

24. Kolovou, G., Kolovou, V. & MAVROGENI, S. Lipidomics in vascular health: Current perspectives. Vascular Health and Risk Management (2015) doi:10.2147/VHRM.S54874.

25. Meikle, P. J. et al. Statin action favors normalization of the plasma lipidome in the atherogenic mixed dyslipidemia of MetS: Potential relevance to statin-associated dysglycemia. J. Lipid Res. (2015) doi:10.1194/jlr.P061143.

26. Teusink, B. et al. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes (2003) doi:10.2337/diabetes.52.3.614.

27. Yeboah, J. et al. Association of plasma sphingomyelin levels and incident coronary heart disease events in an adult population: Multi-ethnic study of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. (2010) doi:10.1161/ATVBAHA.109.199281.

28. Bergheanu, S. C. et al. Lipidomic approach to evaluate rosuvastatin and atorvastatin at various dosages: Investigating differential effects among statins. Curr. Med. Res. Opin. (2008) doi:10.1185/03007990802321709.
29. Lee, H. et al. Regulation of endogenic metabolites by rosuvastatin in hyperlipidemia patients: An integration of metabolomics and lipidomics. Chemistry and Physics of Lipids vol. 214 (Elsevier Ireland Ltd, 2018).

30. Sigruener, A. et al. Glycerophospholipid and sphingolipid species and mortality: The Ludwigshafen risk and cardiovascular health (LURIC) study. PLoS One (2014) doi:10.1371/journal.pone.0085724.

31. Alshehry, Z. H. et al. Plasma Lipidomic Profiles Improve on Traditional Risk Factors for the Prediction of Cardiovascular Events in Type 2 Diabetes Mellitus. Circulation (2016) doi:10.1161/CIRCULATIONAHA.116.023233.

32. Mundra, P. A. et al. Large-scale plasma lipidomic profiling identifies lipids that predict cardiovascular events in secondary prevention. JCI insight (2018) doi:10.1172/jci.insight.121326.

33. Cheng, J. M. et al. Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: Results of the ATHEROREMO-IVUS study. Atherosclerosis (2015) doi:10.1016/j.atherosclerosis.2015.10.022.

34. Anroedh, S. et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. J. Lipid Res. (2018) doi:10.1194/jlr.P081281.

35. Laaksonen, R. et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. Eur. Heart J. (2016) doi:10.1093/eurheartj/ehw148.

36. Catapano, A. L. et al. Lipid-altering efficacy of the ezetimibe/simvastatin single tablet versus rosuvastatin in hypercholesterolemic patients. Curr. Med. Res. Opin. 22, 2041–2053 (2006).

37. Malone, J. K., Kerr, L. F., Campagne, B. N., Sachson, R. A. & Holcombe, J. H. Combined therapy with insulin lispro mix 75/25 plus metformin or insulin glargine plus metformin: A 16-week, randomized, open-label, crossover study in patients with type 2 diabetes beginning insulin therapy. Clin. Ther. 26, 2034–2044 (2004).

38. Pitt, B., Loscalzo, J., Yčas, J. & Raichlen, J. S. Lipid Levels After Acute Coronary Syndromes. J. Am. Coll. Cardiol. (2008) doi:10.1016/j.jacc.2007.11.075.

39. Fonseca, F. A. H. et al. Effects of four antiplatelet/statin combined strategies on immune and inflammatory responses in patients with acute myocardial infarction undergoing pharmacoinvasive strategy: Design and rationale of the B and T Types of Lymphocytes Evaluation in Acute My. Trials 18, (2017).

40. Cao, Z. et al. Evaluation of the Performance of Lipidyzer Platform and Its Application in the Lipidomics Analysis in Mouse Heart and Liver. J. Proteome Res. 19, 2742–2749 (2020).

41. Contrepois, K. et al. Cross-Platform Comparison of Untargeted and Targeted Lipidomics Approaches on Aging Mouse Plasma. Sci. Rep. (2018) doi:10.1038/s41598-018-35807-4.

42. Quell, J. D. et al. Characterization of bulk phosphatidylcholine compositions in human Plasma using Side-Chain resolving lipidomics. Metabolites 9, (2019).

43. Alarcon-Barrera, J. C. et al. Lipid metabolism of leukocytes in the unstimulated and activated states. Anal. Bioanal. Chem. 412, 2353–2363 (2020).
44. Baljit K. Ubhi. Direct Infusion-Tandem Mass Spectrometry (DI-MS/MS) Analysis of Complex Lipids in Human Plasma and Serum Using the Lipidyzer™ Platform. *Clin. Metabolomics* **1730**, 227–236 (2018).

45. Benjamini, Y. Discovering the false discovery rate. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **72**, 405–416 (2010).

46. Saccenti, E., Hoefsloot, H. C. J., Smilde, A. K., Westerhuis, J. A. & Hendriks, M. M. W. B. Reflections on univariate and multivariate analysis of metabolomics data. *Metabolomics* **10**, 361–374 (2014).